Henryk Dancygier

# Clinical Hepatology

## Principles and Practice of Hepatobiliary Diseases

Volume 2

*Foreword by*
Scott L. Friedman

Springer

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## Principles and Practice of Hepatobiliary Diseases

## Volume 2

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# Dedication

*Dedicated to Dr. Herbert Falk (1924–2008).*

*A humanist of great generosity, supporter of scientific advancement and the always visible, but never dominant magnanimous patron of global medical education.*

# Foreword

Modern hepatology would be unrecognizable to those clinicians and pathologists, who founded the field only a generation ago. From a discipline that was largely observational – with few diagnostic tests and even fewer therapies – has emerged an exciting area that is among the most rapidly changing in all of medicine, and which now offers remarkable new tools and treatments. This textbook edited by Professor Dancygier beautifully captures this dynamism of hepatology, and in doing so provides a remarkably complete opus. The work is beautifully laid out exactly as a clinician would think, weaving in the science underlying clinical hepatology with precision and clarity. Standardized and gorgeous drawings, comprehensive tables, and a very consistent style are among its most valuable assets – quite simply, this book is fun to read!

While thoroughly modern, this work still proudly draws upon the roots of our specialty. A strong emphasis on pathology, patterns of injury, clinical presentations of disease, and approaches to clinical problems harken back to hepatology's earliest treatises. The book is particularly reminiscent of early editions of Dame Sheila Sherlock's classic single-author textbook, the fifth edition of which I read cover-to-cover while spending 3 months at the Royal Free Hospital as a medical student in 1977; a signed copy sits proudly on my shelf to this day, and I suspect that many will come to value Professor Dancygier's book for many of its similar virtues. The highly personal stamp of Professor Dancygier infuses this book with cohesion, and conveys the wonders of clinical hepatology. Underscoring another enduring feature of our specialty is the book's transatlantic flavor, with authors from throughout Germany and the USA. This connection between our two countries is also personal – Professor Dancygier's invitation for me to speak at a conference in Munich in 1989 was my first international meeting, and I am proud that our professional association and friendship continue to this day as a result of that first meeting 20 years ago.

I am delighted to be associated with *Clinical Hepatology.* Thus, it is a great personal privilege to introduce this unique and valuable textbook, which is sure to appeal to practitioners of today and ignite a spark of enthusiasm among the hepatologists of tomorrow.

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# Preface

Clinical hepatology is thriving. Hepatology has evolved from a pure diagnostic art to a science in which new treatment options are emerging at a quick pace. At this exciting time, based on the gratifying success of the German Edition of 2003, Springer Publishers have asked me to prepare a US-American/International edition. Gravely miscalculating the amount of labor, I gladly accepted this challenge.

The present book is not merely an updated version of the German edition but a completely new work with new US-American authors, a transatlantic endeavor. In common with its German predecessor, however, it not only aims to provide knowledge in hepatology but also to promote an understanding of liver diseases, and to create joy in dealing with clinical hepatological problems. It is intended for everybody caring for adult patients with hepatobiliary problems, particularly gastroenterologists/hepatologists, internists and clinical pathologists.

My aim was not to provide an encyclopedic behemoth. Instead, I intended to create a comprehensive, up-to-date (references until early 2009 are included), practical and readable book that outlines the current standards of diagnosis and treatment in hepatology and its associated biliary disorders. By elaborating on concepts in hepatology, disease mechanisms, common clinical problems and rare diseases alike it tries to serve the needs not only of the novice in hepatology, but also of the experienced practicing clinician. Ultimately, the success of the book will be determined by its ability to provide answers to clinically relevant questions and to guide clinical decisions.

The competent clinical hepatologist, like hardly any other clinician, has to integrate histopathological, biochemical, immunological, instrumental and clinical skills. The organization of the text follows this principle. It is divided into 3 main parts with 30 sections covering Basic Principles, Clinical Methods, and Hepatobiliary Diseases. Starting from basic concepts the field of clinical hepatology gradually unfolds. Unlike other Hepatology Textbooks I preferred not to include a “stand alone” chapter on liver pathology. Instead hepatopathology has been integrated throughout the entire text as it represents an integral part of clinical hepatology. The first five sections integrate structure and function of the liver and basically provide a general pathology of the liver. The clinical chapters are stringently organized and uniformly structured to enable rapid retrieval of the desired information. In order to enhance readability I have accepted some redundancy, especially in chapters dealing with hepatocellular transporters.

I am indebted to my coauthors, all renowned experts in hepatology. Without their help the creation of a textbook of this volume would not have been possible. My

special thanks go to a young gastroenterologist from Yale, Jason Rogart. He not only served as a proof reader for the contributions by authors whose native language is not English, but also as an author of several chapters and as an editorial assistant. My thanks go also to Ms. Annette Hinze, Meike Stoeck, and Mr. Claus-Dieter Bachem from Springer Publishers who skillfully supported the development of the Textbook. Last but not least I thank my wife Hellena for her endurance and unwavering support. After immerging into the project and resurfacing after finishing the last chapter she was still there.

Offenbach, May 2009 Henryk Dancygier

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Part I Structure and Function of the Liver

Part II Pathophysiology and Morphology of Liver Injury

### B. Clinical Methods

Part III Evaluation of the Patient with Hepatobiliary Disease

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Part IV Diseases of the Liver

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# Malformations and Malpositions of the Liver

55

Henryk Dancygier

## Chapter Outline



## Riedel's Lobe

Riedel's lobe is not a true accessory liver lobe, but rather a caudally oriented tongue-like process of the right liver lobe lateral from the gallbladder [3]. The cause of this malformation, more common in women, is unknown. Histologically liver tissue is normal, and clinically this malformation is asymptomatic. Its clinical significance lies in the potential of being misinterpreted as a tumor.

## Accessory Liver Lobe(s)

An accessory liver lobe (*hepar succenturiatum*) is encountered more often on the right side. It is connected to the right liver lobe by a stalk containing blood vessels and bile ducts. The accessory lobe may be located beneath or above the diaphragm. Usually this malformation is asymptomatic. However, torsion of the stalk may lead to acute right upper quadrant pain, nausea and vomiting. Accessory liver lobes are very rare and are encountered more often in women.

Congenital *hepar lobatum* with the formation of abnormal, coarse lobes may be regarded as an abortive form of an accessory liver lobe.

## Ectopy

Ectopies of the liver (*liver heterotopia* or *hepatic choristoma*) are caused by germ dissemination. In contrast to accessory liver lobes, ectopic liver tissue is disconnected from the main organ. Approximately 50% of ectopic livers are located in the gallbladder, the remainder are

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disseminated in the intra- or retroperitoneal space and in the thoracic cavity. Histologically ectopic livers exhibit a normal lobular architecture and may develop all pathologic changes seen in normal liver [1].

## Extraabdominal Location of the Liver

In congenital defects of the diaphragm the deformed liver can be located partially or totally within the chest. Liver dystopias in the pericardiac space have also been described. Usually these patients manifest additional malformations of the gastrointestinal tract, the lungs, the heart and the thoracic vessels.

Large congenital abdominal wall defects and umbilical hernias are observed in 1:4,000 births, and may be associated with herniation of abdominal organs.

## Intraabdominal Displacement of the Liver

In circumscribed congenital diaphragmatic aplasia the liver may be partially displaced into the chest. Since the organ is still covered by a thin peritoneal and pleural layer, however, the liver technically still lies within the abdominal cavity. A cranial position of the liver is seen more often in acquired diaphragmatic elevation, which can occur in the setting of right sided diaphragmatic paresis.

An insufficiency of hepatic ligaments may lead to *hepatoptosis*. Intestinal loops moving into the newly created free space between liver and diaphragm/abdominal wall characterize *Chilaiditi's syndrome*. Hepatoptosis generally affects women older than 40 years.

The most frequent cause for a caudal displacement of the liver is phrenoptosis, which can be seen in pulmonary emphysema or in marked right sided pleural effusion.

An isolated *transposition* of the liver is rare. Most often it occurs in *total situs inversus*, in which the liver is located in the left upper quadrant. In *partial situs inversus* the liver is located in the median line. This anomaly often is associated with asplenia.

## Agenesis

Complete agenesis of the liver is incompatible with life. The congenital absence of an entire liver lobe is extremely rare, with fewer than 100 cases reported in the literature. Agenesis of the right liver lobe is regarded to be a consequence of a developmental anomaly of the right portal vein branch or a growth failure of the hepatic diverticulum (see Chapter 1). The absence of a hepatic segment or lobe results in compensatory hypertrophy of the remaining liver tissue. In agenesis of the right liver lobe the gallbladder is located above the liver.

Rare cases of *hypoplasia* of the right liver lobe have also been described.

## Atrophy

Atrophy of the entire liver occurs in very old age and in severe chronic hunger disease. Atrophy of a singe lobe or a segment is observed in approximately 5% of diagnostic laparoscopies and in 75% of cases affects the left liver lobe. It is usually due to localized circulatory disturbances or to chronic obstruction of bile flow [2].

## Zahn's Grooves

These are deep grooves (pressure atrophy) of the upper liver surface, predominantly of the right liver lobe. They are caused by pressure of hypertrophied diaphragmatic muscle bundles, predominantly in chronic obstructive pulmonary disease, on the liver parenchyma. Zahn's grooves are found in approximately 10% of all autopsies.

## "Corset Liver"

Corset liver denotes flat, transverse indentations of the right liver lobe that are caused by deformations of the thoracic cage or are due to chronic strangling by a tight corset. Like Zahn's grooves these transverse grooves represent pressure atrophy of hepatic parenchyma.

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# Bile Duct Anomalies

# 56

Henryk Dancygier

## Chapter Outline



See also Chapters 108–110.

Anomalies may affect the intra- and/or extrahepatic bile ducts. They are characterized by an excess (e.g. doubling) or by a deficit of ducts, with variously shaped dilatations and strictures [17].

Important shape variants and anomalies of the gallbladder and bile ducts (with the exception of atresia) are reported in Tables 56.1 and 56.2 and depicted in Figs. 108.1, 108.2 and 109.1.

The most important anomalies of extrahepatic bile ducts are *choledochal cysts*. Spindle-shaped dilatations are more common than round cyst-like dilatations of bile duct segments.

Congenital *bronchobiliary fistulas* are rarities.

Developmental disturbances of the embryonic ductal plate ("ductal plate malformations") form the basis for anomalies termed *fibropolycystic liver diseases* [5, 6]. The bile duct level at which the malformation occurs determines the morphological and clinical picture (Table 56.3).

Fibropolycystic diseases of the liver encompass

- Autosomal recessive and dominant polycystic liver disease
- Congenital hepatic fibrosis
- Caroli's disease
- Caroli's syndrome
- von Meyenburg complexes

The various diseases may present overlapping features, and are often accompanied by renal cysts.

*Polycystic liver disease* is discussed in Chapter 57.

## Congenital Liver Fibrosis

Congenital liver fibrosis (CLF) is an autosomal recessive disease. In most cases it is associated with autosomal

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**Table 56.1** Important anomalies of the gallbladder

| | |
|---|---|
| **Abnormal shape** | • Phrygian cap-like bending of body and fundus<br>• Hour glass gallbladder<br>• Septated gallbladder (longitudinal cystic duplication very rare)<br>• Diverticula: preferentially in neck and fundic region |
| **Abnormal position** | • Left sided gallbladder (e.g. in situs inversus)<br>• Intrahepatic gallbladder<br>• "Pendular gallbladder": fixation at the level of the cystic duct |
| **Numerical aberrations** | • Agenesia: complete absence of the gallbladder anlage (seen in 0.005–0.065% of autopsies)<br>• Aplasia: gallbladder present, but not developed<br>• Hypoplasia: rudimentary gallbladder<br>• Incomplete duplication (Vesica fellea divisa)<br>• Complete duplication and triplication (Vesica fellea duplex et triplex); single case reports in the literature |
| **Heterotopias** | Preferentially in neck region, gastrointestinal mucosa, pancreas, liver |

Source: Adapted from [17]

**Table 56.2** Important bile duct anomalies

| | |
|---|---|
| **Atresia** | See text |
| **Hypoplasia** | Bile ducts with narrowed, but still present lumen |
| **Heterotopias** | Gastric mucosa |
| **Cysts** | Caroli's disease and Caroli's syndrome: see text |
| **Diverticula** | Choledochal diverticula are usually near the papilla |
| **Choledochocele** | Hernia-like evagination of main common bile duct into the duodenum |

Source: Adapted from [17]

**Table 56.3** Fibropolycystic diseases (ductal plate malformations) and level of bile ducts affected

| Disease | Bile duct level |
|---|---|
| **Autosomal recessive polycystic disease** | Interlobular bile ducts |
| **Autosomal dominant polycystic disease** | Peripheral interlobular bile ducts |
| **Congenital hepatic fibrosis** | Interlobular bile ducts |
| **Caroli's disease** | Segmental bile ducts |
| **Caroli's syndrome** | Segmental and interlobular bile ducts |
| **Von Meyenburg complexes** | Peripheral interlobular bile ducts |

Source: Adapted from [6]

recessive (occasionally with autosomal dominant) polycystic liver (kidney) disease. Associations with other syndromes such as *Meckel–Gruber's syndrome* (polydactylia, occipital encephalocele, renal cysts), *Jeune's* and *Ivemark's syndrome* (familial dysplasia of kidneys, liver and pancreas), and with vaginal atresia and tuberous sclerosis have also been reported. CLF may be combined with other anomalies of the liver such as von Meyenburg complexes, choledochal cysts and Caroli's disease. CLF may be associated with a variety of liver tumors (cholangiocellular carcinoma, hepatocellular carcinoma, benign tumors) and be part of COACH (Hypoplasia of *C*erebellar vermis, *O*ligophrenia, congenital *A*taxia, *C*oloboma, *H*epatic fibrosis) syndrome [10].

CLF probably does not represent a uniform nosologic entity, but rather encompasses a spectrum of liver and kidney lesions.

Ductal plate malformation at the level of interlobular bile ducts ("cholangiodysplasia") underlies the *pathogenesis* of CLF.

The *microscopic aspect* of the liver is variable. Fibrous enlargement and bridging of portal tracts containing a variable number of abnormal bile ducts are seen. The bile ducts within the fibrous areas communicate with the remainder of the hepatic bile duct system. The portal venous branches often are hypoplastic, while there is an excess of hepatic artery branches. In some patients the lesions remain stationary for long

periods of time, while in others fibrosis is progressive, and accompanied by cholangitic exacerbations. The slowly progressive destructive cholangitis finally leads to bile duct loss and to "cholangiodysplastic pseudocirrhosis". Circumscribed forms of CLF with only one liver lobe affected have been described [5, 6].

Depending on the rapidity and intensity of the fibrosing and cholangitic processes, different *clinical forms* of CLF may be delineated, i.e. a portal-hypertensive, a cholangitic, and a latent variety. CLF is one of the prototypes of portal hypertension with preserved hepatocellular function. Multiple normal deliveries in a woman with severe portal hypertension due to CLF have been described [13].

## Caroli's Disease and Caroli's Syndrome

### Definition

*Caroli's disease* (CD) is characterized by ectasias of the segmental intrahepatic bile ducts as the sole alteration, while in *Caroli's syndrome* (CS) these lesions are associated with CLF [2, 3, 20].

### Pathogenesis

Pathogenetically, an autosmal recessive ductal plate malformation with consequent dilatation of segmental (and interlobular ducts in CS) intrahepatic bile ducts underlies CD and CS.

### Pathology

The dilatation of the segmental intrahepatic bile ducts generally presents in a diffuse form, but may occasionally involve only a single lobe, commonly the left one. In its simple form, sack-like dilatations of the larger intrahepatic, predominantly segmental, bile ducts are found in CD. The dilated ducts may contain sludge, stones and dysplastic epithelial proliferations [7]. In CS the cysts are accompanied by fibrosis and/or by autosomal recessive polycystic kidney disease. Choledochal cysts are found in approximately 20% of cases.

### Diagnosis

#### Clinical Manifestations

Although the lesions are congenital, the initial manifestation of the disease may not occur until adulthood [8]. The clinical picture is characterized by relapsing cholangitic exacerbations with obstructive jaundice, upper abdominal pain and fever. Progressive remodeling of the liver leads to portal hypertension and its sequelae.

#### Technical Examinations

The biochemical laboratory parameters show a cholestatic pattern. The aminotransferase levels are either normal or only slightly increased.

The diagnosis (cystic dilatations and strictures) is made by abdominal ultrasound and CT, and may be confirmed by MRCP or ERCP. The dilated ducts may contain sludge or stones. In advanced cases morphologic signs of cirrhosis are present.

### Differential Diagnosis

The multiple strictures and spindle-shaped dilatations of bile ducts in primary sclerosing cholangitis are smaller than the sack-like cysts seen in CD and CS. However, intrahepatic bile duct stones may cause strictures with proximal duct dilatation that may be difficult to differentiate from abortive forms of CD.

In Asian patients, bile duct infections with *Ascaris* and *Clonorchis sinensis* as well as intrahepatic hepatolithiasis ("oriental cholangiohepatitis," also known as "recurrent pyogenic cholangitis") must be excluded.

### Course and Prognosis

The disease runs a chronic progressive course and approximately 50% of patients succumb to complications such as sepsis, biliary liver abscesses, portal hypertension or liver failure 1–5 years after the diagnosis has been established. In a longstanding course, amyloidosis may supervene. Seven to 14% of patients develop a cholangiocarcinoma. The occurrence of hepatocellular carcinoma has been reported in single cases.

### Therapy

Cholangitis is treated with repeated courses of antibiotics. Ursodeoxycholic acid (UDCA) may be tried to alleviate cholestasis, however the evidence for the effectiveness of UDCA in this clinical setting is very weak [18].

Endoscopic, percutaneous or combined techniques may be used for stone extraction, dilatation of strictures and placement of stents.

Liver resection is the treatment of choice for CD confined to a single lobe or segment, as it can provide a durable cure and eliminate the potential for cholangitis, lithiasis and carcinoma. In diffuse progressive disease, orthotopic liver transplantation is the only effective long term therapeutic option.

## Von Meyenburg Complexes

Von Meyenburg complexes are microhamartomas of the bile ducts, located within the portal tracts or in their immediate vicinity. A variable number of irregularly shaped and varyingly dilated duct structures are embedded in a fibrous, occasionally hyalinized stroma (Fig. 56.1). The lesions represent partially fibrosed remnants of ductal plate malformations of small, peripherally situated intrahepatic bile ducts and communicate with the remaining bile duct system of the liver.

These small (1–2 mm), often multiple lesions are clinically asymptomatic and are usually incidental laparoscopic findings. They may occur in an otherwise normal liver or be associated with CD or CLF.

## Biliary Atresia

Biliary atresia is an occlusive cholangiopathy that may affect both intra- and extrahepatic parts of the biliary tree.

### Definition

*Extrahepatic biliaray atresia* (EBA) is characterized by a complete absence of duct lumen or loss of duct continuity in one duct segment or in all extrahepatic bile ducts.

In *intrahepatic biliary atresia* (IBA) atresia is usually not complete but the number of interlobular bile ducts is reduced. Thus, IBA is more accurately a hypoplasia rather than a true atresia, which is why this entity is also referred to as "paucity of intrahepatic bile ducts."

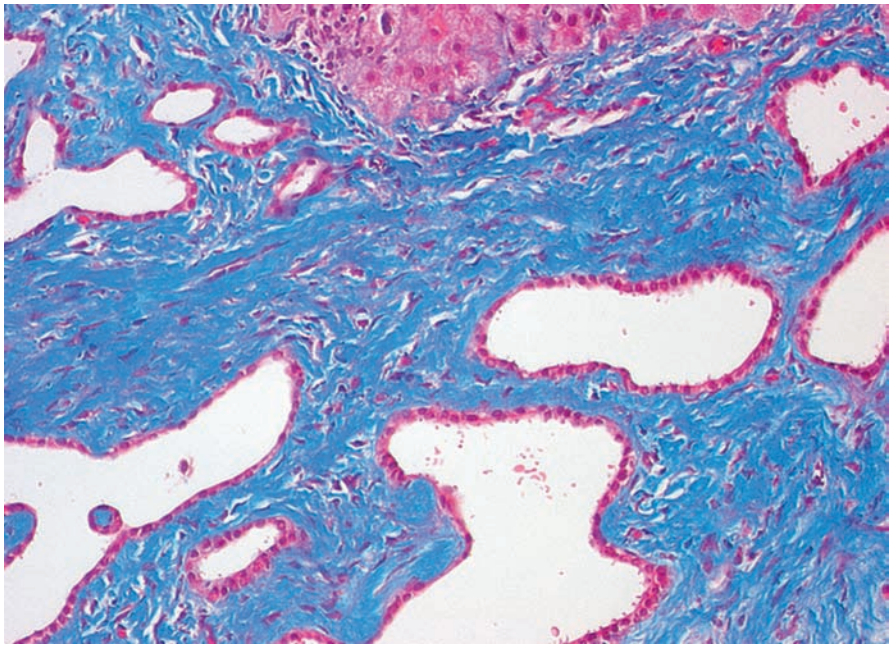

**Fig. 56.1** Von Meyenburg complex. The microhamartoma is composed of small, dilated, irregularly shaped bile ducts that are surrounded by fibrous tissue. Masson trichrome (×200)

## Epidemiology

EBA occurs in 1:10,000 to 13,000 deliveries, more often in female newborns. EBA is responsible for approximately 30–35% of all neonatal cholestasis and in 20% of cases is associated with CLF.

EBA associated with choledochal cyst has recently been suggested to represent a distinct subtype of biliary atresia, characterized by a preponderance of type 1 (see below), by an absence of associated congenital anomalies, and by a relatively good clinical outcome after surgery [15].

## Etiology and Pathogenesis

Embryonic morphogenetic bile duct defects, peri- and postnatal factors have been implicated in the development of EBA. The etiologic hypotheses comprise viral infections (e.g. cytomegalovirus, hepatitis B and C virus), ischemic lesions, toxic injury by bile acids or reflux of pancreatic juice into the bile duct, and an angiofibromatous process. A reoviral etiology has recently been disputed [19]. Immunologically mediated inflammation of bile ducts, involving both cellular and humoral components of autoimmunity, has been suggested to be operative in EBA, and the progressive bile duct injury has been postulated to be due in part to a bile duct epithelia-specific T cell-mediated immune response [12, 16]. All theories, however, are unproven and the exact etiology and pathogenesis of EBA are still unknown. Ultimately a destructive inflammatory-fibrosing cholangiopathy dominates the clinical picture [1, 14].

## Pathology

According to Kasai, various types of EBA can be distinguished (see Fig. 109.2) [9]. 10% of all EBA cases fall upon types I, IIa and IIb that are amenable to corrective surgery. Ninety percent fall upon type III in which a hepatoportoenterostomy (Kasai's procedure) is not possible. In 80% of patients the gallbladder is involved in the atretic process.

Each EBA is also accompanied by alterations of the intrahepatic bile duct system and of liver parenchyma. Even though it is primarily a bile duct disease, EBA is a complex disorder that ultimately affects all structures within the portal tract.

In the first 3–4 weeks of life centrilobular cholestasis with multinucleated giant cells, scattered throughout the parenchyma is seen. An inflammatory cellular infiltration of lobular parenchyma and portal tracts is still lacking in this early phase. During the further course, usually in the 4th–7th week of life the typical changes of extrahepatic bile duct obstruction become evident, with enlargement and rounding of portal tracts, ductular reaction at the porto-lobular interface and a variably dense inflammatory cell infiltrate consisting of neutrophilic granulocytes and lymphocytes. The epithelium of interlobular bile ducts becomes increasingly damaged. Without treatment the inflammatory-fibrosing lesions progress, resulting in loss of portal bile ducts and leading to the development of biliary cirrhosis within several weeks to months. During progression of EBA the portal vein branches within the portal tracts appear increasingly hypoplastic.

## Diagnosis

The diagnosis must be established before the advent of cirrhosis, i.e. usually before the 6th week of life, in order to utilize all opportunities of corrective surgery.

### Clinical Manifestations

EBA presents in two forms, an *embryonic-fetal type* (10–35% of cases), and a *perinatal form* (65–90% of cases). The embryonic-fetal type is characterized by an early onset of neonatal cholestasis, with continuing icterus after neonatal jaundice has abated. The children have acholic stools. This form of EBA also is more often than the perinatal form associated with other anomalies such as poly- and asplenia, cardiovascular malformations, abdominal situs inversus, intestinal malrotations, and vascular abnormalities of the liver. The perinatal form of EBA initially is anicteric and presents with cholestasis in the 4th–8th week of life. Unlike the embryonic-fetal variety an increased incidence of anomalies of other organs is not observed in this form of EBA.

IBA may occur within the context of *Alagille's syndrome* (arteriohepatic dysplasia; see Chapter 85) or as an isolated disease without extrahepatic abnormalities. The nonsyndromatic paucity of bile ducts is a heterogeneous group of disorders and occurs more rarely than Alagille's syndrome.

### Technical Examinations

The laboratory data show a cholestatic enzyme pattern with elevation of AP and γ-GT values. The diagnosis is made with imaging techniques such as abdominal sonography, nuclear imaging, percutaneous transhepatic cholangiography, and MRCP.

Liver biopsy is mandatory. A ratio of the number of interlobular bile duct profiles to the number of portal tracts (a biopsy specimen with at least six portal tracts is required) of <0.5 confirms the diagnosis of IBA.

## *Prognosis and Treatment*

Without treatment 99% of patients die within the first 4 years of life, most of them between the first and second year. The only effective therapeutic modalities are *hepatoportoenterostomy* or *liver transplantation*. However, even after a successful hepatoportoenterostomy, reversal of hepatic parenchymal disease does not occur in most children, and they present serious problems such as malnutrition, growth failure, portal hypertension, osteomalacia and osteoporosis, social and psychological problems. Continuing growth failure after hepatoportoenterostomy is associated with poor clinical outcome [4]. Corticosteroids after hepatoportoenterostomy have a beneficial effect on the rate of bilirubin reduction in the early postoperative period, but do not reduce the need for liver transplantation. In the long term, more than 80% of infants with EBA who are treated with corrective surgery will need liver transplantation [11]. A successful liver transplant offers significantly better prospects.

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# Liver Cysts and Polycystic Liver Disease

# 57

Henryk Dancygier

## Chapter Outline



Liver cysts are etiologically diverse hepatic mass lesions. Despite the fact that not all liver cysts represent developmental anomalies, most are discussed in this section and are subdivided into

- Liver cysts and
- Polycystic liver disease

For the discussion of parasitic cysts the reader is referred to Chapter 66.

## Liver Cysts

### *Definition*

Liver cysts are congenital or acquired fluid filled cavities within the liver parenchyma that are covered by epithelium (*true cysts*) or that do not possess an epithelial lining, but are surrounded by connective tissue (*pseudocysts*).

### *Epidemiology*

The prevalence of liver cysts in the general population is approximately 5% [3]. Congenital hepatic cysts are solitary in 95% of the cases, and are mostly present in the right liver lobe. Women are more frequently affected than men.

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**Table 57.1** Classification of liver cysts

| | |
|---|---|
| **Congenital** | • Solitary<br>• Multiple<br>• Polycystic liver disease<br>• Solitary bile duct cyst<br>• Multiple cystic dilatations of intrahepatic bile ducts |
| **Acquired** | • Traumatic<br>• Inflammatory-infectious[a]<br>Biliary retention cysts in bile duct obstruction<br>Echinococcosis<br>Unilocular (*Echinococcus granulosus*)<br>Multilocular (*Echinococcus alveolaris*)<br>• Neoplastic<br>Dermoid cyst<br>Mucinous cystadenoma/ cystadenocarcinoma<br>Regressive/degenerative changes in primary or metastatic liver tumors |

[a]Occasionally pyogenic and amebic liver abscess or hepatic peliosis may have a cystic appearance

## Etiology

An etiologic classification of liver cysts is reported in Table 57.1.

## Pathogenesis

The pathogenesis of liver cysts is not uniform. Congenital cysts in the context of fibropolycystic diseases result from malformations of the ductal plate. Benign solitary liver cysts are lined by columnar epithelium and probably also represent congenital developmental disorders of intrahepatic bile ducts [5]. Acquired traumatic, neoplastic and infectious liver cysts result from a circumscribed loss of liver tissue with secondary accumulation of fluid. These cysts lack an epithelial lining.

## Diagnosis

### Clinical Manifestations

Congenital liver cysts are usually asymptomatic. Large or multiple cysts may cause a sense of pressure in the upper abdomen and symptoms due to compression of neighbouring organs (see below).

### Technical Examinations

Liver enzymes are usually unremarkable and the biochemical liver profile is not helpful in diagnosing liver cysts. If a marked cholestatic enzyme pattern is present, secondary hepatic parenchymal changes or primary ductal or bilary retention cysts should be considered.

The mainstay of diagnosis are imaging techniques. Cysts as small as 5 mm in diameter are reliably visualized by ultrasound, and are usually found incidentally. The typical sonographic pattern shows a round or oval, anechoic, space occupying lesion with dorsal sound enhancement (Fig. 57.1). *A typical cyst on ultrasound does not require further investigation with CT or MRI*. Only if not all sonographic cyst criteria are met or if the lesion changes its aspect on follow-up examinations, further investigations will be necessary. In a few cases cyst puncture with cytological and microbiological examination of cyst content will be required (caveat: echinococcal cysts and amebic liver abscess prior to antibiotic therapy are contraindications for cyst puncture).

The diagnosis of echinococcal cysts, and of bacterial and amebic liver abscess is discussed in Chapters 65 and 66.

## Differential Diagnosis

The typical sonographic finding in an asymptomatic patient allows for the accurate diagnosis. Echinococcal cysts, retention cysts, Caroli's disease and Caroli's

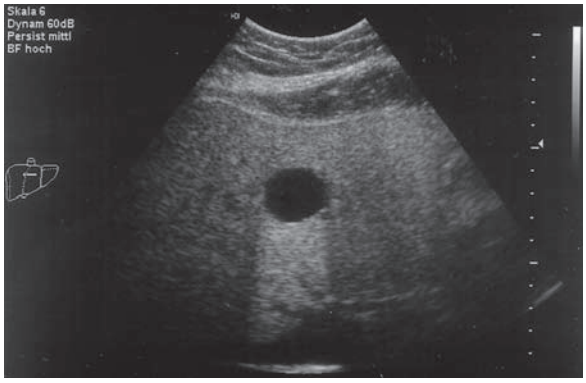


**Fig. 57.1** Typical solitary cyst with dorsal sound enhancement on ultrasound

syndrome, and liver abscesses must be included in the differential diagnosis. These usually cause symptoms.

Regressive changes in neoplastic lesions may impart the lesion a cystic aspect. This is seen especially in metastases from pancreatic and ovarian carcinoma. These "cysts" are usually ill delineated. However, the sonographic aspect does not always allow for a histologic diagnosis, and in some cases a liver biopsy will be necessary.

Mucinous cystadenomas are rare and occur only in women; they may attain a size of up to 20 cm in diameter and a weight of several kilograms.

Traumatic liver cysts are rare. The diagnosis may be deduced from the history of the patient. In these cases usually the levels of serum alkaline phosphatase and total bilirubin are slightly elevated. A bacterial peliosis hepatis occasionally may have a cystic aspect.

Von Meyenburg complexes are usually not seen on ultrasound. However, occasionally they may be larger than 4–5 mm and appear hypo- or anechoic.

### Complications

Hemorrhage or rupture into the peritoneal cavity with peritoneal irritation are very rare, but may occasionally occur after a trauma.

### Natural History and Prognosis

In the vast majority of cases dysontogenetic cysts do not compromise liver function and cause no clinical morbidity or mortality. In polycystic liver disease associated with polycystic kidney disease prognosis is determined by impairment of renal function (see below).

### Therapy

Asymptomatic patients require no treatment. If symptoms occur a percutaneous cyst aspiration may yield temporary relief of mass symptoms. Since cysts usually refill rapidly an attempt at cyst sclerosis with instillation of, for example, alcohol might be considered in non-biliary and non-parasitic cysts. This procedure can be quite painful. Surgical cyst fenestration or partial hepatectomy will only be required in rare cases [6, 12, 17, 20].

## Polycystic Liver Disease

### Definition

*Autosomal dominant polycystic liver disease* (ADPLD) is an inherited disorder characterized by the presence of multiple scattered cysts of biliary origin in the liver parenchyma. It often occurs in association with inherited adult polycystic kidney disease (ADPKD) but also exists as a distinct genetic entity independent from polycystic kidney disease [4, 9, 13, 14, 16, 18].

*Autosomal recessive polycystic kidney disease* (ARPKD) is characterized by the association of renal cysts arising from dilated collecting ducts and congenital hepatic fibrosis.

### Epidemiology

ADPKD is the most frequent hereditary kidney disease. It has an incidence of 1 in 500 to 1 in 5,000 (the incidence of ARPKD is between 1 in 6,000 and 1 in 40,000 births) and is often associated with polycystic liver disease (PLD). PLD is found in approximately 0.6% of autopsies, and 50–70% of cases are associated with ADKPD. Cystic lesions in other organs (e.g. pancreas or spleen) are less often present.

Most reports on the epidemiology of PLD are based on the disease as it manifests in patients with ADPKD. Liver cysts in ADPKD are very rare in children, and there is an age-dependent increase in the frequency of hepatic cysts in patients with ADPKD, from 20% in the third decade to 75% by the seventh decade of life. This suggests that isolated ADPLD seems to be less penetrant in the liver than ADPKD in the kidney. The development of liver cysts in isolated ADPLD may not occur until late in life [14, 18].

### Etiology and Pathogenesis

ADPLD is a genetically heterogeneous disorder involving derangements on at least three different chromosomes. Mutations involving chromosomes 16 and 4

accounting for ADPKD have been well described as have their gene products, polycystin-1 and polycystin-2 [1]. Recently a mutation in the short arm of chromosome 19 (19p13.2–13.1) has been recognized in isolated ADPLD without any associated renal involvement [19].

Cysts arise from dilated biliary microhamartomas (von Meyenburg complexes) and from peribiliary glands [10]. Isolated ADPLD is more severe in women than in men. In addition, there is a positive correlation between the severity of PLD and the number of pregnancies, suggesting a role for estrogens in the pathogenesis of PLD [18].

Recently it has been shown that PLD is caused by mutations in the protein kinase C substrate 80K-H gene, which encodes a protein named hepatocystin. The exact localization and cellular function(s) of hepatocystin are unclear, but recent data are consistent with a role of hepatocystin in carbohydrate processing and quality control of newly synthesized glycoproteins in the endoplasmic reticulum. Thus, defective hepatocystin might lead to altered endoplasmic reticulum processing of some key regulator of cell proliferation, thereby facilitating the formation of multiple cysts [7, 8].

Autocrine and paracrine factors secreted into the cysts such as interleukin-8, epithelial neutrophil attractant 78, interleukin-6, and vascular endothelial growth factor have been suggested to modulate the growth rate of cyst epithelia [15].

A gene mutation on both arms of chromosome 6 is responsible for ARPKD.

## Pathology

ADPLD is characterized by the presence of numerous cysts spread throughout the hepatic parenchyma. Rarely is only one lobe affected. The cysts occur in the portal tracts or in their vicinity, contain clear fluid secreted by their epithelial lining, and do not communicate with the intrahepatic bile duct system. They are often associated with von Meyenburg complexes and fibrosis.

It is not known whether isolated ADPLD is associated with an increased incidence of malformations in other organs. There are, however, data to suggest that structural mitral leaflet abnormalities, aneurysms of the thoracic aorta, intracranial aneurysms and dissection tend to occur more often in individuals with ADPLD [18, 21].

## Diagnosis

### Clinical Manifestations

The clinical profile of isolated ADPLD without kidney disease has not been extensively studied. PLD associated with AKPD is usually asymptomatic, and symptoms of renal disease predominate. Though uncommon, a minority of patients with ADPLD may be symptomatic due to mass effect caused by a very large cyst or a large number of cysts. When this occurs, patients may complain of abdominal pain and distention, early satiety, dyspnea, and back pain [2]. Rarely significant intrahepatic cholestasis and jaundice can occur, as can hepatic venous outflow obstruction or compression of the inferior vena cava [22].

### Technical Examinations

Liver function typically remains normal even in extensive PLD. Minimal elevations of serum alkaline phosphatase and total bilirubin and lower levels of total cholesterol and triglycerides may be present [18]. The latter findings indicate the possibility of a potentially altered liver metabolism in patients with PLD [11].

Imaging with ultrasound or CT is the diagnostic gold standard (see above) (Fig. 57.2).

## Natural History and Prognosis

Despite the sometimes impressive physical and radiologic findings, only a minority of patients will progress to advanced liver disease or develop complications such as hemorrhage, cyst infection, rupture, jaundice, or portal hypertension [2].

## Therapy

Most patients with PLD remain asymptomatic and require no treatment. For management of symptomatic cysts see above. In rare cases liver transplantation will be required in ADPLD.

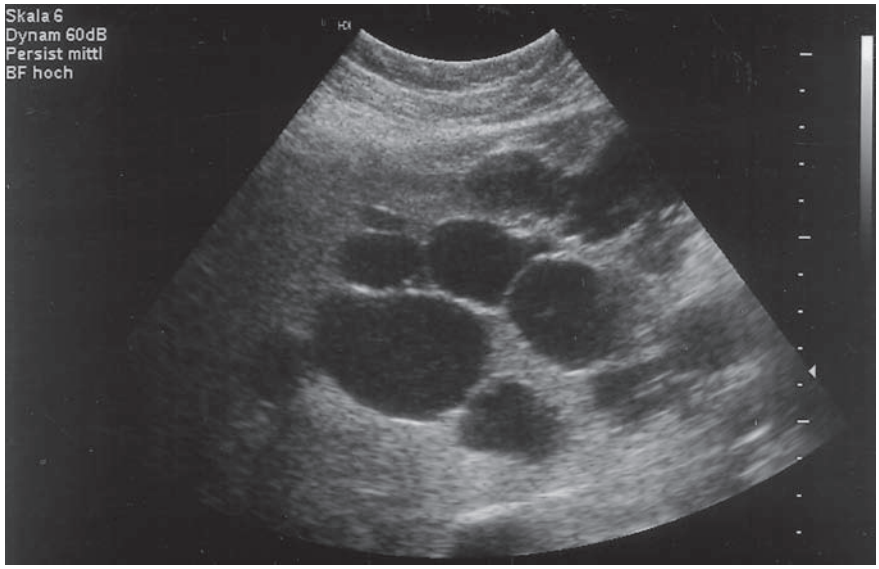


**Fig. 57.2** Polycystic liver disease with multiple cysts of different size

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# Vascular Anomalies

# 58

Henryk Dancygier

In the following paragraphs only a short enumeration of major vascular anomalies encountered in clinical practice is given.

*Aneurysms of the hepatic artery* see Chapter 62.

*Accessory hepatic arteries* derive from the left gastric artery in 60% of cases, and from the superior mesenteric artery in approximately 15% of cases.

*Duplications, hypoplasias, atresias, stenoses* and *aneurysms of the portal vein* are rarities [2].

*Cavernous transformation of the portal vein*, i.e. the formation of an "angiomatous mass" around a partially patent portal vein, is rare as a congenital malformation. It occurs more often as an acquired alteration (see Chapter 61).

A *Cruveilhier-von-Baumgarten disease* denotes the congenital absence of obliteration of the umbilical vein, which implies the lack of the Ligamentum rotundum. The liver may show a mild fibrosis and atrophy, or be entirely normal.

In contrast, a *Cruveilhier-von Baumgarten syndrome* denotes the recanalization of a postpartally (functionally) obliterated umbilical vein in portal hypertension (see Fig. 53.3).

A *fibrous obliteration of the portal vein* and its branches may occur in congenital liver fibrosis.

*Anomalies of the large liver veins* with direct opening into the right or left atrium are very rare and occur most often in the context of complex cardiac malformations.

*Osler-Weber-Rendu's syndrome* (hereditary hemorrhagic telangiectasia) may also affect the small intrahepatic vessels and be associated with intrahepatic telangiectasias, arteriovenous shunts (resulting in shunting from hepatic artery to hepatic veins), and aneurysms [1]. These vascular anomalies cause relapsing parenchymal hemorrhages with reactive fibrosis,

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that during the long course of the disease may result in structural remodeling of the liver. Additionally, hypoperfusion of the peribiliary plexus may result in ischemia of the intrahepatic or extrahepatic bile ducts with the eventual development of biliary strictures or biliary necrosis.

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# Hepatic Veins

# 59

Henryk Dancygier

## Chapter Outline



Disturbances of hepatic venous outflow are usually due to cardiac or pulmonary diseases. However, obstruction to venous outflow may be localized at all levels of the venous outflow tract, from the terminal hepatic venules, via the sublobular veins, the hepatic veins to the inferior vena cava above its junction with the hepatic veins.

## Acute Hepatic Congestion

The sudden occlusion of hepatic venous outflow causes acute liver congestion. It is characterized by a massive centrilobular passive hyperemia usually combined with hypoperfusion. Microscopically, centrilobular hemorrhagic necrosis is seen which is reflected macroscopically by a variegated mottled appearance of the liver. In an uneventful clinical course necrotic hepatocytes and erythrocytes are removed by Kupffer cells. Focal accumulations of ceroid and siderin-storing macrophages may persist for weeks and months, and testify to this phagocytotic activity of Kupffer cells.

Clinically the rapid filling of the liver with blood leads to hepatomegaly, and acute distention of Glisson's capsule causes pain in the right upper abdomen. The alterations in liver enzymes are similar to those seen in ischemic hepatitis (see Chapter 62). Sonographically the intrahepatic veins are pronounced and wide. In right sided heart failure the respiratory oscillations in diameter of the inferior vena cava are restricted.

## Chronic Hepatic Congestion

If the underlying disease process continues, congestion becomes chronic with dilated blood-filled sinusoids, atrophic liver cell plates, and confluent centrilobular

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hemorrhagic necroses. A "reversal of lobular architecture" ensues with portal tracts surrounded by viable parenchyma at the center surrounded by necrotic and hemorrhagic parenchyma. Macroscopically the organ takes on the aspect of a "nutmeg liver". In addition, hypoxic steatosis and cholestasis of the remaining parenchyma may impart an even more variegated aspect to the liver reminiscent of "autumn foliage". With continuing congestion fibrous septa devoid of parenchymal cells develop and connect terminal hepatic venules of neighbouring lobules, leading to "cardiac cirrhosis" which, is not a true cirrhosis but rather a cardiac sclerosis. Marked regenerative nodules are lacking and extrahepatic complications of cirrhosis are rare.

Animal experiments evaluating the hepatic response to right ventricular pressure overload show that along with changes of hepatic architecture a reprogramming of gene expression occurs. Even prior to the development of necrosis and fibrosis, congestion initially leads to a discontinuous and finally to a disappearance of centrilobular expression of enzymes, which become reexpressed in the periportal zone [16].

Clinically an enlarged liver of hard consistency may be palpated. The serum aminotransferases are mildly elevated. On ultrasound the liver displays a mild nonspecific increase and irregularity in echogenicity. With high resolution ultrasound probes (7.5 MHz) a finely undulated liver surface may be visualized in advanced stages.

The diagnosis of chronic hepatic congestion is based on integrating all findings within the clinical context and not on the results of a single examination.

## Budd-Chiari Syndrome

George Budd, an internist at King's College Hospital in London, in his memorable work "Diseases of the Liver" published in 1845 has described the occlusion of liver veins with painful hepatomegaly, refractory ascites, and liver failure. The syndrome has already been mentioned earlier by Rokitansky in 1842, and in 1899 Hans Chiari, a pathologist in Strasbourg, examined three further cases with liver vein thrombosis, assuming they were the result of a primary endophlebitis [8].

### *Definition*

Budd-Chiari syndrome (BCS) is a rare, heterogeneous, and potentially lethal condition related to the obstruction of the hepatic venous outflow tract [43]. The original strict definition relating BCS to the occlusion of liver veins and/or the suprahepatic inferior vena cava, nowadays has been abandoned and the term is applied to most forms of hepatic venous outflow compromise irrespective of the cause or the site of vascular obstruction [23]. However, heart failure and sinusoidal obstruction syndrome, which also impair hepatic venous outflow and share many features with BCS should be considered separately [43].

### *Epidemiology*

Exact data regarding the incidence and prevalence of BCS in Europe and in the USA are not available. In India BCS underlies 7–9% of cases of portal hypertension [12, 38]. Women are more often affected than men, with the average age on presentation being 35 years [42].

### *Etiology and Pathogenesis*

Thrombotic occlusion of the major hepatic veins is the most frequent cause of BCS in the West [11]. Myeloproliferative disorders (MPD) and hypercoagulable states are the leading causes of BCS (approximately 70% of cases). In approximately 20% of patients BCS remains idiopathic. A number of these idiopathic cases do not fulfill the diagnostic criteria for overt MPD but have features suggestive of a latent (occult) MPD based on hyperplastic bone marrow and erythroid progenitor cell culture. A clonal mutation in *JAK2* tyrosine kinase occurs in a high proportion of patients with MPD [35, 37]. *JAK2* is a signal-transduction molecule, which acts downstream of several cytokine receptors including the erythropoietin receptor. The V617F mutation (substitution of phenylalanine for valine at amino acid position 617) gives rise to increased tyrosine kinase activity and allows erythroid precursors to grow in the absence of exogenous erythropoietin. Analysis to

identify *JAK2* mutations provides critical information supporting an (occult) MPD as the cause of a hitherto idiopathic BCS [9, 35, 37].

Recent data also suggest that patients with BCS, including those without an underlying MPD, display a specific gene expression profile that is different from that of normal liver and cirrhosis, and is also different in acute and chronic forms of BCS [34].

The various etiologies of BCS are reported in Table 59.1.

As compared with pure hepatic vein thrombosis, inferior vena cava thrombosis is more common in Asia and Africa. Obstruction of the inferior vena cava by membranes (webs), as well as short-length stenoses, may also be a sequela of thrombosis, and is complicated more commonly by hepatocellular carcinoma.

Short-length stenoses are found in approximately 25% of patients with hepatic vein obstruction and in more than 60% of patients with inferior vena cava obstruction at its hepatic portion ("obliterative hepatocavopathy") [32, 41]. It is not clear why the inferior vena cava is involved so frequently in the Far East and relatively rarely in the West.

BCS may rarely occur in pregnancy, or during infections. It is a common complication of Behcet's disease, in which it is often found with a concomitant thrombosis of the portal vein. Hepatic vein thrombosis in Behcet's disease nearly always represents the extension of inferior vena cava thrombosis [2]. Associations of BCS with celiac disease, hypereosinophilic syndrome, and antiphospholipid syndrome have all been reported [3, 13, 26].

Diffuse involvement of the small hepatic veins (sparing the large veins) is common in paroxysmal nocturnal hemoglobinuria [40].

The occlusion of hepatic venules is generally toxic in origin and secondary to sinusoidal obstruction (sinusoidal obstruction syndrome, see Chapter 60).

BCS has also been classified as primary or secondary. BCS is considered primary when obstruction of the hepatic venous outflow tract is the result of an endoluminal venous lesion such as thrombosis, webs, or endophlebitis. It is considered secondary when the obstruction results from the presence in the lumen of material not originating from the venous system (e.g. tumor, abscess, parasitic cyst) or from extrinsic venous compression without invasion by neighboring space occupying lesions [21, 31]. In practice, extraluminal and intraluminal changes (for example compression leading to thrombosis) are often combined.

BCS causes postsinusoidal portal hypertension, which may lead to complications similar to those observed in cirrhosis. Patients with BCS have activation of vasoactive neurohormonal systems (increased plasma renin activity, aldosterone and norepinephrine levels) and expanded plasma volume. However, in contrast to cirrhotic patients they do not exhibit a hyperdynamic circulation (i.e. systemic vasodilatation, increased cardiac output) [19].

**Table 59.1** Causes of and conditions associated with Budd-Chiari syndrome[a]

| |
|---|
| **Myeloproliferative disorders** (approx. 50% of cases) (primarily polycythemia vera and essential thrombocythemia) |
| • Occult[b] |
| • Classic |
| **Hypercoagulable states** |
| • Antiphospholipid syndrome |
| • Factor V Leiden mutation |
| • Factor II G20210A mutation |
| • Protein C and S deficiency |
| • Antithrombin III deficiency |
| • Plasminogen deficiency |
| • Increased plasma levels of factor VII, VIII and homocystein [10] |
| • Paroxysmal nocturnal hemoglobinuria[c] |
| **Behcet's disease** |
| **Pregnancy** |
| **Oral contraceptives** (only agents with high estrogen content) |
| **Membranous webs of inferior vena cava** |
| • Congenital |
| • Acquired |
| **Hepatic infections** (abscess, echinococcal cyst) |
| **Malignant tumors** (hepatocellular, adrenal, renal carcinomas: direct spread or production of erythropoietin) |
| **Abdominal trauma** |
| **Ulcerative colitis** |
| **Celiac disease** |
| **Sarcoidosis** (granulomatous involvement of hepatic veins) |
| **Hypereosinophilic syndrome** |
| **Idiopathic** (up to 20% of cases) |

[a]Most diseases associated with BCS are combined with underlying thrombophilias.
[b]Occult polycythemia vera may be detected by flow cytometric analysis of autonomous growth of erythroid precursors [20]. *JAK2* mutations detect occult forms of myeloproliferative disorders.
[c]May be easily diagnosed using flow-cytometry for CD55- and CD59-deficient circulating blood cells.

## Pathology

The liver veins may be completely or partially occluded by thrombi which may extend into neighbouring venous vessels. The main histologic features on liver biopsy are congestion, liver cell loss, parenchymal atrophy and fibrosis, all of which predominate in the centrilobular areas. When portal and hepatic veins are simultaneously obstructed the corresponding liver areas undergo infarction and transform into fibrotic scars devoid of liver parenchyma. In insiduously developing BCS, a ductular reaction (not all portal tracts are affected) and the formation of multiple regenerative nodules (nodular regenerative hyperplasia) is common. These nodules are thought to represent the hyperplastic response to focal loss of portal perfusion combined with hyperarterialization in areas with preserved hepatic venous outflow [7, 39, 44].

## Diagnosis

The clinical spectrum of BCS is wide, and depends on the underlying illness, and the extent and rapidity of the obstructive process. It extends from asymptomatic occlusion of hepatic venules to life-threatening and fatal BCS. The diagnosis is based on a high index of suspicion and imaging with ultrasound and MRI must be performed without delay. The diagnosis of BCS follows three major pathways

- Establish the diagnosis
- Find the cause
- Specify the level of obstruction in the hepatic venous outflow tract

### Clinical Manifestations

The presentation of BCS varies, ranging from an asymptomatic condition recognized incidentally to fulminant hepatic failure [17]. According to the rapidity of onset an acute, subacute and a chronic form may be distinguished. Approximately one third of patients present acutely, while the majority have a subacute (weeks) or chronic (months) presentation. Chronic presentation is particularly common in the Far East and in South Africa. Except for descriptive purposes, however, the use of this classification has uncertain clinical utility.

The typical patient reports the onset of abdominal distention (due to the development of ascites) associated with abdominal pain. The prevalence of symptoms varies, with abdominal pain occurring in 23–57%, painful hepatomegaly in 55–89%, leg edema in 32–41%, and jaundice in 6–18% of cases. Dilated and tortuous veins over the abdominal wall and the back occur in 27–49% of patients [43]. If portal hypertension is marked, bleeding from esophageal or gastric varices may occur. Signs of liver failure are noticed only in the most severe cases.

### Laboratory Findings

A rise in aminotransferases, and in lactate and glutamate dehydrogenase levels is nonspecific. Despite the appearance of the above-mentioned symptoms and signs, the parameters of liver synthetic function typically remain normal. This discrepancy should focus one's suspicion to an impairment of hepatovenous outflow.

*JAK2* is a very reliable and noninvasive molecular marker, even for the occult forms of myeloproliferative diseases and should be used as an early test for establishing the cause of BCS [37].

### Imaging Techniques

Imaging techniques are the mainstay in the diagnosis of BCS. A *stepwise approach* is advised. Grey scale sonography and color Doppler imaging combined with pulsed Doppler should be used as first-line, with magnetic resonance imaging reserved as second-line testing. On grey scale sonography intrahepatic or subcapsular hepatic venous collaterals are a sensitive feature of the diagnosis, being found in more than 80% of cases. Extrahepatic portal vein thrombosis is found in up to 20% of patients with BCS [43]. The diagnosis of BCS by pulsed Doppler sonography is accurate in 87% of cases [5].

Direct venography is not necessary for establishing the diagnosis. In planning treatment, however, it

remains the gold standard, permitting the precise delineation of the site and the extent of outflow obstruction. Venography should be reserved for patients in whom interventional therapy is contemplated. Nuclear imaging is nonspecific and insensitive in BCS.

Liver biopsy is not mandatory in patients with suspected BCS. The histologic changes are nonspecific, corresponding to those seen in congestion and circulatory failure. The lesions are distributed unevenly throughout the liver giving rise to sampling error. When liver biopsy is considered in a BCS patient whose coagulation is impaired the transvenous approach should be chosen.

An interesting distinctive feature in BCS is hypertrophy of the caudate lobe, which is found in 80% of patients with occlusion of hepatic veins. The reason for caudate lobe enlargement is its unique blood supply. It receives its venous blood from the right and left branches of the portal vein. The venous outflow from the caudate lobe (segment 1) into the inferior vena cava, however, does not occur through the hepatic vein, but through separate veins (Vv. spigelii). In thrombotic occlusion of all three major hepatic veins the entire venous flow of the liver passes through the caudate lobe causing it to enlarge markedly. The increase in size may be so pronounced as to cause compression with secondary thrombosis of the inferior vena cava.

### *Prognosis and Treatment*

The natural history of BCS is poorly understood. Some patients may recover, however aggravation may occur unpredictably. The survival rates at 1 year and 5 to 10 years are about 60–70% and 10–50%, respecively [45]. With simultaneous portal vein thrombosis the median survival rate is approximately one month! [24] Four independent factors appear to correlate inversely with survival: age of the patient, response of ascites to diuretics, Child-Pugh score, and serum creatinine. Spontaneous repermeation of thrombosed veins with recovery does occur, but is extremely rare.

Hepatocellular carcinoma has been described occasionally in patients with BCS, mostly in those with long-standing obstruction of the inferior vena cava [18, 31, 32]. Differentiating regenerative nodules from HCC without biopsy is extraordinarily difficult.

The management of BCS includes the treatment of the underlying illness, the therapy of complications of portal hypertension, anticoagulation therapy, decompression procedures and liver transplantation [4, 6, 14, 15, 22]. *Therapeutic procedures should be introduced in a stepwise fashion by order of increasing invasiveness, based on the response to previous therapy* rather than on the severity of the patient's condition [36].

Although the evidence for the efficacy of anticoagulation therapy is circumstantial, systemic anticoagulation should be started expeditiously [42, 45]. Especially in patients with extension of thrombosis into the splanchnic venous bed a liberal use of anticoagulants has been advocated [29]. The type and duration of optimal anticoagulation have not been established. Usually intravenous heparin is followed by long-term (life-long?) warfarin. If long-term heparin-based anticoagulation is used low-molecular-weight heparin is generally preferred to unfractionated heparins because of a lower risk of heparin-induced thrombocytopenia and thrombosis.

In severe focal hepatic venous outflow occlusion, angioplasty with or without stenting, and local thrombolysis may be used. The use of systemic thrombolytics is controversial. In severe diffuse forms of BCS decompression with a transhepatic (TIPS) or a surgical portosystemic shunt may be indicated [1, 4, 14, 25, 28, 30].

Fulminant liver failure or severe decompensated disease will require liver transplantation. The survival rate at ten years after transplantation is 70–75%. The risk of recurrence is low when anticoagulant therapy is instituted early [4, 6, 27, 43].

## Central Hyaline Sclerosis

Central hyaline sclerosis (also called sclerosing hyaline necrosis) is characterized by centrilobular parenchymal necrosis and fibrosis leading to obliteration of terminal venules with consequent impairment of venous outflow. The condition is rare and may occur in alcoholic hepatitis where it may cause portal hypertension with ascites without the presence of liver cirrhosis.

## Prolapse of Hepatocytes into the Central Veins

This very rare condition is characterized by a diffuse liver cell hyperplasia with prolapse of hepatocytes into the terminal hepatic venules, resulting in partial occlusion of the vessels. It has been described in patients on long-term methyl-testosterone therapy [33].

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# Sinusoids

# 60

Henryk Dancygier

## Chapter Outline



## Sinusoidal Dilatation

A pure sinusoidal dilatation is observed in approximately 3% of all liver biopsies (excluding congestion, sinusoidal infiltrates, liver cirrhosis or handling artifacts) [3]. Neoplastic and granulomatous diseases (tuberculosis, brucellosis, Crohn's disease, sarcoidosis) are frequent causes of focal sinusoidal dilatation, even in the absence of hepatic infiltration by tumor cells or the deposition of granulomas.

The pathogenesis of sinusoidal ectasia is unknown. Marked dilatation of sinusoids as an isolated histological change, however, should prompt the search for a tumor or a granulomatous disease.

In right sided heart failure and in obstruction of hepatic venous outflow passive dilatation and congestion of centrilobular sinusoids is readily explained by the increased intrasinusoidal hydrostatic pressure. If the process continues, liver cell plates become atrophic, lipofuscin accumulates within hepatocytes, and perisinusoidal fibrosis with deposition of sinusoidal basement membranes develops. As described in Chapter 59, this process leads to cardiac sclerosis ("cirrhose cardiaque").

Dilatation of sinusoids in acinar zones 2 and 3 has been described occasionally in patients on long-term treatment with azathioprine.

The chronic intake of oral contraceptives has been suggested as a possible cause of sinusoidal dilatation, which may assume "dramatic" extensions with atrophy and rupture of liver cell plates and collagenization of the space of Disse (see below). Similar lesions have also been occasionally observed within the context of pregnancy associated liver disorders [13].

Sinusoidal dilatation may also form as a paraneoplastic reaction in malignant lymphoma and occur in the vicinity of liver metastases.

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Recently, isolated sinusoidal dilatation has been described in patients with the antiphospholipid syndrome, suggesting a prothrombotic state as a possible underlying mechanism [11].

## Peliosis Hepatis

### Definition

Hepatic peliosis is characterized by fluid- or blood-filled spaces of various sizes within the hepatic parenchyma that are not lined by epithelium.

### Epidemiology

Hepatic peliosis affects males and females of all age groups equally. Exact data regarding incidence and prevalence are not available.

### Etiology and Pathogenesis

Drugs and toxins are the leading causes of hepatic peliosis (Table 60.1). In addition, peliotic lesions may be seen in tuberculosis, tumor cachexia, and in children dying of suffocation.

A bacillary peliosis in AIDS-patients is caused by infection with *Bartonella (Rochalimaea) henselae* [10].

**Table 60.1** Substances that may cause hepatic peliosis (selection)

| |
|---|
| Anabolic and androgenic steroids |
| • Testosterone |
| • Methyltestosterone |
| • Oxymetholone |
| • Fluoxymesterone |
| • Norethandrolone |
| • Methandrostenolone |
| Oral contraceptives |
| Danazole |
| Azathioprine |
| 6-Thioguanine |
| Tamoxifen |
| Vitamin A (overdose) |
| Vinyl chloride |
| Thorium dioxide (Thorotrast®; not in use anymore) |

The pathogenesis is unknown. Sinusoidal epithelial injury appears to be the pacemaker lesion for the development of peliosis.

### Pathology

The lesions are generally distributed diffusely throughout the liver. In occasional patients isolated peliotic areas with mildly compressed but otherwise normal intervening parenchyma have been described. Microscopically the peliotic lesions are lined by hepatocytes; an endothelial lining is absent. Their hemorrhagic content may be liquid, coagulated or organized, and may communicate with the surrounding sinusoids and terminal hepatic venules.

In bacillary peliosis multiple microorganisms, located extracellularly as well as within round histiocytic cells, may be demonstrated on Warthin-Starry staining. In addition to the lesions described above, a marked vascular proliferation may be present (bacillary angiomatosis) [10].

Extrahepatic peliosis may affect many organs, especially the spleen.

### Diagnosis

#### Signs and Symptoms

The changes are asymptomatic initially. Depending on their size they can cause mass effects and a painful hepatomegaly. Rupture with intraperitoneal bleeding and acute abdominal pain occurs rarely.

#### Laboratory Parameters

Serum concentrations of aminotransferases and cholestatic enzymes may be normal or mildly elevated. The enzyme profile is nonspecific.

#### Imaging Techniques

Imaging techniques (ultrasound, CT, MRI) are the mainstay of morphologic diagnosis of hepatic peliosis. The liver surface may be undulated. On ultrasound the

peliotic lesions appear as localized hypervascularized areas of varying echogenicity [1]. However, the sonographic findings are nonspecific and do not allow one to distinguish peliosis from hepatic metastases or from a multilocular hepatocellular carcinoma.

The diagnosis can be confirmed by liver biopsy and pathogens can be demonstrated by silver staining techniques or PCR.

### Therapy and Prognosis

Potentially causative factors, such as anabolic steroids, should first be discontinued. Peliotic lesions, however, rarely are reversible, and persist even after withdrawal of steroids. Most cases with marked and progressive hepatic peliosis are fatal.

Treatment of bacillary peliosis with erythromycin, clarithromycin or doxycylin yields good results.

In marked localized peliosis, or after intraperitoneal rupture, surgical treatment is indicated.

## Sinusoidal Obstruction Syndrome

Sinusoidal obstruction syndrome (SOS) was formerly called veno-occlusive disease because the obliterative lesions in this condition were often observed in the terminal hepatic venules (central veins) and sublobular veins. However, the primary pathogenetic events affect the sinusoids and not the central veins (i.e. the sinusoidal changes are not the result but the cause of central vein occlusion), hence the term SOS. The involvement of hepatic veins in SOS is not obligatory.

### Definition

SOS is defined as a nonthrombotic obstruction of sinusoidal blood flow.

### Epidemiology

The disease is observed primarily in children and adolescents. It was initially described in the Caribbean and in South America, but SOS is global in distribution. Exact data on incidence and prevalence of SOS are not available.

### Etiology and Pathogenesis

SOS is toxic in origin. The most frequent causes worldwide are bush teas containing pyrrolizidine alkaloids [2, 4]. Natural herbal remedies, such as viper's grass which is used as a tea and a general "tonic," may also cause SOS.

In Western countries SOS is most often associated with bone marrow and hematopoietic cell transplantation, use of immunosuppressive drugs, and graft versus host disease [6, 9, 12]. Further etiologic factors include radiation, aflatoxins, dimethylnitrosamines, and extremely rarely oral contraceptives and hypervitaminosis A [7]. The causes of SOS and the risk factors for SOS after hematopoietic cell transplantation are summarized in Tables 60.2 and 60.3. The prevalence

**Table 60.2** Causes of the sinusoidal obstruction syndrome

| | |
|---|---|
| **Pyrrolizidine alkaloids** | Leading cause of SOS in non-industrialized countries |
| **Stem cell and bone marrow transplantation** (conditioning chemotherapy, whole body irradiation) | Leading cause of SOS in industrialized countries |
| **Chemotherapeutics and immunosuppressants** | • Cyclophosphamide[a]<br>• Busulfan[b]<br>• Carboplatin<br>• BCNU<br>• Actinomycin D<br>• Mithramycin<br>• Dacarbazine<br>• Cytosine arabinoside (cytarabine)<br>• 6-Thioguanine<br>• Azathioprine<br>• Oxaliplatin |
| **Monoclonal antibodies** | Anti-CD33 |
| **Combined chemo- and abdominal radiotherapy,** whole body irradiation | For example, in children with Wilm's tumor |
| **Post liver transplantation** | |

[a]The hepatotoxic metabolite in the glutathione dependent metabolism of cyclophosphamide is acrolein

[b]SOS occurs primarily when busulfan is administered prior to cyclophosphamide

**Table 60.3** Risk factors for the sinusoidal obstruction syndrome in association with stem cell or bone marrow transplantation

| | |
|---|---|
| **Pretransplantation factors** | • Impaired liver function<br>• Liver metastases<br>• Prior liver irradiation<br>• Administration of vancomycin or acyclovir prior to transplantation<br>• Past myeloablation and stem cell transplantation<br>• Past therapy with anti-CD33<br>• Chronic hepatitis C<br>• Protein C deficiency<br>• Factor V Leiden mutation[a]<br>• Prothrombin gene 20210 G-A mutation[a] |
| **Transplantation related factors** | • High dose conditioning regimen<br>• Allogeneous transplantation as compared to autologous transplantation<br>• Conditioning with busulfan (especially when area under curve >1,500 µmol/min/L and combined with cyclophosphamide)<br>• Whole body irradiation, especially when combined with cyclophosphamide<br>• Non-related donor transplants or related, but HLA-mismatched transplants<br>• Methotrexate in the prophylaxis of graft-versus-host disease<br>• Cytomegalovirus infection |

[a]Very weak association with SOS
Source: According to [8]

of SOS within the context of stem cell transplantation strongly depends on the conditioning regimen used prior to the transplant. The new non-myeloablative regimens without cyclophosphamide (e.g., fludarabine with low-dose whole body irradiation) are not hepatotoxic.

The pathogenesis of SOS is not well understood. The initial insult appears to be a poorly defined sinusoidal endothelial cell injury that activates a cascade of events leading to non-thrombotic impairment of microcirculation at the sinusoidal level. Biochemically, sinusoidal endothelial cells are depleted in glutathione. Activation of matrix metalloproteinases with degradation of extracellular matrix and a derivative loss of endothelial cell anchoring may also be pathogenetically relevant [9]. Hypercoagulable states do not seem to be associated with the development SOS.

## Pathology

The earliest lesion in animal experiments (seen on electron microscopy) is swelling and rounding of sinusoidal endothelial cells with loss of endothelial fenestrations and extravasation of red blood cells into the space of Disse. Trichrome stains are best suited for histologic evaluation. The initial histologically visible change is the edematous expansion of the subendothelial space between the basement membrane and the adventitia of terminal hepatic venules and sublobular veins. Fragmented red blood cells and cell debris are found within this space. These venular lesions are accompanied by dilatation and congestion of sinusoids with hemorrhagic necrosis of pericentral liver parenchyma. The architecture of liver cell plates is lost and individual hepatocytes or groups of liver cells may embolize into the lumen of damaged terminal hepatic venules.

Approximately 2 weeks after patients with SOS become symptomatic, activated stellate cells proliferate and extracellular matrix is deposited within the subendothelial and perisinusoidal spaces. The advanced stages of SOS are characterized by collagenization of sinusoids and venules, and progressive and diffuse liver fibrosis. In some cases fibrotic septa bridge terminal venules of neighbouring lobules, imitating cardiac sclerosis.

45% of patients with a mild to moderate SOS and 25% of those with a severe SOS do not show complete occlusion of terminal hepatic venules.

## Diagnosis

A high index of suspicion in the appropriate clinical context (signs of portal hypertension in a patient exposed to pyrrolizidine alkaloids, bush teas, immununosuppressant

agents, post hematopoietic cell transplantation) should lead to the correct diagnosis.

### Signs and Symptoms

Functionally SOS is a disorder of hepatic venous outflow with abdominal distention, ascites, painful hepatomegaly and splenomegaly. The clinical picture varies according to the severity of SOS. Patients may be asymptomatic or develop symptoms and signs of portal hypertension, which typically precede those of parenchymal liver failure. SOS associated with bone marrow transplant usually begins 1–3 weeks after the procedure. The occlusion of centrilobular veins is associated with a more severe clinical picture. However, even 20–30% of patients with occlusion of terminal hepatic venules may be asymptomatic [6, 9].

### Laboratory Parameters

The rise in serum bilirubin and aminotransferase levels is nonspecific. High aminotransferase concentrations are due to centrilobular necrosis and values greater than 750 U/L are assumed to be associated with a poor prognosis.

### Imaging Techniques

Ultrasound, CT and MRI contribute little to the specific diagnosis of SOS, but rather show the consequences of hepatic venous outflow.

Liver biopsy in SOS is often performed by the transvenous route.

## Differential Diagnosis

Graft versus host disease, viral infection, drug injury, ischemic hepatitis and sepsis-associated cholestasis are among the most important differential diagnoses. In graft versus host disease cutaneous and intestinal manifestations usually precede hepatic symptoms. Viral infections include primarily those with herpes simplex, varizella zoster, cytomegalovirus, and Epstein-Barr virus. Ischemic hepatitis occurs in the clinical context of systemic circulatory impairment.

**Table 60.4** Different grades of severity of the sinusoidal obstruction syndrome in association with cyclophosphamide based myeloablation for stem cell transplantation

| | Mild | Moderate | Severe |
|---|---|---|---|
| **Weight gain** (%) | 7 ± 3.5 | 10.1 ± 5.3 | 15.5 ± 9.2 |
| **Maximal bilirubin concentration** (mg/dL) | 4.7 ± 2.9 | 7.9 ± 6.6 | 26 ± 15.2 |
| **Proportion of patients with peripheral edema** (%) | 23 | 70 | 85 |
| **Proportion of patients with ascites** (%) | 5 | 16 | 48 |
| **Mortality on day 100** (all causes) (%) | 3 | 2 | 98 |

Source: Adapted from [6]

## Course and Prognosis

Most patients recover, while some progress to acute liver failure. High serum aminotransferase values, rising creatinine levels, a high hepato-venous pressure gradient, the development of portal vein thrombosis, and diminishing blood oxygen saturation are associated with a poor prognosis. In clinically severe forms mortality rates reach 100%. The patients usually die from renal and cardiac rather than from hepatic failure. In Table 60.4 the different grades of severity of cyclophosphamide-associated SOS are reported.

A subclinical course with demonstrable histologic lesions but without associated symptoms has also been described.

## Treatment

A specific management of SOS is not available and treatment is supportive. All potential causes, such as hepatotoxic conditioning regimens in hematopoietic cell transplantation, must be avoided or eliminated.

Protein C and antithrombin III concentrations are often diminished in SOS. However, administration of antithrombin III is ineffective. In mild cases anticoagulation with intravenous or subcutaneous heparin might be helpful, but solid data supporting this approach are lacking.

In individual cases intravenous infusion of N-acetyl cysteine (50–150 mg/kg body weight/day) over 2–4

weeks has been reported to be beneficial. Data regarding the efficacy of ursodeoxycholic acid in SOS are conflicting.

TIPS or surgical portosystemic shunts have no effect on survival.

## Changes of Sinusoidal Contents

Dilated sinusoids may contain abnormal cells or organic material. Usually these changes are of ancillary clinical importance and can only be demonstrated histologically. Occasionally, however, their presence in a liver biopsy will yield the clue for the diagnosis of a systemic disease.

In *sickle cell anemia* (see Chapter 97) panlobular dilated sinusoids may be packed with deformed red blood cells which often are phagocytosed by Kupffer cells (erythrophagocytosis).

Sinusoids filled with *acanthocytes* hint towards a-β-lipoproteinemia.

*Neutrophilic granulocytes* are seen in the context of systemic leukocytosis and in so-called surgical necroses (i.e. neutophils accumulate in the liver during abdominal operations).

Increased numbers of intrasinusoidal *eosinophils* may be present in generalized allergic reactions to drugs, parasitic infections and in the hypereosinophilic syndrome.

*Lymphocytes* within the sinusoids arranged in a string of pearls-like pattern are typical (but not pathognominic) for infection with Epstein-Barr and cytomegalovirus.

Focal groups of *ceroid storing macrophages* also hint toward viral infections and may be present for months as residual changes after resolution of acute viral hepatitis.

*Plasma cells* are occasionally seen in multiple myeloma and in Waldenström's disease, *mast cells* in systemic mastocytosis, and abnormal *histiocytes* in histiocytosis X.

*Extramedullary hematopoiesis* may persist into neonatal life in neonatal hepatitis. In adults the presence of megakaryocytes and immature white and red blood cells within foci of extramedullary hematopoiesis suggest a myeloproliferative disorder or diffuse metastatic involvement of the bone marrow.

*Neoplastic cells* within the sinusoids are seen in leukemia, malignant lymphoma, and metastatic carcinoma. An infiltration of sinusoids by tumor cells is typical for angiosarcoma and epithelioid hemangioendothelioma (see Chapter 102).

Intrasinusoidal *fibrin deposits* in zone 1 are characteristic of eclampsia and disseminated intravascular coagulation.

*Pathogens*, such as plasmodia, microfilaria (*Wuchereria bancrofti*) or worm larvae (*Strongyloides stercoralis*) may also be encountered within the sinusoids occasionally.

## Perisinusoidal Lesions

*Capillarization of sinusoids* denotes the deposition of collagen fibers within the space of Disse, with or without the formation of a basement membrane. It may occur among others in alcoholic hepatitis (predominantly in zone 3), in chronic viral hepatitis with marked inflammatory activity (predominantly in zone 1), and in drug induced liver damage (e.g., in hypervitaminosis A and during azathioprine and methotrexate therapy). The fibrous tissue within the space of Disse may be visualized by the usual trichrome stains. The morphological demonstration of basement membranes requires immunocytochemical techniques using antibodies against laminin and collagen type IV.

In patients with long-standing diabetes mellitus a noncirrhotic form of sinusoidal fibrosis not associated with nonalcoholic steatohepatitis may be observed. Recently the term "*diabetic hepatosclerosis*" has been proposed for this entity and suggested that it represents a form of *diabetic microangiopathy* affecting the liver [8].

*Amyloid* may be deposited in the branches of hepatic artery or in the space of Disse leading to progressive atrophy of liver cell plates (see Chapter 87) [5]. It can be confirmed by the classical congo-red stain or by immunohistochemistry.

Perisinusoidal deposition of *nonamyloidotic light chains* is rare, and occurs occasionally in chronic renal failure. The deposits are PAS-positive, but do not stain with the usual amyloid stains. They can be visualized by immunocytochemistry using antibodies against κ- or λ-light chains.

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# Portal Vein

# 61

Henryk Dancygier

## Chapter Outline



## Portal Vein Thrombosis

Portal vein thrombosis (PVT) is caused by endothelial injury, either alone or in combination with hypercoagulability and slowed blood flow. In Table 61.1 the most important causes of portal vein thrombosis are reported (see also etiology of Budd–Chiari syndrome in Chapter 59) [3, 8, 11, 19, 22]. The leading underlying disease, which is associated with 25–30% of PVT-cases, is liver cirrhosis. In many of these patients the liver harbors a hepatocellular carcinoma.

PVT may be completely asymptomatic or present with life-threatening complications, such as gastrointestinal hemorrhage and intestinal infarction [1].

### *Acute Portal Vein Thrombosis*

Thrombotic obliteration of the portal vein usually develops insidiously, but may also begin acutely. Acute complete occlusion of the main portal vein trunk is a dramatic clinical event. Due to the abruptness of the process there is no time for the development of collateral vessels and acute PVT may extend to the splenic and mesenteric veins.

The clinical picture is characterized by acute diffuse abdominal pain, hemorrhagic small intestinal infarction, and circulatory shock. *Acute abdominal pain, fever and the rapid development of new or the increase of preexisting ascites in a patient with known cirrhosis should prompt the search for an acute PVT.*

The sudden occlusion of the portal vein or of one of its branches may cause a true or a pseudoinfarct in the liver. Acute occlusion of an intraparenchymal portal vein branch with concomitant impairment of arterial flow leads to the formation of a *pseudoinfarct of Zahn*.

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**Table 61.1** Etiology of portal vein thrombosis[a]

| |
|---|
| **Prehepatic** |
| • Pylephlebitis[b] |
| • Compression of portal vein (e.g. tumors, lymph nodes) |
| • Appendicitis |
| • Diverticulitis |
| • Pancreatitis |
| • Perforated peptic ulcer |
| • Tuberculous lymphadenitis |
| **Intrahepatic** |
| • Liver cirrhosis[c] |
| • Space occupying liver lesions |
| Hepatocellular carcinoma |
| Granuloma, abscess, cysts |
| Nodular transformation |
| Nodular regenerative hyperplasia |
| • Sinusoidal obstruction syndrome (formerly veno-occlusive disease) |
| • Pylephlebitis |
| • Congenital liver fibrosis |
| • Hepatoportal sclerosis (idiopathic portal hypertension) |
| **Posthepatic** |
| • Right heart failure |
| • Constrictive pericarditis |
| • Tumors of right atrium |
| • Obstruction of inferior vena cava (above the entrance of hepatic veins) |
| • Occlusion of hepatic veins |
| **Hypercoagulability** |
| • Protein C and S deficiency |
| • Antiphospholipid syndrome |
| • Antithrombin III deficiency |
| • Factor V Leiden mutation |
| • Factor II (Prothrombin) G20210A mutation |
| • Hyperhomocyteinemia (MTFR mutation) |
| • Increased Factor VIII |
| • Myeloproliferative Diseases (*JAK2* mutations) |
| Polycythemia vera |
| Essential thrombocythemia |
| • Paroxysmal nocturnal hemoglobinuria |
| **Miscellaneous** |
| • Sickle cell anemia |
| • Liver transplantation |
| • Surgical porto-systemic shunt |
| • Malignancy |
| • Blunt trauma |
| • Radiation injury |
| • Antineoplastic chemotherapy |
| • Arsenic intoxication |
| • Oral contraceptives |
| • Pregnancy and potpartum |
| • Neonatal omphalitis |
| **Idiopathic** |

[a]Endothelial injury, slowed blood flow and hypercoagulability combined are pathogenetically important
[b]All causes of pylephlebitis may also cause portal vein thrombosis
[c]Liver cirrhosis is by far the leading disorder underlying portal vein thrombosis

It consists of a sharply demarcated, red-blue, wedge-shaped area (the tip points towards the occluded portal vein branch) of marked hemostasis. The sinusoids are distended and the hepatocytes are severely atrophic but not necrotic, hence the designation pseudoinfarct. The development of a pseudoinfarct of Zahn is clinically silent. Therefore the process usually goes unnoticed or manifests incidentally by a slight and transient rise of aminotransferase concentrations of unknown etiology.

## *Chronic Portal Vein Thrombosis*

Subacute or chronic obliterative lesions of intrahepatic portal vein branches may lead to liver atrophy, with or without nodular regenerative hyperplasia of liver parenchyma [25]. The complete occlusion of a major portal vein branch may result in atrophy of the corresponding segment or lobe of the liver. When PVT develops insidiously, there is usually sufficient time for collateral vessels to develop and a sponge-like, angiomatous network (*cavernous transformation of the portal vein*) forms that maintains hepatic circulation, albeit at a low level. Portal hypertension results in the formation of esophageal varices and splenomegaly. Interestingly, patients with PVT, even in the absence of cirrhosis, may develop subclinical neurological abnormalities compatible with minimal hepatic encephalopathy [14].

The *diagnosis of PVT* is made by ultrasound with Doppler flow studies, CT scanning or MR-angiography (Fig. 61.1). Endoscopic ultrasonography with linear probes also has emerged as a sensitive and specific test to diagnose PVT [10]. A high percentage of patients with cavernous transformation of the portal vein may exhibit concomitant bile duct changes, such as dilatation and compression ("cavernoma-associated cholangiopathy"), which may remain clinically silent or lead to cholestasis or obstructive jaundice. Thus, cavernous transformation may be a rare cause of extrahepatic cholestasisis, and MR-portography may be coupled with MR-cholangiography in patients with PVT [6]. In children cavernous transformation of the portal vein is frequently associated with prehepatic portal hypertension and congenital anomalies, particularly atrial septal defects or malformations of the biliary tract or inferior vena cava [23].

Effective *therapy of PVT* is hampered by the lack of controlled data on which to base clinical decisions. The benefit of local thrombolysis in acute PVT (streptokinase or tissue plasminogen activator) is uncertain.

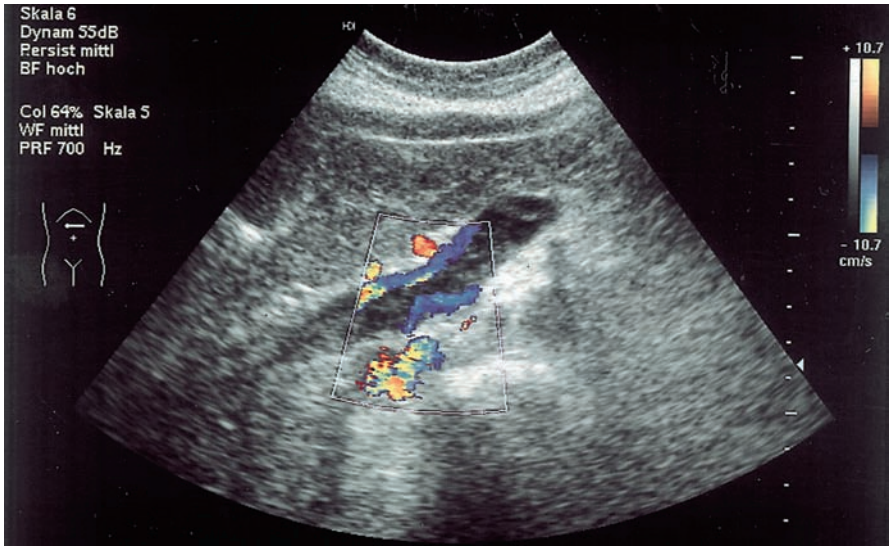


**Fig. 61.1** Color Doppler ultrasound of portal vein thrombosis. The thrombus is partially surrounded by blood

Recanalization of subacute and chronic PVT during anticoagulation for 4 months has been reported, but the study was not controlled and the data were evaluated retrospectively [4]. Therefore, currently there is no compelling evidence for thrombolysis or anticoagulation in patients with PVT. Considering preexisting liver disease, complications of portal hypertension, and comorbidities in most patients with PVT, the risks and benefits of these therapies should be balanced carefully [5].

Anatomic abnormalities causing extrahepatic portal vein obstruction may be amenable to surgical correction.

## Hepatoportal Sclerosis

### *Definition*

Hepatoportal sclerosis (idiopathic portal hypertension, non-cirrhotic portal hypertension; see also Chapter 53) is a disease of unknown etiology which primarily affects the intrahepatic terminal portal venules and leads to atrophy of liver parenchyma as well as to presinusoidal portal hypertension in the absence of cirrhosis.

### *Epidemiology*

Exact data on incidence and prevalence are not available. The disease is very rare in Western countries, and is encountered predominantly in India and in Japan. The incidence in Japan is declining, which may suggest the role of an infectious etiology. In Japan women are affected three times more frequent than men, while in India men are affected more commonly than women. More than 90% of patients are older than 30 years, with a mean age at presentation of 45–50 years [17, 20].

### *Etiology and Pathogenesis*

The cause of hepatoportal sclerosis is unknown. Immunologic alterations, chronic exposure to arsenic compounds, microcirculatory disturbances due to the formation of platelet microthrombi and fibrin emboli, and infectious pathogens (e.g. *Schistosoma*, HIV) have been incriminated [2, 7, 15, 16, 21, 22]. Pathogenetic relationships to Banti's disease (idiopathic splenomegaly with noncirrhotic portal hypertension) have been discussed [13, 22].

### *Pathology*

The terminal portal venules show fibrous intimal thickening (phlebosclerosis) with muscular hypertrophy and fibrosis of the media. The process leads to luminal stenosis and finally to complete obliteration of portal vein branches. The vascular lesions are particularly well visualized by elastic fiber stains. A high incidence of PVT is observed, which is believed to be secondary to the impairment of intrahepatic microcirculation [12].

Obliteration of portal veins is accompanied by marked portal and periportal fibrosis, concentric peribiliary fibroelastosis, sclerosis of the sinusoidal fibrous framework and by parenchymal atrophy. Although obliterative portal venopathy may lead to the formation of nodular regenerative hyperplasia, the basic lobular architecture remains preserved in hepatoportal sclerosis [25]. These changes have to be distinguished from secondary phlebosclerotic changes that may occur in schistosomiasis, primary biliary cirrhosis, and even without an underlying liver disease in old age.

### Diagnosis

The clinical picture is dominated by signs and symptoms of portal hypertension.

The liver enzymes in serum are normal or only slightly elevated. Often a mild hypergammaglobulinemia is observed.

Ultrasound, CT scanning and MRI show the sequelae (portal hypertension, liver atrophy) of the obliterative portal venous lesions but do not visualize the primary disease process itself.

The hepato-venous pressure gradient is normal or only slightly elevated (presinusoidal portal hypertension).

The results of liver biopsy are affected by sampling error since the process does not affect the entire organ uniformly.

### Prognosis and Therapy

The primary disease process is not amenable to therapy. The prognosis largely depends on the efficacy of prophylaxis and treatment of the sequelae of portal hypertension, especially esophageal variceal bleeding (see Chapter 80).

## Portal Vein Inflammation

### Pylephlebitis

Pylephlebitis is a suppurative endophlebitis of the portal vein that may lead to the formation of liver abscesses, mostly in the right liver lobe. It may be associated with portal vein thrombosis.

Pylephlebitis begins as an infective thrombophlebitis of small veins draining an area of infection. Septic thromboemboli lodge in the portal vein and cause inflammation of its wall. The *etiology* is multifaceted (Table 61.2). The most common blood stream isolates are Bacteroides fragilis, aerobic gram negative bacteria, streptococci, staphylococci, citrobacter and yeasts. Enterococci are found rarely [18]. In newborns septic phlebitis of the umbilical vein may extend into the intrahepatic portal vein branches.

Pylephlebitis is a chronic inflammatory disease characterized *clinically* by prolonged fever, night sweats, fatigue and intense malaise [9]. Abdominal pain, when present, is usually not severe. The diagnosis should be suspected in intraabdominal septic disorders with secondary involvement of the liver. Liver abscess and bowel ischemia may complicate pylephlebitis.

**Table 61.2** Causes of suppurative pylephlebitis

| |
|---|
| **Gastrointestinal diseases** |
| • Appendicitis |
| • Ulcerative colitis |
| • Crohn's disease |
| • Diverticulitis of small and large intestine |
| • Infected hemorrhoids |
| • Proctitis |
| • Ulcerated intestinal cancers |
| • Infections (e.g. typhoid fever, abdominal tuberculosis, shigellosis, schistosomiasis) |
| • Umbilical cord infections |
| • Perforating and deeply penetrating peptic ulcers |
| • Post gastrectomy |
| **Hepatobiliary and pancreatic diseases** |
| • Liver abscess |
| • Suppurative cholangitis |
| • Suppurative cholecystitis |
| • Echinococcal cysts |
| • Acute and chronic pancreatitis |
| • Pancreatic abscess (may also result from pylephlebitis) |
| **Suppurative urogenital diseases** |
| **Foreign bodies and trauma** |
| **Miscellaneous** |
| • Subphrenic and subhepatic abscesses |
| • Suppurative mesenterial lymphadenitis |
| • Suppurative retroperitoneal inflammation |
| • Lung abscess, pneumonia, emphysema |
| • Peritoneal carcinomatosis |
| • Bacteremia |
| • Rocky mountain spotted fever |

The laboratory examinations are nonspecific. Marked elevations of inflammatory parameters (ESR, CRP, neutrophil leukocytosis) are accompanied by a mild to moderate rise of aminotransferases, alkaline phosphatase and lactate dehydrogenase. However, a normal leukocyte count and even neutropenia do not exclude pylephlebitis. Blood cultures are mandatory for diagnosis.

The results of imaging are also nonspecific. On ultrasound, echogenicity of the liver is irregular. CT-scanning shows heterogeneous, ill-defined enhancement of parenchyma in the central liver segments. Provided no liver abscess has formed, no space occupying lesions are present. Demonstrating wall irregularities of the portal vein is very difficult. The presence of a thrombus in the portal vein is not sufficient by itself in diagnosing pylephlebitis. Occasionally gas in the portal venous system will be visualized.

*Prognosis* is very severe without adequate treatment. In the preantibiotic era pylephlebitis was universally fatal. Early, targeted antibiotic therapy is the *treatment* of choice. Empiric broad-spectrum antibiotics should be instituted with *one* of the following parenteral regimens while culture results and sensitivities are pending [24].

- Combination therapy

Metronidazole 500 mg i.v. tid

*plus one* of the following,

Ceftriaxone 2 g i.v qd *or*
Cefotaxime 2 g i.v qid *or*
Ciprofloxacin 400 mg i.v. bid *or*
Levofloxacin 500 mg i.v. qd.

or

- Monotherapy

Piperacillin/tazobactam 4.5 g i.v. qid *or*
Ticarcillin-clavulanate 3.1 g i.v. q4h *or*
Ampicillin-sulbactam 3 g i.v. qid *or*
Imipenem 500 mg i.v. qid *or*
Meropenem 1 g i.v. tid or
Ertapenem 1 g i.v. qd.

Most liver abscesses will require drainage, either operatively or percutaneously (ultrasound or CT-guided). The treatment plan should also consider the elimination of potential intraabdominal septic foci.

### *Granulomatous Phlebitis*

Portal vein granulomas may occur in sarcoidosis, schistosomiasis, and in mycobacterium avium intracellulare infection. Mineral oil granulomas are only rarely encountered in the portal vein.

## Portal Vein Gas Embolism

Gas inclusions within the portal vein in adults are frequently caused by vascular mesenterial occlusion and necrotizing enterocolitis. The common pathogenetic mechanism includes intestinal wall defects through which gas-forming pathogens reach the portal venous circulation.

These intravascular gas inclusions are easily distinguished by sonography from air in the bile ducts (pneumobilia) which is regularly present after sphincterotomy. Additionally, patients with portal vein gas emboli usually are severely ill, while pneumobilia occurs in clinically healthy persons.

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# Hepatic Arteries

# 62

Henryk Dancygier

## Chapter Outline



## Congentital Anomalies

The hepatic artery exhibits considerable anatomical variations which usually are not clinically relevant, and therefore are not discussed here. Anomalies of the hepatic artery, however, may attain clinical significance in certain situations such as during operative procedures (inadvertent ligation) or in interpreting angiographic findings (see Chapter 58).

## Hepatic Artery Occlusion

Occlusion of the hepatic artery or of one of its branches may be caused by thrombi, emboli, trauma or by inadvertent ligation. Due to the dual blood supply of the liver, arterial occlusion does not necessarily lead to a hepatic infarct. The consequences of impaired arterial inflow depend primarily on the size of the occluded vessel and the effectiveness of the collateral circulation. Ligation of the proper hepatic artery leads to total liver necrosis if arterial blood flow is not sustained by anastomoses from the gastroduodenal or the right gastric artery. Occlusion of smaller arteries results in focal parenchymal necrosis ("hepatocyte dropout").

Infusion of the antineoplastic agent floxuridine into the hepatic artery may cause an arterial thrombosis or an obliterative arterio- and venopathy of the vessels in the portal tracts. Arterial chemoembolization attempts to derive a therapeutic benefit from iatrogenic occlusion of tumor-feeding vessels. Thrombosis of the hepatic artery or of its branches induced by local infusion of antineoplastic agents may also cause bile duct ischemia with consequent bile duct strictures (see Chapter 52).

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Hyaline fibrin thrombi in small arterial vessels, characteristic of thrombotic thrombocytopenic purpura, may also be found in the liver.

## Hypoxic Hepatitis

See also Chapters 15 and 49.

The term "ischemic hepatitis" was coined in 1979 to refer to a liver injury characterized histologically by centrilobular parenchymal necrosis. Clinically, it is vaguely reminiscent of infectious hepatitis with a sharp increase in serum aminotransferase levels and the presence of anorexia, malaise, jaundice, and tender hepatomegaly [3]. Although the disorder is not due solely to ischemia and is not a hepatitis, the term continues to be used interchangeably with "hypoxic hepatitis" and "shock liver". In view of the etiology, pathogenesis, pathology and clinical features of the disorder, the general term "hypoxic liver injury" would appear to be more appropriate. Nonetheless, in this chapter, all four terms (ischemic hepatitis, hypoxic hepatitis, shock liver, hypoxic liver injury) will be used synonymously.

### *Definition*

Hypoxic hepatitis is defined as an acute liver injury due to an imbalance between hepatic oxygen supply and demand in the absence of other acute causes of liver damage. It is characterized by centrilobular parenchymal necrosis and a massive, but transient, increase in serum aminotransferase levels.

### *Epidemiology*

The incidence of hypoxic liver injury is 0.16% for inpatient admissions, 0.9% for intensive care unit admissions, 2.6% for cardiac care unit (CCU) admissions, and 22% for patients admitted to the CCU with decreased cardiac output [4]. If the cut-off value for serum aminotransferase concentrations defining hypoxic hepatitis is lowered the incidence figures are accordingly higher.

### *Etiology and Pathogenesis*

Hypoxic liver injury has been associated with congestive heart failure, chronic respiratory failure, sepsis, profound anemia, carbon monoxide poisoning, heat stroke, cocaine use, ergotamine poisoning, bacterial endocarditis, anaphylactic reaction, extensive burns and hypotensive states [4, 6]. Patients with chronic respiratory failure and chronic liver congestion are particularly prone to development of hepatic ischemia when a superimposed insult, such as decreased perfusion, hypoxia, or contact with endotoxin, occurs. Although hepatocellular ischemia is the common pathogenetic denominator in all the respective conditions, in clinical practice hypoxic hepatitis cannot be ascribed to only one pathogenetic factor, but generally several factors act in concert. In patients with left-sided heart failure and venous congestion secondary to right-sided heart failure hypoxia of the liver results from decreased arterial hepatic blood flow (ischemia). Chronic respiratory failure aggravates liver hypoxia mainly due to marked hypoxemia. The main contributing cause of hypoxic liver injury in patients with sepsis and septic shock is the increased metabolic (oxygen) demand of hepatocytes along with their inability to extract adequate oxygen from the blood [5, 6, 10]. Endotoxemia from bacterial translocation through the gut will promote hypoxic liver injury, as will a hypermetabolic state from alcohol abuse.

Acute sickle cell crisis in sickle cell anemia may lead to ischemic hepatic changes due to clogging of the sinusoids with damaged erythrocytes. However, marked sickling may be present in the absence of hepatic parenchymal necrosis [1].

### *Pathology*

Hypoxic liver injury predominantly affects centrilobular hepatocytes. Shock liver is the most severe form of ischemic liver cell damage. It is a complex disturbance of hepatic blood circulation occurring in circulatory shock, and is characterized initially by compromised arterial blood flow followed by secondary changes in lobular perfusion and venous outflow. The histopathological features of shock liver are hypoxic centrilobular changes such as loss of glycogen, hypoxic vacuoles, ballooning, and in its most severe form extensive zone

3 hemorrhagic necrosis of liver parenchyma (see Chapter 15). Provided the circulatory compromise has not disrupted the lobular reticulin fiber framework, and periportal cells remain viable, liver regeneration occurs, starting from periportally localized progenitor cells capable of proliferation and differentiation into hepatocytes. Thus, centrilobular ischemic necrosis often does not leave scars behind.

## *Diagnosis*

### Clinical Manifestations

Generally patients with hypoxic liver disease have multiple severe clinical problems, and symptoms are therefore usually related to the underlying disease and to circulatory failure rather than to ischemic liver injury. Patients may complain of right upper quadrant pain (due to the enlarged, congested liver), weakness and shortness of breath.

Hypoxic hepatitis is generally precipitated by an acute event, most commonly arrhythmia, pulmonary edema, and less commonly myocardial infarction. A circulatory shock state, if present, is usually septic or cardiogenic in origin. However, many patients with hypoxic hepatitis have no documented decrease in blood pressure and clinically a shock state is observed in only about 50% of cases [6].

### Laboratory Examinations

Characteristic of hypoxic hepatitis is a massive, sharp but rapidly reversible increase in serum aminotransferase levels. Twelve to 48 h after the initiating event ALT and AST concentrations in serum rise to values up to 20–50–100 times the upper limit of normal (cut off value not exactly defined). Typically aminotransferase levels decrease by 50% within 2–3 days if the causative disturbance is eliminated (see Fig. 49.1). Elevations of aminotransferase concentrations are accompanied by a rise in LDH (often >5,000 U/L) and GLDH. The latter enzyme is a good indicator of centrilobular injury since it is localized preferentially in mitochondria of zone 3 hepatocytes.

Bilirubin values may be slightly elevated but jaundice is not a clinical problem.

### Imaging Techniques

Results of hepatic imaging are nonspecific. Hypoechoic and hypodense irregularities are seen on ultrasound and on CT-scanning respectively. Imaging techniques are applied to exclude other causes of liver damage rather than to diagnose hypoxic liver injury itself.

On liver biopsy various degrees of centrilobular cell damage are seen and can be as severe as extensive centrilobular necrosis. In patients with low serum aminotransferase levels hypoxic liver injury cannot be diagnosed reliably without a biopsy.

## *Differential Diagnosis*

The diagnosis of hypoxic hepatitis requires the described laboratory changes and liver alterations in the appropriate clinical setting. Toxin- or drug-induced hepatitis, viral hepatitis, liver trauma, and pancreatobiliary disease must be excluded. Typically, and in contrast to acute viral or drug-induced hepatitis, in hypoxic hepatitis the markedly elevated liver enzymes return to normal within a few days. Hepatitis due to toxins can cause a marked increase in transaminase levels that reverses rapidly after withdrawal of the inciting agent.

## *Prognosis and Treatment*

The liver in hypoxic hepatitis is usually an innocent bystander that becomes involved in others problems. Treatment should be directed primarily at the underlying disease. Prognosis depends mainly on the underlying illness [7].

# Ischemic Cholangiopathy

See Chapter 52.

# Aneurysms

Hepatic artery aneurysms are usually sac- or spindle-shaped, rarely dissecting. Men are affected four times as often as females. 80% of aneurysms are solitary; in

20% of cases multiple aneurysms are found. 60% of solitary aneurysms affect the common hepatic artery, 30% the right hepatic artery, and in the remainder cases small arterial vessels at the liver hilum are affected. 75% of hepatic artery aneurysms are extrahepatic. Most aneurysms have a size of 2–10 cm at the time of diagnosis [2].

## Etiology

Hepatic artery aneurysms may be due to infections (mycotic aneurysms, syphilis, tuberculosis; <5% of hepatic artery aneurysms), trauma, generalized arteriosclerosis, or vasculitis, or they develop as iatrogenic lesions after, for example, arterial puncture during angiography. The increased use of interventional procedures after blunt abdominal trauma reveals that almost 50% of hepatic artery aneurysms are pseudoaneurysms. True aneurysms occur four times more frequently in the extrahepatic than in the intrahepatic arteries. They usually involve the common hepatic artery, and are associated with arteriosclerosis [9]. More than 50% of patients with polyarteriitis nodosa have multiple small ("miliary") intrahepatic arterial aneurysms.

## Diagnosis

### Clinical Manifestations

Aneurysms of the hepatic artery may be clinically silent, compress neighboring structures, or rupture into the liver or into the peritoneal cavity. Extrinsic compression of the common bile duct results in cholestasis with obstructive jaundice. Intrahepatic rupture causes right upper abdominal pain and may lead to gastrointestinal bleeding from erosion of the aneurysm into the stomach or duodenum. Erosion into the biliary tract occurs in nearly 50% of patients with rupture of an intrahepatic aneurysm. One third of these patients present with the triad of biliary colic, hemobilia, and obstructive jaundice [9]. The diagnosis should be considered in a patient with biliary colic associated with gastrointestinal bleeding. The dissection of the wall of the hepatic artery also causes acute abdominal pain, which may radiate to the right shoulder. This is a rare event and usually is misinterpreted clinically as pain of biliary or pancreatic origin. Rupture of an extrahepatic aneurysm into the peritoneal cavity manifests as abdominal pain associated with hypovolemic shock.

### Imaging Techniques

Ring-like calcifications at the liver hilum on plain abdominal radiography should arouse the suspicion of a hepatic artery aneurysm. The diagnosis is established with color Doppler ultrasound or, increasingly, with MR-angiography (Fig. 62.1). Nowadays, invasive conventional angiography usually is no longer required.

## Prognosis and Therapy

Risk factors for rupture are multiple aneurysms and a nonatherosclerotic etiology. The risk of rupture in relationship to the size of an aneurysm of the hepatic artery is not known. Without treatment almost 20–30% of hepatic artery aneurysms rupture spontaneously into the peritoneal cavity, with a mortality rate approaching 80%. Therefore, as soon as they are detected (usually incidentally) hepatic artery aneurysms should be treated either by ligation of the affected artery, embolization, or stent-graft placement. To avoid hepatic infarction, the portal vein must be patent. Vascular reconstruction is required to prevent hepatic ischemia.

In intrahepatic aneurysms resection of a segment or a lobe of the liver may be indicated.

# Arterioportal Fistula

Arterioportal fistulae occur in connection with aneurysms, tumors, in hereditary hemorrhagic telangiectasia, after trauma and liver biopsy, and as congenital malformations [11].

Pain in the right upper quadrant and a flow murmur are the most common clinical manifestations. Portal hypertension with ascites or variceal bleeding, or signs of hyperdynamic circulation with high-output cardiac failure are rare.

Small fistulae usually occlude spontaneously. Larger arterio-venous fistulae associated with splenomegaly and esophageal varices should be occluded by interventional radiologic techniques or be treated surgically.

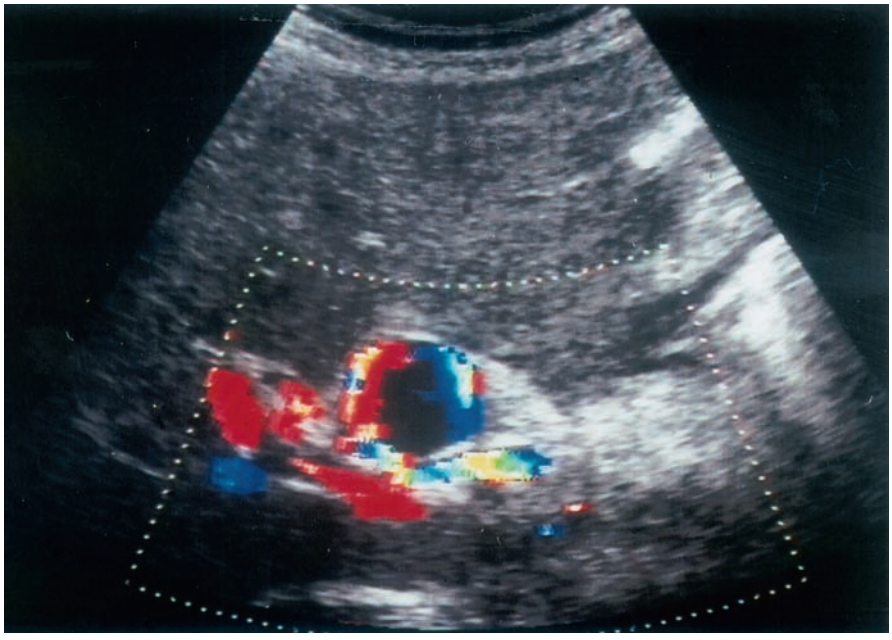

**Fig. 62.1** Aneurysm of the hepatic artery. On conventional grey scale ultrasound the lesion appeared as an anechoic space occupying mass. Color Doppler sonography reveals complete perfusion of the lesion (Courtesy W. Scharnke, MD)

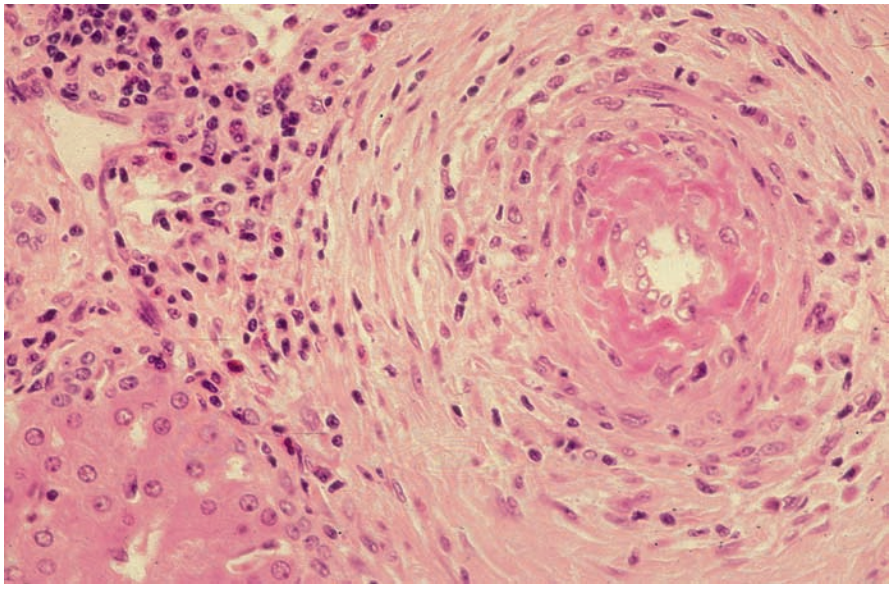

**Fig. 62.2** Polyarteritis nodosa involving a portal branch of the hepatic artery. Hematoxylin & Eosin (×400)

## Arteritis

Inflammation of the hepatic artery is rare. It may occur as a vasculitis affecting primarily the hepatic artery. More commonly, however, the hepatic artery is involved in a generalized vasculitic process in the context of collagen vascular diseases, rheumatic disorders, infections, drug-induced hypersensitivity reactions, or in rejection reaction after orthotopic liver transplantation. Arteritis may lead to thrombotic occlusion and/or the formation of aneurysms of the hepatic artery and its branches.

Hepatic arteries are affected in 60% of patients with *polyarteritis nodosa*; in approximately 15% of cases, a vasculitic-thrombotic occlusion of the hepatic artery or its branches leads to liver infarction (Fig. 62.2). A necrotizing angiitis of the hepatic artery with consequent thrombosis may occasionally be seen in cocaine users.

An obliterative endarteritis of the fine branches of the hepatic artery in the portal tracts with subintimal

accumulation of foamy, lipid containing macrophages (*xanthomatous arteriopathy*) and fibrosis is typical of chronic rejection reaction ("rejection arteriopathy") after liver transplantation. However, it has also been observed in single cases of rapid progressive loss of bile ducts (vanishing bile duct syndrome) [8].

The use of oral contraceptives may lead to endothelial cell hypertrophy and hyperplasia with deposits of acidic mucopolysaccharides in the intima. These changes are observed predominantly in portal tracts adjacent to a hepatocellular adenoma.

## Amyloidosis

Arterial amyloid deposits in the portal tracts have been described in primary (AL) and secondary (AA) amyloidosis, in 68% and in 100% of cases, respectively. See also Chapter 87.

## Vascular Tumors

Hemangiomas, angiomyolipomas, hemangioendotheliomas and angiosarcomas are discussed in Chapters 101 and 102.

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# Viral Infections by Hepatotropic Viruses

# 63

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## Chapter Outline



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# 63.1 Molecular Biology and Immunobiology of Hepatitis Viruses – Approach to Diagnosis and Therapy

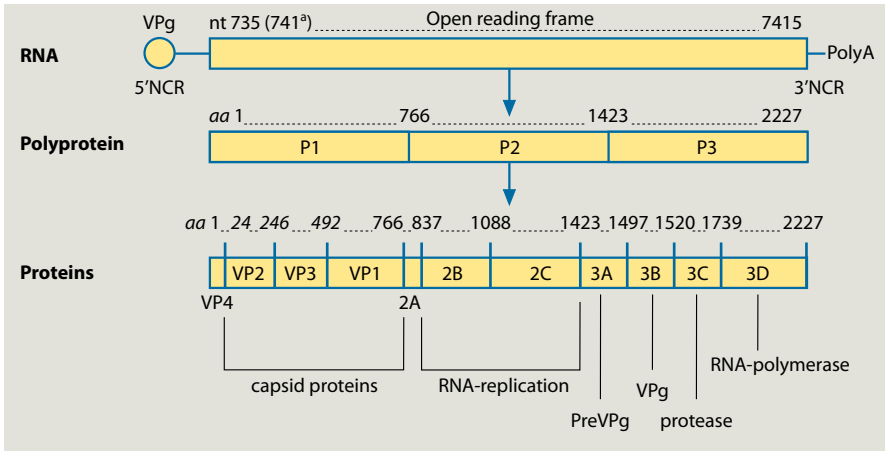
## Molecular Biology of Hepatitis Viruses

### *Molecular Biology of Hepatitis A Virus*

The hepatitis A virus (HAV) was first described by Feinstone et al. in 1973 using electron microscopy to investigate viral particles in the faeces of infected individuals. HAV belongs to the family *Picornavirus*, genus *Hepatovirus*. It is a nonenveloped, small, single strand RNA virus and the causative agent of infectious hepatitis. HAV is transmitted through the faecal-oral route, spreading primarily through close personal contact with an HAV-infected person. After replication in infected hepatocytes, viral particles gain access to the intestine by passing through the biliary tract. Hepatitis A viruses are primarily human pathogens that can be, in contrast to other hepatitis viruses, transmitted to different primates. In addition, they grow in cell culture of primate cells including diploid human fibroblast- and hepatoma-cell lines. The complete genome of wild type HAV was sequenced in 1987. The wild type HAV is relatively resistant against heat and drought, grows slowly in cell culture systems and does not affect the cellular protein synthesis. The wild type virus shows no cytopathic effects as compared to some other viral variants.

#### Morphology, Genome Organization and Replication

HAV is composed of a single strand, 3'-polyadenylated (PolyA), positive-sense RNA genome surrounded by a nonenveloped icosahedral capsid that is approx. 28 nm in diameter. The 7.5 kb large genome consists of a single open reading frame (ORF) with 5'- and 3'-noncoding regions (NCRs) (Fig. 63.1). The 750 bp 5'NCR of the RNA strand comprises the internal



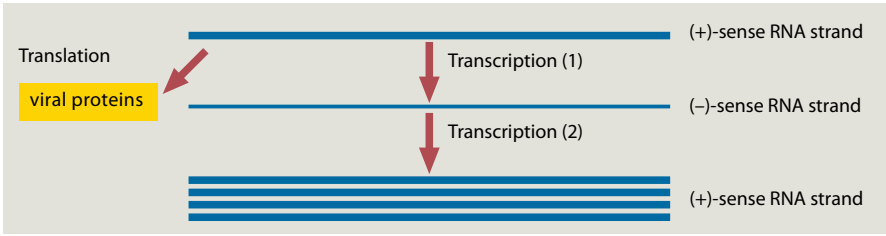




**Fig. 63.1** Genome organization of hepatitis A virus. The different HAV proteins and their function are indicated. The nucleotide positions (nt) or amino acid positions (aa) are indicated. [a]Downstream AUG codon at nt 741 to 743 preferred for translation. Virus-specific protein (VPg); 5'and 3' non-coding region (NCR); polyadenylation signal (PolyA)

**Fig. 63.2** Common replication strategy of HAV, HCV and HEV. First, the genomic (+)-sense RNA strand is transcribed into (–)-sense RNA strand (1). The (–)-sense RNA strand then serves as a template for the synthesis of several RNA genomes (2). In addition, the (+)-sense RNA strand is used as mRNA for the synthesis of virus proteins

ribosome binding site (IRES). In addition, there is a viral protein called virus-specific protein (VPg), bound to the 5'-terminus of the RNA strand, possibly involved in the initiation of RNA synthesis. The 3'NCR consists of 60 nucleotides and is tagged with a polyadenylation (poly-(A)) signal. After translation of the single ORF, the polyprotein is cleaved into one structural protein P1, and two non-structural proteins P2 and P3.

As a common replication strategy of all positive-sense RNA viruses, the genomic RNA functions as mRNA and can be translated immediately upon infection of the host cell. Thereby the virus encoded RNA-dependant RNA-polymerase (RdRp) is translated. The RdRp catalyses the synthesis of both positive- and negative-sense RNA (Fig. 63.2). The P1 protein is cleaved into the four capsid proteins VP1–4 by a virus encoded protease. The capsid proteins assemble to the nucleocapsid in which the positive-sense RNA is packaged. The epitopes conferring antibody recognition are located within the unglycosylated capsid proteins and are probably formed by adjacent regions of different capsid proteins. Therefore, the antigenic properties of the epitopes depend on the conformational folding of the capsid proteins.

## Genotypes and Serotypes

In contrast to other Picornaviruses, HAV shows a highly genetic and antigenic stability. HAV has been classified into four human (I–III and VII) and three simian (IV–VI) genotypes and genotype I and III are further classified into subtypes with sequence variability of less than 7.5% [69]. Within a given genotype sequence, homology is defined to be more than 85%. Most human strains belong to the genotype I, which has been divided into subtypes Ia and Ib. For genotype II only two strains classified as subtypes IIa and IIb are known. A global analysis of the HAV structure did not reveal fundamental antigenic differences. Thus, there is only one serotype of HAV.

## *Molecular Biology of Hepatitis E Virus*

In 1980 the hepatitis E virus (HEV) was identified and assigned to the genus *Hepevirus*. Epidemic outbreaks of acute hepatitis were reported in Asia which were not caused by HAV and therefore referred to be related to a so far unknown orally transmitted Non-A-Non-B hepatitis virus. After its visualization by electron microscopy in the faeces of infected patients, HEV was cloned and sequenced in 1990.

HEV is transmissible to different primates like chimpanzees, cynomolgus monkeys, rhesus monkeys, pigtail monkeys, owl monkeys, tamarins and African green monkeys. Efficient *in vitro* replication of the virus in cell culture is very complex and time-consuming, and cytopathic effects are described.

In the last years it became more and more obvious that HEV circulation in non-HEV-endemic countries could be higher than previously assumed. Anti-HEV-antibodies could be detected in up to 28% of US-citizens but it is unknown whether these antibodies were produced as response to a non-pathogenic HEV infection or as cross reaction to a HEV-related antigen, probably derived from a pig-HEV. Recently a high prevalence of anti-HEV IgG was measured in 209 patients from the Netherlands who all showed clinical symptoms of a hepatitis. All patients were neither abroad nor in contact with anyone who had hepatitis and all were tested negative for hepatitis A, B, and C virus infection. This study provides evidence of locally acquired hepatitis E in The Netherlands. Therefore, in cases of unexplained acute hepatitis, the diagnosis of hepatitis E should be considered even in the absence of foreign travel.

### Morphology and Genome Organization

Based on its morphology the HEV was originally classified within the family *Caliciviruses* (from 1988 to 1998) but because of discrepancies concerning sequence relations it is now the sole member of the genus *Hepevirus* in the family *Hepeviridae*. HEV is a non-enveloped, spherical, positive-sense RNA virus with a diameter ranging from 27 to 34 nm. The HEV genome is shown in Fig. 63.3. The approx. 7.5 kb encompassing RNA contains a 3'-poly(A)-tail, short 5'- and 3'NCR's and three overlapping open reading frames (ORFs). Each ORF is used to express different proteins. ORF1 (5 kb) is located towards the 5'-terminus of the genome and encodes the nonstructural proteins that are required for virus replication including a methyltransferase, a putative papain-like cysteine protease, an RNA-helicase and an RNA-dependent RNA-polymerase (RdRp). ORF2 which does not overlap with ORF1 is located at the 3'-terminus of the genome and encodes the capsid protein with a molecular weight of 71 kDa. ORF2 contains important epitopes that can induce neutralizing antibodies and has been the focus of vaccine development. ORF3 encompasses 369 nucleotides. It begins with the last nucleotide of ORF1 and overlaps with ORF2 with 328 nucleotides. ORF3 encodes a small phosphoprotein (14.5 kDa) which associates with the cytoskeleton, suggesting a possible role in the assembly of virus particles.

### Genotypes and Serotypes

A high genetic heterogeneity that was observed in sequence analysis of HEV isolates from different parts

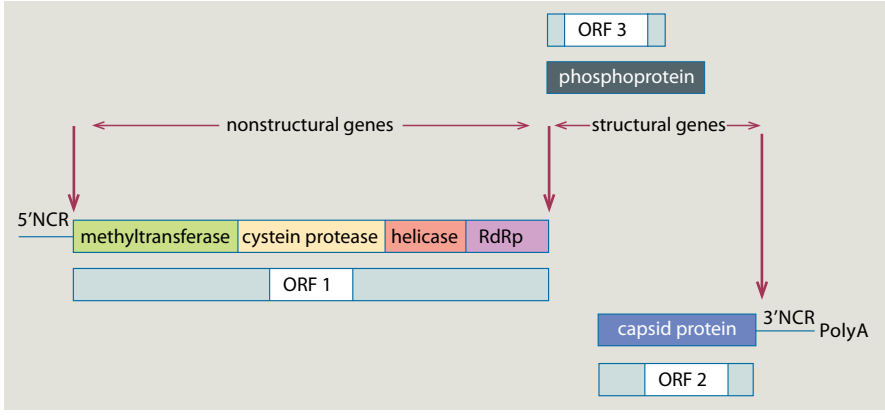



**Fig. 63.3** Genome organization of hepatitis E virus. The partially overlapping three open reading frames (ORFs) and their respective proteins are shown. The 5' and 3' non-coding region (NCR) and the polyadenylation signal (PolyA) at the 3'-terminus of the RNA are indicated. RNA-dependent RNA-polymerase (RdRp)

of the world can be assigned to four different genotypes. Analysis of these different isolates using immune electron microscopy revealed at least one common cross-reactive epitope. Within the epitopes of the Mexican and the Asian isolates amino acid homology in the ORF2 and ORF3 is 90.5% and 73.5%, respectively. In all given genotypes only one serotype is recognized. Thus an efficient vaccine should provide protection against all genotypes. Recently, the safety and efficacy of an HEV recombinant protein (rHEV) vaccine in a phase 2, randomized, double-blind, placebo-controlled trial was evaluated. The study which included 1,796 volunteers from Naples showed a high efficacy of 95% in the prevention of hepatitis E [78]

### Replication of the Hepatitis E Virus

The first step in virus reproduction is translation of the non-structural proteins encoded by the ORF1 which are necessary for virus replication. The viral RNA functions as mRNA and is transcribed to negative-strand RNA which serves as template for replication of new virus genomes and mRNA's (see Fig. 63.2). Upon virion maturation an alkaline domain, located at the N-terminus of the capsid-protein, seems to play an important role in encapsidation of the genome. It is unknown, whether host proteins are involved in virus replication and maturation.

## *Molecular Biology of Hepatitis B Virus*

The human hepatitis B virus (HBV) together with HAV belongs to the oldest known hepatitis viruses. The taxonomy of the most important species of this family is summarized in Table 63.1. HBV belongs to a family of closely related DNA viruses, called *Hepadnaviridae*. The human HBV only infects mature hepatocytes of humans and chimpanzees, and there is only a restricted use of *in vitro* systems such as *in vitro* infection of human hepatocytes. Besides the human pathogenic HBV there are several similar hepatitis viruses pathogenic to animals, such as the woodchuck- or the duck-HBV which are also important model systems for HBV research. HBV replicates primarily in hepatocytes but can also be detected in other tissues and persistent low level viremia is possible after recovery from HBV

**Table 63.1** Taxonomy of the Hepadnaviruses

| Family | Genus | Species |
|---|---|---|
| Hepadnavirus | Orthohepadnavirus[a] | HBV |
| | | WHV |
| | | GSHV |
| | Avihepadnavirus[b] | DHBV |
| | | HHBV |

[a]Orthohepadnavirus: Hepadnaviruses, found in mammalian. *HBV* human hepatitis B virus, *WHV* Woodchuck hepatitis virus, *GSHV* ground squirrel hepatitis virus

[b]Avihepadnavirus: Hepadnaviren, found in birds. *DHBV* duck hepatitis B virus, *HHBV* heron hepatitis B virus

infection. The number of HBV-DNA copies in human sera can vary from $10^{11}$ to $10^{13}$ per mL, whereas the ratio of infectious to non-infectious HBV particles is 1:1,000.

Until the introduction of new efficient cell culture system for the *in vitro* study of HBV infection in 2002, only human hepatocytes or hepatocytes of related primates were susceptible to HBV infection. However the use of human liver material was hampered by the limited availability and the quality of hepatocyte preparation. The observation that hepatocytes of *Tupaia belangeri* are susceptible to HBV and the human hepatocyte-derived cell line HepaRG which supports the full replication cycle of HBV represents a powerful tool to study the mechanism of cell entry as well as many other applications, including drug metabolism [27, 38].

### Genome Organization

The hepatitis B virus is one of a few known non-retroviral viruses which employ reverse transcription as a part of their replication process. The HBV genome is a partially double-stranded circular DNA consisting of approximately 3,200 bp (Fig. 63.4). The negative-sense DNA strand encodes seven different proteins which are expressed from four different, overlapping ORFs. These ORFs, called S, C, P, and X, encode the structure proteins (S and C) and the non-structural proteins (P and X). These proteins are described in detail below. The ORF which encodes the S-proteins, also termed hepatitis-B-S-antigens (HBsAg), comprises three different in-frame start codons. These codons are located in different domains referred to as preS1-domain, preS2-domain or S-domain. Each start codon is used to initiate the translation of one of the three different S-proteins, the large

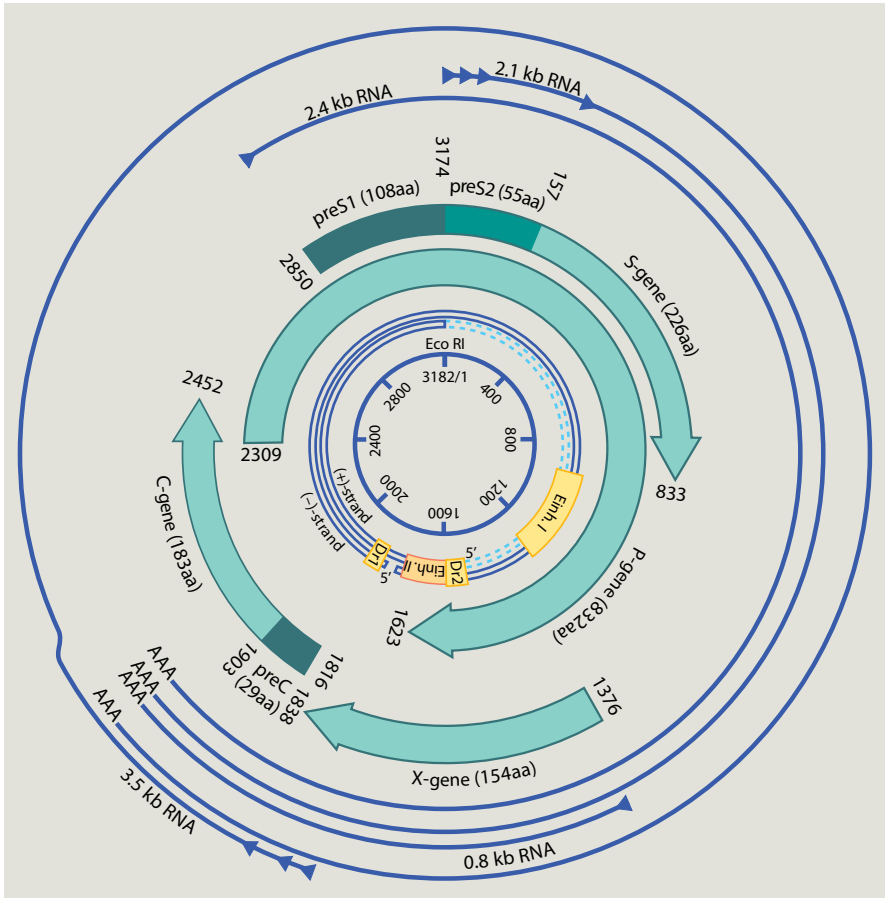



**Fig. 63.4** HBV genome organization. The circular HBV-DNA is indicated with its (−)-DNA strand, the partially overlapping (+)-DNA strand, the DR1/DR2, and the enhancer elements (EnhI and EnhII). The HBV genes preS1/preS2/S, preC/C, P, and X with the nucleotid positions and the length of the encoded proteins are shown. The outer circles represent the three subgenomic RNAs and the pregenomic RNA. The length and the polyadenylation signals (AAA) of each strand is indicated. The triangles in each strand represent the start codon

(L), the middle (M) and the small (S) envelope protein. From the C-ORF the preC-gene, also called hepatitis-B-early-antigen (HBeAg) and the hepatitis-B-C-antigen (HBcAg) or capsid-gene (C-gene) are transcribed. Due to the overlapping nature of the different ORFs more than 50% of the nucleotides are located in more than one HBV-gene. The polymerase (P-protein) is covalently bound to the 5'-terminus of the DNA strand. Further important regions displaying regulatory function in HBV replication are the direct repeats (DR) 1 and DR2 as well as two enhancers (EnhI and EnhII).

The HBV genome is surrounded by an icosahedral capsid that is aproximately 22–25 nm in diameter. Besides the genome, the DNA-polymerase is encapsidated into the nucleocapsid. The viral envelope consists of membranes derived from the endoplasmic reticulum (ER) of the infected cell in which the HBsAg is incorporated. In all infected individuals some small, approximately 22 nm spherical particles, consisting of envelope structures without bearing a nucleocapsid, circulate together with the full infectious viral particles. The concentration of these non-infectious particles, also called the "Australian-antigen", are $10^2$- to $10^3$-fold higher than the concentration of infectious particles.

## HBV Envelope Proteins

The HBV envelope is composed of three related viral surface proteins, the L- (large), M- (middle) and S- (small) envelope-proteins, each, as indicated by the name, with a different molecular weight. They are expressed from one open reading frame containing two promoters and three different in-frame start codons which are located in different domains referred to as preS1-domain, preS2-domain or S-domain. All of the three envelope proteins share the S-domain as common carboxyl-terminus. Depending on which of the three translation initiation sites is used, the L-protein (389 aa or 400 aa), the M- (281 aa), or the S-protein (226 aa) is synthesized (Fig. 63.5). Depending on the glycosylation status, the L-protein exists as 39 or 42 kDa protein,

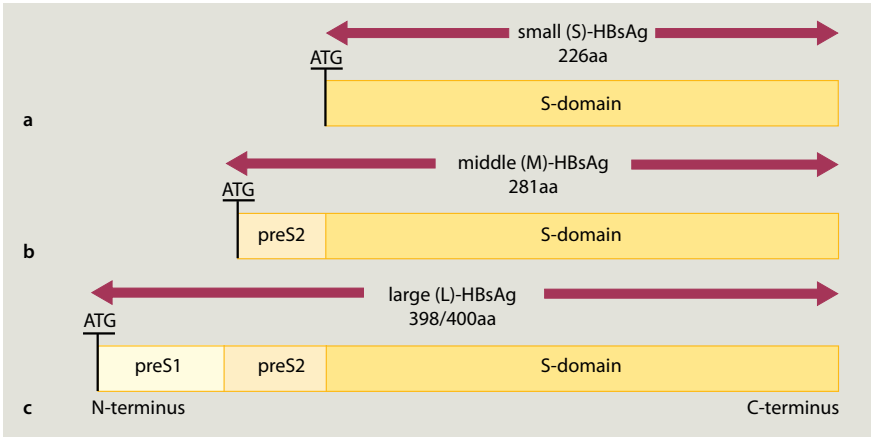



**Fig. 63.5** Linear map of the hepatitis B surface antigens. The open reading frame of the surface proteins provides three internal start-codons (ATG) at the N-terminus. Depending on which of these translation initiation sites is used, the S-protein (A), the M- (B), or L-protein (C) is synthesized. All three surface proteins consist of the same C-terminal sequence (S-domain). Numbers represent the length of each protein

the M-protein as 33 or 36 kDa protein and the S-protein as 24 or 27 kDa protein. The surface proteins are not only incorporated into virion envelopes but are, in addition, secreted in large amounts from infected hepatocytes as spherical and filamentous subviral lipoprotein particles (22 nm in diameter) bearing no nucleocapsid.

Like typical membrane proteins, the surface proteins are synthesized at the endoplasmic reticulum (ER) and are incorporated into the membrane. The transmembrane topology for the M- and the S-proteins are identical. The N-terminus of the proteins is inserted into the lipid bilayer by a signal sequence. A second sequence downstream of the first signal directs the translocation of the polypeptide chain into the ER lumen and anchors it into the membrane. Thereby, the C-terminus is oriented towards the ER lumen whereas the hydrophobic C-terminal domain is embedded into the membrane. This configuration results in the formation of two loops, one oriented towards the ER lumen and one to the cytosol. The luminal loop which is N-glycosylated is known to carry the major epitope for HbsAg [63]. The glycosylation pattern seems not to be involved in epitope formation. In contrast, the epitopes displayed on the surface of preS1- and preS2-antigens are influenced by the glycosylation motifs. For unknown reasons, there are two forms of the L-protein because the transmembrane topology is changing posttranslational. Thus, about half of the L-proteins carry an external and the other half an internal preS-domain.

## HBV Capsid Proteins

The entire open reading-frame of the C-gene contains two in-frame start codons which are used to initiate the synthesis of two proteins, the HBc- and the HBe-antigen (HBcAg, HBeAg). The region in between the first and the second start-codon is termed precore region (preC) and the region between the second start- and the stop-codon is termed core-region (Fig. 63.6). The HBeAg is a nonstructural, secretory protein. It is translated from the preC-ORF as a 25 kDa unglycosylated protein (P25). P25 is directed to the secretory pathway by a 19-amino acid signal sequence at the N-terminus that is cleaved during translocation into the lumen of the endoplasmic reticulum (ER), producing a 22 kDa protein (P22). P22 is further processed in a post-ER compartment by the cleavage of 34 aa from its C-terminus leading to the mature HBeAg. The HBcAg, which is translated as a 21 kDa protein from the C-ORF assembles to the nucleocapsid. The C-terminus of the core-protein consists of several alkaline amino acids which enable the protein to bind RNA and DNA. The HBcAg is not directly detectable in blood samples because no appreciable amounts of core proteins are secreted. However, presentation of the highly antigenic HBcAg through infected cells leads to consistent production of anti-HBc-antibodies which are detectable as a marker of HBV infection in blood screening. Core-proteins that are not completely assembled lack the antigenic activity of a conformational B-cell eptitope or a putative sequential epitope. Besides, the HBcAg is capable of initiating the cellular immune response. Dissolving of core-particles, i.e. by detergent treatment, abolishes the recognition by anti-HBc-antibodies but free core particles P22 can be neutralized by anti-HBe-antibodies. These antibodies also recognize the P25 precursor-form of the HBeAg, which can not assemble to core particles. In contrast to HBcAg, HBeAg and anti-HBeAg-antibodies can be detected in

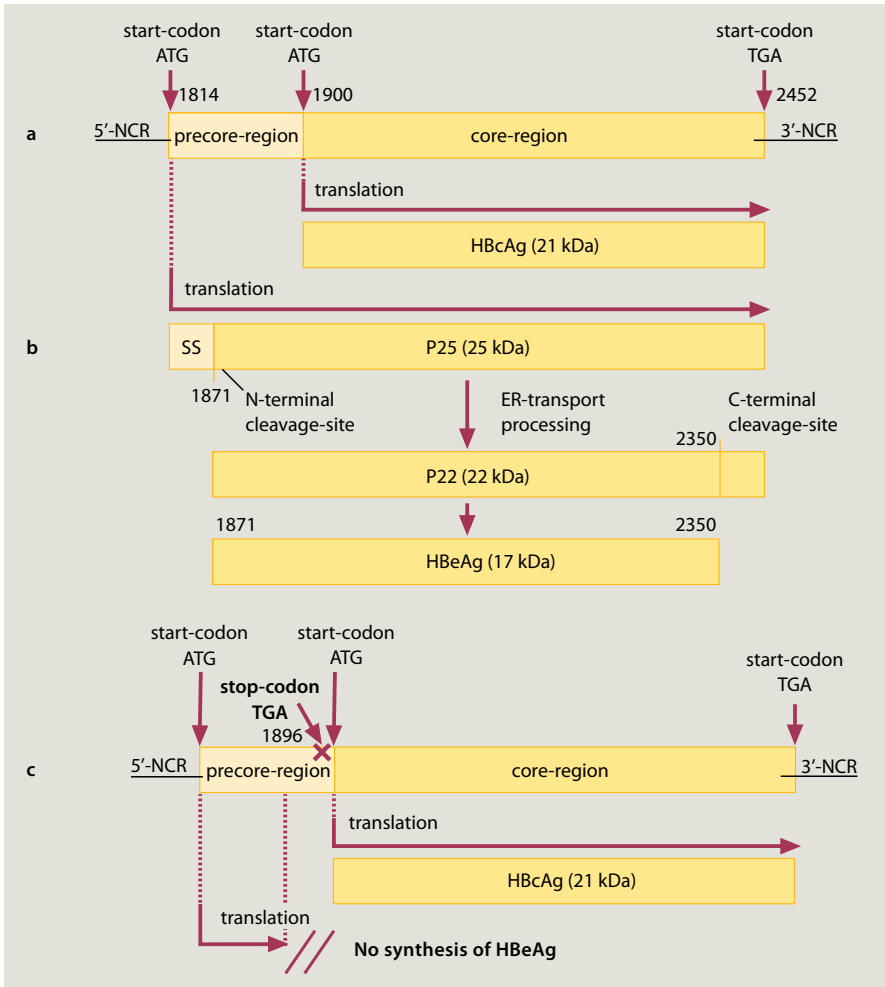



**Fig. 63.6** (**a**) transcription of the HBcAg is initiated at the second start-codon of the ORF. The HBcAg is encoded by the core-gene. (**b**) Transcription of the HBeAg is initated at the first start-codon of the ORF at the 5'-terminus of the precore region. The resulting precursor protein P25 is posttranslationally processed in the ER-compartment by cleavage of the signal sequence (19 aa) from the N-terminus and 34 aa from the C-terminus to yield the HBeAg. (**c**) Precore mutants bear a stop-codon in the precore region leading to inhibition of the synthesis of HBeAg. Numbers represent nucleotide positions

blood samples. The HBeAg is known to have two B-cell epitopes and several HBcAg specific determinants which seem to be masked by the fast binding of soluble HBeAg to serum albumin, alpha-anti-trypsine and immunoglobulins. Thus, HBeAg cannot be eliminated by HBc-antibodies. Alternatively, it is possible that the HBeAg specific antigenic determinants of the core particles are not accessible for antibody detection. Based on murine experimental studies it is assumed that the HBeAg has regulatory functions in the immune response [52, 53]. Mutations in the preC-gene leading to defective or terminated gene transcription which abrogates the synthesis of HBeAg have been described [12]. The most common mutant within the preC-gene is the G to A mutation at nucleotide 1896. This mutation transforms codon 23 from TGG to a TAG stop-codon. HBV-infection with this mutant is highly replicationally efficient indicating that the HBeAg is not necessary for virus replication. Infection with a mutant HBeAg negative isolate lowers the risk for the newborn child to develop chronic infection as compared to wild type HBV transmission. In wild type-HBV infection the development of chronic hepatitis B may be due to the interaction with the HBeAg which has been shown to be responsible for promoting immune tolerance in utero in perinatal infection and to modulate immune response to the HBcAg [16]. In contrast to the preC mutation, mutations in the core promoter have been associated with enhanced pathogenicity, including the development of hepatocellular carcinoma [5, 36]. In addition, core promoter mutations cumulatively enhance viral genome replication *in vitro* [61]. There are several studies which show a correlation of core promoter mutations and fulminant hepatitis, and even a well documented outbreak of fulminant hepatitis after transmission of a core promoter mutant is published [42]. However, a case-controlled study is needed to verify the significance of HBV mutants in fulminant hepatitis B.

## HBV Polymerase

The HBV polymerase, designated pol or P-protein with a molecular weight of 84 kDa, is a multifunctional enzyme with RNA- and DNA-dependent DNA polymerase function. The polymerase is composed of four domains, namely, the terminal protein (TP), the spacer protein, the reverse transcriptase (RT), and the RNaseH. The TP domain is covalently linked to negative-strand DNA which is important for the packaging process of pregenomic RNA into the nucleocapsid and for the initiation of HBV replication. The spacer protein has not been shown to be associated with specific functions. The RT-domain contains the consensus motif for the reverse transcriptase. The RNaseH domain is responsible for the degradation of the pregenomic RNA template in terms of replication.

## HBx-Antigen

The exact function of the 17 kDa HBV-X protein is still unknown. The sequence of the HBV-X protein encoding gene is highly conserved among the genus *Orthohepadnaviridae* which might attribute an essential role to this protein. Several studies have shown that HBV-X can transactivate the transcription of several promoter-enhancer constructs in transfection experiments [77, 83]. In addition, the HBx protein has been proposed as an important oncogenic stimulus for the development of hepatocellular carcinoma in chronically infected individuals [7, 8, 22, 60].

## Genotypes, Serotypes and HBV-Variants

**Genotypes and Serotypes**. Worldwide, HBV isolates have been classified into eight genotypes, A – H. More than 8% divergence in the whole genome sequence or more than 4.1% divergence in the S-gene sequence represents the basis for differentiating various genotypes [58, 59]. Different HBV genotypes have been shown to have a distinct geographic distribution. In Europe, mainly HBV genotypes A and D are present. Genotype A is more prevalent in northern Europe, whereas genotype D is mainly found in the Mediterranean countries and in Eastern Europe [75]. In the USA genotype A, B, C, G, and H are present whereas genotype B and C are also highly prevalent in Asia. Genotype E is only found in Africa and genotype F in South and Central America. Genotype H was recently identified in patients from Central America [4]. Based on the serological heterogeneity of the HBsAg, HBV isolates can be classified into four major subtypes, namely ayw, ayr, adw, and adr. The determinants d, y, w, and r represent different epitopes which are mutually exclusive. Based on the analysis of subdeterminants within ayw/adw and adr, nine subtypes of the virus have been proposed. These include ayw1, ayw2, ayw3, ayw4, ayr, adw2, adw4, adrq$^+$, and adrq$^-$.

**HBV-Variants.** In recent years an increasing number of HBV mutants, found in all of the four HBV ORFs, were described. Most of them seem to be silent without changing the phenotype of the virus. However, some of them have clinical relevance. Pre-core mutants which were mentioned above, on the one hand abrogate the synthesis of HBeAg but on the other hand show enhanced pregenomic encapsulation and initiation of DNA synthesis because the G to A mutation (transformation of codon 23 from TGG to a TAG stop codon) also affects the binding stability [46]. In addition, the pre-core mutant seems to be more prevalent in chronic HBV carriers without clinical, biochemical or histological evidence of liver disease [45, 54]. Additionally, there are also point mutations, short deletions and insertions found within the core promoter region (nucleotides 1634–1782). A double mutation which converts nucleotide 1,765 from A to T and nucleotide 1,767 from G to A is frequently found in the HBV genome. This double mutation within the core removes a nuclear receptor binding site in the core promoter, reduces precore gene expression, and increases the viral replication rate [10]. Further important mutations referring treatment of HBV by nucleoside or nucleotide analogues, are located within the HBsAg between amino acid residues 100 and 160 and result in evasion of the host immune response [24]. Other mutations are described in association with resistance to antiviral agents (DNA polymerase mutations) and hepatocellular carcinogenesis (X mutants). Interestingly, three cases of primary drug resistance were described recently. In three treatment naïve patients who failed primary adefovir therapy a valine at position 233 of the reverse-transcriptase domain, instead of isoleucine (rtI233V) as in the wild type virus, was detected. These variants remained sensitive to tenofovir in vitro [76].

## HBV-Replication Cycle

The 42 nm hepatitis B virion is a complex, spherical, double shelled particle that consists of an outer envelope containing host-derived lipids and S gene polypeptides,

the large (L), middle (M), and small (S) surface proteins, also known as HBsAg. The virus uses the outer envelope for the attachment and entry into the host cell, although numerous potential cellular binding sites for the three HBV-surface proteins have been described in the past. However, none of them have been proven to be a functional receptor (Fig. 63.7). Hepatocytes are known to be the most effective cell type for replicating HBV, but other human cell types have been found to be able to support replication to a lesser degree. The fusion of viral protein and cell membrane allows the release of the inner core or nucleocapsid into the cytosol. The virus-cell fusion seems to be triggered by a conservative region in the N-terminus of its S-domain which includes a hydrophobic sequence [86]. The internalization and the transport of the nucleocapsid to the nucleus is still unclear but there are some studies that the sequence of PLSSIFSRIGDP in the middle of preS2 confers the property of cell permeability [29, 57].

The nucleocapsid, with a diameter of 27 nm, contains the core protein (HBcAg), a 3.2 kb circular, partially double stranded viral DNA genome, and an endogenous DNA polymerase attached to the 5'-terminus of the negative-sense DNA strand. Inside the nucleus, the viral polymerase is removed and converts the partial double stranded DNA into a covalently closed circle DNA (cccDNA). From the cccDNA several genomic and pregenomic RNAs are transcribed

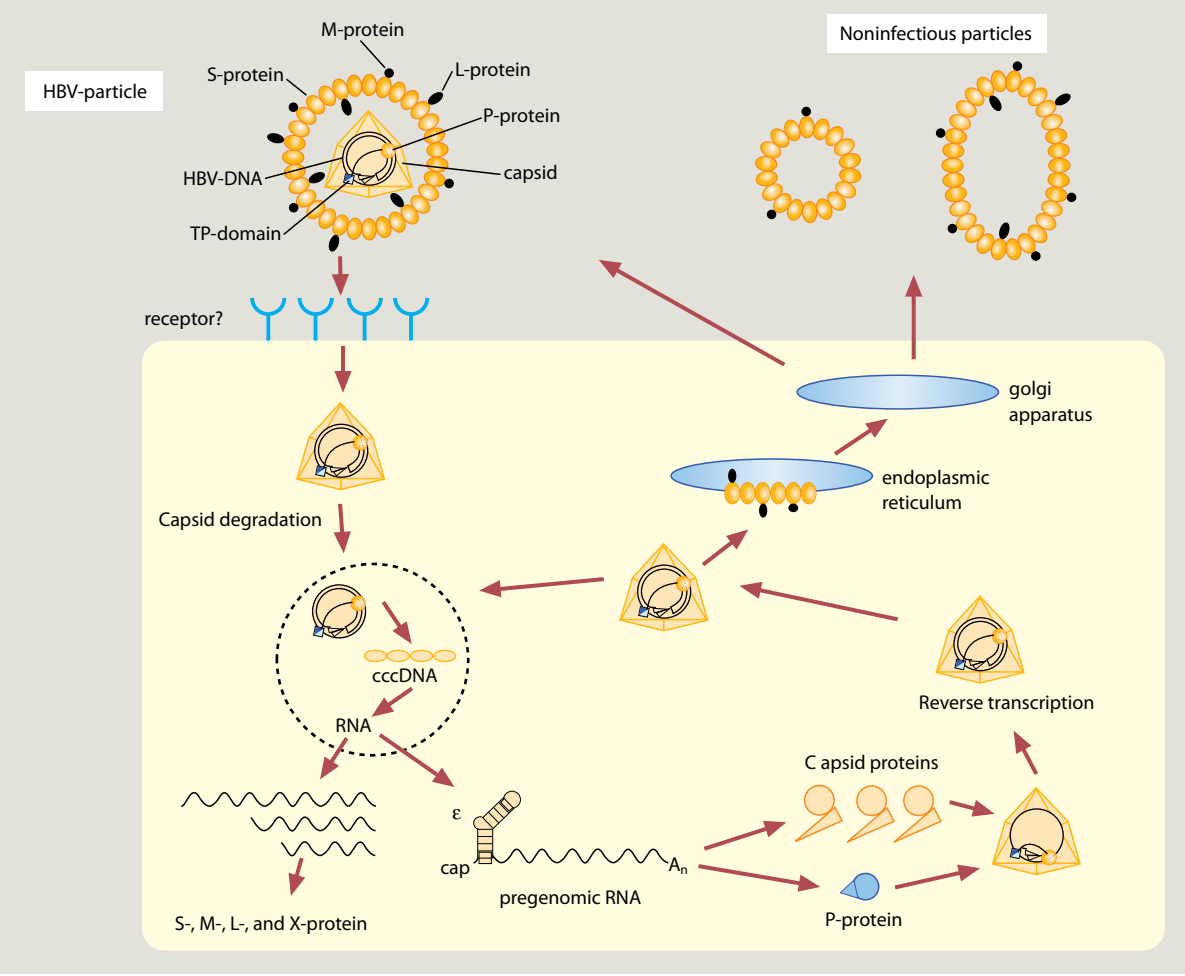



**Fig. 63.7** Replication cycle of the HBV: After entry into the host cell by enveloped virions, the nucleocapsid is released. Inside the cytoplasm of the host cell, the nucleocapsid is degraded. Free DNA is translocated into the nucleus where it is converted to covalently-closed circular DNA (cccDNA). Transcription of cccDNA by the host cellular polymerase II produces several genomic and pregenomic RNAs. All of them contain 5'cap structures and are 3'-terminally poly-adenylated and serve as template for viral gene products. The pregenomic RNA and the P-protein is encapsidated and inside the nucleocapsid the reverse transcription into DNA takes place (for details see text). DNA containing nucleocapsids are then either enveloped and released as infectious HBV viruses, or the DNA is translocated into the nucleus and a new round of HBV replication occurs

by host cellular RNA polymerase II. All RNA-intermediates contain 5'cap structures, are 3'-terminally poly-adenylated, and serve as templates for viral gene products. The mechanisms for the exportation of pregenomic RNAs from the nucleus to the cell cytoplasm, where the translation of the different viral proteins and the capsid assembly take place, are still unclear. Inside the cytoplasm the pregenome and the P-protein are selectively packaged into progeny capsids. The packaging process, which is poorly understood, is triggered by the binding of the P-protein to a special sequence within the pregenome, known as epsilon or encapsidation signal, that forms a hairpin structure. The pregenome serves as a template for the HBV-DNA-negative-strand synthesis (Fig. 63.8). The reverse transcription of the RNA-template starts within

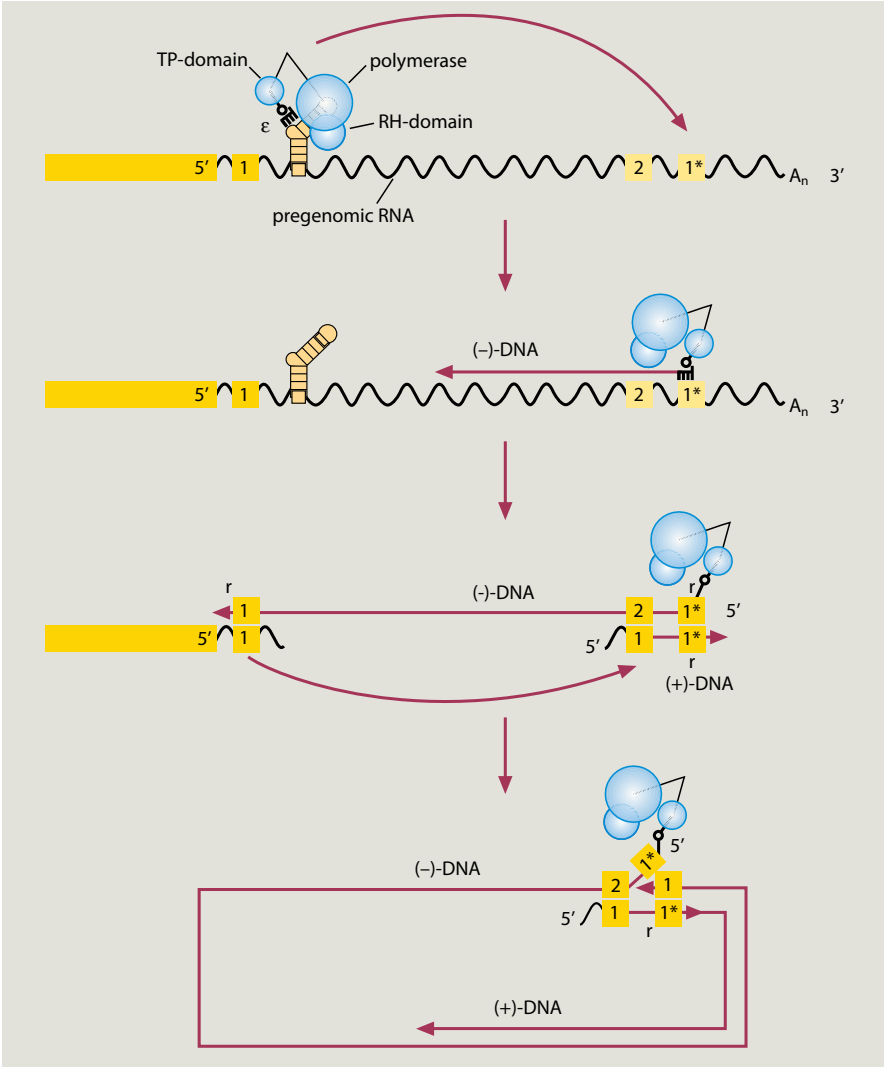



**Fig. 63.8** HBV-DNA-synthesis: The pregenomic RNA serves as template for the HBV-DNA-negative-strand synthesis. The polymerase (polymerase, RNase H-domain and TP-domain) binds to the 5'-terminus of the pregenome at a special structure, called k-loop. The reverse transcription of the RNA-template starts within a region called DR1 (1*) (at the motif 5' UUCA) and is initiated by a protein priming mechanism using a tyrosine located near the amino terminus of the reverse transcriptase itself. After transcription of only four nucleotides, the polymerase switches to the DR1-region of the mRNA and the four nucleotides base pair with the complementary sequences. The pregenome is degraded by the viral RNaseH domain, except for its capped 5'-terminal region including the DR1 sequence. The remaining DR1 sequence serves as primer for the DNA-positive-strand synthesis. The 5'-terminus translocates to a highly similar sequence, termed DR2 (2). The completed HBV-DNA-negative-strand contains a small redundancy, termed r. The growing DNA-positive-strand is transferred from its 5' r to the 3' r of the DNA-negative-strand, enabling further elongation to yield circular DNA

a region called DR1 (motif 5' UUCA) and is initiated by a protein priming mechanism using a tyrosine located near the amino terminus of the reverse transcriptase itself. After transcription of only four nucleotides the polymerase switches to the DR1-region of the mRNA and the four nucleotides base pair with the complementary sequences. The exact mechanisms of polymerase translocation and triggering of DNA-synthesis is still unknown. During the elongation of the DNA-negative-strand towards its 5'-terminus, the pregenome is simultaneously degraded by the viral RNaseH domain, except for its capped 5'-terminal region including the DR1 sequence. The remaining DR1 sequence, which serves now as primer for the DNA-positive-strand synthesis, translocates to a highly similar sequence, termed DR2. The completed HBV-DNA-negative-strand is a copy of the pregenome from its 5'-terminus to the DR1 region and it contains a small redundancy, termed r. A last step towards formation of the initial HBV genome is the circularization of the DNA-strand. The growing DNA-positive-strand is transferred from its 5'r to the 3'r of the DNA-negative-strand, enabling further elongation to yield circular DNA. While inside the nucleocapsid the HBV-genome is replicated, the capsid moves to the post-ER and pre-Golgi membranes where the envelopment by the surface proteins takes place. It is proposed that viral DNA synthesis is associated with a structural change in the capsid, allowing only mature capsids to be enveloped and to finally be secreted [80]. Like typical membrane proteins, the surface proteins are synthesized at the endoplasmic reticulum and incorporated into the membrane. Mature capsids move laterally along intracellular membranes to budding sites where subsequently secretion into the blood occurs. Alternatively to the budding process, retranslocation into the nucleus is possible where a new round of HBV replication starts.

The efficiency of virus replication is quite high and the virus production ranges from $5 \times 10^{10}$ to $1 \times 10^{13}$ virions per day. In spite of this high replication rate the mutational rate is, with a range from $2 \times 10^{-4}$ to $3 \times 10^{-5}$ point mutations per nucleotide per year, relatively low.

## Hepatitis B Immunology

In the early stage of HBV infection a broad-based cellular immune response resulting in activation of the natural killer (NK) cell-system has been demonstrated to be one of the most important factors contributing to virus clearance. Processing and presentation of viral antigens by dendritic cells and macrophages lead to a specific immune reaction. In this process the CD4 T-helper (Th) cells bind with their T-cell receptor to viral antigens which are presented on the surface of HLA-class II molecules. The release of different cytokines by proliferating Th1-cells (IL-2, IFN-γ, TNF-α) and Th2-cells (IL-4, IL-5 and IL-10) enhance the immune response whereas the emphasis of Th1-cells (cellular immune response) and Th2-cells (humoral immune response) may have an important effect on the further development of the HBV-infection. In addition, cytotoxic $CD8^+$ T-cells are activated by HBV antigens bound to HLA-class II molecules and induce apoptosis or lysis of the infected cell. The humoral immune response of the B-cell population is costimulated by the T-cell response and directly by the circulating HBV- antigens.

## Immune Response of Acute and Reactivated HBV Infection

During the course of infection, activated B-cells produce antibodies against the different HBV proteins (HBsAg, HBeAg, HBcAg, P and X) but only the antibodies directed against the envelope proteins (L, M and S) have the capability to neutralize the virus. IgM antibodies which are detectable early during infection and indicate acute hepatitis B are directed against the HBcAg. In addition, antibodies comprising epitopes for the RNaseH-domain of the P-protein can be found during an early stage of infection. Anti-HBe-antibody production is associated with resolution of acute hepatitis B which is associated with a dramatic decrease in virus replication. Finally, in the vast majority of cases (>90%) neutralizing anti-HBs-antibodies are produced which indicate spontaneous resolution of HBV-infection. Clearance of the virus, together with the production of anti-HBs antibodies, is associated with protective immunity for a future HBV infection. Some of the capsid antigens can induce an effective HLA-class II restricted T-cell immune response, which is often associated with HBs-antigen seroconversion. However, a T-cell response directed against the HBs-antigen is detectable at very low levels.

For viral clearance the HLA-class I restricted $CD8^+$-T-cell response directed against HBe/HBc-antigens seems to play a major role. Thus, a spontaneous clearance of acute HBV infection is closely related to an early and strong cytotoxic T-cell response. In addition, an activation is observed. The epitopes which are recognized by the cytotoxic T-cells are located in highly conserved regions of the HBV-genome. Therefore, it is unlikely that mutations in these regions which lead to a lack of $CD8^+$-T-cell response are a plausible explanation for the development of chronic HBV infection.

Instead of a broad antibody response against the different envelope proteins as observed in HBV infection, vaccination with recombinant vaccines induce an immune response only directed against one epitope within the small envelope proteins.

### Immune Response in Chronic Hepatitis B

HBV infection is defined as "chronic" if the HBsAg is detectable for more than 6 months. The importance of T-cell response in virus clearance is underlined by the observation that the development of persistent infection is often associated with a defective cellular immune response. Transmission of HBeAg from a pregnant HBV carrier to the fetus in the majority of the cases is followed by the development of chronic HBV infection because HBeAg is recognized as an autoantigen in the thymus of the fetus. In adult patients manifestation of chronic infection is often combined with a lack of $CD8^+$-T-cell response. For HBV infection itself no cytopathic effects are detectable, and the histological changes and the grade of inflammation correlate with the extent of the T-cell response. Before HBe/antiHBe seroconversion a characteristic increase in the inflammatory activity can be observed, which is combined with an increase of the liver enzymes caused by an enhanced T-cell response.

## *Molecular Biology of Hepatitis D Virus*

Hepatitis-delta virus (HDV) was first described in 1977 in patients with a complicated course of HBV infection. In these patients a new protein was discovered, which was thought to be a new HBV-encoded protein and labeled as the delta antigen. Subsequent research indicated that this antigen was derived from a new virus, named HDV, however, the envelope of HDV is composed of HBV surface antigens. It is known that the HBV surface antigens are part of the HDV envelope and that they are necessary for productive infection. Thus, HDV may be considered to be a satellite virus of HBV. The HDV virion has a spherical structure of approximately 36 nm in diameter and merely differs in the composition of HBV L-, M- or S-proteins. HDV uses the HBV envelope to enter the host cell but the exact receptors are still unknown. The HBV envelope harbors the nucleocapsid consisting of approximately 70 molecules of hepatitis D antigens (HDAg) and the viral RNA genome. The nucleocapsid exists in two different forms, as 24 or 27 kDa capsids. The HDV genome consists of a circular single-stranded RNA molecule of approximately 1.7 kb that displays a high degree of internal base-pairing (approx. 70% of the genome) and a high similarity to plant viroid RNAs (Fig. 63.9). The genome encodes only the HDAg, which is a nuclear, RNA-binding phosphoprotein and which is required for virus replication.

Because HDV has no sequence homology with its helper virus and because it also infects animals, being the only animal virus with a circular RNA genome, it constitutes a unique entity and is assigned to a separate genus, called *Deltaviridae*. The evolution rate of HDV ranges from approximately $2 \times 10^{-2}$ to $7 \times 10^{-3}$ point mutations per nucleotide per year. Multiple ORFs of different length are present on the genomic and on the antigenomic strand of HDV-RNA, but most of their potential gene products have not been detected. The only ORF (ORF5) located on the antigenome, that is preserved among all known isolates, encodes the HDAg [48, 85]. The HDV-RNA can be divided into two domains. One domain represented on both, the genomic and antigenomic RNA, is related to other viroids and displays ribozyme activity. This domain autocatalytically cleaves and ligates itself. Additionally, it shows UV-induced crosslinking of two uracil-nucleotides (U712 and U865) which is a common feature observed in other viroids. The other domain, which is the major part, is located on the antigenome and includes the HDAg encoding ORF (Fig. 63.9).

Recently, a novel system for studying HDV genome replication in cultured cell lines in the absence of HBV was described by stable transfection of a cell line (293-δAg) with a complementary DNA of the HDV sequence [14].

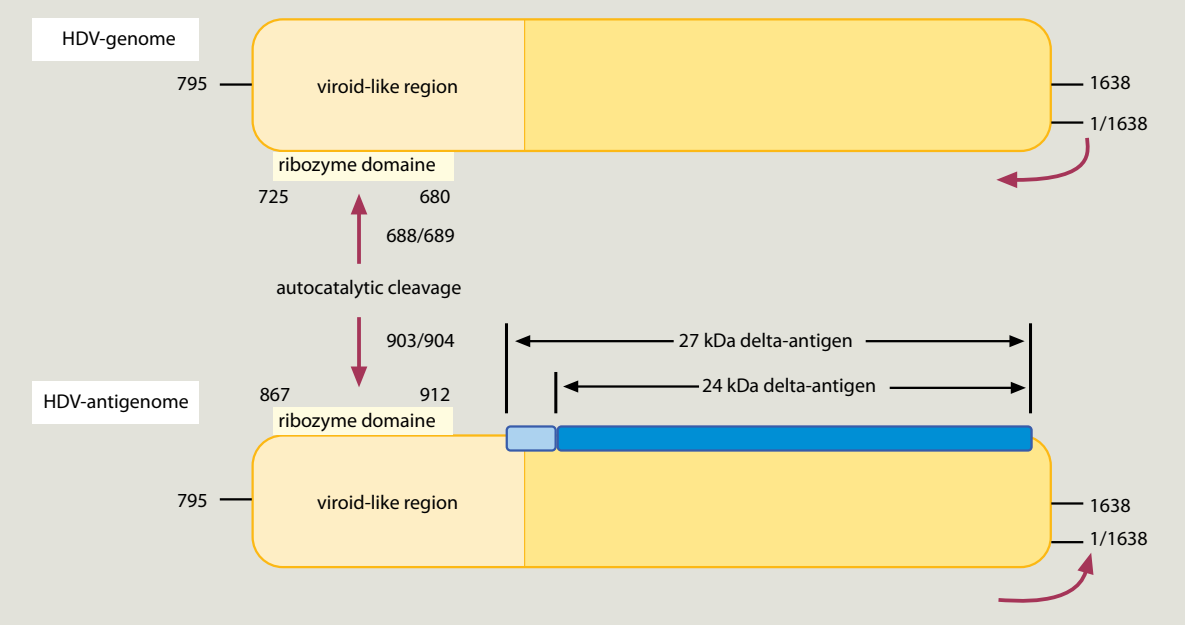



**Fig. 63.9** Schematic graphs of the HDV-RNA structure. The circular HDVgenome (clockwise orientation) and the antigenome (counterclockwise orientation) are shown. The localization of the large and the small HDV-antigens on the antigenome are indicated. Numbers represent nucleotide positions

## Genotypes

Based on sequence homologies, the different HDV isolates can be classified into three different genotypes. Genotype 1 is found in all parts of the world and the sequence homology within this genotype is more than 80%. Genotype 2 is predominantly present in Japan and shares sequence homology of less than 80% with genotype 1. Genotype 3 is mainly present in Peru and Columbia. The sequence similarity is about 60–65% compared to the other genotypes. Infection with HDV genotype 1 might lead more often to the development of liver cirrhosis and hepatocellular carcinoma but the responsibility of sequence heterogeneity for the clinical outcome is still unclear.

## Hepatitis Delta Antigen

HDAg is a RNA-binding phosphoprotein and the major component of the nucleocapsid. It is known to exist as 27 kDa protein referred to as large (L)-HDAg, and as 24 kDa protein, termed small (S)-HDAg. Both proteins have the same amino acid composition although they are translated from two different mRNA-species. At the C-terminus of the L-HDAg 19 additional amino acids may be found because some of the mRNAs are altered at the amber termination codon (from TGA to TGG) and therefore the ORF extends for the additional amino acids. Interestingly, these mutations can be induced by interferon-therapy.

Both proteins contain several structural domains:

One domain has a coiled-coiled structure with classical features of a leucin–zipper sequence, which is important for the dimerization of both proteins to form a nucleocapsid of approximately 70 delta-proteins and a diameter of 19 nm. A second important domain encompasses the nuclear localization sequence which is responsible for the transport of HDAg into the nucleus. The third domain is the RNA-binding domain which confers the ability to HDAg to bind to RNA molecules. The C-terminal extension of the L-HDAg encompasses an isoprenylation signal. Isoprenylation of the C-terminus enables the L-HDAg to interact with the HBV envelope proteins and it might confer the ability to interact with the membrane. In addition, the L-HDAg has been shown to be more phosphorylated than the S-HDAg, but the phosphorylation sites are not yet determined. The L-HDAg is only detectable in later stages of the infection cycle. It

was shown to function as a dominant-negative inhibitor of HDV replication.

## Replication

After infection of the host cell via still unknown receptors and entry mechanisms, the HDV-RNA is translocated to the nucleus where RNA replication takes place. The RNA is replicated by use of cellular transcription machineries, and cellular RNA-dependent RNA-polymerase II (RNA-Pol II) has been implicated as the key enzyme for HDV-RNA replication. HDV-RNA replicates, as known for plant viroids, by a "double-rolling-circle" mechanism. In this model, the circular negative sense RNA is used as template for the synthesis of antigenomic RNA which is copied multiple times. This RNA intermediate, consisting of several HDV genomes, is self cleaved by HDV ribozyme activity into monomeric RNA-strands which are ligated to yield antigenomic, circular RNA. In a second round of rolling circle the antigenomic RNA serves as a template for the synthesis of genomic HDV-RNA. The relative ratio of genomic and antigenomic RNA is approximately 15:1 and each infected cell contains approx. 300,000 copies of HDV-RNA [17]. The replication cycle is shown in Fig. 63.10.

## Immunology

Basically, the immune response in HDV infection is similar to that of HBV infection (see above). Various IgG- and IgM-antibodies can be detected during chronic HDV infection. A decreasing amount of IgM-antibodies in the acute stage of infection is associated with decreasing inflammatory activities and is often an indication of virus clearance, while persistent IgM-antibody levels are characteristics of a chronic infection. However, the main criterion for acute HDV-infection is the detection of HDV-RNA in serum samples. Caused by a not well-known interaction of the HBV replication and gene-expression, the HBV/HDV co-infection typically leads to the suppression of HBV replication with often low or even undetectable levels of HBV-RNA. Consequently, the HBV specific

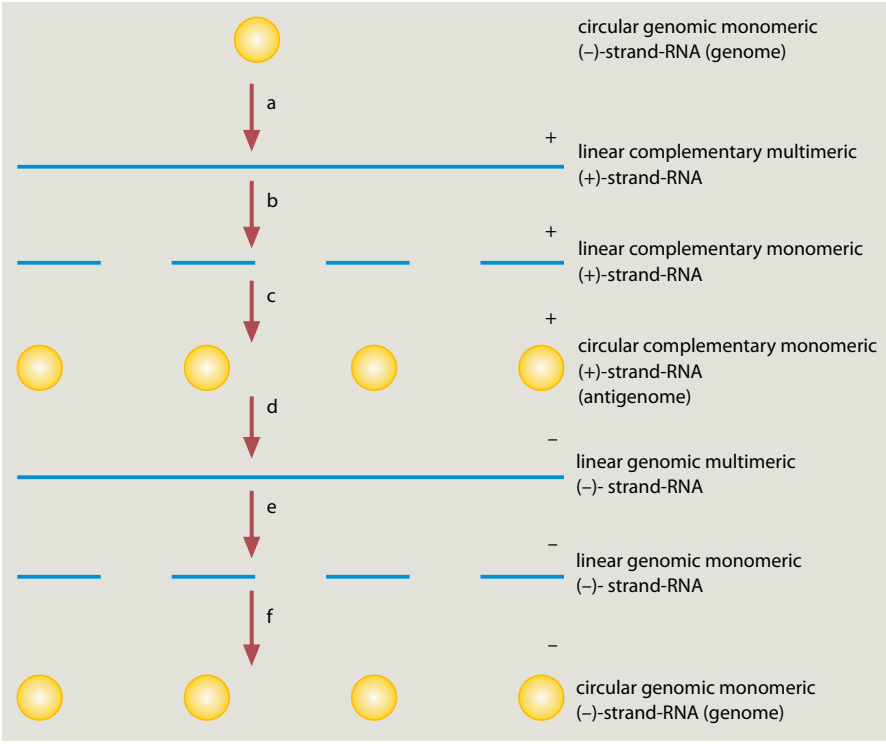



**Fig. 63.10** (**a–f**) Replication of HDV – the double rolling-circle model of HDV-RNA replication. The circular negative sense RNA genome is used as template for the synthesis of a linear antigenomic RNA (**a**) which contains multiple copies of the genome. This RNA intermediate is self cleaved by HDV ribozyme activity into monomeric RNA-strands (**b**) which are ligated to yield antigenomic, circular RNA (**c**). In a second round of rolling circle the antigenomic RNA serves as template for the synthesis of a linear genomic RNA (**d**). This genome intermediate is self cleaved into monomeric RNA-strands (**e**) which are ligated to yield circular genomic RNA (**f**)

mRNA is suppressed and fewer HBV proteins are synthesized. The severe damage to hepatocytes which is often associated with HDV infection may refer to the direct cytopathic effect observed for HDAg. In chronic HBV carriers a specific T-cell response to HDAg in the peripheral blood of individuals with hepatitis delta is related to the decrease of HDV-induced disease activity [56].

### HBV and HDV

HDV infection occurs either as simultaneous co-infection or as superinfection in HBV carriers but, despite its helper function, HDV replicates independently from HBV. Some clinical studies have shown that HDV co-infection influences HBV progression. For chimpanzees it is shown that HDV infection leads to undetectable HBV capsid proteins in liver tissue and decreasing HBV surface protein levels. In some patients HBeAg and even HBsAg may become undetectable after HDV superinfection. In addition, there is some evidence that a co-infection with HDV may suppress molecular markers of HBV infection. In clinical studies it has been shown that the majority of patients with chronic hepatitis B/D have detectable amounts of HBV-antibodies but have undetectable or low HBV-RNA concentrations. Even in liver tissue no significant amounts of HBV-DNA were detectable. These findings need to be confirmed by the use of currently available, more sensitive assays for HBV-DNA detection.

## *Molecular Biology of Hepatitis C Virus*

Since the 1970s there was neither epidemiological nor clinical doubt that additional hepatotropic viruses beside hepatitis A and B existed ("Non-A-Non-B hepatitis"). Numerous attempts to isolate the Non-A-Non-B hepatitis pathogen with classical methods of microbiology were not successful. Experimental data indicated the existence of an RNA virus responsible for an often chronically progressing post transfusion hepatitis. Epidemiologically and clinically this Non-A-Non-B hepatitis entity was differentiated from an enterically transmissible, non chronic Non-A-Non-B acute hepatitis, whose pathogen was already identified in 1980 and termed hepatitis E virus (HEV).

In 1988 isolation of the genome of the parenterally transmissible hepatitis C virus succeeded due to the use of extensive biomolecular techniques. Initially, large amounts of plasma from a chimpanzee artificially infected with the Non-A-Non-B hepatitis pathogen were used for complete RNA extraction. After reverse transcription of the entire RNA, a gene library from cDNA sequences in lambda bacteriophages was established and subsequently suitable proteins were expressed in *E. coli*. Through the screening of more than 1 million of these *in vitro* expressed proteins with sera from patients with clinical and histological proven chronic Non-A-Non-B hepatitis, one clone (5–1–1) was finally found, which apparently expressed viral nucleic acids. This clone was then used as a probe to identify the complete HCV genome.

HCV is classified in the family *Flaviviridae*. Recently a new genus, *Hepaciviridae*, was established for HCV. Unlike other viruses of the *Flaviviridae* (Dengue virus, yellow fever virus, Tick-borne encephalitis virus), HCV is not transmitted through arthropods and a chronic infection is established in the majority of cases.

### Genome Organisation

Hepatitis C virus (HCV) is an enveloped, approximately 9.6 kb, positive-sense, single-stranded RNA virus. After translation from a single large open reading frame (ORF), the polyprotein precursor is cleaved by viral and host peptidases (signal peptidases of the endoplasmic reticulum), resulting in three structural proteins, termed core (p21), envelope 1 (E1, gp31), and envelope 2 (E2, gp70), a protein named p7, which probably forms an ion channel, and six non-structural (NS) proteins, termed NS2 (NS2–3 cysteine protease, p23), NS3 (serine protease/helicase, p70), NS4A (NS3 protease cofactor, p8), NS4B (transmembrane anchor protein, p27), NS5A (replication factor, p58), and NS5B (RNA-dependent RNA-polymerase, p68) (Fig. 63.11). The F protein has been described as being a result of a ribosomal frameshift of the core-encoding genomic region [87]. There is a noncoding region of 324 to 341 nucleotides at the 5'-non coding end (5'NC) containing the internal ribosome entry site (IRES) and a 3'-noncoding (3'NC) region of variable length. The 3'NC region is composed of three sequence elements, a nonconserved highly variable

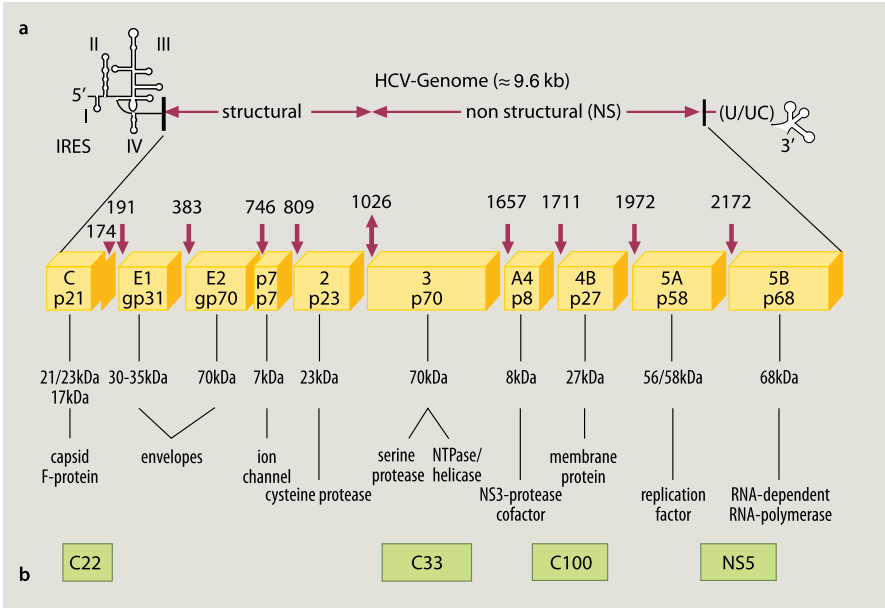



**Fig. 63.11** (**a, b**) HCV genome organisation. (**a**) HCV genome composition with structural and non-structural genes. There are non-coding regions at the 5'end and at the 3'end as well. The translated structural and non-structural proteins are displayed. (**b**) Localization of the epitopes used for antibody detection in commercially assays. In enzyme immunoassays of the 1st generation antibodies against C100 were detected. In tests of the 2nd and 3rd generation antibodies against C22 and C33 and C22, C33 and NS5 were additionally detected, respectively. (C core, E envelope, NS non-structural proteins)

region (30–50 nucleotides), a poly(U • C) tract of variable length (20–200 nucleotides), and a highly conserved 98 nucleotide sequence, designated the 3' X region. The latter forms a three stem-loop structure (SL1 – SL3). An RNA 5'-3' end interaction for initiation of RNA minus-strand synthesis and translation stimulation is supposed for the HCV-RNA, even though it lacks a cap structure at the 5' end and a poly(A) tail at the 3' end, which are typical for many viral RNAs. This interaction can also play a role in switching from RNA translation to negative-strand RNA synthesis. However, the IRES-dependent translation in human hepatoma cell lines is strongly stimulated by the 3'NC region.

## HCV-Quasispecies

A hepatitis C infection *in vivo* is characterized by a heterogeneous population of closely related isolates, the so called quasispecies. The spectrum of quasispecies can change during the normal course of the disease or during treatment. The variability of the virus is due to a high replication rate and a lack of proofreading activity of the RNA-dependent RNA-polymerase. Selection of the isolates *in vivo* occurs as a result of the replication competence of the viruses and through immune selection of the host.

Methodologically, the population of quasispecies from an infected person is best displayed with the hypervariable region 1 (amino acid position 1–27 of E2) of the hepatitis C virus genome. Spontaneous resolution of hepatitis C has been associated with a low quasispecies complexity, whereas a chronic course correlates with early genetic evolution of high quasispecies heterogeneity during the acute phase of infection. Different studies have shown higher virologic response rates to interferon-based HCV treatment in patients with a small quasispecies heterogeneity compared to patients with a high quasispecies diversity and complexity. During antiviral therapy, certain isolates appear to be interferon-sensitive while others are resistant. Conventional cloning and sequencing of the isolates of HCV-quasispecies is the gold standard but time consuming and costly. An alternative method to analyze HCV-quasispecies is the electrophoretic separation of PCR products from the hypervariable region 1. This so called single-stranded conformation polymorphism (SSCP) analysis enables differentiation of DNA fragments with equal length due to sequence specific conformations.

In addition to the investigation of the importance of the HCV-quasispecies of hypervariable region 1 in correlation to interferon-based treatment response, most recently clonal analysis of the HCV-quasispecies was used to characterize potential resistance mutations in

patients undergoing direct antiviral therapies with HCV protease and polymerase inhibitors. Detection of these resistance mutations may be introduced in the routine diagnostic workup after these new treatment options are approved for standard treatment of hepatitis C.

### Interferon-sensitivity Determining Region in the NS5A Gene

Infection with hepatitis C virus genotype 1 is associated with inferior virologic response rates to an IFN-α-based treatment in comparison with HCV genotypes 2 and 3. Sequence comparisons from Japanese interferon-sensitive and -resistant HCV 1b isolates showed that amino acid sequences between codon 2209 and 2248 of the ORF within the non-structural (NS)-5A protein are associated with treatment response. HCV-1b-isolates with four or more mutations in this interferon-sensitivity determining region (ISDR) named area, compared to the HCV 1b prototype sequence, were sensitive to interferon while the HCV 1b prototype isolates were resistant to interferon. Isolates with one to three amino acid changes showed a variable response to IFN-α. In addition, an inverse correlation between an increasing number of mutations within the ISDR and the HCV-RNA concentration at baseline was observed. Subsequently, the correlation of ISDR mutations with treatment response was investigated from different groups in Japan, Europe and the United States. Studies from Japan generally were able to confirm the strong correlation between ISDR mutations and treatment response. Groups from Europe, and the U.S. initially were not able to verify the importance of ISDR mutations for IFN alfa sensitivity and it turned out in subsequent studies that HCV 1b isolates with multiple mutations within the ISDR are rare in Europe and the U.S. as compared with those found in Japan. In further studies with larger patient cohorts, as well as in a meta-analysis, the positive correlation of an increasing number of ISDR mutations with sustained virologic response to IFN alfa-based therapy could be confirmed. However, the functional background for this correlation still is unknown.

### Structural HCV Proteins

The amino-terminal one third of the polyprotein encodes the HCV structural proteins. They are proteolytically cleaved through host signal peptidases to obtain the highly basic core (C) protein and glycoproteins E1 and E2. The C protein is an approx. 21 kDa RNA-binding phosphoprotein needed for encapsidation, which is localized near the endoplasmic reticulum. The core protein is essential for expression of E1 and E2. Recently, the new HCV F protein has been described. Its expression results of a ribosomal frameshift within the capsid-encoding sequence. The function of the F protein is not yet known.

The envelope proteins E1 and E2 are 30 – 35 and 70 kDa glycoproteins forming a stable heterodimer. A 27 amino acid hyper variable region (HVR1) is located at the amino-terminus of E2 and the HVR1 is assumed to be important for establishment of chronic HCV infection. Despite the high level of amino acid variability in HVR1, there is an overall conservation of basic residues that are important for viral entry [11]. Epitopes for specific binding of neutralizing antibodies were found within the HVR1. Due to the lack of proofreading activity of the HCV RNA-dependent RNA-polymerase and a continuous immunologic pressure, isolates with changing amino acid sequences within the HVR1 develop during HCV infection. Antibodies directed against the HVR1 from previous HCV isolates cannot bind to subsequently produced modified HVR1 sequences, resulting in a selection process which is termed immune-escape. The hypothesis of immune selection is supported by the finding of a lower HVR1 variability in immune suppressed patients with HCV infection than in immune competent patients. Chimpanzees with cured HCV infection could be re-infected with the same HCV isolate. However, in chimpanzees infected with a single consensus HCV clone, no mutations within the HVR1 were detected within a 1 year follow-up period. Thus, further mechanisms of persistence of HCV infection have to be assumed. For both E1 and E2, hydrophobic anchor domains are located at the carboxy-terminal end of the proteins. These domains are important for integration in the membrane of the endoplasmic reticulum. The 7 kDa protein p7 is supposed to form an ion channel and is probably involved in several steps of virus production [6].

### Non-structural HCV Proteins

The NS2/3 junction is cleaved by the NS2 autoprotease while all other non-structural proteins are processed by the NS3/4A serine protease. The carboxy-terminal two thirds of the hydrophobic 23 kDa NS2 protein contains

the catalytic triad of a cysteine protease. These residues are necessary for NS2/3 cleavage as well as for the downstream expression of the NS3 serine protease domain, although NS3–4A protease activity is dispensable for NS2/3 processing [44]. NS3 is a 70 kDa multifunctional protein, with an N-terminal serine protease domain and a C-terminal RNA helicase/NTPase domain. The NS3 serine protease is involved in the autocatalytic NS3/4A cleavage. For cleaving of NS4A/B, NS4B/5A, and NS5A/B the NS3 protein has to form a complex with the NS4A protein. NS4A is a small protein of 54 amino acids and represents an important cofactor for the NS3/4A protease. In addition, it is involved in phosphorylation of the NS5A protein. The integral membrane protein NS4B is involved in the membranous web formation and has been proposed to serve as a scaffold for replication complex assembly. Several adaptive mutations for replication efficiency in the replicon model have been mapped to this protein, and NS4B has been found to encode a GTPase activity that may be related to its membrane-altering ability. NS5A is a mainly hydrophilic phosphoprotein. It can be detected in a phosphorylated (56 kDa) and a hyperphosphorylated (58 kDa) form. NS5A possesses an N-terminal amphipathic α-helix that mediates membrane association. In addition, it is supposed to be involved in protein–protein interactions required for composition of a functional replication complex. This helix is followed by three domains (I–III). Domain I (N-terminal) coordinates a single zinc atom per protein molecule. Its crystal structure has recently been resolved, and reveals a novel protein fold, a zinc coordination motif, and a dimerization interface. In addition, the ISDR within the NS5A protein was clinically shown to be involved in sensitivity to interferon-based antiviral therapy (see above). The cytoplasmic perinuclear located NS5B protein (68 kDa) exhibits highly conserved motifs which are typical for its function as RNA-dependent RNA-polymerase. A non-HCV-specific RNA polymerase activity was shown *in vitro*.

## Genotypes and Subtypes

Up to now a minimum of eight HCV isolates were sequenced completely and several other isolates were sequenced partially. Due to sequence comparisons worldwide, six HCV genotypes (type 1–6) with nucleotide sequence differences of more than 30% were distinguished. Inside these groups several subtypes can be defined (subtype a, b, c, etc.). The homology of subtypes is between 80–90%. Between isolates of the HCV quasispecies in a given patient, amino acid sequence differences of a maximum of approximately 10% are detectable.

In Europe the prevalent genotypes are HCV-1b, HCV-1a, HCV-2b, and HCV-3a. HCV-1a is the main genotype in the U.S. followed by HCV-1b, HCV-2a, and HCV 3a. An obvious difference in the distribution of HCV genotypes exists between Japan and Western countries. In Japan the prevalence of HCV-1a is less than 1 – 2% and HCV-3 genotypes are rare as well, with HCV-1b and HCV-2 dominating. Genotypes HCV-5 and HCV-6 are detected mainly in South Africa and Hong Kong, while HCV-4 appears in Egypt and the Middle East. Co-infections with several HCV genotypes and superinfections with heterologous HCV genotypes are described especially in polytransfused, hemophilic patients, and IV drug abusers.

While there is no evidence of a different natural course of HCV infection in patients infected with different subtypes or genotypes, diagnosis of hepatitis C virus genotypes is important for predicting response to antiviral therapy.

## Replication Cycle

During replication of the hepatitis C virus the genomic (+)-strand RNA is first transcribed to a (−)-strand RNA. Based on this (−)-strand RNA several (+)-strand RNAs can be retranscribed (Fig. 63.12). The replication takes place in a membrane-associated complex derived from the endoplasmic reticulum (ER) and has been designated "membranous web". All HCV proteins are associated with this web-structure directly or indirectly. While the major location of replication are the hepatocytes in the liver, additional extrahepatic replication in B- and T-lymphocytes was suspected. HCV entry in host cells is a clathrin-dependent process which requires a low pH compartment. The HCV envelope protein E2 binds the tetraspanin CD81 which is expressed in hepatocytes and B lymphocytes amongst others and CD81 was shown to be an essential receptor for HCV entry. However, CD81 is present on multiple cell types which can not be infected with HCV and so further receptors must be involved in HCV entry. One of them is the low density lipoprotein (LDL) receptor. The receptor binds to virus associated LDL particles and mediates internalization. This is a common process in the *Flaviviridae* family. A

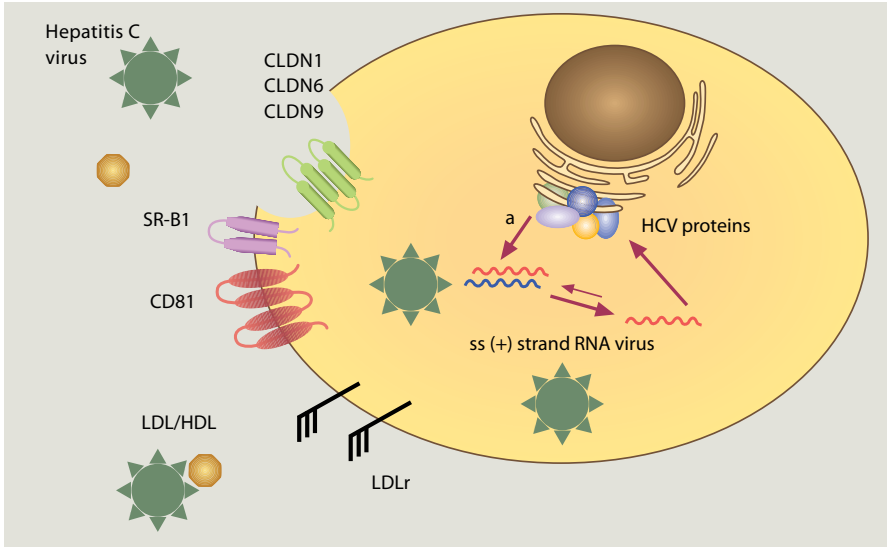



**Fig. 63.12** Viral entry and replication cycle of HCV

binding of the hepatitis C virus envelope glycoprotein E2, independent from the virus isolate, is mediated by the human scavenger receptor class B type I (SR-BI). SR-BI enhances HCV entry in a high density lipoprotein (HDL) dependent manner [74]. Another HCV co-receptor, which functions in a later step of entry, is the integral membrane protein Claudin-1 (CLDN1) [21]. Two further members of the Claudin family, namely CLDN6 and CLDN9, function as additional co-receptors for HCV [90]. They are expressed in liver and peripheral blood mononuclear cells (PBMCs) unlike CLDN1, which is expressed only in liver (Fig. 63.12). Recently, occludin was described as the last essential receptor for HCV entry. The transcription accuracy of the HCV polymerase is low and leads to a high variability of newly generated HCV isolates (HCV quasispecies). The mutation rate is approx. $2 \times 10^{-3}$ mutations/nucleotide position/year similar to other RNA viruses. Despite the high genetic variability, functions of HCV proteins remain stable. For proper function of the internal ribosome entry site of the 5'NC region a high sequence identity is mandatory, which most likely is ensured through negative selection of dysfunctional isolates during replication.

## Diagnostics of Hepatitis Viruses

### *Hepatitis A*

Clinical routine diagnostics of HAV infection are based on detection of anti-HAV-antibodies in serum samples. Serological markers during the natural course of HAV infection are shown in Fig. 63.13. In addition, highly sensitive detection of HAV-RNA by nucleic acid amplification methods (standard RT-PCR, real-time RT-PCR, hybridization techniques) can be performed. Analysis of faecal samples is possible by using electron microscopy, and antigen assays. However, while HAV antigens are easily detectable during the incubation phase of HAV-infection, detection rates are less than 50% after clinical symptoms become apparent and HAV virions are excreted in faeces. Diagnosis of HAV infection is reviewed in detail by Nainan et al. [55].

#### Anti-HAV-Antibodies and Nucleic Acid Amplification

Detection of anti-HAV-IgM antibodies is used as the primary marker of an acute infection. Resolved HAV-infection is characterized by anti-HAV IgG antibodies in the absence of IgM antibodies. In addition, after successful vaccination anti-HAV IgG antibodies are detectable which are directed against structural HAV proteins expressed during vaccination. Antibody levels after vaccination are typical significantly lower than after resolved HAV infection. Assays for total anti-HAV antibodies have a sensitivity of 10–20 IU/L.

Several commercial test systems from various companies (Abbott, Roche Diagnostics, Organon, Technica, Sorin) are available. A number of methods are used to detect the virus-specific antibody classes (IgG, IgM), including radioimmunoassay, immunochemical staining, enzyme-linked immunosorbent assay, immunoblotting, and dot blot immunogold filtration.

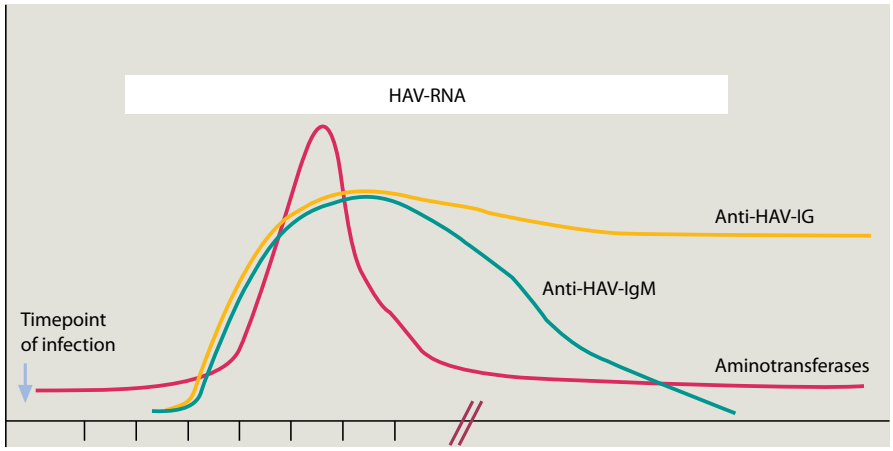



**Fig. 63.13** Serological and clinical progression of an infection with HAV

Nucleic acid detection assays are more sensitive than immunoassays for viral antigens or antibody detection systems. The virus can be detected by Southern blotting, nucleic acid hybridization, reverse transcription-PCR (RT-PCR) and antigen-capture RT-PCR. More recently, a real-time RT-PCR assay (TaqMan assay) has been developed for the rapid detection of all human HAV genotypes using highly conserved sequences within the 5'YTP [35]. This assay detects HAV in environmental, food, and clinical samples with a sensitivity of 0.5 infectious units per milliliter (IU/mL) of HAV and 40 copies of a synthetic transcript.

### Conclusion for HAV Diagnosis

For routine purposes HAV infection is diagnosed by anti-HAV-antibodies (IgM and IgG) in serum samples. Presence of anti-HAV IgM antibodies indicate acute HAV infection. Anti-HAV IgM antibodies are detectable with the beginning of clinical symptoms of acute hepatitis A. Antibody concentrations increase during the first month of clinically overt hepatitis A and may be detectable for a maximum of 1 year. In rare cases persistent detection of (low level) anti-HAV IgM antibodies have been reported. Anti-HAV-IgG-antibody levels increase together with IgM-concentration and in the majority of cases remain detectable the whole life.

In patients with resolved HAV infection, and after active or passive immunization, only anti-HAV IgG antibodies are detectable. Before HAV vaccination is performed, blood screening for total HAV antibodies is recommended.

Assays for detection of HAV antigens and HAV-RNA (radioimmunoassay, immunochemical staining, enzyme-linked immunosorbent assay, electron microscopy, hybridization methods, RT-PCR) are not used for routine diagnosis but are important for epidemiological investigations of routes of infections during epidemics and for detection of HAV in water and blood products

## *Hepatitis E*

Diagnosis of HEV is possible by detection of HEV-antibodies (IgA, IgM, and IgG) and viral RNA in serum and fecal samples. In addition, electron microscopy is a possible tool to detect HEV. Currently only the antibody-detection systems are relevant for routine clinical purposes. In Fig. 63.14 the serological and molecular patterns of HEV infection are illustrated.

### Anti-HEV Antibodies and Nucleic Acid Amplification

For routine diagnosis, HEV antibody detection systems like enzyme immunoassays, western blots or indirect immunoassays are used. Recombinant or synthesized HEV antigens with immune-dominant epitopes derived from the structural proteins of two isolates from Burma and Mexico are typically used in these assays. In patients with acute HEV infection these assays have a sensitivity of 90–95%. Anti-HEV IgM antibodies become positive during the early stage of clinical overt acute hepatitis E and after 1 year of infection are still detectable in approximately

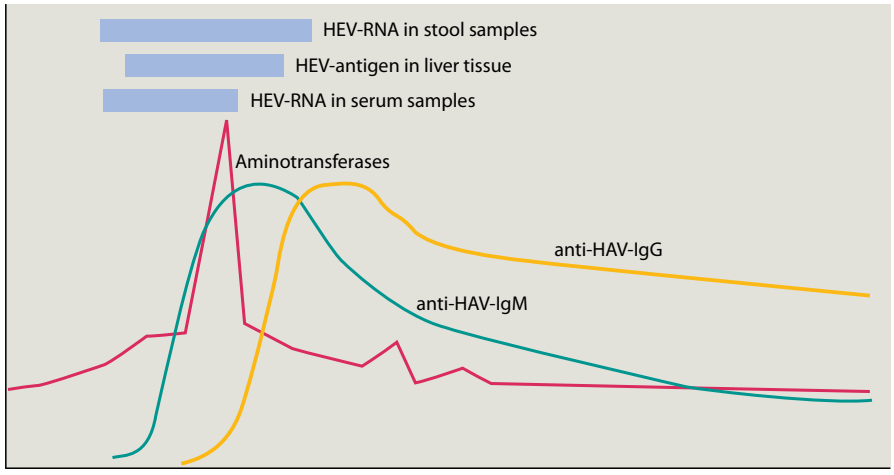



**Fig. 63.14** Progression of HEV infection

40% of patients. IgG antibodies become detectable some days after the IgM antibodies during acute hepatitis E, and IgG antibodies remain detectable for many years (47% after 14 years). The presence of HEV-IgG antibodies either indicates acute or resolved HEV infection. For epidemiological purposes the use of highly sensitive assays is important because antibody-concentrations may already be very low after some months of infection.

The direct detection of HEV-RNA from serum and fecal samples using a "nested-RT-PCR" system has been shown to be highly sensitive. Detection of HEV-RNA is possible during the incubation phase and within the first days of clinically overt hepatitis E. Direct detection of HEV by electron microscopy is also only possible before clinical evidence of acute hepatitis E and during the first days of icteric hepatitis. A minimal concentration of at least $10^7$ particles/mL is necessary. Electron microscopy is not used for routine diagnostics.

### Conclusion for HEV Diagnosis

In western countries HEV infection typically is suspected in patients with acute hepatitis and negative markers for HAV, HBV/HDV, and HCV infection. In addition, a history of traveling to endemic areas should be sought. However, numerous cases of acute hepatitis E have been reported without a travel history. Similar to hepatitis A, diagnosis of acute hepatitis E is easily confirmed by specific detection of anti-HEV-IgM antibodies. Chronic courses of infections are unknown. Positive test results for HEV-IgG antibodies indicate either an acute or a resolved infection. In addition, the presence of an ongoing HEV infection can be confirmed by HEV-RNA detection in serum samples.

## *Hepatitis B*

Diagnosis of hepatitis B infection is based on serological and molecular test systems. Over the last few years the measurement of HBV-DNA levels has become the most reliable method used for accurate diagnosis and prognosis of acute and chronic HBV infection. The recently introduced real-time PCR technique represents the method of choice compared to previous, conventional endpoint PCR due to a very sensitive quantification of the viral load over a wide dynamic range. Molecular-based methods are used to aid in the management of HBV infection, and are helpful for monitoring patients during therapy.

An infection with HBV should be excluded in all patients with acute or chronic liver diseases, in particular in patients with liver cirrhosis. Table 63.2 summarizes the serological and molecular parameters used in HBV diagnosis. The serological features during different courses of hepatitis B virus infection are shown in Fig. 63.15.

### Serological Diagnosis of HBV

During the natural course of HBV infection antibodies against different virus antigens are produced, i.e. HBc-, HBe-, and HBs-antibodies. In addition to these serological markers used in clinical practice, there are some antigens with their respective antibodies which have no clinical relevance (e.g. preS1Ag/anti-preS1-antibodies,

**Table 63.2** Meaning of serological and molecular virus markers for HBV diagnosis

| | |
|---|---|
| Anti-HBs | Resolved hepatitis B or successful vaccination |
| Anti-HBc-IgM | Acute or passed HBV infection; sometimes in acute episodes of chronic infection |
| Anti-HBc-IgG | Acute or past HBV infection, well established screening parameter for HBV |
| Anti-HBe | In wild type HBV infection: change between the high-replication stage to the low-level replication stage of HBV infection |
| HBsAg | Early marker of acute HBV infection, indication for chronic infection. Screening parameter for HBV infection |
| HBeAg | Indirect parameter of high virus replication and high infectivity |
| HBV-DNA (quantitative) | Direct parameter of virus replication and infectivity |
| HBV-DNA (qualitative) | No use for diagnosis |

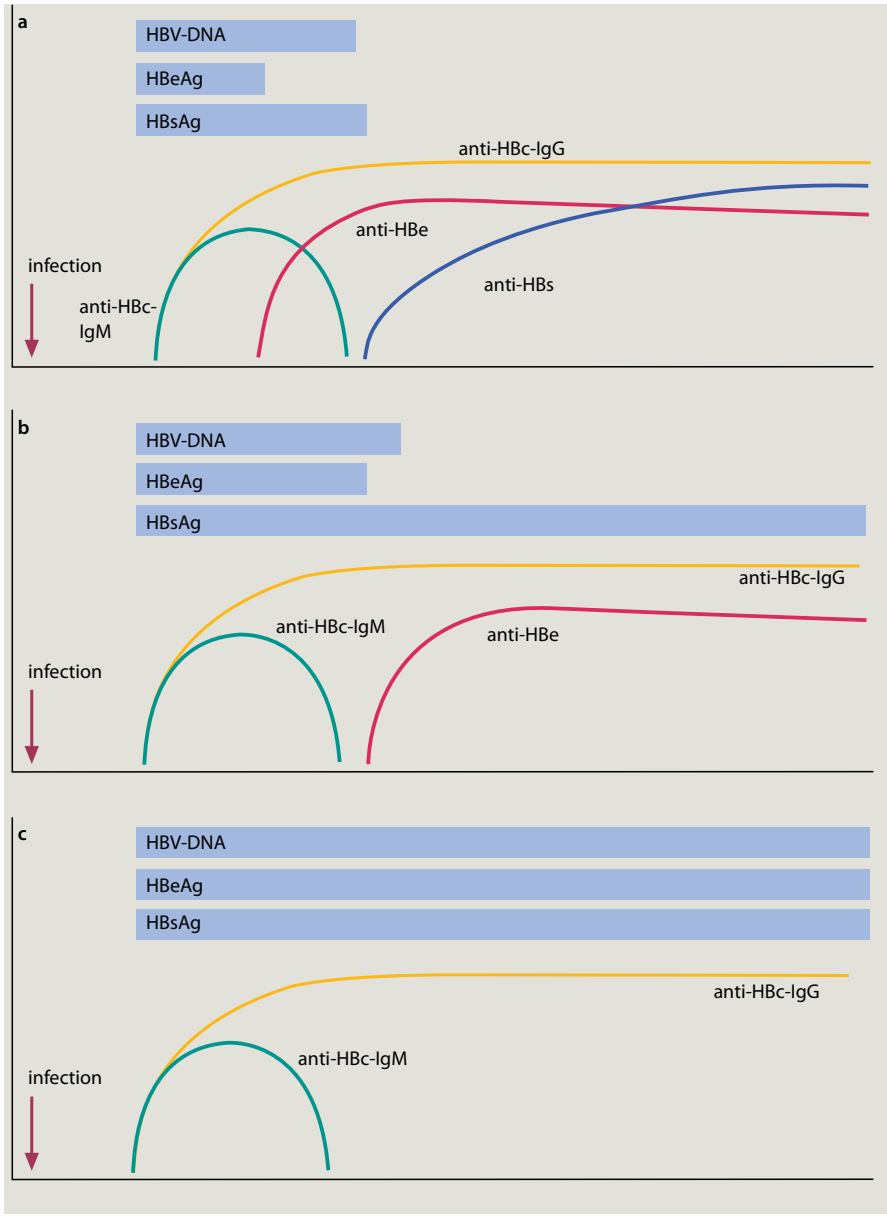



**Fig. 63.15** **a–c** Serological progression of the HBV infection. (**a**) Acute HBV infection with virus clearance. (**b**) Chronic HBV infection with seroconversion from HBeAg to antibody-production. (**c**) Chronic HBV infection without HBeAg seroconversion

preS2Ag/anti-preS2-antibodies, HBxAg/anti-HBx-antibodies, and HBpolAg/anti-HBpol-antibodies). During HBV infection the production of certain antibodies indicates elimination of the respective antigens from serum, an event dubbed "seroconversion." After HBV infection occurs, nearly all patients develop anti-HBc-antibodies. HBc-antibodies may remain detectable over several years or decades after HBV infection and are therefore used as a screening marker. For discrimination between acute, resolved or chronic HBV infection in anti-HBc-positive patients, analysis of additional parameters e.g. HBsAg, anti-HBs-antibodies, HBeAg, and anti-HBe-antibodies should be performed. Different forms of HBV progression and the presence of HBV mutants often hamper serological diagnosis.

### HBsAg and Anti-HBs-Antibodies

In acute HBV infection, surface antigens are detectable several weeks after infection and its appearance coincides with the onset of clinical symptoms. HBsAg is incorporated into complete HBV particles as well as into non-infectious particles. It can be found in almost all body fluids. Hepatitis B surface antigens are a very early serological marker of HBV infection. They are already detectable in the incubation period of the infection. In only a few HBV infected patients surface antigens remain undetectable, e.g. in patients with fulminant hepatic failure and in HBs-antigen escape mutants. The quantification of surface-protein-concentrations after 4 weeks of infection may allow for a prediction of HBV progression. Chronic HBV infection is defined by the persistence of HBs-antigens for more than 6 months.

Different immunological based systems (immune diffusion, enzyme immunoassay, enzyme-linked immunosorbent assay, radioimmunoassay) are available to detect HBsAg. Commercially available assays are highly sensitive and specific for surface antigens. The test reproducibility is higher than 99%. At present, monoclonal antibodies used in the different assays are directed against different HBsAg-epitopes. In patients with very high HBs-antigen serum concentrations (above 100 ng/mL) the saturation of detector-antibodies is a limitating factor in the exact quantification; also, the specificity of some antibodies to the different HBV genotypes may cause problems. In addition to the simple HBsAg detection, it is also possible to determine HBV-stereotypes (adw, ayw, adr, ayr) which is only important for epidemiological research and is not performed in routine clinical practice. The respective test systems are based on serotype-specific monoclonal antibodies. Commercial assays are not available.

**Table 63.3** Anti-HBs titer: control and refreshing of vaccination

| Anti-HBs | Refreshing |
|---|---|
| < 10 IE/L | Immediately |
| < 100 IE/L | 3–6 months |
| < 1,000 IE/L | 1 year |
| < 10,000 IE/L | 3 years |
| > 10,000 IE/L | 5 years |

The immune response against the hepatitis B surface antigens is, due to the different forms of HBs-antigens (preS1, preS2, S) and the presence of numerous epitopes (a, d-y, w-r, etc.), highly heterogeneous. During the initial course of HBV infection a subtype-specific immunity is detectable which subsequently is extended to other HBV subtypes. Anti-HBs antibody response is an important parameter to distinguish between ongoing and resolved infection as well as to determine the success of vaccination (see Table 63.3). Presence of anti-HBs-antibodies which are produced typically 2–3 months after disappearance of HBs-antigen, indicates spontaneous resolution. In patients with past HBV infection anti-HBs-antibody titer can fall below the assay's limit of detection. Multiple different assays for detection of anti-HBs antibodies are commercially available. Most of them have a detection limit between 2 and 10 IU anti-HBs/L. False positive results may be obtained in approx. 2% of cases.

### PreS-Ag and Anti-PreS-Antibodies

During acute HBV infection, virus replication is associated with expression of both preS-antigens, while development of anti-preS antibodies is an early indicator of viral clearance. During resolution of HBV infection, preS1Ag is eliminated initially, followed by the preS2- and the S-Ag. Subsequently the respective antibodies are produced in the same order. Measurement of preS-Ag is clinically irrelevant while the detection of anti-preS1 and anti-preS2 antibodies maybe useful to narrow the diagnostic window between HBsAg elimination and production of anti-HBs-antibodies. Both preS antigens are detectable by monoclonal antibodies. Anti-preS1 and anti-preS2 are recognized on the basis of sequential epitopes by recombinant peptides in immunoblot assays. Commercial assays for anti-preS1 or anti-preS2 are not available.

### Anti-HBc-Antibodies

Detection of anti-HBc-antibodies is used as a screening parameter for HBV in blood-samples and body fluids. Anti-HBc-antibodies are produced early after HBV infection and typically are lifelong persistent. In addition, no anti-HBc-antibodies are produced after hepatitis B vaccination. Rarely, especially in immunosuppressed patients and in cases with assay-specific escape mutations, no anti-HBc-antibodies may be detectable. During acute infection, it is possible that due to a diagnostic window between HBsAg elimination and anti-HBs-antibody production, anti-HBc-antibodies but neither HBsAg nor anti-HBs-antibodies are detectable. A similar situation with anti-HBc as the only marker of hepatitis B is possible many years after resolved HBV infection when HBs-antigen is undetectable and anti-HBs titers fell below the detection limit of the assay. Routine diagnostics of numerous available anti-HBc detection assays is based on immunological technologies using recombinant HBcAg.

Anti-HBc-IgM antibodies are already detectable in the incubation period and maximum concentrations typically are observed in the third week after infection. Six months after HBV infection anti-HBc-IgM antibodies become undetectable. In addition, anti-HBc-IgM antibodies may be present during acute episodes of chronic HBV infection. In asymptomatic HBV carriers typically no anti-HBc-IgM antibodies are detectable. Quantitative and qualitative measurement of anti-HBc-IgM antibodies can be performed by several enzyme immunoassays.

### HBeAg and Anti-HBe-Antibodies

Detection of HBeAg usually is associated with high HBV-DNA concentrations. Accordingly, seroconversion from HBeAg to anti-HBe-antibody production typically indicates a change from a high-replicative to low-replicative HBV infection. However, due to a number of HBe-antigen mutants (precore/core promoter mutation), detection of anti-HBe-antibodies cannot be used to determine low-replicative HBV infection with low HBV-DNA levels. For monitoring of the activity of HBV infection and response to antiviral therapy, measurement of HBV-DNA concentration represents the only reliable parameter. In patients with HBeAg positive HBV infection, monitoring of HBeAg and anti-HBe-antibody production nevertheless is an important tool to determine durable response to antiviral therapy. Several highly sensitive and specific quantitative and qualitative detection immunoassays for HBe-Ag and anti-HBe-antibodies are commercially available.

## Molecular Diagnosis of HBV

HBV-DNA is a direct marker of viremia, hence of potential infectivity. In addition, in the early stage of infection the HBV-DNA can be observed two to four weeks before HBsAg is detectable. Furthermore, HBV-DNA is used to determine the activity of HBV infection, to select patients for antiviral therapy, and to monitor treatment response. Qualitative and quantitative detection of HBV-DNA in peripheral blood is performed by signal amplification ('branched DNA' technology) or target amplification (including standard and real-time PCR as well as transcription-mediated amplification [TMA]) based assays. Commercial assays include semi-automated bDNA signal amplification (*Versant HBV-DNA 3.0 (bDNA) former Bayer now Siemens Diagnostics*), semi-automated quantitative PCR (*Cobas Amplicor HBV Monitor, Roche Diagnostics*) and real time PCR (*Abbott RealTime HBV test, Abbott; Artus HBV PCR Kit, Qiagen* and *Cobas TaqMan 48 HBV Assay, Roche Diagnostics*). Real-time PCR technology schematically is shown in Fig. 63.22 and is explained in detail below. The real-time PCR technology represents the reliable method for HBV-DNA measurement compared to previous, conventional endpoint PCR due to a very sensitive quantification of the viral load over a wide dynamic range.

### Qualitative HBV-PCR

Qualitative PCR is a highly sensitive method to detect HBV-DNA without determination of HBV-DNA viral load (Fig. 63.16). The assay basically consists of three main steps: (i) sample preparation (ii) target amplification and (iii) detection. Sample preparation involves detergent lysis of the virus followed by the hybridization of the free viral nucleic acid with capture oligonucleotides complementary to highly conserved regions

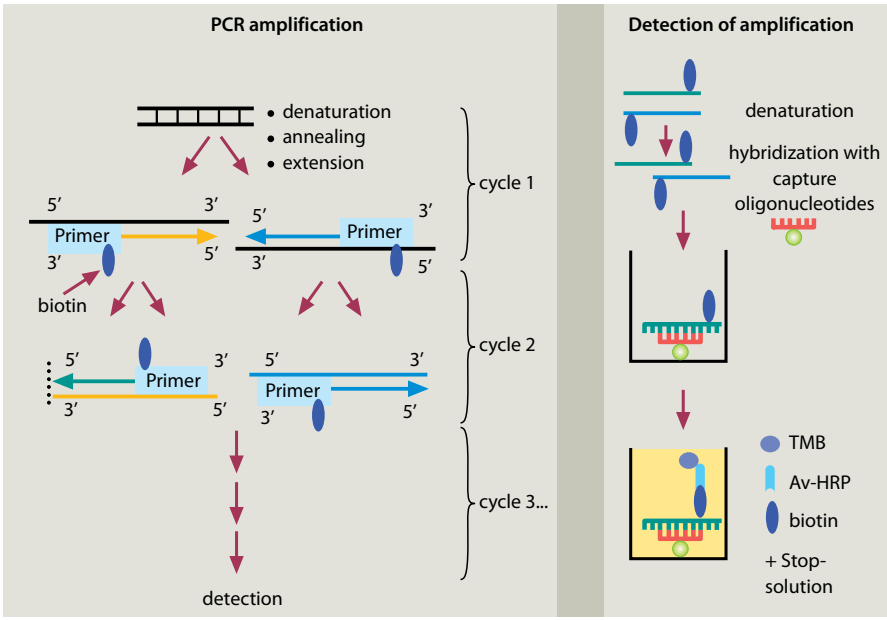



**Fig. 63.16** Diagram of a qualitative polymerase-chain reaction (PCR). The three major steps of a PCR consist of denaturation, annealing and extension. In the denaturation step single stranded DNA is generated by melting the DNA double-strand with high temperatures up to 96°C. In the annealing step two short oligonucleotides (primer) which are complement to the 3'- and 5'-terminus of the target gene bind with high affinity to the nucleotide sequence. The extension step is performed by a heat resistance DNA-polymerase. By adding desoxynucleotidtriphoshates (dNTPs) the bases (complementary to the template) are coupled to the primer on the 3' side of each nascent strand. Because both strands are copied during PCR, the number of copies per cycle of the target gene increases exponentially. For RNA amplification it is necessary to convert the RNA to complementary DNA (cDNA) in a one-step process using dNTPs and the enzyme reverse transcriptase. After PCR, the amplified DNA can be detected by several methods, e.g. electrophoretic DNA separation and staining with ethidiumbromide or detection using enzyme-labeled-synthetic oligonucleotides and chemiluminescent substrates that result in a detectable light emission. Here, the primers are labeled with biotin which is detected by avidin conjugated horseradish peroxidase (AV-HRP). The analysis is based on the oxidation of the chromogenic substrate 3,5,3',5'-tetramethylbenzidine (TMB) by the AV-HRP which results in an intermediate compound with an absorbance maximum at a wavelength of 450 nm

of the HBV genome. Positive results by qualitative HBV-DNA PCR may be obtained in almost all HBsAg-carriers as well as in 5–10% of patients with resolved infection. Differentiation between HBV infection with high- and low-level replication, is impossible by qualitative HBV-DNA assays.

## Quantitative Methods for HBV-DNA Measurement

Several assays for quantitative measurement of HBV-DNA have been developed. However, until recently results of the different assays (pg/mL, copies/mL, genome equivalents/mL) were not directly comparable due to the lack of adjustment to an international HBV-DNA standard and differences in the dynamic ranges of the assays.

### *Cobas Amplicor™ HBV-Monitor*

The first standardized commercially available and CE-marked test system, based on quantitative PCR was the Cobas Amplicor™ HBV Monitor assay (CAHBM, Roche Diagnostics). This assay is calibrated to international units (IU/mL) on the basis of the first WHO HBV international standard code 79/796. The CAHBM assay encompasses the co-amplification of HBV template together with an internal standard (QS) added at known concentrations during the nucleic acid extraction step. Detection of the template is performed in a subsequent enzyme-linked immunosorbent assay (Fig. 63.17). The system involves a manual DNA extraction. The assay has a sensitivity of approx. 74 IU/mL (200 copies/mL) with a linear range up to 38,000 IU/mL (200,000 copies/mL).

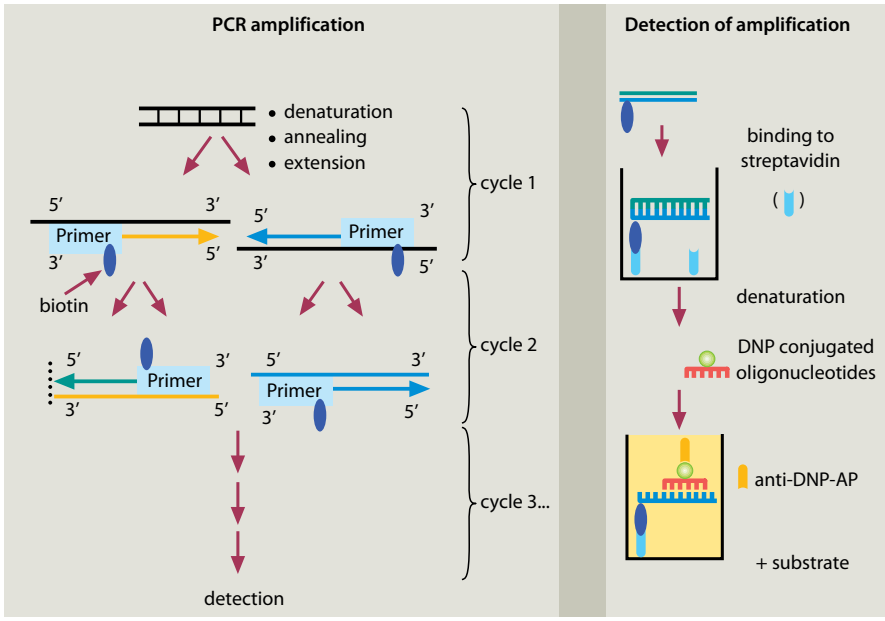



**Fig. 63.17** Quantitative PCR (Amplicor HBV monitor). The Amplicor HBV MONITOR test is an *in vitro* nucleic acid amplification test for the quantification of HBV-DNA in human serum or plasma. The amplified DNA is hybridized with dinitrophenyl- (DNP) oligonucleotides. After binding of DNP to alkaline phosphatase conjugated antibodies against DNP (anti-DNP-AP), the addition of a substrate results in a light emission that is directly related to the amount of HBV-DNA. As a reference, an internal standard with known HBV-DNA concentration is used

### *Branched HBV-DNA (bDNA) Assay*

The bDNA assay (Versant HBV-DNA 3.0 Assay, Siemens Diagnostics) is based on a signal amplification technology (Fig. 63.18). The branched DNA-complex is generated by a sandwich nucleic acid procedure. After lysis of the HBV virions and release of nucleic acids the negative-sense HBV-DNA strand binds to immobilized capture-oligonucleotides. The oligonucleotides bind to conserved DNA regions throughout the entire HBV genome. In a second step, several target-oligonucleotides bind to the free ends of HBV-DNA strands. Finally, multiple copies of an alkaline phosphatase-labeled probe are hybridized to the immobilized complex. Detection is achieved by incubating the alkaline phosphatase-bound complex with a chemiluminescent substrate. Light emission directly is related to the amount of HBV-DNA in each sample. The HBV bDNA assay is highly specific, however, the assay has a limited sensitivity with a limit of detection of 357 IU/mL (2,000 copies/mL). The upper limit of detection is $18 \times 10^6$ IU/mL ($1 \times 10^8$ copies/mL). The linear range for HBV-DNA quantification is from 357 IU/mL to $18 \times 10^6$ IU/mL.

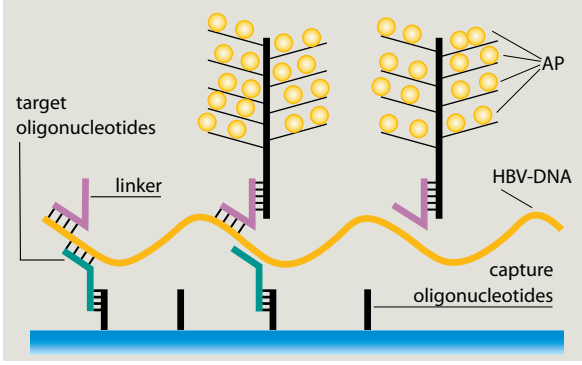



**Fig. 63.18** The Versant HBV-DNA 3.0 (bDNA) technology. The bDNA-complex is generated by a sandwich nucleic acid procedure. The negative-sense DNA strand binds to immobilized capture-oligonucleotides. The oligonucleotides are complement to a 3'-region of the HBs-gene. In a second step, several target-oligonucleotides bind to the HBV-DNA strand. In a last step multiple copies of an alkaline phosphatase (AP)-labeled probe are then hybridized to the immobilized complex. Detection is achieved by incubating the alkaline phosphatase-bound complex with a chemiluminescent substrate. Light emission is directly related to the amount of HBV-DNA in each sample

### *Real Time PCR: Cobas Ampliprep/Cobas TaqMan Assay*

HBV-DNA extraction from plasma-samples is performed fully automated using the Cobas Ampliprep instrument and the quantification of DNA is performed by real time PCR using the Cobas TaqMan. Recently, an evaluation of this CE-labeled assay showed a nearly 7 log IU/mL dynamic range up to $1.1 \times 10^8$ IU/mL, an assay sensitivity (95% hit-rate) of 4–12 IU/mL and an

equivalent detection of genotypes A - G including a prevalent pre-core mutant [2, 31]. The manufacturers of new diagnostic molecular assays use the international standard for quantitation of HBV-DNA. Therefore, the results obtained by the new Cobas Ampliprep/Cobas TaqMan Assay are solely expressed as international units of HBV-DNA per milliliter.

#### *Real Time PCR: Abbott RealTime HBV Assay*

In 2007 a further real time based assay, called Abbott RealTime HBV test (North Chicago, IL, USA), for monitoring HBV viral load in patients was approved with the CE label in Europe. The Abbott RealTime HBV test, developed for use on the Abbott ***m***2000™ automated instrument system, is designed for the quantitation of HBV in human plasma or serum from patients known to be infected with the virus and is suitable to measuring all known HBV genotypes A - H. The quantitation of mutations within the polymerase, the precore/core are not yet included. The sensitivity of the assay is 10 IU/mL with up to 8.5 log IU/mL linear range.

#### *Real Time PCR: Artus HBV PCR Kit*

The *artus* HBV PCR Kit (CE labeled) constitutes a ready-to-use system for the detection of HBV-DNA by real time PCR. For detection, different instruments are available: (i) the LightCycler® 1.1/1.2/1.5/2.0 *(artus HBV LC PCR KIT),* (ii) the artus 3000™ or Rotor Gene™ 3000 *(artus HBV RG PCR KIT)* or, the (iii) ABI Prism® *(artus HBV TM PCR KIT)*. The artus HBV Kit contains reagents and enzymes for the specific amplification of a 134 bp region of the HBV genome and for the direct detection of the specific amplicon with the different instruments. The test is suitable to detect all known HBV genotypes A-H with high sensitivity. The sensitivity of the kit depends on the DNA purification method and the analytical instrument used for detection. The linear range of the assay performed with the Rotor Gene™ 3000 is 0.02 IU/µL to 1 × 108 IU/µL.

### Conclusion for HBV Diagnosis

#### Acute HBV Infection

The most important serological marker for acute HBV infection is the presence of anti-HBc-IgM antibodies. Anti-HBc-IgM antibodies are produced by all patients with acute hepatitis B and are already detectable during the incubation period of infection. In patients with fulminant acute hepatitis B, anti-HBc-antibodies may be the only serological marker. Usually, anti-HBc-IgM antibodies become undetectable 6 months after HBV infection. HBeAg typically appears together with the first clinical symptoms and intra-individually a good correlation with the HBV-DNA concentration is observed. HBsAg also appears during the incubation period and together with anti-HBc-IgM antibodies represents a marker of an early-stage of infection. In contrast to HBeAg, HBs-antigens are detectable throughout the entire course of ongoing hepatitis B and only disappear together with resolution of HBV infection. The period between the disappearance of the hepatitis B surface antigens and the production of the respective antibodies (anti-HBs) is called the diagnostic window. With the exception of rare mutants which may lead to the production of HBsAg and anti-HBs-antibodies at the same time, the detection of surface antibodies is a reliable marker for spontaneous resolution of HBV infection.

#### Chronic HBV Infection

Definition of chronic HBV infection is based on the detection of HBs-antigen for more than 6 months. Thus, HBsAg is a useful screening parameter for diagnosis of chronic hepatitis B. However, the presence of HBs-antigen alone is insufficient to predict the progression of HBV infection. For differentiation of highly active and low active chronic hepatitis B, quantification of HBV-DNA needs to be performed (Table 63.4). In patients with highly replicative chronic hepatitis B, alanineaminotransferase activity is increased and inflammation in liver histology can be observed. In highly replicative wild type HBV infection HBeAg is detectable while anti-HBe-antibodies are negative. A seroconversion from HBeAg to anti-HBe-antibodies is associated with a decrease of HBV-DNA concentrations from a high to low replication level. However, a significant number of patients are infected with HBe-antigen negative HBV-precore/core promotor-mutants. These mutants are associated with high HBV-DNA levels and elevated liver enzymes. Many of these mutants bear a stop-codon in the precore region blocking the synthesis of HBeAg. Initially these mutants were considered to be limited to the Mediterranean area but epidemiological studies have shown an increasing presence of these mutants also in northern European countries [37].

**Table 63.4** Different forms of chronic HBV infection

| | DNA (PCR) HBV (qualitative) | DNA HBV quantitative (virus copies/mL) | Serology HBsAg | Serology HBeAg | Transaminases | Histology | Therapy indication |
|---|---|---|---|---|---|---|---|
| Low-replication Level | + | $< 10^4$ | + | – | Normal, sometimes marginal or slightly increased | Normal, minimal inflammatory | –[a,b] |
| | | | | +[c] | increased | Inflammatory activity, fibrosis | +[a] |
| High-replication level | + | $> 10^4$ | + | –[d] | | Inflammatory activity, fibrosis | |
| | | | | +/–[e] | normal | Low activity | –[a] |

[a]The decision to start antiviral therapy should be made predominantly by considering the clinical presentation, the transaminase levels and the histological changes. Treatment may be also indicated in patients with low viral load ($<10^4$ virus copies/mL)
[b]Routine surveillance necessary; in case that the transaminase activity and viral load increases, a therapy indication should be reconsidered
[c]Wild type
[d]HBeAg-negative mutant
[e]In immune tolerant patients with HBV infection, high HBV-DNA titer are present, while the transaminase activity is normal. Perinatally infected patients often develop an immune tolerant form of hepatitis B. After weeks or even years the infection can switch to a high-level replication

In patients with low replicative wild type HBV infection usually HBeAg is undetectable, aminotransaminase levels are normal, and no significant inflammatory activities are detected in the liver. Recently, in a large prospective study a positive correlation was detected between probability of development of liver cirrhosis as well as hepatocellular carcinoma and HBV-DNA concentration [15, 33]. On the basis of this study the cut off between high- and low-replicative chronic hepatitis B was determined at approximately 10,000 copies/mL (2,000 IU/mL). In all patients with a viral load above 10,000 copies/mL treatment indication should be evaluated on the basis of liver enzymes, histologic inflammation and fibrosis score as well as extrahepatic manifestions of hepatitis B and risk for development of hepatocellular carcinoma [18]. Additionally, in patients with advanced liver fibrosis or cirrhosis and detectable HBV-DNA irrespective of the viral load, antiviral therapy should be initiated.

The primary aim of antiviral therapy in HBe-Ag positive chronic hepatitis B is to achieve a seroconversion from HBeAg to the development of anti-HBeAg antibodies, which typically is associated with long term low HBV-DNA levels even after treatment discontinuation. In patients with HBe-Ag negative highly replicative chronic hepatitis B long term treatment for continuous control of HBV-DNA replication is typically needed.

## *Hepatitis D*

HDV infection depends on the presence of HBV infection. Thus, detectable HBsAg is necessary for diagnosis of hepatitis D. Fig. 63.19 shows the different serological pattern of HBV/HDV co- and super-infection. Clinically, diagnosis of HDV infection is performed by the detection of anti-HD antibodies, which in the majority of cases indicate active hepatitis delta infection. However, for proof of replicative HDV infection and accurate monitoring of efficacy and outcome of antiviral therapy, measurement of HDV-RNA by different molecular techniques, such as HDV-RNA hybridization and RT-PCR, is required.

### Hepatitis Delta Serology

For detection of total HDV-antibodies (IgG, IgM, IgA) as a screening for acute, chronic or past HDV infection several immunoassays are commercially available. These assays rely on the binding of anti-HDV antibodies to fixed hepatitis delta-antigen. With several tests a simultaneous detection of anti-HDV antibodies and hepatitis delta antigen is possible. However, these assays have a limited sensitivity.

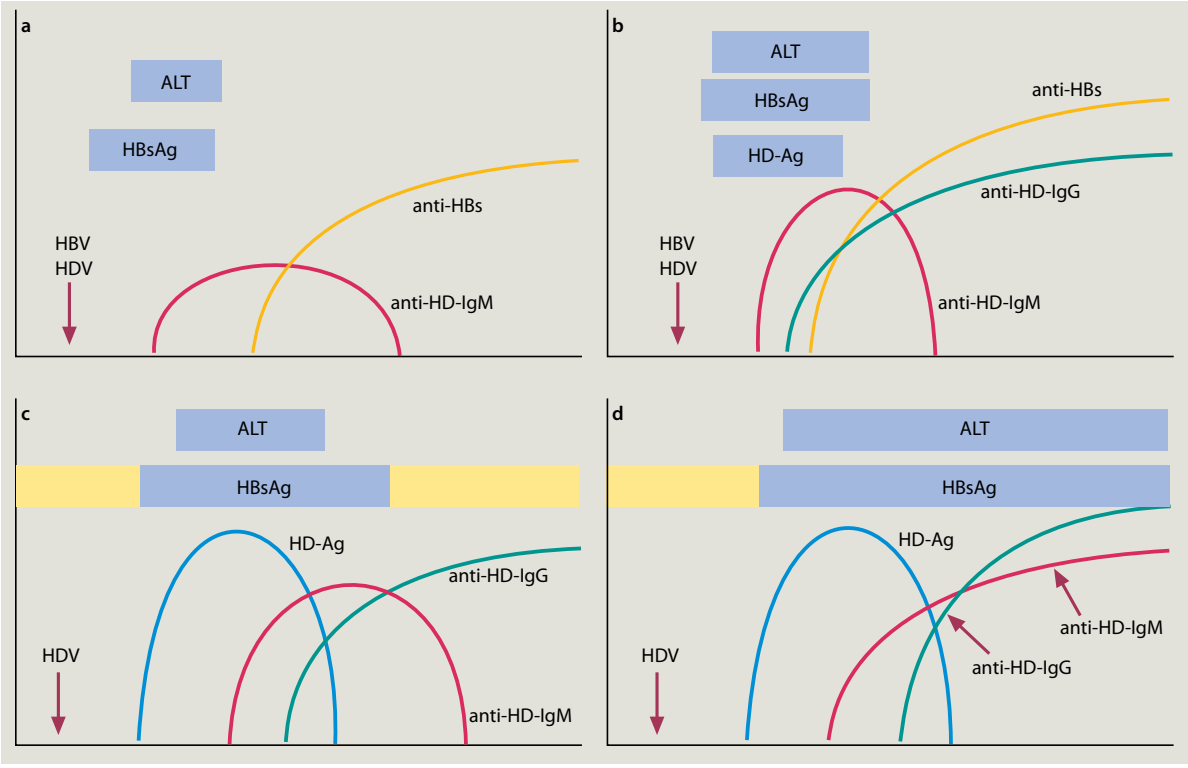



**Fig. 63.19** (**a–d**) Serological and clinical progression of HDV infection (**a**) and HBV/HDV coinfection (**b**). In most cases a coinfection can be cleared. The HDVAg is undetectable **(a)** or detectable (**b**) in the serum. In HBV/HDV coinfection, the serum ALT concentration often shows a biphasic progression. (**c, d**) Superinfection of an HBsAg carrier with HDV. The superinfection can be cleared (**c**) or can develop to a chronic infection (**d**). The gray shadow of the HBsAg bar represents the serum concentration of HBsAg whereas light gray represents high concentration. ALT Alanine aminotransferase (Modified from Zakim and Boyer 1996)

Anti-HDV-IgM antibodies are detectable in almost all patients with active HDV infections (co-, or superinfection). Concentrations of anti-HD-IgM antibodies correlate with hepatitis delta antigen expression-levels and with the inflammatory activity observed in liver tissue. Due to the low level replication of hepatitis delta in patients with HBV-HDV co-infection, typically only low concentrations of anti-HDV-IgM antibodies are detectable, which decrease rapidly after spontaneous resolution. In self-limiting HBV/HDV co-infections anti-HDV-IgM antibodies frequently represent the only marker of infection. Anti-HDV-IgM antibodies are detectable by immunoenzyme assays which are commercially available.

In patients with HDV superinfection, anti-HBcAg IgM antibodies are typically negative, whereas, anti-HD-IgM antibodies and/or serum HDV-Ag are present during the acute phase followed by high titers of anti-HDV-IgG antibodies, persistent HDV-RNA and intrahepatic hepatitis delta antigen.

For direct detection of the HDV-Ag several immunoassays are commercially available. The antigen detection in serum samples is hindered by the fact that the nucleocapsid is encapsidated into the HBV envelope and has to be released prior to detection. In addition, circulation of hepatitis delta virus is only detectable for approximately 2 weeks (if at all); during the development of anti-HDV-antibodies, HDV antigen becomes undetectable. Serum HDV-Ag determination by immunoblot is a specific and sensitive test that is reactive in more than 70% of patients with chronic hepatitis delta. The assay, however, is technically difficult, time-consuming, and cannot be used for the routine detection of HDV-Ag.

### Molecular Diagnosis of Hepatitis Delta

RT-PCR based on TaqMan technology is currently the most sensitive assay for detecting HDV viremia and

the state of the art method for monitoring viral load in HDV patients during interferon alfa therapy [41]. Furthermore, a preliminary study which indicates that PEG-IFN alfa is safe and efficient for treatment of chronic HDV is also based on RT-PCR measurements [13].

### Morphological HDV Diagnosis

Hepatitis delta antigen primarily is expressed in the nuclei of hepatocytes and is rarely detectable in serum samples. Hepatitis delta antigen is the first marker of HDV replication and is observed in the liver of patients with co- and super-infection after 4 – 20 and 3 – 6 weeks, respectively. In addition, HDV-RNA is detectable in liver tissue by RT-PCR or hybridization techniques.

### Conclusion for HDV Diagnosis

Serological or molecular-based diagnosis of hepatitis delta is indicated in all patients positive for HBsAg. In addition, patients with fulminant or acute hepatitis should be screened for HDV-infection. The first marker of HDV infection is the expression of the hepatitis delta antigen in liver tissue. During the incubation period, hepatitis delta virions are released into the bloodstream. In serum samples initially anti-HDV-IgM- and subsequently – IgG-antibodies are detectable. Diagnosis of hepatitis delta relies on the detection of anti-HDV-antibodies. In addition, it is also possible to measure HDV-Ag or HDV-RNA concentration.

For differentiation of acute and chronic HDV infection IgG and IgM antibody response levels are used. In acute HDV infection predominantly anti-HDV-IgM-antibodies are observed, while in patients with chronic infection both anti-IgM- and anti-IgG-antibodies are present. The antibody-response largely varies from patient to patient (see Fig. 63.19). HBV-HDV co-infected patients have only transient and low HDV-specific antibodies. In patients with HDV superinfection anti-HDV-IgM-antibodies and/or serum hepatitis delta antigen are high during the acute phase followed by high titers of IgG, persistent HDV-RNA and intrahepatic HDV-Ag. For optimized monitoring and determination of outcome of antiviral therapy measurement of HDV-RNA is required. However, HDV-RNA RT-PCR is not yet widely available in clinical practice.

## *Hepatitis C*

Hepatitis-C-virus (HCV-) diagnostics should be performed in all patients with increased aminotransferase levels and in patients with chronic liver disease or liver cirrhosis of unknown aetiology, particularly if transmission risks (e.g. administration of blood or blood products, intravenous or nasal drug abuse, tattoos, piercing, needle stick injuries etc.) are present. Chronic hepatitis C virus infection typically is associated with non-specific symptoms such as fatigue and intermittent right upper abdomen pain which rarely directly lead to diagnosis of HCV-infection.

In case of suspicion of chronic hepatitis C, screening is performed by anti-HCV-antibody testing (Table 63.5). Current 2nd and 3rd generation HCV antibody tests very rarely produce false-positive or false-negative results. For confirmation of ongoing HCV infection in patients with positive anti-HCV antibodies HCV-RNA testing is required. Assays for detection of HCV core antigens have been developed but are associated with a limited sensitivity. Determination of HCV-genotype and quantitative measurement of serum HCV-RNA concentrations before and during antiviral therapy is useful to predict outcome and to determine duration of therapy.

In patients with suspected acute hepatitis C analysis of HCV-RNA should be performed, because anti-HCV-antibodies in many cases are not detectable until several weeks after infection. Anti-HCV-IgM tests cannot narrow this diagnostic window considerably. HCV-RNA detection by RT-PCR or TMA are highly sensitive procedures, which in all cases become positive within the first 2 weeks after HCV transmission.

**Table 63.5** Comparison of serological and molecular tests for diagnostics of hepatitis C

| | Anti-HCV | HCV-RNA |
|---|---|---|
| Detection of acute infections | approx. after 4–6 weeks | within the 1st week |
| Differentiation cured/ chronic infection | +/– | + |
| Diagnostics in immuno-incompetent patients | +/– | + |
| Diagnostics in newborns | +/– | + |
| Reinfection after organ transplantation | – | + |
| Virus quantification | – | + |
| Organ specific detection | – | + |

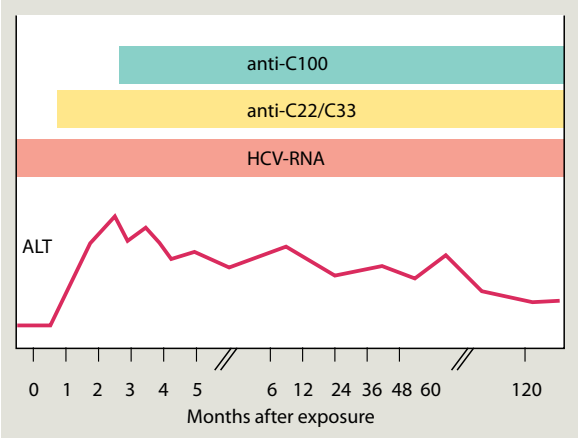



**Fig. 63.20** Course of a chronic HCV infection. The first marker of HCV infection is detectable HCV-RNA in the serum, followed by an increase of alanine aminotransferases (ALT) and detectable HCV antibodies. Antibodies against epitopes of different HCV regions typically occur at different times during the infection. The first occurring antibody is C22, the second one is C33, and then as third antibody C100 is detectable

Serological and molecular characteristics of HCV-infection are shown in Fig. 63.20.

## Serological HCV Diagnostics

### Enzyme Immunoassays (IgG and IgM)

Enzyme immunoassays (EIA) of the first generation detected serum antibodies directed against epitopes of a non-structural protein in the NS4 gene (C100). Test systems of the first generation had a sensitivity of approximately 70 – 80% and especially in patients with high immunoglobulin levels, they often showed false-positive results. EIAs of the second generation detect antibodies directed against structural proteins (core-antigen, C22) and non-structural proteins of the NS3 and NS4 region (C33, C100). With these assays more than 95% of HCV-infected patients were tested positive and they are more specific than tests of the first generation. Furthermore, in cases of acute infection they turn out to be positive several weeks earlier. Antibody tests of the third generation were complemented with recombinant proteins of the NS5-region. However, the improved sensitivity of the EIAs of the third generation is instead due to a higher reactivity of the NS3-antigen. The implementation of the NS5-antigen could even increase the number of false-positive results. Anti-HCV-antibodies have been detected in saliva and urine as well. Several commercial assays are available (e.g. Ortho Diagnostic Systems, Sanofi Diagnostics Pasteur, Abbott).

With EIAs of the third generation HCV antibodies can be detected approx. 6 weeks after transmission of the virus compared with approx. 10 weeks with tests of the second and approx. 16 weeks with tests of the first generation. The diagnostic window between HCV infection and positive HCV antibodies can be narrowed by detection of HCV-specific IgM-antibodies in only a few cases. Not all patients with an acute HCV infection develop anti-HCV-IgM-antibodies. In addition, anti-HCV-IgM-antibodies may be present in patients with acute HCV infection as well as intermittently during chronic hepatitis C.

Anti-HCV-IgG-antibodies normally persist in chronically infected patients. Exceptions were described for HIV-positive patients with chronic hepatitis C. After clearance of HCV infection, anti-HCV-IgG-antibodies are still detectable but may fall below the assays' limit of detection after many years.

### Confirmation Tests

To affirm positive EIA results, several immunoblot techniques with different membrane associated recombinant HCV proteins are available (so called RIBA-[recombinant immunoblot assay-] systems, Ortho/Chiron; Matrix HCV, Abbott; INNO-LIA™-HCV Ab III, Innogenetics). However, these assays are rarely routinely required and confirmation of ongoing HCV infection is best performed by direct detection of HCV-specific RNA with reverse transcription and polymerase chain reaction (RT-PCR), transcription mediated amplification (TMA) or signal amplification methods.

Immunoblots are based on a modification of the EIA. Anti-HCV-antibodies bind recombinant hepatitis-C-virus proteins to build an antigen-antibody-complex. After a washing step for unbound antibodies the human-IgG can be detected immunoenzymatically. In 20% of cases of blood donors with a positive anti-HCV-EIA but negative or ambiguous immunoblot result, HCV-RNA is detected by RT-PCR. Consequently, these confirmation tests are only useful in populations with low HCV prevalence, but for clinical practice in patients with liver diseases they are dispensable.

## Molecular Based HCV Detection Methods

Present recommendations for the management of interferon-alfa based treatment in patients with chronic HCV infection are based on HCV-RNA measurements before, during, and after antiviral therapy. Currently, this treatment leads to sustained virologic response with negative HCV-RNA in serum 24 weeks after the end of therapy in 54 – 56% of the patients. To improve therapy outcome, future developments are aiming for individualization of treatment duration on the basis of HCV genotype as well as HCV-RNA quantification at baseline and early during therapy. Both HCV genotype and pretreatment hepatitis C viremia were consistently identified as predictive factors for the outcome of therapy in multivariate analyses. Changes in HCV-RNA serum concentrations during the early phase of interferon-based therapy have been analyzed by complex models of viral kinetics and applied to the prediction of treatment outcomes. Approximately 1 – 2 days after the first drug application, inhibition of virus release and intracellular virus production results in first phase rapid HCV-RNA reduction which is followed by a flatter second phase decline [62]. For management of current PEG-IFN/ribavirin based standard treatment HCV-RNA measurements at week 4, 12, and 24 are used. In patients with a decline of less than 2 log HCV-RNA IU/mL after 12 weeks or detectable HCV-RNA concentrations at week 24 therapy is discontinued due to a minimal chance of achieving a sustained virologic response (1 – 2%). In subgroups of patients with low baseline viremia (<400,000 – 800,000 IU/mL) and rapid virologic response (HCV-RNA undetectable/< 50 IU/mL) after 4 weeks, treatment duration can be shortened to 16 (genotype 2, 3) or 24 weeks (genotype 1).

Several qualitative and quantitative commercial standardized systems for measuring HCV-RNA concentration in blood samples are available (see below) which differ in analytical sensitivities and dynamic ranges. The different available assays are based on amplification methods like target (reverse transcription [RT]-PCR and transcription- mediated amplification [TMA]) or signal amplification techniques (branched DNA [bDNA]). A major disadvantage of the assays based on conventional PCR methods is a limited linear range with the need to dilute those samples which are above the upper limit of detection. This time-consuming limitation has been resolved by introduction of molecular assays based on real-time PCR technology. Recently, two highly sensitive real-time PCR based assays for HCV RNA quantification (*COBAS TaqMan*™, Roche Molecular Systems, Pleasanton, CA, USA and *Abbott RealTime HCV test*, Abbott Diagnostics, North Chicago, IL, USA) were introduced. Both are approved by the EU (CE certification). While commercial use of HCV RNA assays in the European Union is restricted to devices and kits with CE certification, in the U.S. specialized laboratories may use non-certified HCV RNA tests provided that the requirements of a quality assurance are fulfilled. In Tables 63.6 and 63.7 the current marked status of each test system (FDA and/or CE certification) are summarized. Results of HCV-RNA assays are calibrated in international units (IU/mL) on the basis of the first WHO HCV international standard (96/720).

**Table 63.6** Qualitative test systems for HCV RNA detection

| Name of the assay | Distributor | Technology | Marked status |
|---|---|---|---|
| Amplicor™ HCV 2.0 | Roche Molecular Systems | PCR | FDA, CE |
| Versant™ HCV | Siemens | TMA | FDA, CE |

**Table 63.7** Quantitative test systems for HCV RNA detection

| Name of the assay | Distributor | Technology | Marked status |
|---|---|---|---|
| Amplicor™ HCV Monitor 2.0 | Roche Molecular Systems | PCR | CE |
| HCV-SuperQuant™ | National Genetics Institute | PCR | / |
| Versant™ HCV-RNA 3.0 | Siemens | bDNA | FDA, CE |
| COBAS Ampliprep/ COBAS TaqMan | Roche Molecular Systems | Realtime PCR | FDA, CE |
| Abbott RealTime HCV | Abbott Diagnostics | Realtime PCR | CE |

### Qualitative HCV-RNA Detection

#### *Reverse Transcription-Polymerase Chain Reaction (RT-PCR)*

The first test systems to confirm ongoing, replicating hepatitis C were based on RT-PCR. Due to their lower

detection limits in comparison with quantitative HCV-RNA assays, qualitative HCV-RNA tests are used for diagnosis of acute hepatitis C in which HCV-RNA concentrations are fluctuating and may be very low. In addition, they are used for confirmation of virologic response during, at the end, and after antiviral therapy.

The oligonucleotides used for HCV-cDNA generation are complement to the highly conserved 5'NCR region. An improved sensitivity (and specificity) could be achieved by the introduction of a nested PCR. The amplification of a target sequence within a nested PCR is performed in two steps. In a first PCR, the target sequence is amplified using an outer oligonucleotide primer pair. The amplified DNA serves in a second PCR as a template to amplify the target sequence with an inner primer pair, which is complement to the 3'- and 5'-terminus of the target sequence. However, in a two step PCR system the PCR tube has to be opened after the first step to transfer the DNA into a new PCR-reaction mix. Thus, there is an increased risk of contamination and generation of false-positive results.

### *Qualitative RT-PCR (Amplicor™ HCV 2.0, Roche Diagnostics)*

By the end of 1993 a first standardized RT-PCR based assay for detection of HCV-RNA was introduced, called Amplicor™ HCV (Roche Molecular Systems, Pleasanton, CA, USA). The Amplicor™ HCV, a FDA- and CE-accredited system is a combined single tube-, single enzyme-, single primer set RT-PCR assay. Under suitable buffer conditions, the DNA polymerase of *Thermus thermophilus* used in this assay, function as reverse transcriptase as well as DNA polymerase. This allows the performance of the RT-PCR in a one-step procedure without opening the reaction tube. In addition, to reduce potential contamination, the assay incorporates an internal control for monitoring the assay performance. Currently, a second version of this assay is available, called HCV Amplicor 2.0 assay which reliable detects HCV-RNA concentrations down to 50 IU/mL.

### *Transcription-Mediated Amplification (TMA, Versant™, Siemens)*

The TMA (Fig. 63.21) is an isothermal and autocatalytic method to amplify target sequences with FDA and CE market status. The TMA reaction-mix contains two oligonucleotides, one RNA polymerase, and one reverse transcriptase. In a first step a T7-promoter-primer binds to the target RNA. The reverse transcriptase produces a RNA/DNA double-strand. Due to the RNaseH activity of the reverse transcriptase the RNA-strand is degraded. The second primer binds to the DNA copy and produces a DNA/DNA double-strand including the T7-promoter. The RNA-polymerase recognizes the T7-promoter and produces 100–1,000 RNA-amplicons which are then returned to a new amplification cycle.

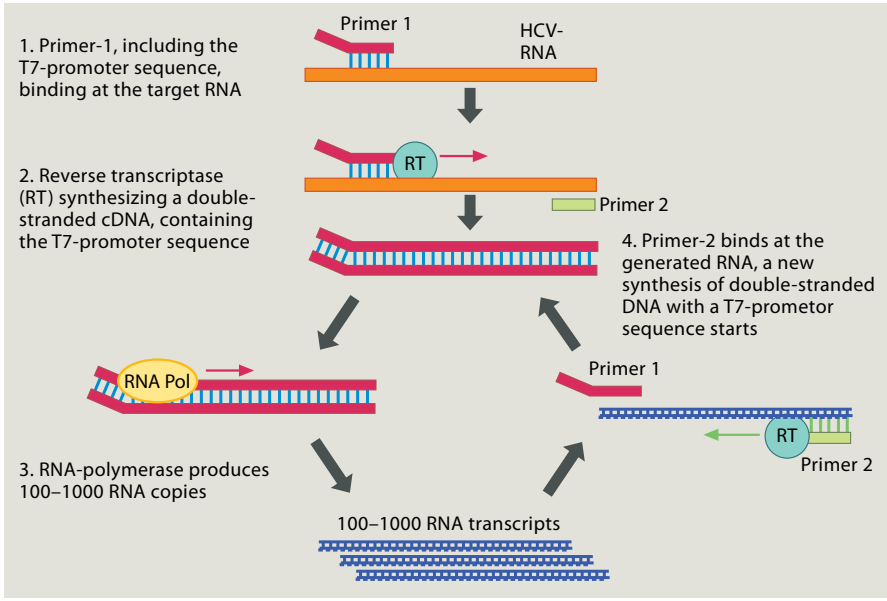



**Fig. 63.21** Principle of transcription mediated amplification (TMA, Bayer Diagnostics). For details, see text

Within 1 hour, approx. ten billion amplicons are produced. The amplified DNA is detected by liquid hybridization. Thereby labeled DNA-probes hybridize with the target sequence. The hybridized probes are detected in a chemiluminescence assay. Due to its extreme high sensitivity the TMA-based assay (lower detection 5 – 10 IU/mL) is able to detect residual HCV-RNA amounts not observed by standard RT-PCR-based tests (lower detection limit 50 IU/mL) [70].

## Quantitative HCV-RNA Detection

### *Cobas Amplicor™ HCV-Monitor (Roche Diagnostics)*

The further development of the qualitative Amplicor™ HCV assay resulted in a semiautomated, quantitative detection assay, called COBAS (complete bioanalytical system) Amplicor™ HCV Monitor 2.0 assay with improved performance characteristics. The dynamic range of the CE-labeled Amplicor™ HCV Monitor 2.0 assay is 600 – 500,000 IU/mL. While the amplification efficiency of the first version of the Amplicor™ HCV Monitor depended on the genotype, the version 2.0 assay works equally for all HCV genotypes and has a specificity of approximately 100%.

### *Branched DNA-Hybridising Assay (Versant™ HCV-RNA Assay (bDNA), Siemens)*

Bayer Diagnostics (Tarrytown N.Y.) established the semiautomated Versant™ HCV-RNA Assay (bDNA) for quantification of HCV-RNA which is now distributed by Siemens. The Versant™ HCV-RNA Assay is FDA and CE-accredited. Since version 2.0 all six major HCV genotypes are equally quantified. The current version 3.0 of the bDNA assay has a lower detection limit of 615 IU/mL and a linear quantification with low inter- and intra-assay variability up to 8,000,000 IU/mL. In contrast to the amplification of the HCV cDNA with RT-PCR, the HCV-RNA is detected with several hybridizing reactions coupled in the branched DNA technology (signal amplification). For a description of bDNA methodology see above (Diagnostics of hepatitis B) (see Fig. 63.18).

### *HCV-SuperQuant™ (National Genetics Institute, NGI)*

The current version of the HCV-SuperQuant™-assay, which is neither FDA- nor CE-approved, is based on RT-PCR combined with detection of HCV cDNA by southern blotting. The HCV-RNA is transcribed into cDNA which is transferred to a membrane. After hybridization with a digoxigenin-labeled probe the HCV DNA is detected. The HCV-SuperQuant™ has a dynamic range of 39 – 2.0 × $10^6$ IU/mL. In combination with a second assay called UltraQual™ PCR, also provided by NGI, a mean sensitivity of 1 – 1.6 IU/mL can be achieved. This assay is FDA approved for donor screening of source plasma in pools of up to 512.

### *Real-Time Based HCV-RNA Detection Assays*

Recently, HCV quantification assays based on real-time PCR were approved by Roche Molecular Systems (Pleasanton, CA, USA) and Abbott Laboratories (North Chicago, IL, USA). The real-time technology, shown in Fig. 63.22, is used to simultaneously quantify and amplify a target DNA sequence. Real-time PCR methods have the advantage of linear quantification over a

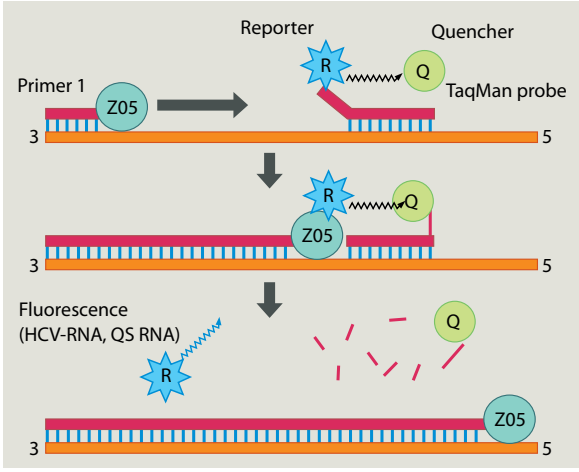



**Fig. 63.22** Real-time PCR (COBAS Ampliprep/COBAS TaqMan). The real-time technology is used to simultaneously quantify and amplify a target DNA sequence. For HCV, the detection is performed with dual-labeled oligonucleotides specific to the 5'NCR of the HCV genome. The 5'-terminus of these oligonucleotides are linked to a fluorescent molecule whereas the 3'-part is labeled with a quenching molecule. During PCR, the oligonucleotides hybridize with the target DNA and are degraded by the nuclease activity of the Z05 DNA polymerase. The reporter molecule and the quencher molecule are thereby separated from each other and the fluorescence of the reporter molecule is increased. As internal control a non-infectious RNA construct of known concentration is added to the samples, named QS RNA (Quantitation standard)

broad dynamic range, together with an integrated, automated detection system. To meet the needs of a high throughput diagnostic laboratory, completely automated versions of these assays have been produced.

### *COBAS TaqMan (CAP/CTM, Roche Diagnostics)*

Within the FDA as well as CE-labeled COBAS Ampliprep/COBAS TaqMan (CAP/CTM) assay, the Ampliprep instrument is used for HCV-RNA extraction from serum- or plasma-samples using magnetic particles. Alternatively, HCV-RNA preparation can be performed manually with glass fiber columns using the high pure system viral nucleic acid kit (HPS). The two major steps of the COBAS TaqMan assay are (i) reverse transcription of the target RNA to generate cDNA and (ii) simultaneous PCR amplification of target cDNA and detection. The detection is performed with dual-labeled oligonucleotides specific to the 5'NCR of the HCV genome and specific for the template of an internal control (synthetic RNA for binding the same primer as for HCV-RNA), used as test standard. The 5'-terminus of these oligonucleotides are linked to a fluorescent molecule whereas the 3'-part is labeled with a quenching molecule. During PCR, the oligonucleotides hybridize with the target DNA and are degraded by the nuclease acitivity of the Z05 DNA polymerase. Thereby the reporter molecule and the quencher molecule are separated from each other and the fluorescence of the reporter molecule is increased. The fluorescence signals are subsequently analyzed by the AmpliLink software (Fig. 63.22).

The first generation of the HPS/CTM assay with manual HCV-RNA extraction was restricted to genotype 1 and 6 only, due to significant under-quantification of genotype 2, 3, 4 and 5 samples. In a second generation of the HPS/CTM an equal quantification of all six major HCV genotypes was reported. The current version of the CAP/CTM assay shows equal quantification of HCV genotype 1, 2, 3, 5, and 6 samples while for genotype 4 probes a slight under-quantification in comparison with the COBAS Amplicor HCV Monitor assay was described in several studies. For both, the HPS/CTM and the CAP/CTM assay, a lower detection limit of approximately 10 IU/mL and a linear amplification of HCV-RNA from approximately 30 up to 10,000,000 IU/mL was reported [71]. For the CAP/CTM assay a first fully automated system including HCV-RNA preparation, amplification and detection for high throughput HCV-RNA quantification is available.

### *Abbott RealTime HCV Test (North Chicago, IL, USA)*

The Abbott RealTime HCV test with CE market status is developed for use on the Abbott m2000™ automated instrument system and is designed for the precise measurement of HCV-RNA in human plasma or serum samples. HCV-RNA is extracted using magnetic particles. The detection system is also based on dual-labeled oligonucleotides specific for the 5'NCR of the HCV genome. The free oligonucleotides are not fluorescent, because reporter and quencher molecules are very close to each other due to their random coiled structure. After hybridization with the target sequence the fluorescent molecule is separated from the quenching molecule and the light emission can be measured. For calculation of quantitative results, the Abbott m2000rt instrument compares the data with a stored calibration curve based on an internal standard (IC). The IC target sequence is derived from the hydroxypyruvate reductase gene from the pumpkin plant *Curcurbita pepo*. Recently, an evaluation of the Abbott real-time HCV assay showed a high sensitivity (lower detection limit 12 IU/mL), a high specificity of approx. 100% and an equal linear quantification of all HCV genotypes up to $10^6$ IU/mL [50].

## HCV Typing Methods

HCV isolates can be divided into six genotypes (genotype 1 – 6) and multiple subtypes (a, b, c). In Europe, HCV subtypes 1, 2, 3, and 4 are most prevalent. Determination of the HCV genotype before initiation of antiviral therapy is suggested for prediction of antiviral response and determination of treatment duration which are significantly different for genotype 1 versus genotype 2 or 3 infected patients.

### *Genotyping by Sequence Analysis*

Goldstandard for HCV genotyping is the sequencing of the HCV isolates. For geno-/ subtyping the sequencing analysis of the 5'non-translated region is not sufficient. It has to be complemented with analyses of the coding regions. Non-overlapping evolutionary distances could be validated for the nonstructural (NS-)5B and other HCV coding regions for isolates, subtypes and genotypes.

Several typing systems are available. Using reverse hybridizing systems, type-specific PCR, hybridizing reactions with specific oligonucleotides and HCV-RNA sequencing. The commercial assays use reverse-hybridization and sequencing methods with the nucleotide sequence information from the 5'noncoding region, the core gene and the NS5 gene.

*Reverse Hybridizing Assay (Versant™ HCV Genotype 2.0 System (LiPA), Siemens)*

This method identifies the genotypes HCV-1 to HCV-6 and more than 15 different subtypes. The high specificity of this assay is based on the simultaneous detection of 5'NTR and core regions, which minimizes the risk of false-positive results. The ability to differentiate subtype HCV-1a and HCV-1b improved up to 96.8% in the second version of the assay which contributes mainly to the higher yield of positive results. But there are still some problems present in subtyping genotype 2 and 4. The Versant™ HCV Genotype 2.0 System (LiPA) is the most widely used HCV genotyping assay.

*Trugene (TRUGENE® HCV 5'NC Genotyping Kit, Siemens)*

The Trugene assay determines HCV type and subtype based on nucleotide sequence analysis of the 5'NC region of the genome. Positive results are obtained in approximately 81%. But accurate subtype determination is limited due to the insufficient diversity of the 5'NC region. The TRUGENE® NS5B HCV Genotyping assay is currently under development.

### Morphological

The specific determination of HCV infection in the liver with histopathological methods is problematic. *In situ* hybridizing methods for detection of HCV-RNA are described, but they are technically difficult, particularly with the formalin-fixed paraffin material. Detection of HCV-RNA via PCR is possible on paraffin-fixed embedded tissues as well as on native material. For an immunohistochemistry different antibodies against HCV-coding proteins were described. Antibodies for application for formalin-fixed material are tested and commercially available. However, there are some technical problems with nonspecific background staining. In some cases, detection of HCV antigens in liver biopsy samples from serological antibody as well as HCV-RNA negative cases were was described. Conversely, however, the detection of HCV antigens may be negative in tissues of patients with positive HCV antibodies and detectable HCV-RNA.

### Conclusion for HCV Diagnosis

In patients with suspected chronic hepatitis C, screening for HCV antibodies is the method of choice. Verification of positive anti-HCV-antibodies by confirmation immunoblot assays generally is not necessary. The currently used HCV antibody assays of the second and third generation rarely produce false-positive results.

For discrimination of ongoing from past HCV infection in patients with positive anti-HCV-antibodies, HCV-RNA measurement is required. In patients with suspected acute hepatitis C a direct detection of HCV-RNA by highly sensitive amplification methods (RT-PCR or TMA) should always be performed, as anti-HCV-antibodies are in most cases not detectable until several weeks after infection. Anti-HCV-IgM tests cannot narrow this diagnostic window.

Before initiation of interferon-based antiviral therapy determination of HCV genotype and HCV-RNA viral load is used for prediction of treatment response and determination of treatment duration.

During antiviral therapy a quantitative and qualitative HCV-RNA measurement is performed for monitoring of treatment response, determination of treatment duration and early discontinuation in non-responders. The primary aim of interferon/ribavirin combination therapy is eradication of HCV infection with negative HCV-RNA 6 months after the end-of-treatment (sustained virologic response) which is associated with normalization of serum aminotransferases, improvement of liver histology and reduction of the risk for development of hepatocellular carcinoma.

## Immunological and Direct Antiviral Treatment Approaches in Viral Hepatitis

Infection with hepatitis B, B/D or C virus leads to an impairment of the liver parenchyma and promotes the development of long term complications such as liver cirrhosis or hepatocellular carcinoma. For treatment of chronic hepatitis B, (pegylated) interferon alfa and the

nucleos(t)ide analogues lamivudine, adefovir, entecavir, and telbivudine are licensed at present. For treatment of chronic hepatitis delta infection only, (pegylated) interferon alfa is available. The standard treatment for patients with chronic hepatitis C is pegylated interferon alfa in combination with the nucleoside analogue ribavirin. However, in a significant number of patients these treatment options lead to an insufficient response. Hence, an improvement with new antiviral therapies is required.

The majority of studies focus on the development of direct antiviral drugs. One possibility is the inhibition of virus-encoded enzymes (e.g. inhibitors of the protease, helicase and the RNA-dependent RNA-polymerase of hepatitis C virus or polymerase of hepatitis B virus). Substances which bind specifically at the viral genome are antisense-oligonucleotides and ribozymes. Inhibitors of the internal ribosome entry site (IRES) of HCV can also prevent transcription and translation of the viral genome. Another approach is the development of therapeutic vaccines.

**Table 63.8** Nucleos(t)ide analogs in hepatitis B treatment

| Substance | Efficacy | Status | Side effects |
|---|---|---|---|
| Aciclovir (ACV) | – | Zovirax® | neuropathy |
| Adefovir Dipivoxil (PMEA) | + | Hepsera® | (nephrotoxicity?) |
| Didanosin (ddl) | – | Videx® | neuropathy, lactic acidosis |
| Emtricitabine | + | Phase II | |
| Entecavir (ETV) | + | Baraclude® | |
| Famciclovir/ Penciclovir | + | Famvir® | nephrotoxicity |
| Fialuridin (FIAU)[a] | + | Phase II | multiple organ failure |
| Ganciclovir (DHPG) | (+) | Cymeven® | neutropenia |
| Lamivudine (LAM, 3TC) | + | Zeffix® | |
| Lobucavir[b] | + | Phase II | carcinogenicity |
| Pradefovir | + | Phase II | |
| Telbivudine (LdT) | + | Sebivo® | |
| Tenofovir (TDF)[c] | + | Viread® | |
| Zalcitabin (ddC) | – | HIVID® | neuropathy |
| Zidovidin (AZT) | – | Retrovir® | myopathy, steatosis |

[a]Fialuridin showed a delayed toxicity in Phase II-studies with serious mitochondrial functional disturbance and lethal outcome

[b]Lobucavir has proved cancerogenic in animal tests, further clinical development was stopped

[c]Lamivudine, Emtricitabine and Tenofovir are approved for treatment of HIV infection

## *Chronic Hepatitis B*

### Nucleos(t)ide Analogs

One approach in treating chronic hepatitis B is the use of nucleoside or nucleotide analogs, which inhibit the hepatitis B encoded reverse-transcriptase/polymerase. Nucleos(t)ide analogs are false nucleotides which, after incorporation during replication, lead to a chain-termination. Treatment with direct antiviral drugs may lead to the selection of viral isolates with resistant mutations. Table 63.8 gives an overview of nucleos(t)ide analogs currently used for treatment of chronic hepatitis B.

#### Lamivudine

Lamivudine is a deoxycytidine analog that is active against HIV and hepatitis B virus. Lamivudine was the first direct antiviral to be approved for HBV infection. In patients with chronic hepatitis B, lamivudine profoundly suppresses HBV replication [34]. Within the cells lamivudine is metabolized to the triphosphate, which is responsible for the antiviral activity through inhibition of the DNA polymerase. With increasing treatment duration, resistance mutants (e.g. the so called YMDD-HBV-mutant), may be selected. The YMDD motif (tyrosine, methionine, aspartate, aspartate) is a highly conserved amino acid sequence involved in deoxynucleoside triphosphate (dNTP) binding in the catalytic site of a number of RNA-dependent DNA polymerases, including hepatitis B virus (HBV) DNA polymerase. In approximately 50% of the patients, resistant mutants associated with increasing HBV-DNA viral loads are selected after 2 years of treatment with lamivudine.

#### Adefovir Dipivoxil

Adefovir [9-(2-phosphonomethoxy-ethyl)-adenine] (PMEA) is an acyclic nucleotide phosphonate, which does not have to be activated through intracellular

phosphorylation. The oral prodrug of adefovir is adefovir dipivoxil (bis-[POM]-PMEA). In addition to the competitive inhibition of the viral polymerase and an obligatory chain-determination after integration in the viral RNA, adefovir induces activation of natural killer cells and production of alpha- and beta-interferon. Adefovir dipivoxil is approved for the treatment of wild type and lamivudine resistant hepatitis B virus infection and no cross resistance was described between lamivudine and adefovir. However, treatment with adefovir is also associated with the development of resistance, and in approximately 10% of the patients loss of efficacy with increasing HBV-DNA levels after 2 years of therapy has been described.

### Tenofovir

Tenofovir disoproxil fumarate (TDF), which is chemically closely related to adefovir and which is approved for treatment of HIV infected patients, has recently been shown to also be effective in patients with HBV infection. TDF was shown to reduce HBV-DNA more rapidly, more consistently, and in a significantly higher proportion of patients compared to lamivudine and adefovir dipivoxil. TDF is active in wild type and lamivudine resistant hepatitis B. So far, development of resistance to TDF has not been reported in patients treated with HBV infection. In 2008 tenofovir was approved for treatment of chronic hepatitis B.

### Entecavir

Entecavir (ETV) is a carbo-cyclic guanosine analog which also is highly effective against hepatitis B virus polymerase. Inhibition of hepatitis B virus replication has been shown *in vitro* and *in vivo*. Several polymerase assays showed that entecavir inhibits reverse transcription and the DNA-dependent DNA synthesis, but most notably the replication-initiating priming reaction via the HBV polymerase is blocked. Entecavir can also lead to suppression of HBV replication in patients with lamivudine resistant YMDD-HBV-mutants. Entecavir resistance requires pre-existing lamivudine resistance and additional mutations in the HBV reverse transcriptase/polymerase. Entecavir appears to be a stronger inhibitor of HBV replication than either lamivudine or adefovir [39, 81]. Due to the need of at least two mutations, reported rates of development of resistance are very low (approximately 1% after 4 years). Entecavir is approved for treatment of naïve and lamivudine resistant patients with chronic hepatitis B.

### Telbivudine

Telbivudine (L-deoxythymidine or LdT) is a nucleoside analog that selectively inhibits HBV replication. It has demonstrated potent activity against hepatitis B in phase 3 studies and recently was approved for treatment of chronic hepatitis B. The response rate is significantly higher compared with lamivudine. Telbivudine is well tolerated and shows no mitochondrial toxicity and no dose-limiting side effects. Development of resistance is somewhat slower than for lamivudine, with approximately 20% of patients with selection of resistance mutants after 2 years of treatment.

## Immunomodulatory Drugs

### Protein Vaccination

An immunomodulatory approach to the treatment of chronic hepatitis B infection is the use of vaccines. The major aim of this approach is to overcome the immune tolerance present in chronic hepatitis B virus infection. In initial clinical studies, some patients showed a decrease in HBV-DNA serum levels below the detection limit of quantitative assays after injection of the small and middle HBsAg. Additional patients showed significantly decreased HBV-DNA serum concentrations. However, the seroconversion rate from HBeAg to anti-HBe or HBsAg to anti-HBs, respectively, was not higher in the treated patients than expected during natural clinical course. Complications or increasing aminotransferases were not reported.

Furthermore, experiments with immunodominant T-cell-epitopes of the HBcAg were performed. For this approach, a lipopeptide-vaccine (CY-1899) was designed consisting of HBV core antigen peptide 18 – 27 as the CTL epitope, tetanus toxoid peptide 830 – 843 as the T helper peptide, and two palmitic acid molecules as lipids. A dose escalation trial showed that this vaccine was safe and able to induce a primary HBV-specific

CTL response [84]. A subsequent study showed that administration of the single-epitope vaccine, CY-1899, initiated CTL activity, which, however, was significantly lower than that observed during spontaneous HBV clearance. This low-level CTL activity was not associated with a decline in viral load, and no significant changes in liver biochemistry or viral serologic markers were observed during follow-up [79].

### DNA Vaccination (Genetic Immunization, Genetic Vaccination)

Genetic immunization is based on intravenous, intramuscular or subcutaneous injection of plasmid DNA encoding HBV antigens. The so-called DNA vaccines induce immune responses against antigens synthesized *in vivo* after introduction of DNAs encoding antigen sequences. In the context of class I and class II MHC molecules, the endogenous synthesis of antigen leads to appropriate antigen-presentation which in turn will result in strong humoral and cellular immune responses (Fig. 63.23). The genetic vaccination can be applied prophylactically to uninfected people as well as to treat chronically infected patients. Studies in animal models showed a humoral and cellular immune response after injection of HBsAg or HBcAg. Particularly, the cellular immune response via virus specific cytotoxic T-lymphocytes (CTL) induced by DNA vaccination is very effective. An advantage is that the peptides are produced within the cells and thus are processed in a natural and optimal way. In conjunction with MHC class I molecules they build a potent immune response against already infected hepatocytes. Phase I and II clinical studies showed that the vaccine was safe and well tolerated. In people who were positive for the HLA class I A2 allele, the vaccine also induced antigen-specific $CD8^+$ T cells that bound HLA-A2/ $HBsAg_{335–343}$ tetramers, secreted IFN-γ, and lysed target cells presenting an HBsAg CTL epitope. Enumeration of HBsAg-specific T cells producing cytokines indicated preferential induction of a type 1 T helper cell response. These results provide the first demonstration of a DNA vaccine inducing protective antibody titers and both humoral and cell-mediated immune responses in humans.

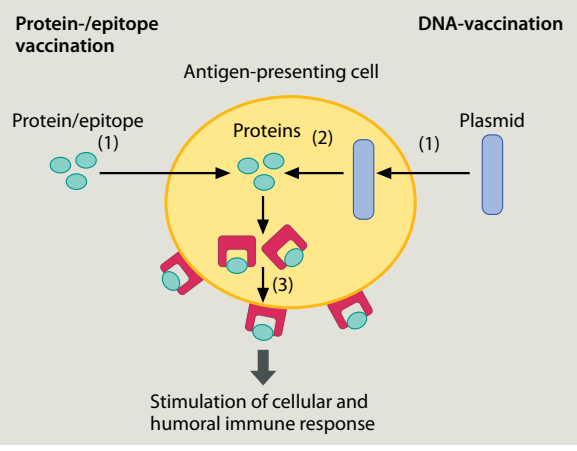



**Fig. 63.23** Therapeutic protein- or DNA-vaccination. For a protein-/epitope vaccination proteins or peptides were transfected in the cell (1), presented at the cell surface (3) and recognized by $CD4^+$ -T-cells. For a DNA-vaccination viral proteins were translated (2) from the transfected plasmid-DNA (1) and represented at the cell surface (3)

### Monoclonal Antibodies

Another specific antiviral therapy is based on the use of monoclonal antibodies directed against structural HBV proteins. Two monoclonal antibodies against two different epitopes from the HBsAg were tested as monotherapy in phase I studies on patients chronically infected with hepatitis B.

### Adoptive T Cell Transfer

The transfer of immunoreactive cells is another possibility of immunomodulatory therapy (adoptive T cell transfer). However, some difficulties, in particular MHC incompatibility and graft-versus-host-reactions, are at present challenging obstacles to overcome. The assumption that chronic hepatitis B infection can be cured through transfer of immunoreactive cells is based upon the observation that patients with chronic hepatitis B infection became HBsAg-negative 1 – 3 years after bone marrow transplantation from anti-HBs- and anti-HBc-positive donors.

## Drugs Specific Against Viral RNA/DNA

### Antisense-Oligonucleotides

Antisense-oligonucleotides are small pieces of DNA or RNA which are complementary to conserved regions

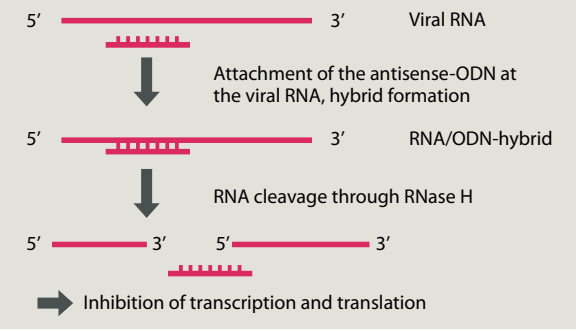



**Fig. 63.24** Mechanism of action of antisense-oligodeoxynucleotides (antisense-ODN). Antisense-oligodeoxynucleotides hybridize with viral RNA

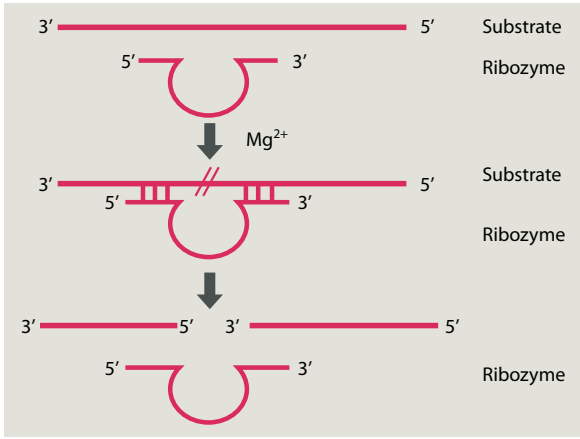



**Fig. 63.25** Catalytic cleavage through a "hammerhead-ribozyme". The ribozyme binds at the complementary sequence of the substrate. Cleavage occurs in the presence of $Mg^{2+}$

of a target-RNA (Fig. 63.24). Due to formation of hybrids with the target-RNA, all steps of the viral cycle with a single-stranded genome can be blocked. Several mechanisms of action are discussed. The binding between mRNA and ribosomes and/or mRNA initiation factors can be inhibited. Inhibition of the formation of the ribosomal subunit and the following translation may also be of importance. Destruction of the viral RNA through a cellular ribonuclease (RNase H) is possible with DNA-antisense-molecules as well. In addition, non-sequence specific mechanisms were reported.

To specifically bind to a determined genome sequence instead of natural nucleic acid molecules, the antisense-oligonucleotides must have a length of at least 11 – 15 nucleotides. A problem with the development of antisense-oligonucleotides is that the synthetic molecules can be degraded by cellular enzymes. Attempts to avoid this degradation by chemical modification have been made. Proper transport of the oligonucleotides into the target cells is problematic as well.

Antisense strategies have been tested successfully *in vitro* with a number of different viruses. Antisense-oligonucleotides cause a decrease of HBV-DNA levels in cell culture models and in animal models (duck and woodchuck).

## Ribozymes

Ribozymes (ribonucleic acid and enzyme) are naturally occurring RNA molecules, which catalyze the sequence specific cleavage of a target RNA. One ribozyme can cleave several target RNAs. The smallest and best characterized ribozymes, beside the so called "hairpin ribozymes," are the "hammerhead ribozymes". They consist of three helices: the variable helices I and III hybridize with the complementary parts of the target RNA, and the conserved helix II forms the catalytic active center (Fig. 63.25).

Ribozymes can be produced synthetically. They can be designed in a way that they are able to hybridize with specific parts of RNAs and cleave the RNA at these sites. A problem is the fast degradation of unmodified ribozymes in serum. Nuclease-stable ribozymes can be designed through chemical modifications.

Cell culture systems showed that hairpin ribozymes, directed against different sites of the pregenomic HBV RNA, recognize the RNA and cleave it specifically. An efficient cleavage of HBV RNA through hammerhead ribozymes was shown only in cell-free systems or cell lysates. One explanation may be the intracellular protein-RNA interaction or the low $Mg^{2+}$ concentration. Until now, no therapeutic potent ribozyme mediated cleavage of HBV nucleic acids has been demonstrated.

## Dominant-Negative Mutants

The biological effect of a wild type-(wt-) gene product can be inhibited by an overexpressing mutant (inactive) gene product. In other words, the inactive mutant gene product dominates the active wt-product (dominant-negative).

Dominant-negative mutants against the hepatitis B virus genome were generated and their effect was demonstrated *in vitro*. The core-coding part of the gene was thereby fused with the surface-gene, and via adenoviral vectors introduced in a cell system which was already transiently transfected with replication competent HBV vector constructs. Due to the dominant-negative core-surface-HBV-mutants, an inhibition of the HBV replication of more than 90% was observed. The inhibition is most likely achieved through blocking of the encapsulation of the viral genome. The antiviral approach with dominant-negative mutants seems to be relatively insensitive against sequence variations. Thus, the risk of developing resistant virus isolates is thought to be less likely than for ribozymes or antisense-oligonucleotides.

## *Chronic Hepatitis C*

### Inhibitors of the Inosine Monophosphate Dehydrogenase

Inosine monophosphate dehydrogenase (IMPDH) is a cellular enzyme needed for formation of guanosine nucleotides. IMPDH exists in two isoforms (I and II) which are identical in 84%.

#### VX-497

VX-497 is a potent, reversible uncompetitive IMPDH inhibitor which is orally available. The monotherapy with VX-497 leads to an effect against a variety of DNA or RNA viruses. In combination with interferon-alfa an additive antiviral effect is demonstrated. A phase II study in patients with HCV infection showed a significant decrease in aminotransferases, but viremia did not change.

#### Ribavirin

Ribavirin (1-ß-D-ribofuranosyl-1,2,4-triazole-3-carboxamide) is a guanosine analogue that possesses broad-spectrum antiviral activity against several RNA and DNA viruses in vitro. An initial pilot study investigating the utility of ribavirin in patients with chronic hepatitis C was published in 1991 [67]. Subsequently, several controlled trials showed that ribavirin monotherapy led to a decline in alanine aminotransferases levels in a significant proportion of patients [20]. However, in the majority of patients no significant reduction of HCV-RNA was detected, suggesting that ribavirin monotherapy has no or only marginal effects on HCV replication *in vivo* in several patients. Despite these unsuccessful studies with monotherapy, ribavirin was combined with (PEG)-IFN alfa, which surprisingly led to substantially improved sustained virologic response rates in patients with chronic hepatitis C. The benefit of adding ribavirin to interferon-alfa based antiviral therapy results from a sharper second phase decline and mainly the prevention of virologic relapse. However, the exact antiviral mechanisms of ribavirin are poorly understood, and currently four different mechanisms of action have been proposed: (i) enhancement of the host adaptive antiviral immune response, (ii) inhibition of host IMPDH, (iii) direct inhibition of HCV NS5B RNA-dependent RNA-polymerase, and, more recently, (iv) RNA virus mutagenesis and "error catastrophe" [23, 47, 66]. For the latter hypothesis, it has been reported that ribavirin acts as an RNA virus mutagen, thereby leading to an increased mutational frequency that exceeds the mutational threshold of viral fitness and drives RNA viruses into error catastrophe [32].

### CpG-Oligo(deoxy)nucleotides

CpG-motifs are non-methylated cytidine-guanosine-dinucleotides surrounded by certain bases. They occur much more frequently in viral or bacterial DNA than in human DNA. Thus, CpG-motifs turn out to be a characteristic and quantitative differentiating factor between human and viral or bacterial DNA, respectively. They can be recognized by the human immune system and they activate immune response mainly via stimulation of Th1-response with the activation of natural killer cells and dendritic cells. B-cell-response is stimulated via CpG-motifs as well.

Synthetically produced oligonucleotides including CpG-motifs can simulate an infection and hence activate the immune system. A potent CpG-motif was identified, and modified nuclease-stable CpG-oligonucleotides were developed. Potential applications are tumour diseases,

infectious diseases (immunomodulation, prophylactic or therapeutic vaccination), allergies, and bronchial asthma.

The number of CpG-motifs in the vaccine can be increased or decreased. An increase of CpG-motifs can be reasonable if the activity of the vaccine should be raised. A decrease of the CpG-motifs leads to a reduced immune response and can be applied if genes should be inserted in the genome.

## Vaccinations

Hepatitis C virus exhibits a high genetic variability with multiple genotypes and subtypes, furthermore it shows significant intra-individual viral heterogeneity (quasispecies); therefore, development of an effective active immune prophylaxis (prophylactic vaccination) against HCV has not been successful so far. The purpose of a therapeutic vaccination is to induce a strong immune response with consecutive elimination of the virus (see Fig. 63.23).

Vaccines can consist of isolated or recombinant produced viral proteins, peptides, virus-like particles, "naked" DNA or recombinant produced viruses. An effective HCV vaccine should be effective against all HCV-genotypes and -subtypes. Highly conserved regions such as the HCV-core-antigen are potentially suitable target epitopes. Additionally, studies with the variable envelope-(E1- and E2-)antigen have been performed. Anti-E2-antibodies recognizing epitopes within the hypervariable region (HVR) 1 showed neutralizing effects in chimpanzees. In addition to induction of high titer cross-reactive antibodies, a multi-specific cellular immune response is likely important for curing the disease.

### DNA Vaccines

For administration of DNA vaccines, intramuscular, subcutaneous or intravenous injection of plasmid-DNAs is performed. The plasmid-DNA is taken up by the host cells followed by intracellular expression of the appropriate (viral) protein. The subsequent presentation of antigens stimulates the cellular and humoral immune response.

Initial studies with genetic immunization against HCV showed that through the application of certain gene sequences from the HCV genome in a host, a humoral as well as a cellular immune response was induced. Animal studies showed that vaccines with the conserved nucleocapsid-(core)region and the hypervariable region (HVR) 1, but also with non-structural proteins (NS3, NS4, and NS5), are able to induce an immune response. This immune response can be increased through additional administration of immunomodulatory cytokines. Chimpanzees were successfully infected with HCV despite previous vaccination with a DNA coding for part of the E2 protein. However, the clinical course of the infection was relatively mild and self limiting in both cases. In patients with chronic hepatitis C, studies with therapeutic DNA vaccination have not yet been published.

### Protein Vaccines

Another approach of vaccination is the application of viral antigens (protein vaccination). Particularly, studies with the envelope-(E1- und E2-)antigen have been performed. In chimpanzees a vaccine from recombinant envelope antigens induced an antibody response and CD4-T-cell response, which inhibited chronification of the infection. Another protein vaccine, containing a purified genotype HCV-1b envelope-protein (INNOVAX C, Innogenetics), was investigated in a clinical study in patients with chronic hepatitis C. Because there was no statistically significant effect observed between patients and placebo groups, further development of the vaccine was stopped.

### Epitope Vaccine

Epitope vaccination is performed by the transfection of immunodominant epitopes into the target cells. To increase immunogenicity the epitopes are bound to a carrier complex (e.g. PADRE™, Epimmune). An epitope vaccine containing several immunodominant T-cell-epitopes which should stimulate $CD4^+$- and $CD8^+$-responses is in preclinical development at present and a phase I study is planned (Epimmune). Another therapeutic peptide vaccine is IC41 (Intercell). It consists of five synthetic peptides harbouring HCV CD4 and CD8 T-cell epitopes and the synthetic adjuvant poly-L-arginine. A clinical phase II study determined a HCV-specific IFNγ secreting $CD4^+$ and $CD8^+$

T cell response in chronic HCV patients. Additional phase II studies are currently underway. Collaboration between CSL and Chiron provides a vaccine which has proved to be safe and well-tolerated. It induced cellular immune responses in initial trials, so a phase II study is currently planned.

### Amantadine and Rimantadine

Amantadine (L-Aminoadamantan, Infex®), a tricyclic amine, and its analogue Rimantadine (Flumadine™) are two potential antiviral agents for the treatment of chronic hepatitis C. Amantadine has antiviral activity against a broad range of viruses including influenza A virus infection. In patients with chronic hepatitis C, amantadine had no antiviral effect when used as monotherapy in several studies [3]. Placebo-controlled trials with the combination treatment of IFN-α with or without amantadine have shown no significantly higher sustained virologic response rates in patients who received amantadine [89]. Meta-analyses of all studies with IFN-α in combination with amantadine showed contradictory results [49]. Triple therapy with PEG-IFN, ribavirin, and amantadine showed improved sustained virologic response rates in patients who were previously nonresponders but showed no significant benefit in naïve patients or relapsers in comparison with standard combination therapy [9]. Additional studies with PEG-IFN, ribavirin, and higher doses of amantadine to verify a potential antiviral effect of amantadine in patients with chronic hepatitis C virus genotype 1 infection have been completed, however, showed no additive antiviral efficacy. As a potential target for amantadine, the HCV p7 protein was identified [26]. The HCV p7 protein was identified as being a calcium ion channel in artificial lipid bilayers and in membranes of mammalian cells. Amantadine abrogates the activity of this channel *in vitro*, similar to its ability to inhibit the M2 protein, an integral membrane protein of influenza A virus [28]. In a recent study, the clinical importance of amino acid variations within the HCV p7 protein for the response to IFN-α-based antiviral therapy with and without amantadine in patients with HCV genotype 1 infection was investigated. Overall, the HCV p7 protein was highly conserved, and no significant association of amino acid variations within HCV p7 with the virologic treatment response in patients who received amantadine was observed [51]. Taken together, there is currently no evidence for a clear additional antiviral effect of amantadine for first-line therapy of patients with chronic hepatitis C. Finally, most recently no antiviral effect of amantadine (as demonstrated by a lack of reduction of HCV-RNA levels) was detectable in the HCV replicon system.

### Specific Enzyme Inhibitors

An important focus for new antiviral treatment options in HCV infection is the development of direct antiviral drugs which specifically interact with the HCV replication cycle (specifically targeted antiviral therapy in hepatitis C, STAT-C). Based on the availability of an *in vitro* HCV replicon system and the detailed knowledge about the structure and function of HCV proteins, in recent years a subset of new compounds comprising direct inhibitors of HCV enzymes like protease, helicase, and polymerase were developed. The most advanced, newest antiviral agents are directed against the HCV NS3/4A serine protease and the HCV-specific NS5B RNA-dependent RNA-polymerase (Fig. 63.26). Different antiviral agents for the specific inhibition of the HCV NS3/4A protease and the HCV NS5B polymerase are currently in phase 1/phase 2, and phase 3 trials for treatment of patients with chronic hepatitis C, and multiple additional direct antiviral drugs are in preclinical development. So far, results of phase 2 studies are available for two protease inhibitors (VX-950/telaprevir, and SCH503034/boceprevir) and two polymerase inhibitors (R1479, and HCV-796).

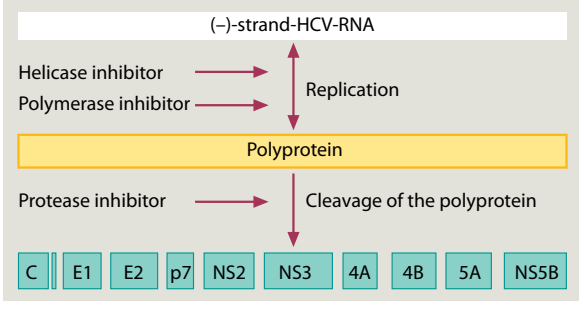



**Fig. 63.26** Mechanisms of action of HCV helicase-, polymerase- and protease-inhibitors. The inhibitors inhibit different steps of the viral replication cycle (transcription, translation, and cleavage of the polyprotein)

### Helicase Inhibitors

For translation and replication the HCV-RNA needs to be unwound. This process is catalyzed through a helicase which is part of the HCV NS3 protein. Specific inhibitors of the HCV helicase are under development. However, clinical results are not yet available.

### Protease Inhibitors

Like other RNA viruses, HCV expresses its genetic information in a single polyprotein which has to be cleaved in structural and non-structural proteins by cellular and viral proteases. During short-term monotherapy with the protease inhibitors BILN2061 (2 days), SCH503034, and VX-950 (14 days), a decrease in HCV-RNA concentrations of between 1.5 and 4 $\log_{10}$ HCV-RNA IU/mL was observed in patients with chronic HCV genotype 1 infection [30, 64, 73]. BILN2061 and VX-950 especially showed high antiviral efficacy, with HCV-RNA levels below the detection limit of a highly sensitive assay (<10 IU/mL) in a significant number of patients at the end of the 2 – 14 days therapy. However, after the end of dosing, HCV-RNA levels increased to concentrations at baseline in all patients [40, 65, 88]. For the protease inhibitor BILN2061, studies of patients infected with genotypes 2 and 3 were published. Interestingly, a generally less pronounced antiviral activity in patients infected with genotypes 2 and 3 was observed. This demonstrates a high possibility of specificity of direct antiviral drugs for a certain genotype caused by the substantial HCV sequence variability between different genotypes [30, 68]. In comparison with IFN-α-based therapy, the antiviral efficacy for blocking viral production is significantly higher in patients treated with the protease inhibitor BILN2061 (99% versus 55 – 95%). Combination therapy of SCH503034 or VX-950 with PEG-IFN leads to a greater decline of HCV-RNA viral levels compared with protease inhibitor monotherapy, and viral breakthrough due to protease inhibitor resistance is prevented efficiently. Due to the error-prone nature of the HCV RNA-dependent RNA-polymerase together with highly efficient replication, the selection of isolates that are resistant to compounds such as HCV NS3/4A protease and NS5B polymerase inhibitors could be a major limitation for the efficiency of direct antiviral therapies in patients with chronic hepatitis C. In the subgenomic HCV replicon system, different mutations in the HCV NS3 serine protease domain (R155Q, A156S/T, D168A/V, T54A, and V170A) that confer different levels of resistance to BILN 2061, VX-950, and SCH503034 were identified [43, 82]. In patients treated with the protease inhibitor VX-950, selection of resistant variants with viral breakthrough during a 14-day treatment period was observed. Through highly sensitive sequence analysis of the HCV quasispecies before treatment initiation, at the end of therapy, and during follow-up, different amino acid positions (V36, T54, R155, and A156) with mutations that confer different levels of resistance to VX-950 were described [72]. For treatment with SCH503034, analysis of the HCV quasispecies during therapy revealed imutations at amino acid positions V36, T54, V55, R155, A156, and V170. By direct sequencing, the selection of HCV isolates with mutations at position T54 in single patients was reported. The question of whether the combination of different direct antiviral drugs or combination therapies of direct antiviral drugs with PEG-IFN and ribavirin will shorten treatment duration, prevent the development of resistance, or significantly improve sustained virologic response rates, will be investigated in future studies.

### Polymerase Inhibitors

Hepatitis C virus replicates by a virus encoded (NS5B) RNA-dependent RNA-polymerase. Thus, inhibition of the HCV-specific RNA-dependent RNA-polymerase is another approach in the development of direct antiviral drugs. The HCV nucleoside RNA-polymerase inhibitor NM283 is the oral prodrug of 2'-*C*-methyl-cytidine (NM107) and is cleaved to the free nucleoside that is converted to the active triphosphate by the cellular machinery. NM107 specifically inhibits HCV replication in the HCV replicon assay *in vitro*. No *in vivo* resistance data for NM283 are available yet, but it was shown that the 2'-*C*-methyl-nucleoside NM107 is susceptible to resistance development, as shown in replicon systems and isolated polymerase assays. Sequence analysis of the HCV NS5B gene of several drug-resistant replicons defined a single replacement of the highly conserved serine 282 with threonine (S282T) that confers resistance to nucleosides containing 2'-methyl functionality. Results of phase 1/phase 2 studies with the HCV NS5B RNA polymerase inhibitor valopicitabine (NM283) showed a decrease in the HCV-RNA concentration of 1

to 2 $\log_{10}$ HCV-RNA IU/mL in the treatment of naïve patients or IFN-α nonresponders [1]. Interim analysis of ongoing studies also showed improved treatment efficacy for combination therapy with NM283 and PEG-IFN. However, no improvement of sustained virologic response rates for naïve or non-responder patients was demonstrated in these studies. In addition, significant gastrointestinal side effects have been reported and the further development of NM283 was stopped recently.

HCV-769 is a non-nucleoside inhibitor of the HCV RNA-dependent RNA-polymerase. It demonstrated initial rapid antiviral activity with approximately 2 log declines of HCV-RNA concentrations in treatment-naïve patients with HCV infection during a 14-days study. Subsequent increases of plasma HCV-RNA levels during HCV-796 monotherapy appear to be associated with selection of viral variants with reduced susceptibility to the inhibitor. The major variant expresses a C316Y substitution in NS5B, and remains susceptible to interferon and ribavirin *in vitro*. Phase 2 clinical studies with combination therapy with PEG-IFN and ribavirin have been stopped due to significant increases in transaminase levels in patients treated with HCV-796.

R1626, the oral prodrug of 4'-acido-cytidine (R1479), is another nucleoside inhibitor of the HCV NS5B polymerase, currently in development. In a phase I study R1626 monotherapy greater viral reductions than those described for other polymerase inhibitors were observed in patients with chronic genotype 1 hepatitis C. With the highest dose (4,500 mg BID) a mean decline of 3.7 $\log_{10}$ HCV-RNA IU/mL within 2 weeks of treatment was reported. A significant drop in hemoglobin levels was observed especially with the highest dose, and a phase II study with a lower dose in combination with PEG-interferon alfa 2a and ribavirin was initiated.

Taken together, only limited data on HCV polymerase inhibitors are available today. So far, the antiviral activity of the drugs tested in patients with chronic hepatitis C seems to be generally weaker than that of several HCV protease inhibitors, but this may be overcome by increased specificity and higher doses of HCV polymerase inhibitors.

## Drugs Specifically Directed Against Viral RNA

Compounds which are directed specifically against viral RNA are inhibitors of the internal ribosome entry site (IRES), antisense-oligonucleotides and ribozymes.

### Inhibitors of the Internal Ribosome Entry Site

Translation of HCV-RNA starts after ribosomes bind at the internal ribosome entry site (IRES) which is located in the 5'NTR. By contrast, translation of cellular mRNA is cap-dependent.

A short RNA (inhibitor RNA, iRNA), isolated from *Saccharomyces cerevisiae,* inhibits the cap-independent, IRES-mediated translation of several viruses. The iRNA has no effect on cap-dependent translation of cellular mRNAs [19]. These iRNA seems to inhibit translation not via antisense mechanisms but through binding at certain cellular proteins. The iRNA probably has a stable secondary structure which complies with the viral 5'NTR partly. VGX-410C (VGX Pharmaceuticals) represents the first drug in the class of HCV IRES inhibitors. It is an orally active, small-molecule drug. VGX-410C suppresses HCV translation and blocks the HCV replication process. As all the genotypes of HCV use the same pathway, this drug target should be effective for all HCV genotypes. After a phase II study dvelopement of VGX-410C was discontinued due to insufficient efficacy.

### Antisense-Oligodeoxynucleotides

Antisense-oligodeoxynucleotides (ODNs) are small, single-stranded DNA molecules complementary to specific sequences in viral RNA or DNA. They have shown great efficacy in selective inhibition of gene expression [25]. They can be targeted at elements critical for the translation of genes, resulting in translation arrest. The main mechanism of inhibition at RNA viruses involves induction of RNase H activity capable of cleaving RNA in RNA-DNA hybrids (Fig. 63.24). Natural A-ODNs without alteration of the structure are quickly degraded by nucleases present in serum. Chemical modifications confer nuclease resistance. Therefore, oxygen in the phosphate residue is replaced by sulphur, methyl or benzyl residues (S-ODN, M-ODN, B-ODN).

Capable target sequences for A-ODNs are highly conserved RNA sections like the 5'NTR region of the hepatitis C virus. 5'NTR and core region complementary antisense-oligodeoxynucleotides (mostly S-ODNs) can inhibit translation and replication of the hepatitis C virus to 80 – 96% *in vitro*.

A 20-base phosphorothioate antisense oligodeoxynucleotide (ISIS 14803, ISIS Pharmaceutical/Elan) for

treatment of chronic hepatitis C was under investigation in phase I/II studies. In preclinical studies the substance led to specific reduction of HCV-RNA in cell culture. Since 1999, first antisense-oligonucleotides have been approved in Europe for treatment of CMV-retinitis in AIDS patients (Formivirsen, Vitravene, ISIS Pharmaceuticals).

### Ribozymes

Specially built ribozymes target highly conserved regions within the hepatitis C genome. It is important that the target sequence for the ribozyme is single-stranded *in vivo* to avoid intramolecular hybridising. Moreover, the target sequence should be sterically well accessible. Suitable target sequences in HCV are the internal ribosome entry site in the 5'NTR region or the core gene. The HCV genome possesses only one open reading frame. Thus, inhibition of the internal ribosome entry site could inhibit the complete protein expression. Initial studies showed efficient catalytic activity of hammerhead-ribozymes cleaving HCV-RNA *in vitro*. In human HCV infected hepatocytes, (adenoviral-)expressed ribozymes reduced or even eliminated HCV-RNA.

A nuclease-resistant hammerhead-ribozyme directed against the 5'NTR was developed by Ribozyme Pharmaceuticals (LY 466700, Heptazyme™). An accumulation in murine hepatocytes and sinus endothelial cells was shown. Cell cultures with HCV-5'NTR-polio virus-chimera exhibit inhibition of replication of more than 90%. Clinical studies were halted due to toxic side effects in primates.

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## 63.2 Pathology of Acute and Chronic Viral Hepatitis

The hepatic response to viral infection, particularly by the hepatitis viruses A-E, is predominantly immunologically mediated and characterized pathologically by hepatocellular damage accompanied by infiltrates of immune effector cells [27]. This response has been well characterized morphologically following the onset of the acute hepatitis and later, if chronic viral infection ensues [53]. The diagnosis of acute viral hepatitis is usually established serologically and only rarely by biopsy. In contrast, for chronic viral hepatitis the liver biopsy has historically been considered the "gold standard" for direct morphological assessment of the degree of parenchymal injury and inflammation, and the potential complications of fibrosis or cirrhosis. Several classification systems for chronic hepatitis are now available to express this information in semiquantitative form, providing separate scores for the *grade* of necroinflammation and for the *stage* of fibrosis or cirrhosis [10]. The recent advent of non-invasive surrogate tests for necroinflammation and fibrosis (or cirrhosis) in chronic hepatitis has also been predicated on having morphologic data against which to evaluate the specificity and sensitivity of such tests [11, 31, 35].

### General Features of Acute Viral Hepatitis

In full-blown acute viral hepatitis the liver is grossly enlarged and swollen owing to the influx of immune cells and varying degrees of liver-cell ballooning. Microscopically there is diffuse inflammation involving portal tracts and the lobular parenchyma (Fig. 63.27). Expression of viral antigens on hepatocytes in the context of major histocompatibility antigens provokes a primary cytotoxic T-lymphocyte (CTL) response mediated by CD8-positive suppressor/cytotoxic lymphocytes. These infiltrating small T-lymphocytes along with scattered other effector cells, including occasional plasma cells and eosinophils, are found within portal tracts as well as within the sinusoids where they induce hepatocellular damage and death, thereby interrupting the normal orderly pattern of radiating cords of hepatocytes. The resulting appearance is one of lobular disarray and unrest, with lymphocytes closely apposed to hepatocytes undergoing ballooning and apoptosis, a process termed *spotty necrosis*. This is an active milieu of cytokine release as well as granzyme-perforin and FAS-FAS ligand activity [27, 56]. Kupffer cells engaged in phagocytosis of dead hepatocytes accumulate within lobular necroinflammatory foci, their lysosomal ceroid constituents staining tan-brown with hematoxylin and eosin (H&E)

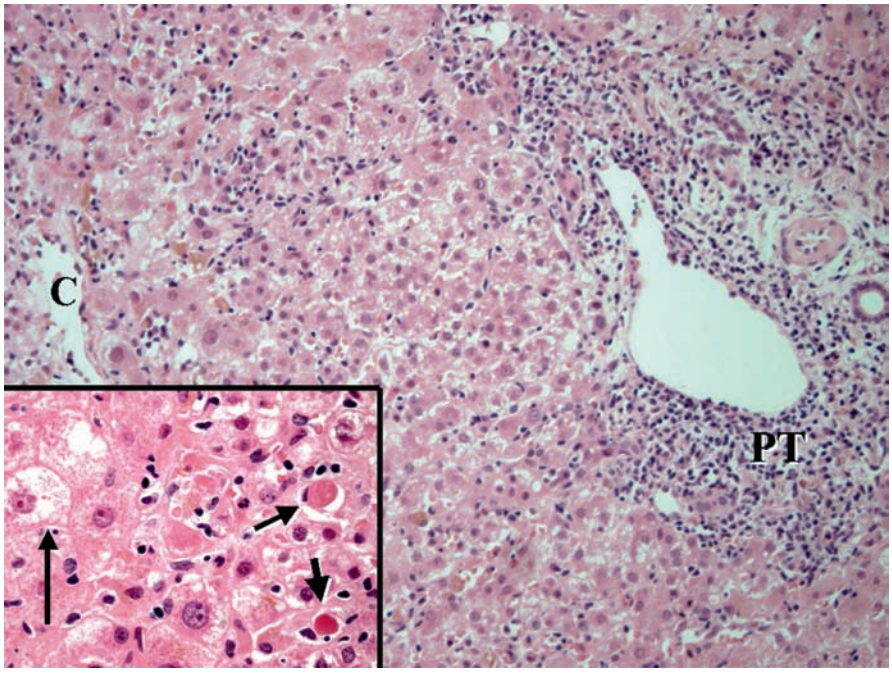


**Fig. 63.27** Acute viral hepatitis. There is diffuse inflammation involving the portal tract (PT) and the lobular parenchyma. Lobular disarray is present due to interruption of liver-cell plates by mononuclear cells (predominantly lymphocytes) in combination with variable hepatocyte ballooning. C. central vein. Inset: Higher magnification showing ballooned hepatocytes (long arrow) and apoptotic bodies (short arrows). (Hematoxylin and eosin stain)

stain and purple-red with DPAS (diastase-pretreated periodic acid-Schiff stain). Centrilobular regions often show the most pronounced necroinflammation. Cholestasis may be absent or minimal, or, as in certain cases of acute hepatitis A and E, unusually prominent.

More severe forms of acute hepatitis may be associated with several types of *confluent necrosis* in which foci of spotty necrosis merge to form contiguous regions of inflammation, hepatocyte loss and parenchymal collapse. Such forms of confluent necrosis include *bridging hepatic necrosis* (necrosis extending between portal tracts or between portal tracts and central veins), *multilobular necrosis* (extending between adjacent lobules) and *massive hepatic necrosis* (Fig. 63.28) [9]. The latter form is usually manifested clinically as *fulminant hepatitis* and results in diffuse loss of hepatic parenchyma and a grossly shrunken, underweight liver with a wrinkled capsule due to the loss of underlying liver tissue. Reticulin staining is helpful in demonstrating the extent of parenchymal collapse. With multilobular and massive hepatic necrosis, activation of progenitor cells at the edges of portal tracts leads to a prominent *ductular reaction,* sometimes referred to as proliferation of neocholangioles (Fig. 63.29) [46]. Centrilobular and midzonal regions, by contrast, are pale-staining and devoid of viable hepatocytes, with scattered residual lymphocytes, ceroid-laden Kupffer cells and variable sinusoidal endothelial disruption and erythrocyte extravasation. The term *submassive necrosis* is applied to cases of severe acute hepatitis with extensive confluent necrosis which progress over a more protracted clinical course of sometimes several months. In these cases, the liver is grossly underweight and shows broad areas of parenchymal collapse alternating with regenerative nodules and varied degrees of early fibrosis.

The major pathological *differential diagnosis* includes drug hepatotoxicity and autoimmune hepatitis. Idiosyncratic liver damage due to a variety of drugs can produce similar or identical changes to those seen in acute viral hepatitis (e.g., isoniazid, nitrofurantoin, alphamethyldopa) [23]. Herbal and alternative agents as well as recreational drugs should also be considered in this category [52]. Excessive numbers of eosinophils, formation of non-caseating granulomas, bile duct damage and steatosis are additional features which may point specifically to drug causation. Although autoimmune hepatitis may occasionally present with the histopathological changes of an acute hepatitis, it more often is associated with the pathological features of chronic hepatitis evolving toward cirrhosis. Nevertheless, certain features reflecting the inherently chronic nature of autoimmune liver injury may be visible. Prominence of inflammation not only within portal tracts but also

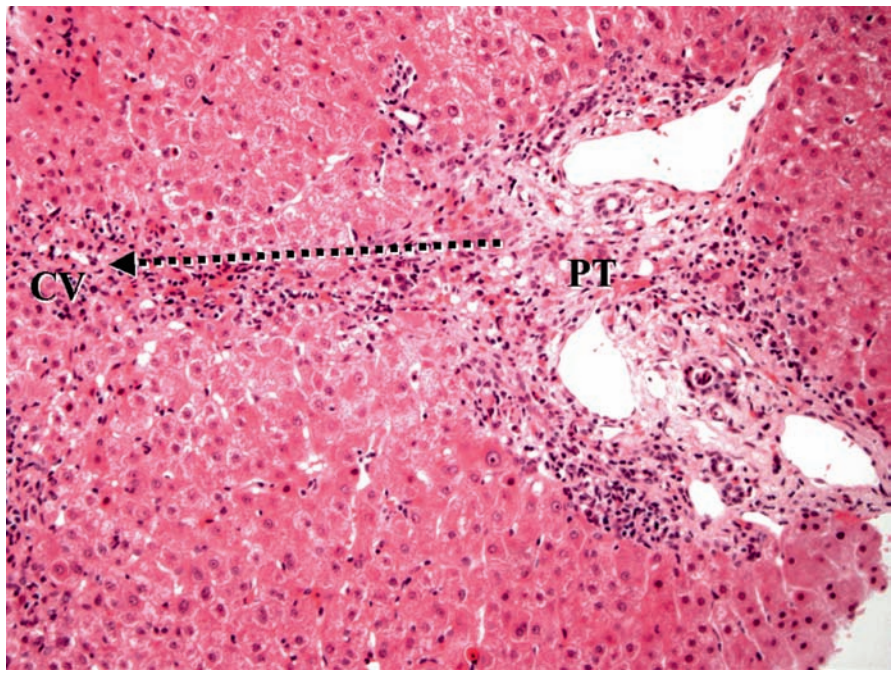


**Fig. 63.28** Bridging hepatic necrosis. A bridge of confluent necrosis is present (directly under the dashed arrow), extending from the portal tract (PT) toward the central vein (CV). (Hematoxylin and eosin stain)

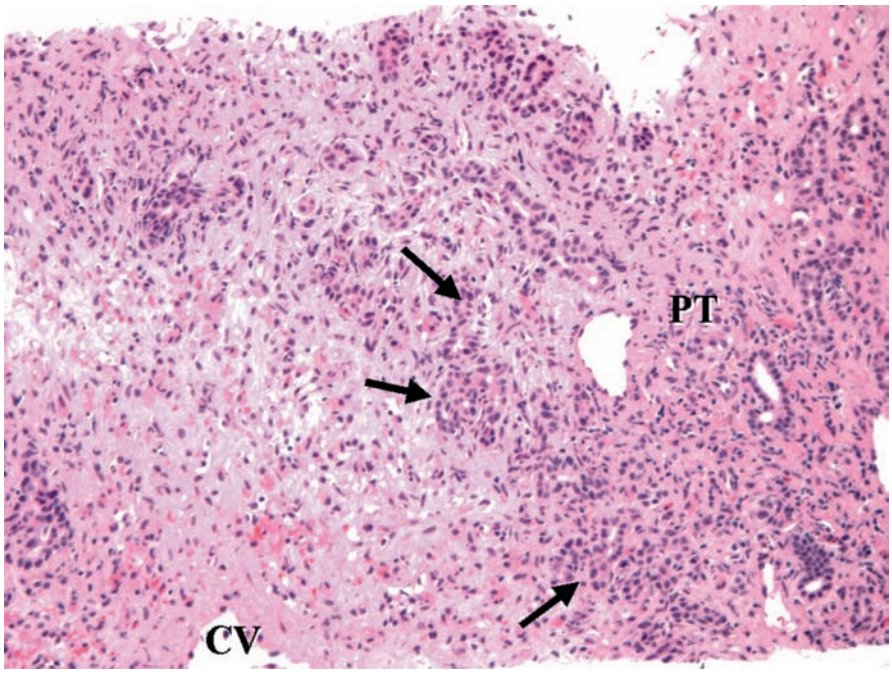


**Fig. 63.29** Massive hepatic necrosis. There is complete loss of liver parenchyma from the edge of the portal tract (PT) to the central vein (CV), with only residual sinusoidal endothelium and inflammatory cells visible. Note the prominent periportal proliferation of bile ductular structures (ductular reaction) from progenitor cells (arrows). (Hematoxylin and eosin stain)

eroding into the periportal parenchyma as interface hepatitis is a helpful finding. Regenerative liver-cell rosettes may also form in the periportal regions affected by interface hepatitis. Prominence or predominance of plasma cells in clusters within the portal and periportal infiltrates and within the lobules is also characteristic of autoimmune liver disease. Transformation of hepatocytes into multinucleated giant cells is an unusual finding in adults ("*postinfantile giant cell hepatitis*") and in the pathological setting of an acute hepatitis is another indication for exclusion of autoimmune hepatitis [19].

## General Features of Chronic Viral Hepatitis

Chronic hepatitis is defined as inflammation of the liver continuing without improvement for 6 months or longer [34]. The time frame for observation of the histopathological changes of chronic hepatitis is usually years to decades after the initial acute viral hepatitis. The inflammation, hepatocellular injury and potential fibrosis (or cirrhosis) vary considerably from case to case [28]. In some instances the activity is minimal, with only sparse lymphocytes within portal tracts and a few scattered lobular apoptotic bodies (Fig. 63.30a). Other cases with more substantial (mild, moderate or marked) activity show denser portal tract infiltrates of lymphocytes with scattered plasma cells and a few eosinophils, with extension of the infiltrates into the periportal regions, a process formerly called piecemeal necrosis, now termed *interface hepatitis* (Fig. 63.30b) [16, 30]. Close inspection of regions of interface hepatitis typically show lymphocytes and plasma cells closely apposed to or replacing the periportal hepatocytes, and apoptotic bodies are sometimes evident. If interface hepatitis is identified, such regions require further examination with connective tissue stains (trichrome and reticulin methods) in order to determine whether or not fibrosis has developed at these sites. Hepatocytes in periportal regions may become oncocytic, appearing highly eosinophilic and granular due to mitochondrial hyperplasia [37].

Periportal fibrous scarring associated with interface hepatitis often emerges in tandem with a ductular reaction (proliferation of bile ductular structures) derived from periportal progenitor cells, as has been demonstrated in chronic hepatitis C [13, 21]. This ductular reaction can be highlighted by immunostaining for cytokeratin 7 since the ductular structures bear the cytokeratin 7-positive biliary immunophenotype.

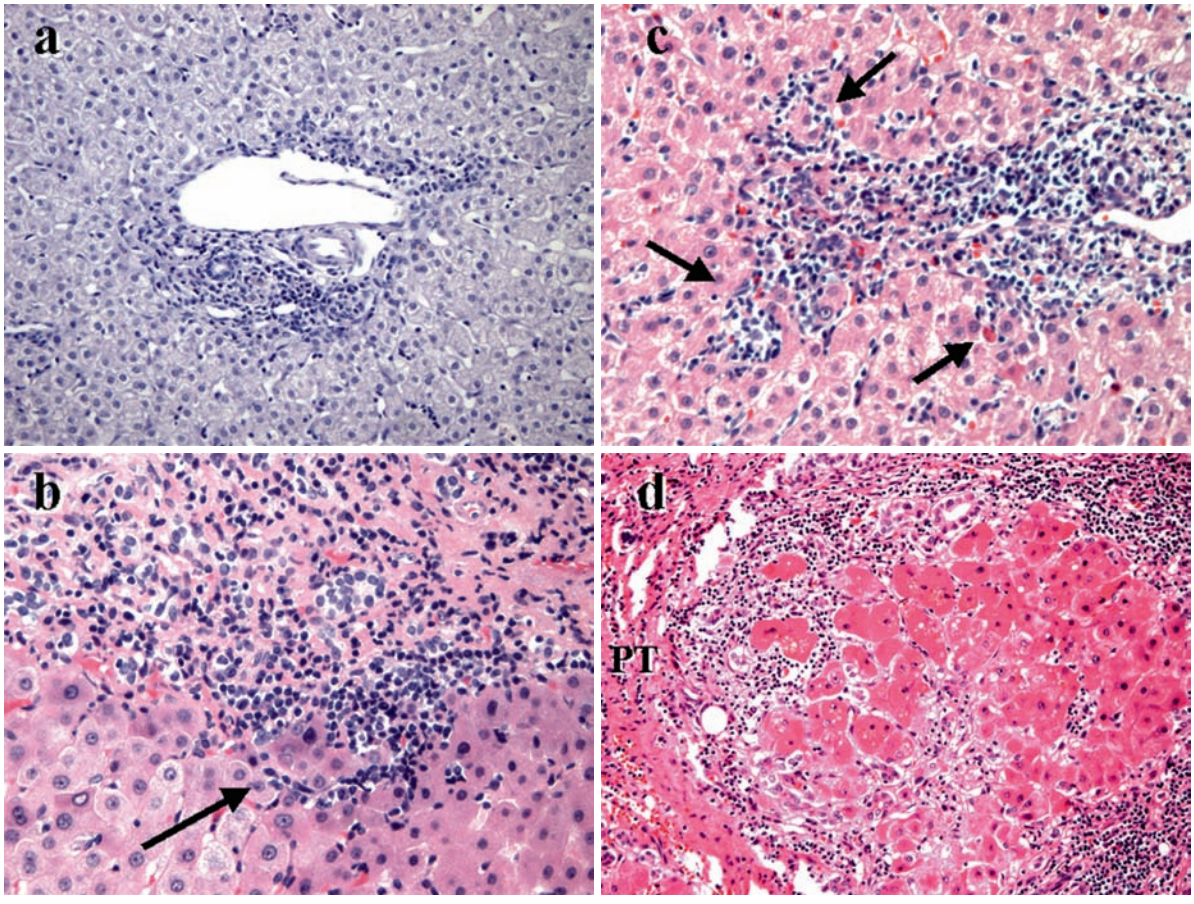


**Fig. 63.30** Grades of necroinflammatory activity in chronic hepatitis. (**a**) Minimal activity, with inflammation confined to the portal tract at center, with quiescent lobular parenchyma. (**b**) Mild activity, with a focus of interface hepatitis (arrow). (**c**) Moderate activity, with multiple foci of periportal interface hepatitis (arrows). (**d**) marked activity, with circumferential interface hepatitis at the edges of a cirrhotic nodule. There is also marked activity within the center of the parenchymal nodule. The majority of the hepatocytes in this nodule have undergone oncocytic change and are deeply eosinophilic. PT = portal tract. (All panels, hematoxylin and eosin stain)

Lymphoid structures, including lymphoid aggregates and lymphoid follicles with germinal centers may also form within portal tract connective tissue, frequently near bile ducts or surrounding them [40]. Lymphoid aggregates and follicles are seen most often in chronic hepatitis C, though they less frequently are present in chronic hepatitis B and autoimmune hepatitis.

The lobular activity in chronic viral hepatitis is usually much less prominent than in acute hepatitis. This activity is typified by scattered foci of necroinflammation with gaps in the liver-cell plates where intrasinusoidal clusters of lymphocytes, several Kupffer cells and a few apoptotic bodies or apoptotic fragments are localized. Variable hepatocyte ballooning is seen. In some cases the lobular unrest is disproportionately high in comparison to relatively sparse portal inflammation, a lesion originally called "chronic lobular hepatitis" [43]. Flares of lobular activity may accompany increases in serum aminotransferases in exacerbations of the immune response to the underlying chronic viral infection. Alternatively, excessive lobular necroinflammation may signify reactivation of previously quiescent known or occult chronic viral infection with resultant active viral replication (e.g., following chemotherapy or immunosuppression), or, in the case of chronic hepatitis B, clearance of serum HBeAg with the development of antibody to HBeAg (HBeAg-to-anti-HBe transition) or superimposed delta virus (HDV) infection. Bridging hepatic necrosis, multilobular necrosis or even massive hepatic necrosis may develop if the

chronic hepatitis is particularly severe.

Foci of hepatocellular atypia designated liver-cell dysplasia (LCD) may arise in the setting of chronic viral hepatitis. The most frequently found type of LCD was first described in chronic hepatitis B virus carriers and features enlarged hepatocytes with nuclear abnormalities, including hyperchromatism, prominent nucleoli and chromatin, multinucleation, multiple nucleoli and vesicular pseudo-inclusions (Fig. 63.31) [2]. Large cell dysplasia or large cell change, as this has been designated, is associated with a greatly increased risk of hepatocellular carcinoma, though its status as a direct "pre-neoplastic" lesion is controversial [36, 41]. A second type of LCD designated small cell dysplasia (with atypical nuclei in small hepatocytes) is considered by many to be a direct precursor lesion [59]. As is evident from the preceding discussion, a given liver biopsy specimen in chronic viral hepatitis requires evaluation of the many components of the necroinflammatory response, including the degree of portal, periportal and lobular inflammation as well as the type and severity of hepatocellular changes.

Architectural alterations in chronic hepatitis stem from the development of varied degrees of portal and periportal fibrosis combined with regenerative hyperplasia of periportal hepatocytes. Activation of hepatic stellate cells and portal myofibroblasts may cause an overall increase in portal connective tissue, irregular stellate periportal scars, bridging fibrous septa linking portal tracts, or diffuse fibrosis circumscribing cirrhotic regenerative nodules (Fig. 63.32) [22, 45]. The process of hepatic fibrogenesis is relatively slow, and potentially subject to dissolution through the activity of tissue matrix metalloproteinases, even with regression of cirrhosis in some cases [51, 54].

## Pathology of Specific Hepatitis Viruses

The features of acute and chronic viral hepatitis described earlier show broad similarities among the hepatitis viruses. However, certain morphologic features help distinguish among these agents.

### *Hepatitis A Virus (HAV)*

Since HAV results only in acute hepatitis, the uncomplicated case results in the generic changes of an acute hepatitis. However, two common patterns have been cited. The first is characterized by periportal interface

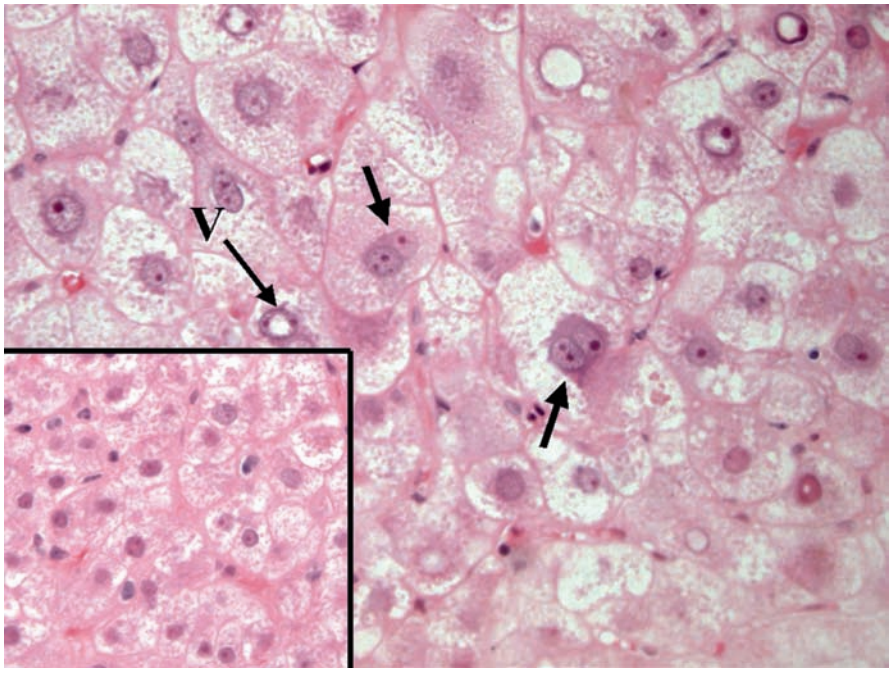


**Fig. 63.31** Liver-cell dysplasia, large cell type (large cell change). Compared to the non-dysplastic hepatocytes from the same case shown in the inset, large cell dysplasia shows enlarged hepatocytes with multiple nuclei and prominent nucleoli (arrows) and intranuclear vesicular inclusions (V). (Hematoxylin and eosin stain)

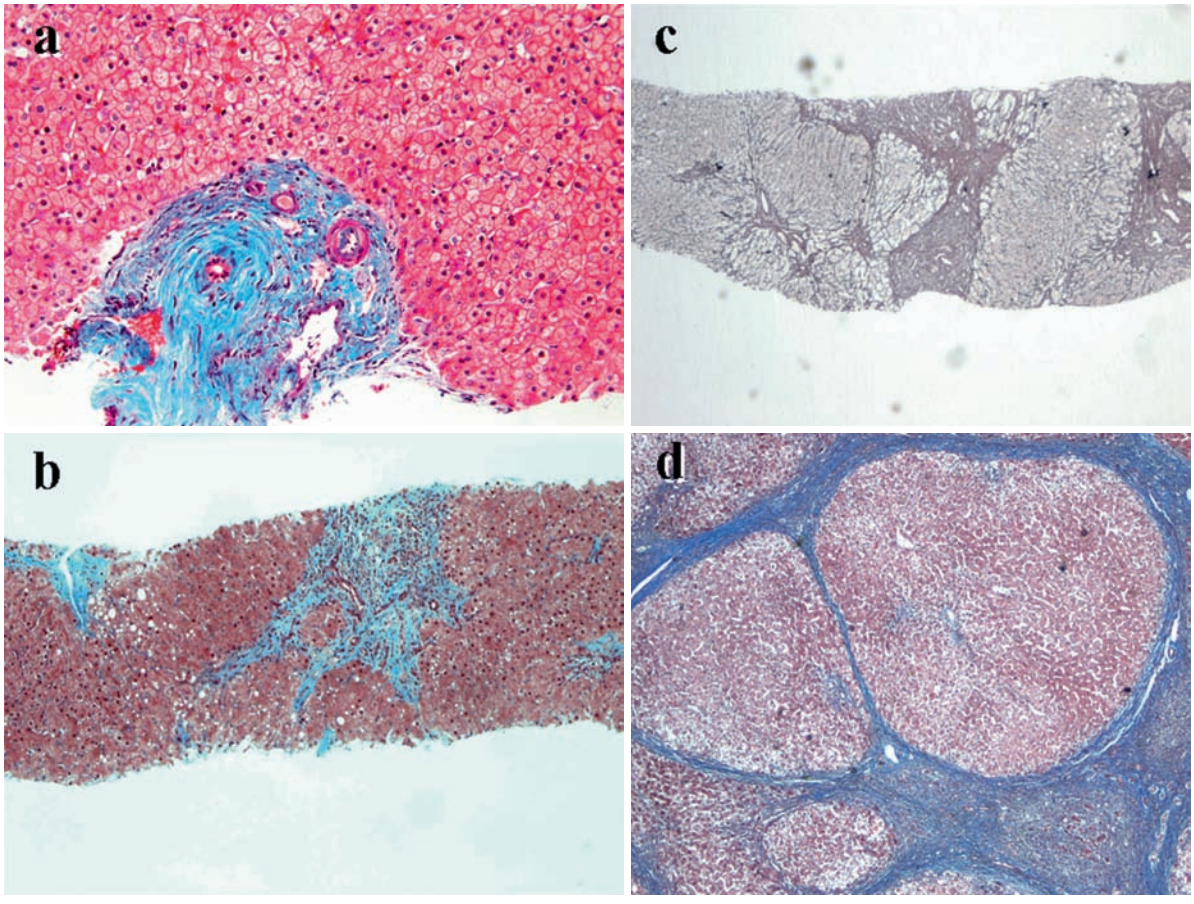


**Fig. 63.32** Stages of fibrosis in chronic hepatitis. (**a**) Stage 1 (minimal fibrosis) shows expansion of the portal connective tissue in a rounded mass. (**b**) Stage 2 (mild fibrosis) shows irregular stellate periportal fibrosis. (**c**) Stage 3 (moderate fibrosis with architectural distortion) shows extensive bridging fibrosis and early regenerative nodules, without a fully developed cirrhosis. (**d**) Stage 4 (cirrhosis) demonstrates regenerative nodules entirely circumscribed by fibrosis. (**a**, **b** and d: trichrome stain; **c**: reticulin stain)

hepatitis with prominent plasma cells, thereby resembling aspects of autoimmune hepatitis. The second is a cholestatic hepatitis with perivenular cholestasis and minor or no liver-cell necrosis (Fig. 63.33) [1, 55]. The two patterns may be combined. Fibrin-ring granulomas have also been reported in hepatitis A [60].

## *Hepatitis B Virus (HBV)*

Acute hepatitis B resembles other forms of viral hepatitis histopathologically. The rapidity of immune elimination of virus-infected cells in the acute disease typically results in negative immunostaining for either hepatitis surface or core antigens.

Chronic hepatitis B, on the other hand, has a number of distinctive features. The ground-glass cytoplasmic inclusions seen on hematoxylin and eosin stain within hepatocytes reflect surface antigen (HBsAg) protein synthesized in the endoplasmic reticulum (Fig. 63.34) [26]. These pale pink, homogeneous inclusions resembling "frosted glass" may occupy nearly the entire cell, or portions of the hepatocyte cytoplasm, and frequently appear separated from the cell membrane by an empty space. The distribution of such inclusions varies considerably; in some cases only focal inclusions can be identified, while in others numerous inclusions are found, sometimes in adjacent clusters of hepatocytes. The inclusions can be stained with either Shikata's orcein stain or with the Victoria blue method as well as by specific immunoperoxidase

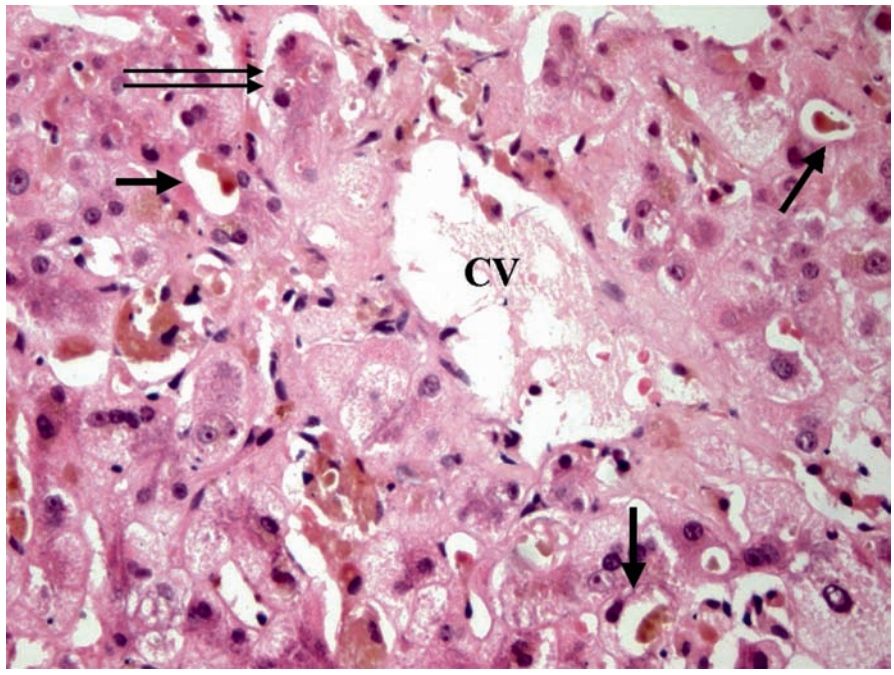


**Fig. 63.33** Acute hepatitis A. Cholestasis is prominent near the central vein (CV), with bile present in hepatocytes (double arrow), within bile canaliculi (single arrows) and within sinusoidal Kupffer cells, seen to the left of the vein. (Hematoxylin and eosin stain)

techniques (Fig. 63.34) [50]. With active viral replication core antigen (HBcAg) can be immunohistochemically identified within hepatocyte nuclei (in some cases also within the cytoplasm) and on H&E stain sometimes renders a pale blue homogeneous nuclear appearance described as "sanded nuclei" (Figs. 63.34d and e) [7]. Immunostain methods for early antigen (HBeAg) and for X antigen (HBXAg) are also available, the former, like HBcAg, consistent with viral replication, while the latter is associated with integration of HBV genome into the host genome [12, 58].

The routine histopathology of chronic hepatitis B spans the entire spectrum of possible changes seen in chronic hepatitis, from minimal portal tract and lobular inflammation to extensive portal and periportal interface and lobular hepatitis and evolution to cirrhosis. In children and younger individuals who acquired HBV infection by vertical transmission, inflammation and hepatocellular damage is often minimal or mild in the early years. Chronic HBV carriers with superimposed HDV infection show worsening of lobular and periportal changes (see Hepatitis D Virus, below).

In the setting of liver transplantation for HBV-related liver disease, recurrence of HBV infection in the allograft may show several morphologic patterns. In most cases, early recurrence is evident in the form of a lobular hepatitis with little portal inflammation. By 6 months or later the characteristic portal or portal and periportal inflammatory pattern of chronic hepatitis B becomes re-established. An unusual pattern of disease termed "fibrosing cholestatic hepatitis" develops due to immunosuppression in a minority of graft recipients with resultant graft failure, sometimes within several months of transplantation [15]. Serum HBV DNA titers are high and there is unchecked viral replication within the liver, as evidenced by extensive immunostain positivity for HBcAg. Severe cholestasis and hepatocyte ballooning, extensive ground-glass inclusions within hepatocytes, periportal fibrosis and a prominent ductular reaction (highlighted with immunostain for cytokeratin 7) are the chief morphologic changes. Similar changes may also occur in cardiac or renal graft recipients with underlying chronic hepatitis B. A related immunosuppression lesion, "steatoviral hepatitis", with cytopathic damage by marked HBV replication results in extensive liver-cell ballooning accompanied by large droplet steatosis [42].

## *Hepatitis C Virus (HCV)*

The pathologic features of chronic hepatitis C are well described, including the predilection for formation of

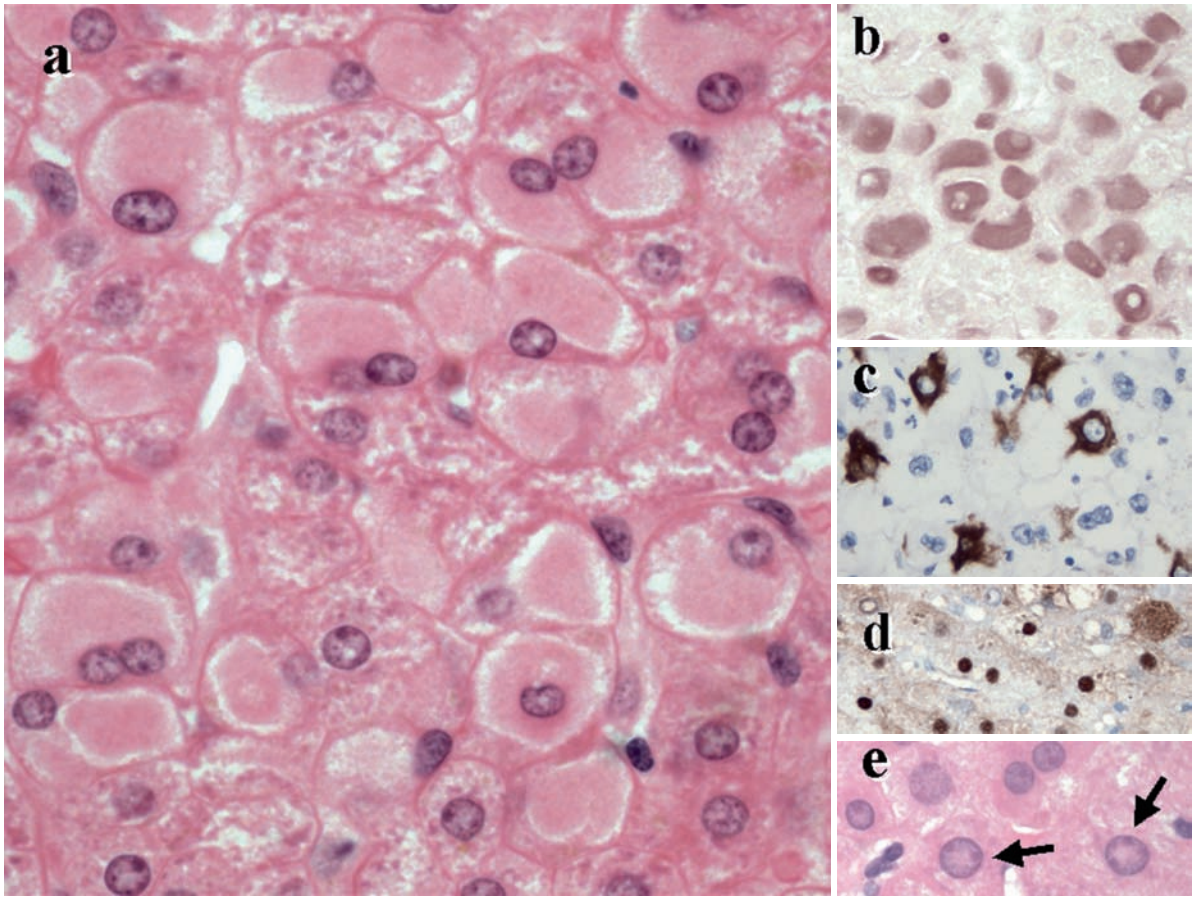


**Fig. 63.34** Chronic hepatitis B. **(a)** Numerous ground-glass cytoplasmic inclusions are present in hepatocytes, representing hepatitis B surface antigen. **(b)** Intracellular inclusions are positive on orcein stain. **(c)** immunostain for hepatitis B surface antigen demonstrates positive cytoplasmic inclusions. **(d)** immunostain for hepatitis B core antigen shows many positive hepatocyte nuclei and cytoplasmic positivity in a single cell at upper right. **(e)** "sanded nuclei" (arrows) with pale nuclear staining contain hepatitis B core particles. (**a** and **e**: hematoxylin and eosin stain; panel **b**: orcein stain; panels **c** and **d**: specific immunoperoxidase)

lymphoid structures (lymphoid aggregates and/or follicles) within portal tracts (Fig. 63.35) [3, 20, 39, 49]. These B-cell containing structures are readily recognized because of their density (in contrast to the more dispersed lymphoid cells in the remainder of the portal tract connective tissue) and by their proximity to interlobular bile ducts [40]. Large droplet steatosis in hepatocytes is also often present, either because of infection with genotype 3 (wherein the HCV core protein interferes with lipoprotein assembly and secretion) or because of other risks for fatty liver disease such as insulin resistance, obesity or ethanol use [8]. Damaged bile ducts may be present, although they are relatively infrequent in comparison to steatosis or lymphoid aggregate/follicle formation and, unlike primary biliary cirrhosis, are not usually associated with duct destruction. In many cases the necroinflammation is mild, with immune cells confined within portal tracts or with only mild foci of interface hepatitis. The lobular activity of chronic hepatitis C is typically in the form of scattered apoptotic bodies with random necroinflammatory foci (Fig. 63.35). As with chronic hepatitis B, the entire spectrum of chronic hepatitis, including fibrosis and cirrhosis may be seen with chronic hepatitis C. The lack of a uniformly reproducible commercial immunostain for HCV in paraffin-embedded samples has impaired diagnostic use of immunohistochemistry for chronic hepatitis C, although selective use of specially prepared monoclonal antibodies with and without frozen sections has been reported [24].

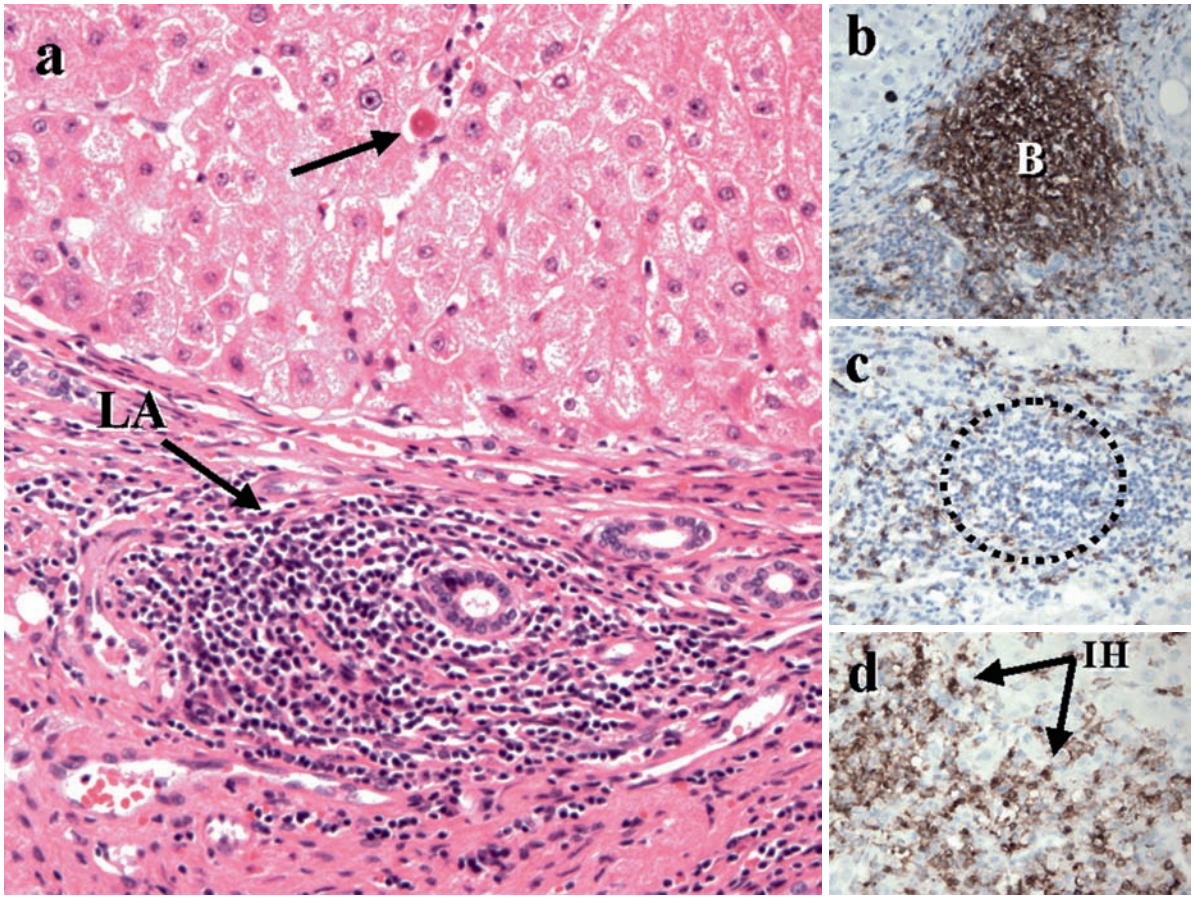


**Fig. 63.35** Chronic hepatitis C. (**a**) A characteristic lymphoid aggregate (LA) is seen within the portal tract, surrounding a bile duct. An apoptotic body and focal inflammation are seen in the parenchyma (arrow). (**b–d**) The specific lymphocyte subpopulations constituting lymphoid aggregates in chronic hepatitis C are shown with specific immunoperoxidase staining. (**b**) A dense core of B cells is highlighted with immunostain for CD20 (**c**); CD8-positive suppressor/cytotoxic T cells are highlighted in the area surrounding the B cell region (outside the dashed circle); (**d**) areas of interface hepatitis (IH) feature predominantly CD4 helper T cells. (**a**: hematoxylin and eosin; **b–d**: specific immunoperoxidase for CD 20, CD8 and CD4)

Recurrent disease following liver transplantation is characterized in early months by lobular activity and excessive numbers of apoptotic bodies, with later months bringing the characteristic portal tract inflammatory cell infiltrates [25, 47]. Lymphoid structures in portal tracts may form relatively early, even within the first few months. Distinguishing recurrent chronic hepatitis C from acute cellular rejection of the allograft is a common diagnostic dilemma, since bile duct damage is a shared feature of both processes. However, the presence of portal lymphoid aggregates and periportal interface hepatitis, lobular activity with apoptotic bodies, absent or only sparse bile duct damage and relatively homogeneous lymphoplasmacytic infiltrates (compared to the more pleomorphic, sometimes eosinophil-rich infiltrates of rejection) and absence of classical diffuse portal vein and/or central vein endotheliitis point to recurrent chronic hepatitis C [18]. A minority of cases of recurrence show pronounced cholestasis and hepatocellular ballooning with a pattern of periportal fibrosis and ductular reaction closely resembling fibrosing cholestatic hepatitis B (though without ground-glass inclusions) (Fig. 63.36) [17]. This pattern is associated with very high serum HCV RNA titers. This pattern has also been reported in HCV-positive recipients of cardiac and renal allografts.

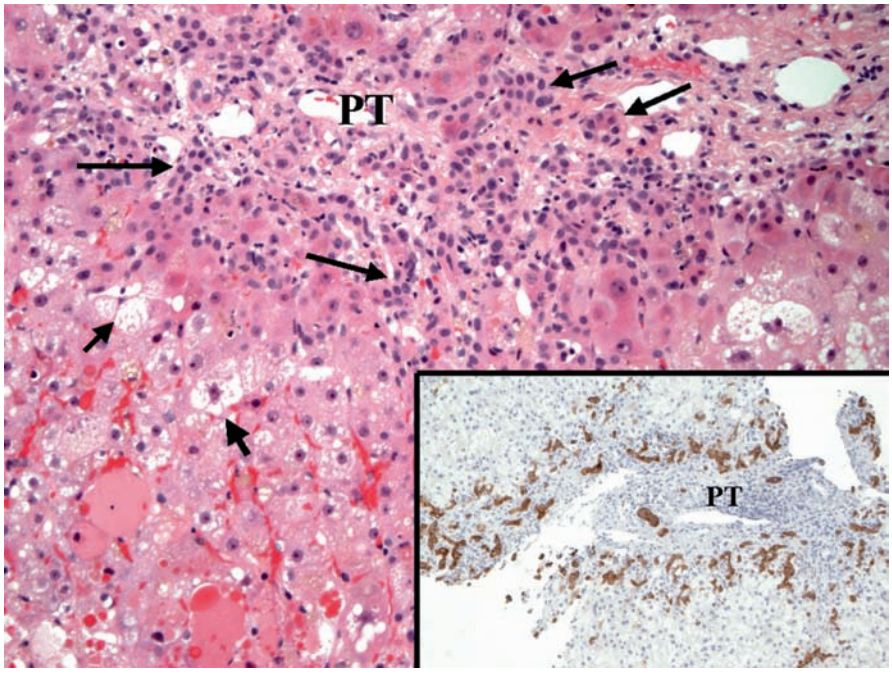


**Fig. 63.36** Fibrosing cholestatic hepatitis C after liver transplantation. This form of recurrent hepatitis C shows prominent proliferation of bile ductular structures at the edges of portal tracts (PT) with extensive hepatocyte ballooning (short arrows) and intracellular cholestasis. (Hematoxylin and eosin stain). Inset: Immunostain for cytokeratin 7 demonstrates the prominent ductular reaction in the periportal region surrounding the portal tract (PT). (Specific immunoperoxidase)

### *Hepatitis D Virus (HDV)*

Features of acute and chronic HDV infection encompass the generic spectrum of changes described earlier for acute and chronic viral hepatitis. Both interface hepatitis and lobular activity tend to be high in chronic hepatitis D. Microvesicular steatosis may be present in some cases [38, 44]. Immunostain for HDAg, like HBcAg, demonstrates nuclear positivity. Hepatocyte nuclei containing HDAg may show the "sanded" appearance seen with HBV intranuclear core antigen.

### *Hepatitis E Virus (HEV)*

The acute disease may demonstrate typical diffuse portal tract and lobular changes of acute viral hepatitis, or a cholestatic form with bile canalicular cholestasis and cholestatic liver-cell rosettes predominating, sometimes with neutrophils admixed in the inflammatory infiltrates [57]. This form is histologically similar to cholestatic acute hepatitis A.

## Grading and Staging of Chronic Hepatitis

The pathologic diagnosis of chronic hepatitis has traditionally relied on descriptive terms to convey the degree of inflammation and hepatocellular damage as well as the presence of fibrosis or cirrhosis in liver specimens. Diagnostic formulations such as "chronic hepatitis C with mild activity and mild periportal fibrosis" or "chronic hepatitis B with marked activity and developing cirrhosis" are examples. During the past 50 years since Menghini popularized the use of percutaneous needle biopsies in the evaluation of liver disease, classification systems for chronic hepatitis have evolved [10, 16]. The current practice of *semi-quantitative scoring* to provide a numerical grade of necroinflammation and stage of fibrosis (or cirrhosis) has certain advantages for the purpose of antiviral drug trials, assessment of therapeutic response and comparison between interval liver biopsies in a person with chronic viral hepatitis. This type of scoring assigns progressively increasing numbers to increasing degrees of severity, usually ranging from 0 to 4. The first semi-quantitative scoring system developed by Knodell and colleagues in 1981 provided a total *histological*

**Table 63.9** A simple system for scoring necroinflammatory activity in chronic hepatitis

| Grade | Portal/periportal activity | Lobular activity |
|---|---|---|
| 0 | None or minimal | None |
| 1 | Portal inflammation (CPH) | Inflammation but no necrosis |
| 2 | Mild piecemeal necrosis (mild CAH) | Focal necrosis or acidophil bodies |
| 3 | Moderate piecemeal necrosis (moderate CAH) | Severe focal cell damage |
| 4 | Severe piecemeal necrosis (severe CAH) | Damage includes bridging necrosis |

A score of 0 for portal activity and 2, 3 or 4 for lobular activity corresponds to the current category of chronic lobular hepatitis (CLH)
Source: Reproduced from [48]. With permission from Elsevier and the European Association for the Study of the Liver.
Piecemeal necrosis is now designated "interface hepatitis". *CPH* chronic persistent hepatitis; *CAH* chronic active hepatitis.

**Table 63.10** A scoring system for fibrosis and cirrhosis

| Grade | Fibrosis |
|---|---|
| 1 | None |
| 2 | Enlarged, fibrotic portal tracts |
| 3 | Periportal or portal-portal septa but intact architecture |
| 4 | Fibrosis with architectural distortion but no obvious cirrhosis |
| 5 | Probable or definite cirrhosis |

Alternatively, cirrhosis can be separately scored from fibrosis, into the following categories: probably absent; developing; suspected; present; cannot be assessed.
Source: Reproduced from [48]. With permission from Elsevier and the European Association for the Study of the Liver

*activity index (HAI)*, a score which incorporated both the degree of necroinflammation and the degree of fibrosis or cirrhosis in a given specimen [32]. Other systems have included the Scheuer method, Batts and Ludwig system, METAVIR systems, and a modified HAI developed by Ishak et al. (Tables 63.9 and 63.10 ; see also Chapter 29) [4, 5, 6, 29, 48]. Each system has its advocates, and individual centers have adopted one or another of these as best suits both clinical and research needs. One of the best graphic depictions of the principles of grading and staging is found in the publication by Batts and Ludwig (Figs 63.37 and 63.38) [4]. Histopathologic examples are shown in Figs. 63.30 and 63.32.

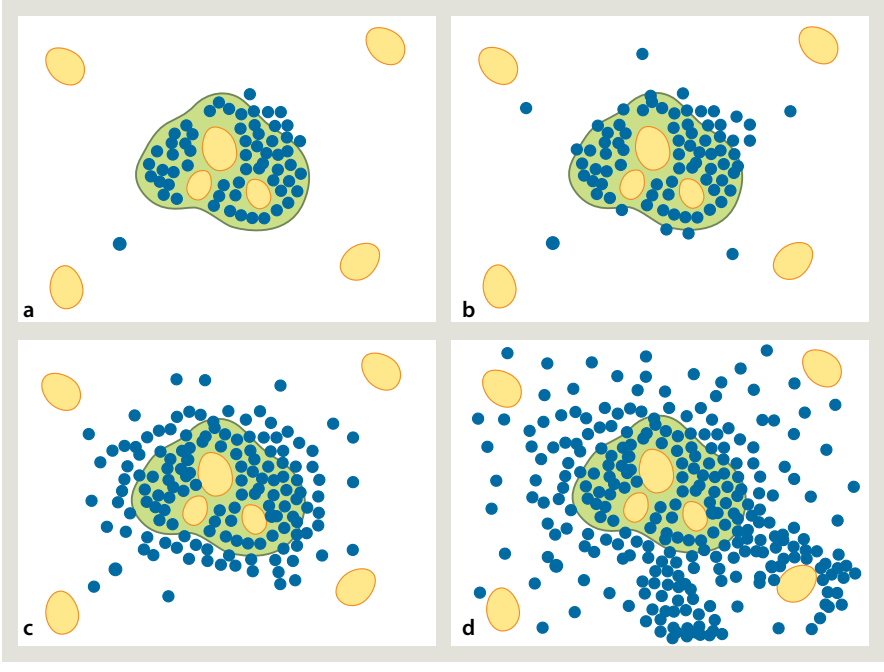


**Fig. 63.37** Batts and Ludwig grading of chronic hepatitis. Schematic diagram. (**a**) Minimal activity (grade 1) with mild portal inflammation but scant piecemeal necrosis and no lobular necrosis. (**b**) Mild activity (grade 2) with mild portal inflammation, piecemeal necrosis, and scant lobular spotty necrosis. (**c**) Moderate activity (grade 3) with moderate portal inflammation, piecemeal necrosis, and lobular spotty necrosis. (**d**) Severe activity (grade 4) with marked portal inflammation, brisk piecemeal necrosis, considerable spotty necrosis, and areas of confluent necrosis resulting in bridging. When discrepancies exist between piecemeal and lobular necrosis, the more severe lesion should determine the grade. Reproduced from [4]. With permission of the American Journal of Surgical Pathology)

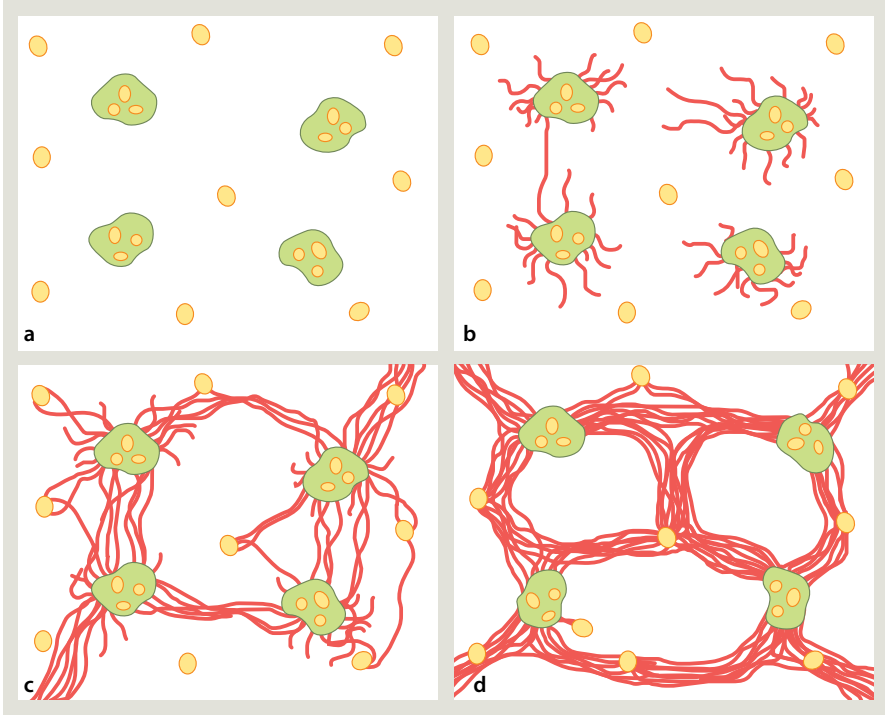


**Fig. 63.38** Batts and Ludwig staging of chronic hepatitis. Schematic diagram. (**a**) Portal fibrosis (stage 1) characterized by mild fibrous expansion of portal tracts. (**b**) Periportal fibrosis (stage 2) showing fine strands of connective tissue in zone 1 with only rare portal-portal septa. (**c**) Septal fibrosis (stage 3) manifested by connective tissue bridges that link portal tracts with other portal tracts and central veins, minimally distorted architecture, but no regenerative nodules. (**d**) Cirrhosis (stage 4) showing bridging fibrosis and nodular regeneration. Reproduced from [4]. With permission of the American Journal of Surgical Pathology)

Several caveats should be borne in mind in considering semi-quantitative grading and staging in chronic viral hepatitis. First, the liver sample must be an adequate size and representative of changes presumed to be present throughout the liver [50]. For percutaneous needle biopsies this is now widely accepted as a 2.0 cm length biopsy core which includes a minimum of 11 portal tracts [14]. Second, at a given institution, the system adopted should be used by a single pathologist or several pathologists who are thoroughly versed in the scoring methods (so as to minimize the incidence of inter- and intra-observer variations). Clinicians at individual centers must also understand the information conveyed in the specific system. Third, the scores generated by grading and staging are *categorical data* rather than absolute measurements (such as enzyme activities obtained in serum tests for alanine aminotransferase or alkaline phosphatase) and should be evaluated with statistical methods appropriate for this type of data [33].

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## 63.3 Hepatitis A to E. Epidemiology, Clinical Manifestations, Prevention and Therapy

The spectrum of viral hepatitis ranges from an unapparent infection to fulminant hepatic failure, from a subclinically persistent infection to chronic progressive liver disease ultimately resulting in liver cirrhosis and hepatocellular carcinoma.

The diagnosis is based on a detailed history and on the interpretation of laboratory parameters, such as liver enzymes, serological and molecular biological parameters. Additionally, a history regarding occupational and drug history, foreign travel, blood transfusions, operations, tattoos, dental treatments, intravenous drug use and sexual practices should be obtained. The diagnosis is established by determining viral antigen and antibody profiles in serum. In the acute phase of the disease molecular biological techniques are of major importance (see Section 63.1).

Histological examination of the liver is no longer necessary to diagnose acute viral hepatitis. Ultrasound, CT scanning and MRI are also not required to confirm the diagnosis of acute hepatitis. Their primary importance lies in the follow up of chronic liver disease, the detection of cirrhosis and signs of portal hypertension, and especially in the early detection of focal liver lesions, such as regenerative, dysplastic or neoplastic nodules.

If elevated aminotransferase levels fail to normalize and liver injury persists 6 months after the onset of acute hepatitis, a chronic hepatitis is evolving. In the follow up of patients with chronic hepatitis liver biopsy still is firmly established as the only method that allows for grading and staging of the disease (see Chapter 29 and Section 63.2).

Chronic hepatitis is an etiologically multifaceted disease. Viral etiologies (HBV, HDV, HCV) must be distinguished from autoimmune causes (characterized by the absence of viral markers and the presence of autoantibodies in serum; see Chapter 72). Rarely, drug-induced liver injury may result in chronic hepatitis (see Chapter 93).

Despite the development of sensitive assays for the detection of hepatitis viruses, the etiology of acute hepatitis remains obscure in up to 20% of cases. Among the non-(A-E) viruses suggested as causative agents of sporadic acute hepatitis were hepatitis GB virus C (GBV-C; initially called hepatitis G virus), TT virus (TTV), and SEN virus (a novel DNA virus distantly related to TTV).

Recent evidence, however, suggests that GBV-C, TTV and SENV are not responsible for the majority of sporadic acute non-(A-E) hepatitis cases. Thus the existence of hitherto still unknown hepatitis agents must be postulated as agents of sporadic acute hepatitis [15].

## Hepatitis A

### *Epidemiology*

Hepatitis A occurs globally, and is among the most frequent infections and the commonest causes of acute hepatitis worldwide (Fig. 63.39). The disease is endemic, with seroprevalance rates approaching nearly 100% in areas with relatively low socioeconomic standards, such as Africa, the Southeast Mediterranean, the Middle East, Bulgaria, Romania, the former states of the Soviet Union, India and large parts of South East Asia and South America.

In many Western countries the acute disease and death due to hepatitis A must be reported to the health authorities. While in the past approximately 5,000–6,000 new cases per year were reported in Germany, a marked decrease of new infections has been observed in the last years. Some 12–20 patients die from hepatitis A in Germany yearly. While in the developing countries exposure to hepatitis A virus (HAV) and infection followed by immunity occurs already in childhood, in economically developed countries improved hygienic and sanitation standards led to a rapid decline of endemic infection (endemicity 2–5%) and to a change in the epidemiological profile. Hepatitis A increasingly affects older adults, in whom it often follows a more severe clinical course than in infants. The low population immunity creates the potential for epidemics resulting from food- and water-borne transmission.

In the United States, the incidence rate of hepatitis A has dramatically declined to historically low levels during the past several years. With 7,700 reported cases, the 2003 rate was the lowest recorded in 40 years of surveillance. Adjusting for underreporting and asymptomatic infections, the estimated number of new infections was 61,000 and the 2004 rate of 1.9 reported cases per 100,000 represented a further decrease (Fig. 63.40) [3, 18].

HAV is relatively stable and remains infectious on environmental surfaces for at least 1 month. It survives

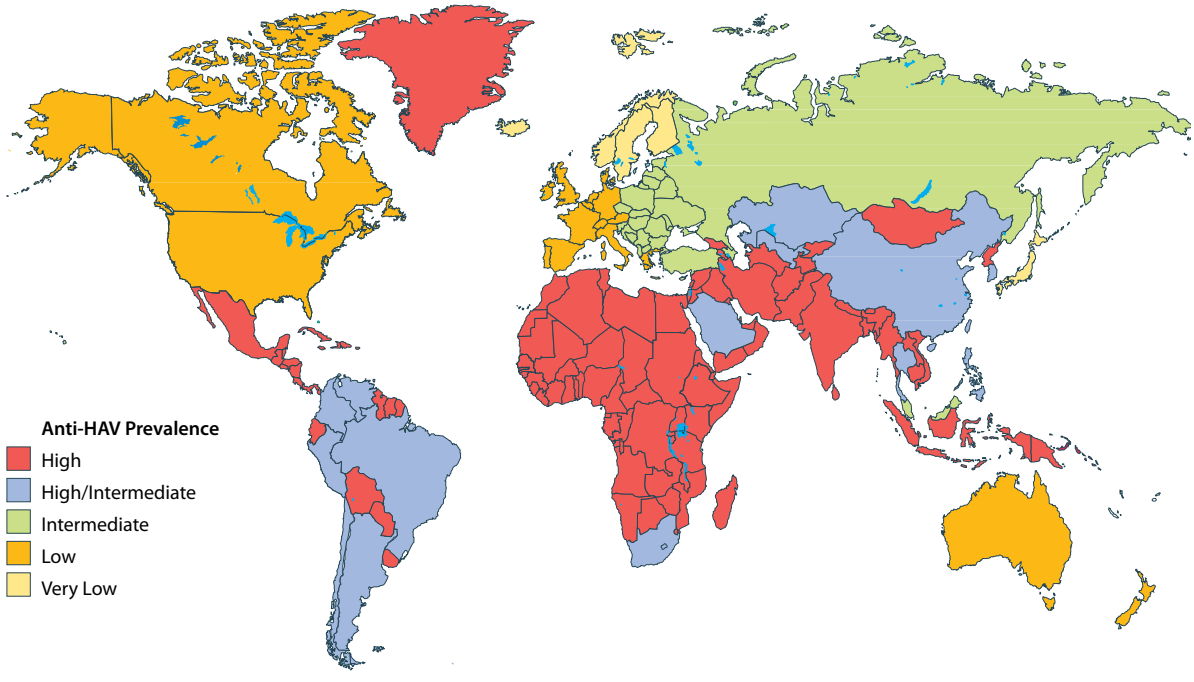


**Fig. 63.39** Geographic distribution of hepatitis A virus infection

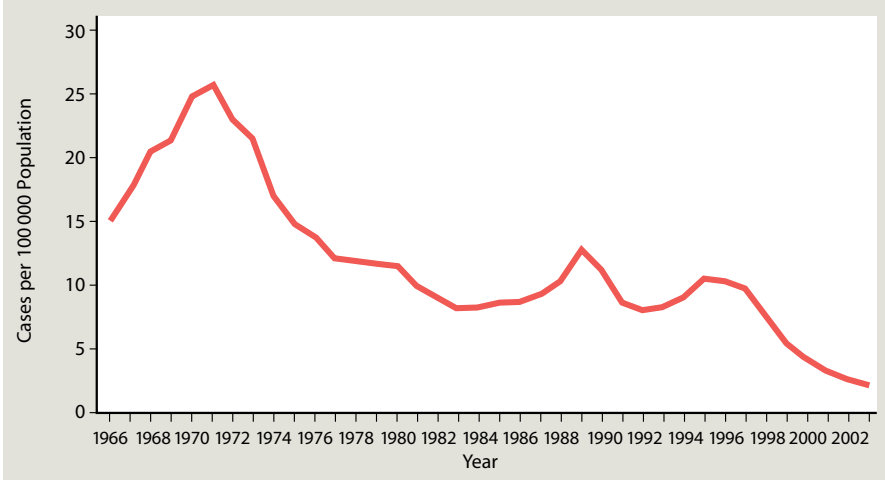


**Fig. 63.40** Hepatitis A incidence by year (1996–2003) in the US (According to [18])

for extended periods in seawater, fresh water, wastewater, and soil. The virus is resistant to freezing, detergents, and acids, but it is inactivated by formalin and chlorine and by temperatures higher than 85°C (185°F). Household bleach is sufficient to inactivate HAV.

*Transmission* of HAV is by the fecal–oral route, either by direct contact with a person who is infected with HAV or by ingestion of food or water that has been contaminated with the virus. Contaminated food is identified as the source of transmission for less than 5% of cases reported in the United States [6]. However, foods imported from communities where transmission of HAV remains common can be sources of exposure and outbreaks of hepatitis A as a result of contamination caused during harvesting or processing [8, 19]. Transmission typically occurs when food is contaminated by an infected food-service worker at the point of sale or service.

The main epidemiologic sources of HAV infection in the USA are depicted in Fig. 63.41. The most common causes of HAV infection are personal contact with infected persons and transmission in day care centers. Children and infants can shed HAV for longer periods than adults. Because even asymptomatic infected children may shed HAV in their stools for up to 6 months, infection in children often initiates and perpetuates community-wide outbreaks. Chronic shedding of HAV in feces does not occur.

Consumption of shellfish harvested from contaminated waters is a significant source of infection outside the United States. The acquisition of hepatitis A through travel to endemic regions is epidemiologically important because of its impact on perpetuating infection in countries with a low incidence of HAV infection, such as Western Europe and the United States.

In the United States and Central Europe there is no occupational risk for health care, child care, or food service workers to acquire HAV infection.

The *incubation period* of viral hepatitis A is 15–50 days, with an average of 30 days. The virus replicates only in hepatocytes and gastrointestinal epithelial cells. During the late incubation period and the preicteric phase of the disease, viral particles are released into blood and bile. HAV may be demonstrated in the stool 1–2 weeks prior to the clinical onset of hepatitis. *Peak infectivity correlates with the greatest viral excretion in the stool during the two weeks before the onset*

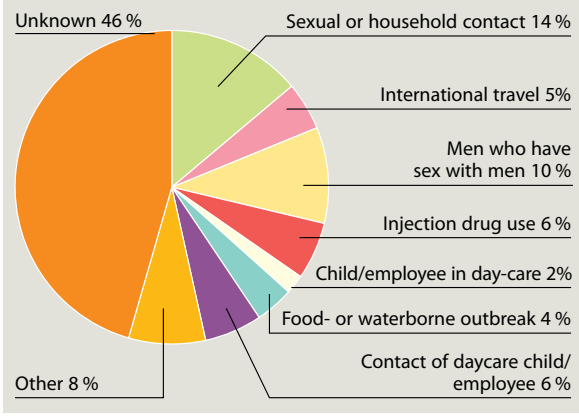


**Fig. 63.41** Risk factors associated with reported hepatitis A in the United States (1990–2000)

*of jaundice or elevation of aminotransferases*. With the appearance of jaundice, viremia, viral excretion in stool, and infectivity decrease, despite the persistence of HAV in the liver. Healthy adult patients are non-contagious by 2–3 weeks after the onset of illness, but children and immunocompromised persons may remain contagious for up to 6 months [3].

Due to viremia, which occurs soon after infection and persists throughout the period of liver enzyme elevation, parenteral transmission of HAV through blood and blood products is possible. However, in clinical practice the transmission of HAV by transfusion of blood products collected during the viremic phase is an exceedingly rare event.

## Pathogenesis

Symptomatic hepatitis A is the clinical expression of the destruction of liver cells by the host's cell-mediated immune response. HAV-specific $CD8^+$ T lymphocytes and natural killer cells mediate liver cell death and may induce apoptosis. Direct cytopathic effects of HAV play a minor role. Circulating anti-HAV antibodies probably inhibit reinfection of hepatocytes and, together with T cell dependent lysis of infected hepatocytes, ultimately lead to elimination of HAV infection. However, the precise pathophysiologic mechanisms operating in HAV infection are still largely unknown.

## Clinical Presentation

### Symptoms and Signs

The presence and severity of symptoms in patients with HAV infection is related to the patient's age. Fifty to 90% of children younger than 6 years of age with acute hepatitis A remain asymptomatic, whereas 70% of infected adults develop symptoms. More than 80% of adults with hepatitis A are ill for up to 8 weeks. In adult patients, approximately 1 week prior to the onset of jaundice (*preicteric phase*), the disease begins with non-specific constitutional and gastrointestinal symptoms, such as fever (38–39°C), malaise, headaches, arthralgias, myalgias, anorexia, nausea, vomiting, and disturbances of taste and smell sensation. Smokers sense a passing aversion to nicotine. In children with symptomatic HAV infection flu-like symptoms with pharyngitis, cough, coryza and photophobia may appear (Table 63.11). The *icteric phase*, which lasts for 4–30 days, is heralded by bilirubinuria with darkening of the urine followed within a few days by acholic, pale, clay-colored stools and then by the appearance of scleral icterus and skin jaundice. Upon the development of jaundice the prodromal symptoms usually subside. Fatigue, anorexia and slight nausea, however, may persist causing a mild weight loss of 2.5–5 kg. The patient complains of a dull sense of pressure in the right upper quadrant caused by distention of the liver capsule. He or she senses his or her liver ("Organgefühl"). In a substantial number of cases hepatitis A remains anicteric.

On physical examination there is moderate hepatomegaly and tenderness over the liver with palpation. A mild splenomegaly is present. Ten to 20% of patients with acute hepatitis A have posterior cervical lymphadenopathy. Occasionally spider nevi may occur, but disappear during convalescence. In patients with

**Table 63.11** Symptoms and signs in acute viral hepatitis

| | |
|---|---|
| **Constitutional symptoms** | • Fever 38–39°C<br>• Fatigue, adynamia<br>• Headache, arthralgias<br>• Pruritus |
| **Gastrointestinal symptoms** | • Decreased appetite<br>• Nausea and vomiting<br>• Mild weight loss (2.5–5 kg)<br>• Disturbances of taste and olfactory sense<br>• Right upper quadrant pain |
| **Flu-like symptoms** | • Pharyngitis<br>• Coryza<br>• Cough<br>• Photosensitivity |
| **Jaundice** | • Scleral icterus and skin jaundice<br>– Darkening of urine<br>– Discoloration of stool |
| **Signs** | • Painful hepatomegaly (stretching of liver capsule)<br>• Mild splenomegaly<br>• Cervical lymphadenopathy (10–20%)<br>• Spider nevi (rare) |

Source: Adapted from [3]

**Table 63.12** Extrahepatic manifestations of hepatitis A virus infection

| | |
|---|---|
| **Gastrointestinal findings** | • Acalculous cholecystitis<br>• Acute pancreatitis |
| **Hematologic findings** | • Aplastic anemia<br>• Autoimmune hemolysis<br>• Autoimmune thrombocytopenic purpura<br>• Hemolysis (in patients with glucose-6-phosphate dehydrogenase deficiency)<br>• Red cell aplasia |
| **Neurologic findings** | • Guillain-Barré syndrome<br>• Mononeuritis<br>• Mononeuritis multiplex<br>• Postviral encephalitis<br>• Transverse myelitis |
| **Renal findings** | • Acute tubular necrosis<br>• Interstitial nephritis<br>• Mesangial proliferative glomerulonephritis<br>• Nephrotic syndrome |
| **Other findings** | • Cutaneous leukocytoclastic vasculitis<br>• Cryoglobulinemia<br>• Reactive arthritis |

elevated temperature a relative bradycardia may be observed. In some patients a mild and transient steatorrhea may occur. *Extrahepatic manifestations* are rare and are mainly observed in cholestatic or relapsing hepatitis A (Table 63.12) [2].

Acute HAV infection during pregnancy is associated with a high risk of maternal complications and preterm labor [5].

### Laboratory Findings

In the acute phase, serum ALT and AST levels are markedly elevated (ALT > AST; 500 to > 5,000 U/L) and return gradually to normal over several weeks. The degree of aminotransferase elevation does not correlate with the extent of liver injury. In jaundiced patients serum bilirubin levels rise from 5 to 20 mg/dL and remain elevated despite falling aminotransferase levels. Approximately 50% of bilirubin is conjugated, the remainder is unconjugated. Provided a hemolytic anemia has been excluded, sustained elevations of serum bilirubin to values greater than 20 mg/dL are associated with a more severe clinical course. Serum alkaline phosphatase levels are normal or only slightly elevated. As a rule, serum iron concentrations are elevated during the acute phase of the disease, thought to mirror hepatocyte injury with release of intracellular iron. Despite the acute hepatic inflammation, inflammatory markers such as CRP and ESR are typically normal, since the synthesis of acute phase proteins is downregulated in the acutely inflamed liver (Table 63.13).

A transient neutropenia and lymphocytopenia are often observed during the acute phase of hepatitis A, followed by a relative lymphocytosis. Altered coagulation parameters, especially a prolonged prothrombin time, hint toward a serious prognosis, and are due to a reduced synthetic capacity of the liver because of extensive hepatocellular necrosis. During the acute phase of the disease IgG and IgM are moderately elevated in approximately 30% of patients. In some patients a microhematuria and a mild proteinuria are observed.

As in other acute viral infections, smooth muscle antibodies, antibodies against cell nuclei or other cell components, as well as low titre elevations of rheumatoid factor may occur. These changes are transient and non-specific and must not be mistaken for autoimmune hepatitis.

## *Diagnosis*

The diagnosis of viral hepatitis A is suspected on clinical grounds and confirmed by serologic methods (see Section 63.1). The symptoms described above in a person having close personal contact with an infected person, in men who have sex with men, in individuals who have travelled to endemic areas, in a person with contact to a child or employee in a child care center, or in persons involved in a known food-borne outbreak should arouse the suspicion of hepatitis A.

The presumptive diagnosis is corroborated by high serum aminotransferase levels (up to several thousand U/L), with ALT typically higher than AST, and confirmed by demonstrating IgM directed against HAV. A positive IgM test in a person without symptoms of acute viral hepatitis and with only slightly elevated serum aminotransferases (<–100 U/L), however, is likely to be false positive. Serum anti-

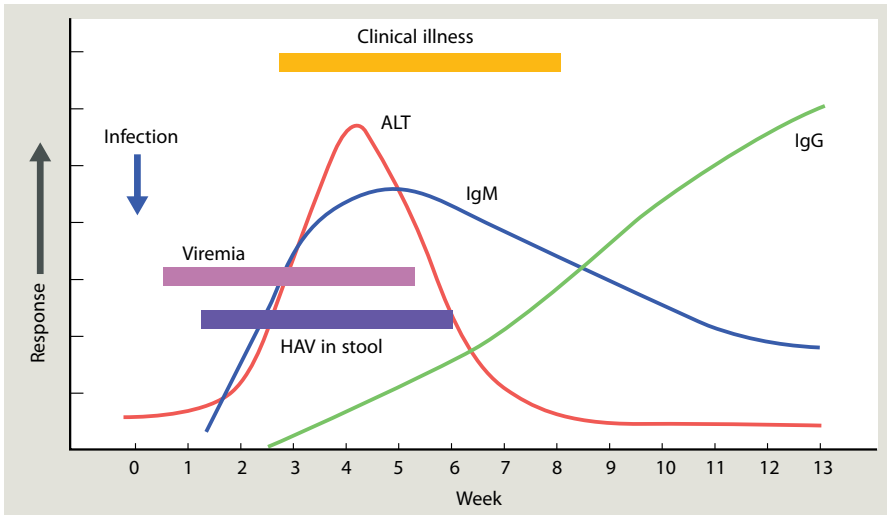


**Fig. 63.42** Timeline of hepatitis A manifestations (according to [3]). ALT Alanine aminotransferase, Ig Immunoglobulin

**Table 63.13** Laboratory findings in patients with acute viral hepatitis A and B

| Parameter | Change | Comments |
|---|---|---|
| **AST, ALT** | ↑↑↑ (400–4,000 U/L) | Injury and death of liver cells |
| | ALT > AST | No strict correlation between ALT and AST levels and extent of hepatocyte injury or death |
| **Bilirubin** | ↑↑ (5–20 mg/dL) | Prognostic parameter, if elevation long-standing |
| **Alkaline Phosphatase** | ↔ – (↑) | Markedly elevated levels in cholestatic variant of acute hepatitis |
| **Prothrombin Time** | Prolongation | Prognostic parameter |
| **Serum Albumin** | ↔ – ↓ | Prognostic parameter |
| | | Decreased only in complicated cases |
| **IgG and IgM** | ↑ | In 30% of patients in the acute phase |
| **Autoantibodies** | Low titer elevation of SMA, ANA, and rheumatoid factor | Nonspecific |
| **ESR and CRP** | ↔ | Typically normal despite necroinflammatory reaction. Hepatic synthesis of acute phase proteins, such as CRP, is downregulated |
| **Serum iron** | ↑ | Expression of cell death |
| **Neutropenia, atypical lymphocytes** | Lymphocytopenia followed by relative lymphocytosis | Differential diagnosis: infectious mononucleosis |
| **Steatorrhea** | Mild and transient | Rare |
| **Microhematuria** | | Rare |
| **Proteinnuria** | Minimal | Sporadic |

HAV IgM usually may be detected 5–10 days before the onset of symptoms, and its titre remains elevated for 4–6 months. Soon after the rise of anti-HAV IgM, anti-HAV IgG begins to rise and is present throughout the person's lifetime, conferring immunity (Fig. 63.42).

HAV RNA can be detected in blood and stool during the acute phase of infection by PCR. However, in clinical practice this method is not required to diagnose hepatitis A.

## *Differential Diagnosis*

Hepatitis caused by other hepatotropic and non hepatotropic viruses, toxoplasmosis, cholestatic and drug induced or alcoholic liver diseases may resemble hepatitis A both clinically and chemically. Immune serologic diagnosis allows for rapid differentiation of HAV infection from these entities.

If right upper quadrant pain and gastrointestinal symptoms predominate, the differential diagnosis must

include acute cholecystitis, choledocholithiasis with obstructive jaundice, and ascending cholangitis. Rarely hepatic metastases or metabolic diseases, such as Wilson's disease or $\alpha_1$-antitrypsin deficiency, may cause diagnostic confusion. Ischemic hepatitis, as can be seen in severe heart and pulmonary failure, may mimic acute hepatitis. However, the different clinical scenarios and the rapid fall of markedly increased aminotransferase levels within a few days favors a diagnosis of ischemic hepatitis (Table 63.14).

**Table 63.14** Differential diagnosis of acute viral hepatitis A and B

| | |
|---|---|
| **Hepatitis caused by hepatotropic viruses** | • HAV<br>• HEV<br>• HBV<br>• HDV<br>• HCV<br>• TTV<br>• GBV (?) |
| **Hepatitis caused by non hepatotropic viruses** | • EBV<br>• CMV<br>• HSV<br>• Coxsackie-Virus |
| **Autoimmune diseases** | • Autoimmune hepatitis<br>• Systemic lupus erythematosus |
| **Protozoal infections** | • e.g. Toxoplasmosis |
| **Bacterial infections** | • Leptospirosis<br>• Rocky Mountain Spotted Fever<br>• Sepsis |
| **Reactions to drugs** | • Acetaminophen<br>• Antiseizure medications<br>• Isoniazid<br>• Oral contraceptives<br>• Rifampin<br>• Sulfonamides |
| **Cholestatic liver disease** | • Drug or toxin induced hepatopathy<br>• Alcoholic liver disease<br>• Choledocholithiasis<br>• Acute cholecystitis<br>• Ascending cholangitis |
| **Metabolic diseases** | • Hemochromatosis<br>• Wilson's disease<br>• $\alpha_1$-antitrypsin deficiency |
| **Cardiovascular diseases** | • Heart failure<br>• Severe hypotonia, shock (ischemic hepatitis) |

## *Natural Course and Prognosis*

In the vast majority of patients hepatitis A is an acute self limited disease that resolves completely within 1–2 months without sequelae. *An asymptomatic HAV carrier state and chronic HAV-induced liver disease do not occur*. However, a prolonged or relapsing course lasting several months may occur in 10–20% of symptomatic, mainly older, patients with comorbidities. Furthermore, HAV superinfection is associated with a high risk of liver failure and death in patients with underlying chronic liver disease, such as chronic hepatitis B and C [12].

### Cholestatic Hepatitis

This uncommon variant of hepatitis A is characterized by prolonged jaundice and pruritus persisting for several months. Serum bilirubin concentrations may exceed 20 mg/dL, whereas serum aminotransferase levels may decline toward normal despite the presence of cholestasis. Somewhat surprisingly, serum alkaline phosphatase concentrations are increased in only some patients with cholestatic hepatitis [9]. A short course of low dose corticosteroid treatment, e.g. prednisolone 30 mg qd, tapering over 3 weeks, may shorten the duration of jaundice and pruritus, but this approach is debatable in view of the excellent prognosis for spontaneous, complete resolution. In rare cases acute but transient oliguric renal failure may complicate cholestatic hepatitis.

### Relapsing Hepatitis

In 4–20% of patients, weeks to months after apparent resolution of hepatitis A, a symptomatic relapse and liver-test abnormalities may recur. This atypical course with multiple relapses seems to be especially common in children. The relapses are clinically mild and despite the protracted course, the prognosis for complete recovery is excellent. In a small number of affected patients extrahepatic manifestations, such as arthritis, vasculitis and cryoglobulinemia may be seen.

### Fulminant Hepatitis and Acute Liver Failure

Acute liver failure due to HAV infection is an uncommon but potentially lethal illness [14]. Overall, fewer than 1% of patients with acute hepatitis A experience a

fulminant course characterized by worsening jaundice, decreasing hepatic synthetic capacity and development of encephalopathy. Fulminant courses with high case-fatality rates primarily occur in intravenous drug users with underlying liver damage, and in older patients with comorbid conditions [13]. Fulminant hepatitis A has been observed during pregnancy or in association with HAV superinfection in patients with chronic hepatitis B and C [16]. The overall fatality rate is 0.3% but increases to 2.5% in older persons, and to 6.4% in intravenous drug-users, most of them HCV-coinfected alcohol users and some HCV/HIV coinfected [13]. More than 70% of persons dying from hepatitis A are older than 50 years, although this group makes up for only approximately 8% of all HAV infected persons. Clinical manifestations and laboratory alterations correspond to acute liver failure and most patients will require emergency liver transplant. Features that favor listing for liver transplantation include jaundice lasting more than 7 days prior to the onset of encephalopathy, serum bilirubin concentration > 17 mg/dL, serum ALT < 2,600 IU/L, prothrombin time > 25 s, creatinine > 2.0 mg/dL, and the need for intubation and administration of pressors [14].

## Prevention

HAV infected patients are contagious from 1 to 2 weeks before the onset of jaundice up until 1 week after. Infection with HAV is best avoided by adhering to sanitation and hygienic measures, such as thorough handwashing after defecation, heating foods appropriately and avoiding water and foods from endemic areas. Vaccination against hepatitis A and B is the most effective means of preventing sexual transmission of hepatitis A and B. Disease reporting is required of physicians, laboratories, and school officials and should trigger investigation to determine the source of infection, identify exposed persons, and provide postexposure prophylaxis without delay.

### Passive Immunization

Standard immune globulin obtained from pooled human plasma of more than 1,000 donors contains HAV antibody levels (approximately 100 IU/L) sufficient to provide short-term protection against infection with HAV. Pregnancy and lactation are not contraindications for the administration of immune globulin. In persons with selective IgA deficiency, anaphylaxis may occur after repeated intramuscular applications of immune globulin. Passive immunization should be avoided within 2–3 weeks after administration of live, attenuated vaccines because it decreases their immunogenicity. On the other hand, vaccines based on live attenuated viruses (e.g. measles, mumps, rubella, varicella) should be delayed until 3–5 months after intramuscular immune globulin injection.

**Preexposure Prophylaxis**. Preexposure prophylaxis is recommended for travelers to countries with intermediate (10–19 diseases/100,000 inhabitants: Eastern Europe, Greece, Turkey, countries of the former Soviet Union) and high prevalence of hepatitis A (≥ 20 diseases/100,000 inhabitants: Mexico, Central and South America, Greenland, Africa, Middle East, Asia [except Japan]).

*Immune globulin*, 0.06 mL/kg (corresponding to approximately 5 mL in adults and 2 mL in children up to 20 kg body weight), is administered intramuscularly before the start of travel. When using *hyperimmune hepatitis A immune globulin,* 2 mL for adults and 1 mL for children up to 20 kg body weight will suffice. The peak anti-HAV titer achieved by passive immunization is 150 IU/L. Preexposure prophylaxis is effective in 80–85% of cases and the *protection provided by immune globulin 0.06 mL/kg lasts for 3–5 months* [7]. Immune globulin should be administered by intramuscular injection into either the deltoid or gluteal muscle. Prophylaxis is recommended for persons traveling to any part of the world except Canada, Western Europe, Japan, New Zealand and Australia. If the departure is scheduled within 4 weeks, preexposure passive immunization and vaccination should be administered concomitantly.

If the sojourn in an endemic area is continued for longer than 3 months or if repeated travels lead to renewed exposures, intramusucular immune globulin 0.06 mL/kg is repeated after 4–5 months. Further administration of immune globulin is not necessary, since by that time most travelers will have acquired natural immunity through contact with HAV.

One dose of hepatitis A vaccine administered at any time before traveling to an endemic area also provides adequate protection (see below) [17].

**Postexposure prophylaxis**. Administration of immune globulin within 2 weeks after close contact with an infected person is 69–89% effective

in preventing symptomatic infection or attenuating symptoms and reducing viral transmission. Previously unimmunized contacts of a patient with acute hepatitis A should receive intramuscular globulin without delay and serologic testing for anti-HAV antibodies. Household contacts of patients with acute hepatitis A are protected against infection by intramuscular administration of immune globulin 0.02 mL/kg. Since 2–3 weeks after the onset of illness the patient is no longer contagious, injection of immune globulin does not need to be repeated. The duration of protection provided by 0.02 mL/kg immune globulin is 1–2 months.

Recommendations for postexposure prophylaxis are summarized in Table 63.15.

**Table 63.15** Recommendations for postexposure prophylaxis in contacts of patients with hepatitis A

| |
|---|
| **Postexposure prophylaxis should be considered in persons who:** |
| • Live in the same house, share a room, care for the patient |
| • Are engaged in intimate contacts with the patient |
| • Share illicit drugs with the patient |
| • Are in contact with the patient in a food service establishment, in which the patient handled foods served without further cooking during the time the patient was infective |
| • Work at or attend a child care center, where hepatitis A has been diagnosed in an employee, in a child, or in household contacts of two or more enrolled children |
| • Work at or attend a school or a health care setting in which multiple persons with hepatitis A virus have been identified |
| **Postexposure prophylaxis is not recommended in:** |
| • Casual contacts of the patient |
| • Hospital staff members |
| • Persons with immunoglobulin A deficiency |
| • Persons in the same school, work, or health care setting in which only one case of hepatitis A has been identified, unless otherwise indicated in the recommendations above |

Source: Adapted from [3]

## Active Immunization

Despite the presence of at least three genotypes, HAV expresses only one single serotype of limited antigenic variability. This allowed the development of a formalin inactivated whole virus vaccine that confers immunity against all probable HAV strains. Active immunization with inactivated whole virus vaccines further reduces the incidence of HAV infection and eliminates the need for passive pre- and postexposure prophylaxis. Antibodies elicited by the vaccine have the same neutralizing capacity as antibodies acquired during natural infection. They are directed against a three dimensional conformation created by juxtapositioning of binding sites on two capsid-polypeptides. The production of a recombinant vaccine is hampered by the fact that the resultant capsid-polypeptides do not arrange in the desired three-dimensional conformation. Work on the development of a recombinant vaccine by cloning HAV is in progress.

Two types of vaccines for HAV, introduced in 1995 and 1996 are presently available in the United States in pediatric/adolescent and adult formulation [1, 10].

**VAQTA®**. Each 0.5 mL dose contains approximately 25 Units of formalin-inactivated hepatitis A virus antigen adsorbed onto aluminium hydroxyphosphate sulphate, in 0.9% NaCl. The formulation does not contain a preservative. Vaccination schedule consists of two doses, administered on a 0, 6–18 months schedule. VAQTA® is contraindicated in persons with known hypersensitivity to any component of the vaccine.

**Havrix®**. A 0.5 mL pediatric dose contains 360 or 720 ELISA Units of formalin-inactivated hepatitis A viral antigen adsorbed to aluminium hydroxide. The adult dose contains 1,440 ELISA Units/mL. The formulation contains 0.5% 2-phenoxyethanol as a preservative. Vaccination schedule consists of two doses, administered on a 0, 6–12 month schedule. Havrix® is contraindicated in persons with known hypersensitivity to any component of the vaccine.

Table 63.16 summarizes the information on VAQTA® and Havirx®. The indications for HAV vaccination are summarized in Table 63.17.

In addition to HAV mono-vaccines, a combined inactivated hepatitis A and recombinant hepatitis B vaccine is approved in the United States for persons ≥ 18 years old (**Twinrix®**). A formulation for children is available in many other countries. 1 mL of the vaccine contains 720 ELISA Units hepatitis A antigen and 20 µg HBsAg. The vaccination schedule is 0, 1 and 6 months (see chapter "Hepatitis B").

Hepatitis A vaccines are highly immunogenic with 97–100% of children, adolescents and adults having protective levels of antibody within 1 month after receiving the first dose and 100% after the second dose. Post-vaccination testing is not recommended. Antibodies persist for at least 5–8 years; protection, however, lasts for at least 20 years. Thus, it seems that in addition to specific protective antibodies other mechanisms, such

**Table 63.16** Recommended dosages of hepatitis A vaccines

| Vaccine | Age (years) | Dose | Volume (mL) | Application | Dose schedule |
|---|---|---|---|---|---|
| **Havrix®** | 2–18 | 720 (ELISA Units) | 0.5 | i.m. (s.c.) | 2 doses, 6–12 mo apart |
| | > 18 | 1,440 (ELISA Units) | 1.0 | | 2 doses, 6–12 mo apart |
| **VAQTA®** | 2–18 | 25 (Units) | 0.5 | i.m. | 2 doses, 6–18 mo apart |
| | > 18 | 50 (Units) | 1.0 | | 2 doses, 6–18 mo apart |

**Table 63.17** Persons at increased risk for hepatitis A who should be vaccinated against hepatitis A (According to [3, 4, 7])

- Persons traveling to or working in countries with high or intermediate prevalence of hepatitis A (Central and South America, Mexico, Asia (except Japan), Africa, Eastern Europe)[a]
- Universal hepatitis A vaccination should be implemented and integrated into routine childhood immunization schedule at age 1–2 years. As of 2006 vaccination is mandatory for school entry in Alaska, Arizona, Nevada, New Mexico, Oklahoma, Texas, and Utah.
- Unimmunised persons, especially children in communities where outbreaks of hepatitis A are occurring, e.g. day-care centers
- Men who have sex with men
- Illicit drug users
- Persons receiving clotting factor concentrates
- Persons with chronic liver disease (hepatitis B, hepatitis C) or who have received or will receive a liver transplant
- Laboratory personnel who work with HAV-infected primates or who work with HAV in research laboratories

[a]Further information is available at http://www.cdc.gov/ncidod/diseases/hepatitis/a/vax/index.htm and at http://www.cdc.gov/ncidod/diseases/hepatitis/a/prevalence.htm

as cellular memory, contribute to protection against HAV infection. Decreased seroconversion rates are seen in old and immunosuppressed persons, e.g. organ transplant recipients and patients on dialysis.

Vaccination against HAV is well tolerated. The most common side effects are tenderness at the injection site (50%), headache (15%) and malaise (7%). No special precautions for immunocompromised persons are needed. Inactivated vaccines generally do not interfere with the immune response to other inactivated or live vaccines and vaccination against HAV infection can be administered concomitantly with M-M-R II (measles, mumps, and rubella live vaccine).

Recent data also confirm the protective efficacy of hepatitis A vaccine in *postexposure prophylaxis* when administered within 8–14 days after exposure to HAV [11, 17]. Thus for healthy persons under age 40, immunoglobulin is no longer required in this setting [17]. In older patients hepatitis A vaccine still should be administered simultaneously with passive immunization.

## *Therapy*

Since acute hepatitis A resolves completely without sequelae in nearly all patients, a specific antiviral therapy is neither necessary nor available. Treatment is supportive. Rigorous bed rest and a "liver diet" do not have an impact on the clinical course of the disease. The patient should observe a balanced diet. Avoidance of hepatotoxins such as alcohol and acetaminophen is axiomatic. Patients with signs of fulminant hepatitis and with hepatic failure require immediate hospitalization and evaluation for liver transplant.

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# Hepatitis B

The hepatitis B virus (HBV) is a hepatotropic virus that belongs to the family of Hepadnaviridae (Flaviviridae). It may cause acute and chronic hepatitis as well as cirrhosis and hepatocellular carcinoma (HCC) [139].

## *Definition*

*Acute hepatitis B* is the initial infection of the liver by HBV with biochemical, virological and histological evidence of liver injury.

*Chronic hepatitis B* is an infection of the liver caused by HBV, persisting for more than 6 months, and charactarized by biochemical and virological evidence of active viral infection and histologic liver injury.

## *Epidemiology*

HBV was discovered in 1966. It represents one of the most prevalent infectious agents in man and is a global health problem with approximately 2 billion people infected worldwide, and 350–400 million (representing 5–7% of the total world population) suffering from chronic HBV infection [138]. Worldwide, chronic HBV infection is a leading cause of cirrhosis, HCC (60% of all cases of liver cancer worldwide are caused by viral hepatitis B), and death, particularly in moderate and highly endemic countries. Between 1979 and 1998 a fourfold increase in age-adjusted death rates due to HBV was reported [117]. Approximately one million deaths worldwide are attributable annualy to HBV infection [139, 227]. Although the incidence of acute HBV infection in the general US population has declined by 70% over the last decade, approximately 70,000 people have acute hepatitis B each year and an estimated 1.25 million persons are infected with HBV [171].

In Western countries HBV is acquired primarily in adulthood, whereas in Asia and most of Africa, the virus is acquired perinatally or in childhood. In highly endemic regions in Africa, the Far East as well as in large parts of South America more than half of the entire population is expected to be infected with HBV during their lifetime. The prevalence of HBV infection

in these geographical areas lies between 10% and 20%, and 8% of the population are chronic HBV carriers due to perinatal (vertical) transmission from the mother and horizontal transmission from child to child.

Chronic HBV infection is highly endemic in most Asian countries. In the USA Asians make up 3.6% of the US population, or approximately ten million people. Asian immigrants from endemic areas have a high prevalence of HBV infection comparable to their native country, whereas those born in the United States have a reported prevalence of 1.6%. Among the Asian American population in New York City 23% have detectable serum HBsAg.

By contrast, the prevalence of HBV infection is 3–5% in Southern Europe and 0.2–0.5% in Central Europe, Scandinavia, Australia and in the USA. HBV prevalence is substantially higher (circa 30%) in patients with Down syndrome, lepromatous leprosy, polyarteritis nodosa, leukemias, Hodgkin's lymphoma, i.v. drug users, and in patients on renal dialysis. In non-vaccinated health care workers HBV prevalence rates are approximately 15%.

Different *HBV genotypes* (see Section 63.1) have distinct geographical distributions (Table 63.18). HBV genotypes B and C are most prevalent in highly endemic areas where vertical transmission is the primary means of transmission. In contrast, HBV genotypes A, D, E, F, and G are found in areas where horizontal or sexual transmission of HBV is more common.

**Table 63.18** Hepatitis B virus genotypes, subtypes and their geographic distribution

| Genotype | Subtypes | Distribution |
|---|---|---|
| **A** | *adw2, ayw1* | Northwestern Europe, Spain, Poland, North America, India, Central Africa, Brazil |
| **B** | *adw2, ayw1* | East and Far East Asia, Indonesia, Pacific Islands |
| **C** | *adw2, adqr +, adrq-* | Eats and Far East Asia, Pacific Islands, Australia, USA, Brazil |
| **D** | *ayr* | Globally distributed with highest prevalence in Mediterranean, Middle East, Iran, India, Africa, North America |
| **E** | *ayw2, ayw3* | West and South Africa |
| **F** | *ayw4* | South and Central America, Polynesia, Alaska |
| **G** | *adw4q-, adw2, ayw4, adw2* | France, USA |

Recently, a new strain has been described in one American and two Nicaraguan patients, and the designation of **genotype H** has been proposed.
Source: Adapted from [154, 245]

HBV circulates in high concentrations in blood, but HBsAg has been demonstrated in nearly all body fluids of infected persons, including saliva, tears, vaginal secretion, urine, semen and breast milk. Stool is probably not infectious. HBV antigens and HBV DNA have been demonstrated in lymph nodes, bone marrow, circulating lymphocytes, spleen and pancreas. Although HBV is not cytopathic and does not injur these tissues and cells, these extrahepatic sites may become a source of reinfection and reappearance of HBV infection in the transplanted liver. Health care workers (surgeons, pathologists, dentists, laboratory workers, renal dialysis technicians, etc.) are markedly at risk for acquiring acute hepatitis B, and in this group of persons hepatitis B is currently the most important *occupational disease* ahead of tuberculosis, hepatitis A and C. *HBsAg positive medical personnel observing the usual protective measures do not seem to pose a danger of transmitting HBV to patients*.

*Transmission* is parenteral from mother-to-child (vertical or perinatal transmission) or by percutaneous or mucosal exposure to infectious bodily fluids (horizontal transmission). Perinatal infection of infants from infected mothers and horizontal infection early in childhood from exposure to HBsAg–positive family members are the main routes of HBV transmission in highly endemic areas. Perinatal transmission occurs mainly with HBsAg carriers and women with acute hepatitis B in late pregnancy or in the early postpartal period. The likelihood of transmission correlates with the viral load. Approximately 10% of infections are already acquired in utero.

In regions such as subSaharan Africa, tribal rituals, such as scarifications, might also be a potent source of infection. In regions of low endemicity, such as Western countries, hepatitis B is primarily a disease of adolescents and adults as a result of high-risk sexual behavior and injection drug use. Sexual transmission is ascribed to minute mucosal injuries, which are always present and allow the virus to gain entrance to the circulation. Especially in regions with low HBV endemicity sexual transmission represents one of the major routes of infection. The risk of sexual transmission is much higher for HBV than for HCV. Direct inoculation by accidental needle-stick injury with contaminated syringes, tattoos, piercing, and acupuncture represent

other routes of aquiring HBV. Transfusion related hepatitis B is extremely rare since screening for hepatitis B is routine in most transfusion centers. The risk of transmitting HBV with blood or blood-products is estimated to be 1:100,000–1:1,000,000. Droplet infection does not play a role in HBV transmission.

Different HBV genotypes may be preferentially transmitted by different modes. Thus, genotype C may have been responsible for most perinatal transmission, given that seroconversion from HBeAg occurs decades later than in other genotypes [156].

Due to the parenteral route of transmission and the relatively low rate of endemic infection, hepatitis B in the US and in Europe is a disease that affects primarily certain risk groups, such as medical personnel, patients with Down syndrome, frequent recipients of blood or blood products (hemophiliacs), persons with infectious or malignant diseases (leukemias, malignant lymphomas, polyarteritis nodosa), patients on hemodialysis, organ transplant recipients, drug addicts, prisoners, men having sex with men, and prostitutes. Spouses and family members of acutely infected persons are also at increased risk. The majority of infections coincide with the start of sexual contact, i.e. juveniles and young adults with no typical risk behaviour are affected (see indications for vaccination).

HBV can survive in the environment for 7 days or more [9].

The *incubation period* is 6 weeks to 6 months and depends primarily on the dose of infectious HBV particles. The patient remains contagious from the time of infection until complete healing of hepatitis B (loss of HBsAg and development of anti HBs).

## Pathogenesis

The molecular biology and the life cycle of HBV are discussed in Section 63.1. HBV entry into hepatocytes requires the initial attachment of HBV envelope protein-derived lipopeptides to the carbohydrate side chains of hepatocyte-associated heparan sulfate proteoglycans as attachment receptors. This interaction initializes the multistep entry process [56, 209]. Key steps in the replication of HBV are illustrated in Fig. 63.6.

HBV is noncytopathic for hepatocytes. *It is the dynamic interplay between HBV, hepatocytes and the host's innate and adaptive immune responses that determines the outcome of HBV infection*. The efficacy of virus-specific T cells determines whether viral clearance or viral persistence will prevail. Vigorous HBV immune clearance is mirrored by liver damage and clinically acute hepatitis. Recovery from hepatitis B is associated with long-term persistence of cytotoxic T lymphocytes (CTL) that actively maintain CTL responses for life.

Patients with weak or totally undetectable HBV specific T cell responses will progress to chronic hepatitis B. Thus, inadequate innate and adaptive immune response (inadequate clearance) accounts for persistent HBV infection [250]. However, even after resolution of acute or chronic hepatitis B, replicative HBV intermediates can persist in the liver under immunologic control, conferring a risk of reactivation following immunosuppressive or chemotherapy [240].

In acute hepatitis B $CD4^+$ and $CD8^+$ T cell responses are strong and multispecific. A few weeks after the infection virus specific $CD4^+$ and $CD8^+$ T cells appear in the peripheral blood and accumulate in the liver. Activated $CD4^+$ T cells recognize viral fragments in association with HLA class II antigens on the surface of antigen presenting cells, secrete cytokines and stimulate macrophages, $CD8^+$ T cells and B-cells to proliferate. Resolution of hepatitis B is characterized by the appearance of neutralizing and protective antibodies against HBsAg.

$CD8^+$ T cells are the primary immune effector cells in hepatitis B. Intrahepatic HBV-specific $CD8^+$ T cells remain detectable in the liver after HBsAg seroconversion [217]. Several HBV peptide fragments, after being processed within the cell, are expressed on the surface of hepatocytes in association with HLA class I antigens and are recognized by HLA class I restricted $CD8^+$ T cells. This interaction between HBV infected hepatocytes and CTL activates death receptors, such as Fas-ligands, stimulates the secretion of cytokines and perforin by $CD8^+$ T cells and results in apoptosis of the infected hepatocytes (see Chapters 18 and 23). In addition, HBV itself enhances tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) cytotoxicity by increasing its TRAIL/death receptor expression [110]. $CD8^+$ T cells also secrete cytokines that impair the replicative cycle of HBV by purging HBV replicative intermediates from the cytoplasm and covalently closed circular viral DNA from the nucleus of infected cells. This noncytopathic antiviral mechanism contributes

to viral clearance. The differences in cytokine production by HBV-specific T cells in blood and liver may explain the capacity of HBV to persist in the absence of significant hepatic destruction [28, 84, 85]. *Acute hepatitis is the clinical expression of a vigorous immune response which is associated histologically with a marked, self-limited necroinflammatory activity that in the majority of patients results in the elimination of HBV infected hepatocytes and resolution of acute hepatitis*.

Chronic hepatitis B is characterized by T cell failure with weak mono- or oligospecific T cell responses. Impaired T cell immune control fails to eliminate HBV and results in viral persistence and protracted hepatic necroinflammation. Not infrequently in chronic hepatitis B, T cell immune responses are missing completely, except for episodes of acute exacerbation. The mechanisms contributing to T cell failure and viral persistence are poorly understood. Quantitative and qualitative T cell defects occur. Primary inability of T cells to mount a virus specific response, T cell dysfunction, T cell exhaustion, insufficient accumulation (homing) of T cells in the liver, deranged secretion of antiviral cytokines by T cells, the occurrence of viral escape mutants, genetic factors and immune tolerance in perinatally acquired HBV infection are some of the mechanisms currently discussed [178]. In addition, regulatory T cells might suppress HBV-specific T helper cells and impair dendritic cell function (crucial for antigen presentation), and contribute thereby to the evasion of HBV from an adequate immune response, leading to viral persistence and to disease chronicity [218, 235].

HBV covalently closed circular (ccc) DNA plays a key role in viral persistence. It is a replicative form of HBV DNA that is incorporated into the nucleosome to form a non-integrated mini-chromosome (see Section 63.1). It persists as a stable episome, but may be reactivated and act as a template for the transcription of viral genes. Clearance of cccDNA reservoirs is thought to be the limiting factor for complete elimination of HBV from infected cells [276].

### Hepatitis B Stages

Based on the interaction between the patient's immune response and HBV infected hepatocytes, HBV infection may be subdivided into various phases:

- Immune tolerant phase
- Immune clearance phase
- Low or non-replicative phase and
- Reactivation phase (Fig. 63.43) [65]

The *immune tolerant phase* is characterized by a lack of or a very weak immune response against infected hepatocytes and by high viral replication (high serum HBV DNA levels). Liver biopsy shows only minimal changes with low histologic necroinflammatory activity, no or only very mild fibrosis, and serum ALT values are normal or only slightly increased. The patients are generally asymptomatic. The immune tolerant phase is seen primarily in patients with perinatally acquired chronic hepatitis B, and may last with minimal to absent hepatic necroinflammatory activity for the first 20–30 years of HBV infection; this quiescence may be related to the acquisition of core deletions. Loss of tolerance is characterized by clearance of HBV or by a rapid transition to an inactive carrier state in two-thirds of patients, and to chronic hepatitis in one third of patients [3]. Patients who acquire the disease during adolescence or adulthood have no immune tolerant phase. Instead the disease progresses directly to immune clearance. Thus, in adult-acquired disease, the early phase of infection often is accompanied by marked disease activity, with increased ALT levels, whereas in perinatally acquired disease, patients tend to be immune tolerant with normal ALT levels. Chronic hepatitis is much more likely to follow infection acquired in the neonatal period and in childhood (90%) than in adult life (< 5%).

During the *immune clearance phase,* immune mediated lysis of infected hepatocytes takes place mirrored

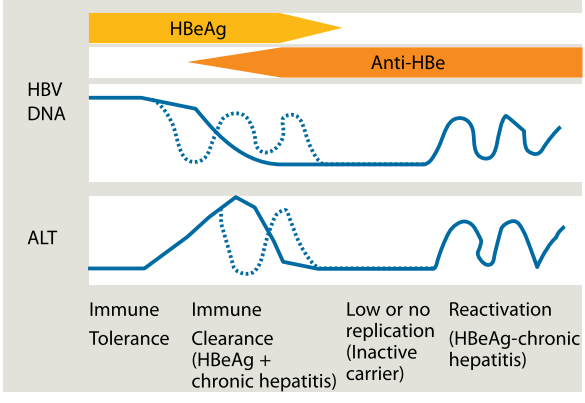


**Fig. 63.43** Stages of HBV infection (Adapted from [65])

by clinical reactivation with increased and fluctuating serum ALT levels and declining HBV DNA concentrations in serum. Marked necroinflammatory activity is present and there is evidence of fibrogenesis on liver histology. This phase may last for months to years and successful viral clearance is usually marked by seroconversion from HBeAg to antiHBe and by loss of HBV DNA from serum.

Most patients who mount a successful immune reaction against HBV infected hepatocytes proceed to the *low* or *nonreplicative phase* characterized by seroconversion from HBeAg to anti-HBe, by a further decline of HBV DNA to low or undetectable levels and normalization of serum ALT levels. These biochemical changes are accompanied by resolution of liver necroinflammation. The persistence of HBsAg characterizes the "asymptomatic HBsAg carrier" (inactive HBsAg carrier state) while conversion from HBsAg to anti-HBs denotes "healing of hepatitis B".

The low or nonreplicative phase may last for a lifetime, but in some patients *reactivation of HBV replication* occurs. Levels of HBV DNA, with or without HBeAg seroreversion, and ALT concentrations rise again.

Even in patients with resolved infection and undetectable serum levels of HBV, the virus may persist for decades and become the source of reactivation, for example during severe immune suppression, such as cancer chemotherapy or after organ transplantation. HBV cccDNA is responsible for viral persistence in the natural course of chronic HBV infection and serves as the template for the production of new HBV (see above and Section 63.1). The levels of both HBV cccDNA, a marker of HBV persistence, and pregenomic RNA, an indicator of viral replication, in the liver of chronically infected patients correlate with viral activity and the phase of HBV infection [136].

## *Clinical Manifestations*

### Acute Hepatitis B

The acute disease begins with a nonspecific prodromal stage characterized by fatigue, malaise, fever and anorexia, occasionally accompanied by arthralgias and right upper quadrant pain. The symptoms and signs correspond to those of hepatitis A (Table 63.11). In 5–10% of patients a serum sickness-like syndrome develops with skin rash, urticaria, angioedema, arthritis, high fevers (39.5–40°C) and infrequent hematuria and proteinuria. After a few days, while the patient begins to feel better, jaundice develops, accompanied by darkening of urine and discoloration of stool.

In approximately two thirds of adult patients the disease runs an inapparent or anicteric course. In perinatally infected newborns and in immunosuppressed individuals the prevalence of asymptomatic HBV infections is even higher. Acute HBV infection in children may rarely present with lymphadenopathy and a non-pruritic skin rash on the face, extremities and buttocks, a condition known as *acrodermatitis papulosa (eruptiva) infantum* or *Gianotti-Crosti syndrome*.

The laboratory findings in acute hepatitis B are similar to those of hepatitis A and are summarized in Table 63.13. For immune serologic parameters see Section 63.1. Elevated aminotrasferase levels usually normalize gradually within several weeks.

### Chronic Hepatitis B

Persistence of hepatitis B surface antigen (HBsAg) in the circulation for more than 6 months defines chronic hepatitis B, which is more likely to follow infections acquired in childhood than those acquired in adult life. Liver damage in chronic hepatitis B may vary from mild chronic inflammation to severe hepatitis progressing to cirrhosis or hepatocellular carcinoma.

The patient with chronic HBV infection may be asymptomatic or may complain of mild nausea, decreased appetite, and fatigue. A mild hepatomegaly may be the only pathologic finding on physical examination. The serum aminotransferase levels are slightly elevated (usually less than ten times the upper limit of normal; concentration of ALT is typically higher than that of AST; with the development of cirrhosis AST levels become greater than ALT levels). Serum levels of alkaline phosphatase are generally within normal limits or only slightly elevated. Acute exacerbations may manifest by intermittent elevations of serum bilirubin levels (up to 3–10 mg/dL), and in patients with established cirrhosis may lead to hepatic decompensation with the development of ascites, edema, gastrointestinal bleeding and hepatic encephalopathy.

In both acute and in chronic hepatitis B autoantibodies, e.g. ANA, AMA, SMA, rheumatoid factor may appear

in up to 50% of patients. In contrast to autoimmune hepatitis, autoantibody titers in HBV infection are low and they represent immunologic epiphenomena [48].

The aminotransferase levels do not correlate with necroinflammatory activity and stage of fibrosis, and approximately one third of patients with persistently normal or only mildly elevated ALT levels in chronic HBV infection have significant fibrosis and inflammation on liver biopsy [132, 229]. Noninvasive predictive models of liver fibrosis based on serum biochemical markers have been developed, but have not gained wide clinical acceptance, and liver biopsy remains the gold standard for grading and staging of chronic hepatitis B [99, 176, 273].

## Extrahepatic Manifestations

Extrahepatic manifestations may occur in the prodromal phase of acute disease and are seen in 10–20% of patients in the chronic phase of HBV infection [251]. The main extrahepatic manifestations are HBV associated *glomerulonephritis* and *polyarteritis nodosa*. However, cutaneous *leucocytoclastic vasculitis* with palpable purpura, *synovitis*, *urticaria, polyneuropathy* with sensory-motor deficiency, *sicca syndrome*, *aplastic anemia*, *autoimmune thyroiditis* or *ulcerative colitis* may also be seen rarely in association with hepatitis B. A *mixed cryoglobulinemia* occurs in up to 15% of cases, but is mainly seen in chronic HCV infection. *Non-Hodgkin's lymphoma* has been reported to develop nearly three times more likely in chronic HBV-infected patients than in those without HBV infection [232]. There seems to be an independent association between HBV infection with *gestational diabetes mellitus* and HBsAg carriers have increased risk of antepartum hemorrhage, and threatened preterm labor [135, 230]. There seems to be no relationship between HBV genotypes and the presence of extrahepatic manifestations [20].

### HBV-associated Glomerulonephritis

This is a rare immune complex disease occuring predominantly in HBV endemic areas. Patients mostly present with signs and symptoms of the nephrotic syndrome. Rarely the initial presentation is that of acute renal failure accompanied by signs of cutaneous vasculitis, polyarteritis nodosa or cryoglobulinemia.

Three forms of glomerulonephritis are associated with HBV infection: (1) membranous glomerulonephritis, (2) membranoproliferative glomerulonephritis, and (3) immunoglobulin A nephropathy [130, 131].

*Membranous glomerulonephritis* (MGN) is the most common form and primarily affects children. It is rare in children without HBV infection or systemic lupus erythematosus. But in adult white Americans, membranous nephropathy is the most common pattern of idiopathic nephrotic syndrome. Deposition of subepithelial antigen-antibody-complexes (mainly HBeAg and anti-HBe) is thought to be pathogenetically responsible for the clinical manifestations of HBV-associated MGN. After several months to years, HBV-associated MGN heals in nearly all (95%) affected children. This is usually accompanied by seroconversion of HBeAg to anti-HBe. Resolution of renal disease, however, is relatively uncommon in adults.

*Membranoproliferative glomerulonephritis* (MPGN) occurs more often in adults, with 80% of MPGN cases being associated with chronic hepatitis C rather than HBV infection. HBV-associated MPGN is characterized by immune-complex deposits (containing HBsAg and HBeAg) in the mesangium and subepithelial space. Some of the capillary loops appear normal, with thin basement membranes, while the basement membranes of other capillary loops appear fragmented. Having both abnormal and normal segments of the glomerulus is typical of secondary forms of MPGN and is usually caused by HBV or HCV. MPGN is generally associated with activation of the complement pathway with reduced serum levels of C3 and C4. Rheumatoid factor may be present in serum. The typical clinical presentation is with nephrotic range proteinuria. In a few cases HBV-associated MPGN may run a rapidly progressive course to renal insufficiency, but there is limited information on the natural course of HBV-associated MPGN in adults. The impact of antiviral therapy on the prognosis of HBV-associated renal disease is uncertain. A meta-regression analysis, however, showed a significant link between HBeAg clearance and proteinuria remission after interferon therapy, supporting the role of HBV in the pathogenesis of renal disease [60].

*Immunoglobulin A nephropathy* is seen most often in children and in Asians.

### Polyarteritis Nodosa

Fewer than 1% of HBV infected patients present with signs of polyarteritis nodosa (PAN), but 20–30% of patients with PAN are HBsAg positive. It typically occurs within 4 months after the onset of HBV infection and the presence of HBV-related disease is suggested by finding HBsAg, HBeAg, and HBV DNA in serum. Deposits of HBsAg, immunoglobulins and complement components are present in the vessel walls of small and medium-sized arterioles. These immune deposits activate the complement pathway which leads to injury of the vessel wall with formation of fibrinoid necrosis and perivascular cellular infiltrates, ultimately resulting in vessel obstruction and organ ischemia. The cardinal clinical features are arterial hypertension, arthralgias, arthritis, skin rash, abdominal pain, fever and eosinophilia. In severe cases the disease may progress to a diffuse necrotizing multisystem vasculitis affecting the kidneys, the gastrointestinal tract, the peripheral and central nervous system. The clinical manifestations are the same as in PAN not associated with HBV [86]. There is no association between the severity of liver disease and that of HBV-associated PAN. Patients with HBV-related PAN may benefit from antiviral therapy.

## *Diagnosis*

HBV infection is usually diagnosed by the detection of HBsAg in serum. Hepatitis B viremia is established by detecting HBV-DNA or by demonstrating HBeAg in serum. However, *the presence of anti-HBe does not indicate absence of viral replication*. Acute HBV infection is usually diagnosed by the presence of HBsAg and anti HBc-IgM in serum. The serologic and molecular diagnosis of HBV infection is discussed in Section 63.1 (Fig. 63.15). Liver biopsy is not required to diagnose acute hepatitis B.

Serum aminotransferases are markedly elevated (up to several thousends U/mL) with ALT usually being higher than AST in acute hepatitis B. They gradually normalize over several weeks in uncomplicated cases. Typically the levels of aminotransferases are raised only moderately (less than ten times the upper limit of normal) in patients with chronic hepatitis B, but patients may also have near normal or persistently normal values, despite ongoing inflammation in the liver. Chronic hepatitis B is a dynamic disease and the levels of aminotransferases usually fluctuate. As in acute hepatitis B, the levels of ALT are higher than those of AST. However, with progression to cirrhosis, the ALT/AST ratio may reverse.

Imaging modalities, such as ultrasound, CT-scanning and MRI do not play a role in the diagnosis of acute or chronic hepatitis, except in the differential diagnosis of elevated liver enzymes, excluding for example space-occupying lesions, and in demonstrating progression to cirrhosis. In chronic hepatitis B, enlarged perihepatic lymph nodes have been reported to be an indicator for histologic and biochemical inflammatory activity in the liver [39].

Liver biopsy still is the gold standard in grading and staging of chronic hepatitis. Recent attempts at predicting necroinflammation, significant fibrosis, and cirrhosis with noninvasive methods in patients with chronic HBV infection are encouraging. Thus, noninvasive markers, such as HBV DNA levels, AST, alkaline phosphatase, and platelet count reliably predicted hepatic necroinflammation and significant fibrosis, and the age-spleen-platelet ratio index (ASPRI) was accurate in predicting cirrhosis in patients with chronic hepatitis B [118, 174]. If these data are confirmed, these simple noninvasive models have the potential to reduce the number of liver biopsies in patients with chronic hepatitis B (see also Chapter 28).

## *Differential Diagnosis*

The differential diagnosis includes hepatitis caused by other hepatotropic (HAV, HEV, HDV, HCV) and non-hepatotropic (EBV, CMV, HSV, Coxsackie-virus) viruses. Of particular importance is the recognition of concomitant HDV infection (coinfection, superinfection), since HDV may have an impact on the natural course of HBV infection. Acute exacerbations of chronic hepatitis B, deteriorating liver function in HBV infected patients or persistent liver injury, despite HBeAg loss, should prompt one to consider an accompanying HDV infection. Around 25% of patients with autoimmune hepatitis have an acute clinical onset of the disease with signs and symptoms that may mimick acute viral hepatitis. Cholestatic liver

diseases, e.g. drug, toxin and alcohol-induced liver injury, acute cholecystitis, obstructive jaundice due to common bile duct stone(s) and an ascending, suppurative cholangitis fall into the broader range of differential diagnoses of acute viral hepatitis (Table 63.14).

## *Major Patterns of Chronic Hepatitis B*

The definitions, diagnostic criteria and clinical terms used in HBV infection are summarized in Table 63.19. The major patterns of chronic hepatitis B comprise

- HBeAg-positive chronic hepatitis B
- HBeAg-negative chronic hepatitis B
- Inactive HBsAg carrier state
- Occult HBV infection

HBV replicates via an RNA intermediate, using a reverse transcriptase that appears to lack a proofreading function. Therefore, HBV exhibits a higher mutational frequency than most DNA viruses, and individulas chronically infected with HBV harbor the virus as a mixture of viral quasispecies with a dominant strain (see Section 63.1) [80].

These natural polymorphisms of HBV are called "variants" as they describe variations observed within an individual and between populations compared with published wild type strains. Thus, *HBV variants* are naturally occurring HBV subspecies that are present in a large proportion of patients independent of external selection pressures. The most common HBV variants are the pre-core stop codon (G1896A) and core-promoter variants (A1762T + G1764A) that lead to reduced or abolished HBeAg secretion.

In contrast, point mutations in the HBV genome arising from internal or external selection pressure, for example the host immune response or exerted by a drug, give rise to *HBV mutants*. These mutants may be resistant to the pressure inducing agent(s) and may "escape" the drug's action. A variety of precore/core mutants have been described. The two well-defined precore mutations include a stop codon mutation at nucleotide (nt) 1,896 (or codon 28) (resulting in the cessation of HBeAg expression) and mutations in the basal core promoter (BCP) at nt 1,762 and nt 1,764 (resulting in diminished production of HBeAg and a resultant increased host immune response).

Marked geographic differences in the prevalence of precore stop mutant viruses have been noted. 12–27% of isolates from US and European patients with chronic active hepatitis B, and 47–60% of patients in Asia, Africa, Southern Europe, and the Middle East exhibit these precore stop mutations [104].

Precore stop codon variants can be found in patients with a wide spectrum of liver disease, and they may or may not be pathogenic. There is no general agreement whether or not precore stop codon variants are associated with more severe liver disease than wild-type strains. *Core promoter mutations appear to cause more serious liver disease* and have been implicated with the development of HCC [262].

Although testing for precore and core promoter variants is becoming more widespread in research studies, it is not recommended for routine clinical management as the role of these variants in the natural history of HBV and the response to antiviral therapy remains unclear.

### HBeAg-Positive Chronic Hepatitis B

Patients with HBeAg-positive chronic hepatitis B usually present in the third or fourth decade of life. HBeAg-positive chronic hepatitis B typically is associated with high levels of circulating HBV-DNA (> $10^{5–7}$ copies/mL, up to $10^{10}$ copies/mL), variable elevations of ALT and histological activity. HBeAg seroconversion occurs at an annual rate of 10–15% in adults with elevated ALT. Approximately 65% of patients eventually undergo seroconversion from HBeAg to anti-HBe associated with reduction of HBV-DNA replication [66a]. Spontaneous and treatment-induced seroconversion rates are higher in patients with raised ALT and genotype B (vs C) and genotype D (vs A) (see below). Frequently an acute exacerbation, lasting 2 to 4 months, precedes HBeAg seroconversion. Seroconversion from HBeAg to anti-HBe with significant reduction of HBV replication (to less than $10^4$ copies/mL) marks the transition of chronic hepatitis B to the inactive HBsAg carrier state and is usually associated with biochemical and histologic remission, and a diminished risk of disease progression. However, in a small percentage of patients ALT and HBV DNA levels remain elevated. These patients as well as those undergoing reactivation of hepatitis B after HBeAg seroconversion constitute the group of patients with HBeAg-negative chronic hepatitis B.

**Table 63.19** Definitions, diagnostic criteria and clinical terms used in HBV infection[a]

| Definitions | Diagnostic criteria |
|---|---|
| **Chronic hepatitis B** | |
| Chronic necroinflammatory liver disease caused by persistent HBV infection | 1. HBsAg positive > 6 months<br>2. Serum HBV DNA > 20,000 IU/mL (> $10^5$ copies/mL)<br>3. Persistent or intermittent elevations of ALT/AST levels<br>4. Liver biopsy showing chronic hepatitis (necroinflammatory score ≥ 4)[b] |
| *Chronic hepatitis B may be subdivided into*: | |
| HBeAg positive chronic hepatitis B | HBeAg positive, anti HBe negative |
| HBeAg negative chronic hepatitis B | HBeAg negative, anti HBe positive[c] |
| **Inactive HBsAg carrier state**<br>Persistent HBV infection of the liver without significant ongoing necroinflammatory activity | 1. HBsAg positive > 6 months<br>2. HBeAg negative, anti HBe positive<br>3. Serum HBV DNA < 20,000 IU/mL (< $10^5$ copies/mL)<br>4. Persistently normal ALT and AST levels<br>5. Liver biopsy showing absence of significant hepatitis (necroinflammatory score < 4)[b] |
| **Resolved hepatitis B**<br>Previous HBV infection without further virological, biochemical, or histological evidence of active virus infection or disease | 1. Previous known history of acute or chronic hepatitis B or the presence of anti HBc ± anti HBs<br>2. HBsAg negative<br>3. Undetectable serum HBV-DNA[d]<br>4. Normal ALT levels |
| **Reactivation of hepatitis B** | Reappearance of active necroinflammatory disease of the liver in a person known to have the inactive HBsAg carrier state or resolved hepatitis B |
| **Acute exacerbation or flare of hepatitis B** | Intermittent elevations of aminotransferase activity to more than ten times the upper limit of normal and more than two times the baseline value[e] |
| **HBeAg clearance** | Loss of HBeAg in a person who was previously HBeAg positive |
| **HBeAg seroconversion** | Loss of HBeAg and detection of anti HBe in a person who was previously HBeAg positive and anti HBe negative, associated with a decrease in serum HBV DNA levels to less than 20,000 IU/mL (< $10^5$ copies/mL) |
| **HBeAg reversion** | Reappearance of HBeAg in a person who was previously HBeAg negative and anti HBe positive; occurs rarely (< 5%) |
| **Primary treatment failure** | Reduction of serum HBV DNA levels by less than 1 $\log_{10}$ IU/mL from baseline at week 12 |
| **Complete virologic response**[f] | Negative HBV DNA by a sensitive assay (< 60 IU/mL or < 300 copies/mL) |
| **Partial virologic response** | HBV DNA levels < 2,000 IU/mL (4 $\log_{10}$ copies/mL) |
| **Inadequate virologic response** | HBV DNA levels ≥ 2,000 IU/mL (4 $\log_{10}$ copies/mL) |

[a]Adapted from references 114 and 115
[b]Liver biopsy optional
[c]Most of these patients have precore or core promoter variants
[d]Very low levels (10 IU/mL) can be detected using molecular assays
[e]Most clinicians would regard elevations of ALT levels > 5 times ULN compatible with an acute exacerbation of chronic hepatitis B
[f]Measurement of the HBV DNA levels at week 24 is considered essential to characterize virologic responses as complete, partial, or inadequate

## HBeAg-negative Chronic Hepatitis B

Over the course of the last two decades an increasing prevalence of HBeAg negative chronic hepatitis B has been recognized. Nowadays, as many as 50% of patients with chronic HBV infection worldwide have undetectable HBeAg, and 70–90% of unselected adult chronic HBV carriers from Italy and France are HBeAg negative at diagnosis [66a]. Patients with HBeAg-negative chronic hepatitis B are HBsAg positive and anti-HBe positive. HBeAg-negative chronic hepatitis usually represents a late phase in the natural history of chronic HBV infection rather than a de novo infection with naturally occuring HBV variants that do not

produce HBeAg. Acute infection with the precore mutant rarely, if ever, leads to chronicity [34]. For reasons that are not yet known, replication-competent HBV variants with mutations in the precore or core promoter regions preventing or down-regulating HBeAg production may be selected during or after HBeAg seroconversion. The precore variant is usually found in association with HBV genotype D. Therefore, HBeAg negative chronic hepatitis is common in the Mediterranean and in the Middle East, where genotype D predominates. Basic core promoter mutations are not genotype dependent and are seen in Asia, where both genotypes B and C are common.

Absence of HBeAg in serum is due to genotypic changes preventing expression of HBeAg. The most common mutation that prevents HBeAg production is a guanine (G) to adenine (A) change at nucleotide 1,896 (G1896A) which creates a stop codon in the precore region of the HBV genome that prematurely terminates synthesis of HBeAg as well as mutations in the basic core promoter region which down-regulate HBeAg synthesis at the transcriptional level. Despite their inability to synthesize HBeAg, these patients may display a high HBV replicative activity.

Compared to patients with HBeAg-positive chronic hepatitis, those with HBeAg-negative chronic hepatitis are usually older and have lower levels of serum HBV DNA ($10^4$–$10^8$ copies/mL). Males predominate and the reported male/female ratio ranges from 3.9 to 17 [65]. However, many patients with HBeAg-negative chronic hepatitis have wide fluctuations in both HBV DNA and serum ALT levels. Despite lower HBV DNA levels, HBeAg-negative patients may have histologically severe and progressive chronic hepatitis, which is more active and advanced than in patients with HBeAg-positive chronic hepatitis. HBeAg-negative patients also are more likely to have cirrhosis at the time of their first presentation. Spontaneous sustained remissions of disease activity are rare. Viremia and necroinflammatory changes improve on treatment, but sustained antiviral responses are difficult to achieve in HBeAg-negative chronic hepatitis B.

### Inactive HBsAg Carrier State

The inactive HBsAg carrier state denotes a remission in disease activity, and is diagnosed by the absence of HBeAg, the presence of anti-HBe, undetectable or low levels of HBV DNA in PCR-based assays, repeatedly normal ALT levels, and minimal or no necroinflammation, slight fibrosis, or even normal histology on biopsy. Health related quality of life in asymptomatic carriers is comparable to healthy people, and prognosis is usually benign [181]. Spontaneous reactivation, however, may occur with a very low risk of progression to cirrhosis and HCC, which is strongly related to ongoing high levels of HBV replication independently of HBeAg status [66]. Thus, HBeAg-negative patients are not a homogeneous group and seroconversion to anti-HBe is not necessarily a marker of remission [151]. It may be accompanied by HBV genomic variability and by varying degrees of HBV replication. Long-term longitudinal studies (up to 23 years) of adult inactive carriers have reported that 15–24% developed HBeAg negative chronic hepatitis and 1–17% had sustained reversion back to HBeAg positivity [44, 66]. Since patients with HBeAg-negative chronic hepatitis have wide fluctuations in serum ALT and 20–30% of patients with histologically documented chronic inflammation have persistently normal ALT levels, the distinction between inactive carriers and HBeAg-negative, anti-HBe positive patients with progressive disease can be difficult without longitudinal follow-up studies and liver biopsy [19, 122].

Apoptotic caspases are activated in viral hepatitis and serum caspase activity has been suggested as a sensitive marker of early liver injury. Recently it has been shown that serum caspase activity is strongly associated with the presence of liver injury in patients with HBeAg-negative chronic HBV infection. Measurements of caspase-generated fragments of cytokeratin-18 seem to be a useful marker for differentiation between the inactive HBV carrier state and HBeAg-negative chronic inflammation [183]. These data, however, must be confirmed, before they can be implemented into clinical practice.

### Occult Hepatitis B Virus infection

The long-lasting persistence of HBV genomes (HBV DNA detectable only by ultrasensitive HBV DNA amplification techniques; < 200 IU/mL) in liver tissue or in serum in HBsAg negative individuals is termed occult HBV infection [196]. On the basis of the HBV antibody profile, occult HBV infection may be distinguished as seropositive (anti-HBc and/or anti-HBs positive) or seronegative (anti-HBc and anti-HBs negative). Eighty percent of occult HBV infections are seropositive, 20% are negative for all serum markers of HBV infection.

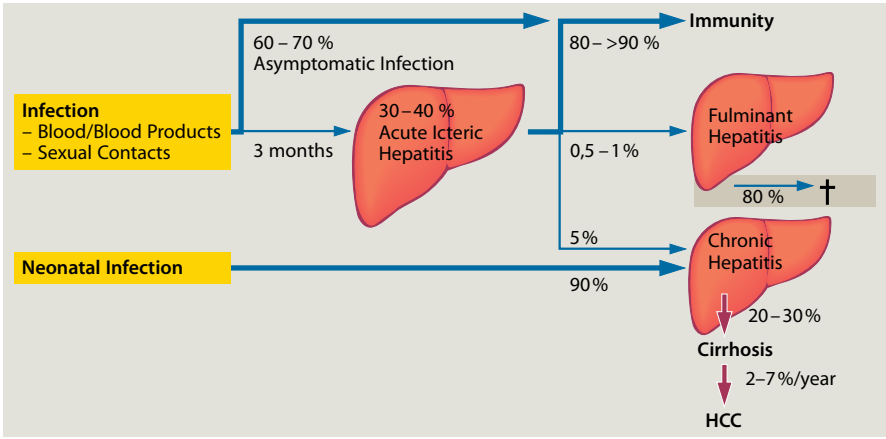


**Fig. 63.44** Natural course of HBV infection. Infection acquired during the perinatal/neonatal period leads to chronic hepatitis in approximately 90% of patients, while HBV infection acquired in adult life is followed by chronic hepatitis in less than 5% of patients

In a study in a North American community-based population the prevalence of occult HBV infection was 18% in those with serologic evidence of previous HBV infection and 8.1% in HBV seronegative individuals. Age, gender and liver biochemistry findings did not identify those with occult HBV, and S-variants were present in the majority of individuals with occult HBV [173].

By examining liver tissue from a large series of HBsAg negative Italian individuals with no clinical and biochemical evidence of liver disease, occult HBV infection was revealed in approximately 16% of the Italian general population (!), and was significantly associated with the anti-HBc positive status [197].

HCV infected patients are considered the group of individuals with the highest prevalence of occult HBV infection. Data on occult HBV prevalence in HIV positive individuals are wildly divergent.

Occult HBV infection is mostly due to a strong suppression of viral replication and gene expression, but in some cases may be associated with mutant viruses undetectable by HBsAg assays. The exact mechanisms responsible for the inhibition of HBV genome are unknown. The host's immune surveillance, coinfection with other agents and virus interference as well as epigenetic factors suppressing HBV replication and transcription at the level of nuclear HBV cccDNA might be important in causing occult HBV infection. The stability and long-term persistence of viral cccDNA molecules together with the long half-life of hepatocytes imply that HBV infection, once it has occurred, may possibly continue for life.

Occult HBV infection has important clinical implications. Despite HBsAg unreactivity, multiple replication-competent HBV variants accumulate in the liver of occult HBV infected patients and HBV in serum and liver maintains its infectious and pro-oncogenic properties [190, 191]. Patients with occult HBV infection may transmit HBV by blood transfusion or organ transplantation. Any patient with occult HBV receiving systemic chemo-, radio- or immunosuppressive therapy is potentially at risk for HBV reactivation. Moreover, there is evidence to suggest that, despite the lack of overt infection, the persistence of even small amounts of HBV can enhance the progression to liver fibrosis, is common among patients with cryptogenic liver cirrhosis, and can favor the development of HCC [22, 195].

## *Natural Course and Prognosis*

The natural course of HBV infection is outlined in Fig. 63.44. Infections acquired during adulthood usually follow a benign course, with less than 5% progressing to chronic hepatitis B, whereas 90% of perinatally and neonatally acquired infections become chronic and carry the risk of further progression to liver cirrhosis and HCC [66a].

### Acute Hepatitis B

In 95% of immunocompetent adult patients acute hepatitis B is a self-limited disease and heals without sequelae after 3–4 months, conferring lifelong immunity. Only up to 5% of adult patients develop chronic hepatitis. Perinatally acquired HBV infection is mostly subclinical and asymptomatic, but 90–95% of children develop chronic hepatitis. However, even after serologic recovery with loss of HBsAg and development of

anti-HBs and complete clinical recovery from acute self-limited hepatitis B, in some patients occult HBV infection may persist in the liver accompanied by abnormal liver histology for years [272].

### Fulminant Hepatitis B

Fulminant hepatic failure is defined as the onset of coagulopathy and encephalopathy within 8 weeks of presentation of acute hepatitis. An overly exuberant immune response to HBV antigens is believed to account for the rapid and severe lysis of infected hepatocytes. A fulminant course of acute hepatitis B is very rare and is observed in approximately 0.1–0.5% of patients (1% in hospitalized patients). On the other hand, acute HBV infection accounts for 8.6% of cases of acute liver failure in the United States [224]. The risk increases with age and with preexisting morbid conditions. Coinfection with hepatitis D is a particularly frequent association. The role of HBV genotypes in fulminant hepatitis B is controversial. A fulminant exacerbation of chronic HBV infection can occur after abrupt discontinuation of immunosuppressive therapy for other conditions or of nucleos(t)ide treatment of chronic hepatitis B, and is considered to result from a rebound immune response of the host. Clinical features and laboratory parameters are those of acute liver failure (see Chapter 78). The prothrombin time is considered to be the best prognostic marker.

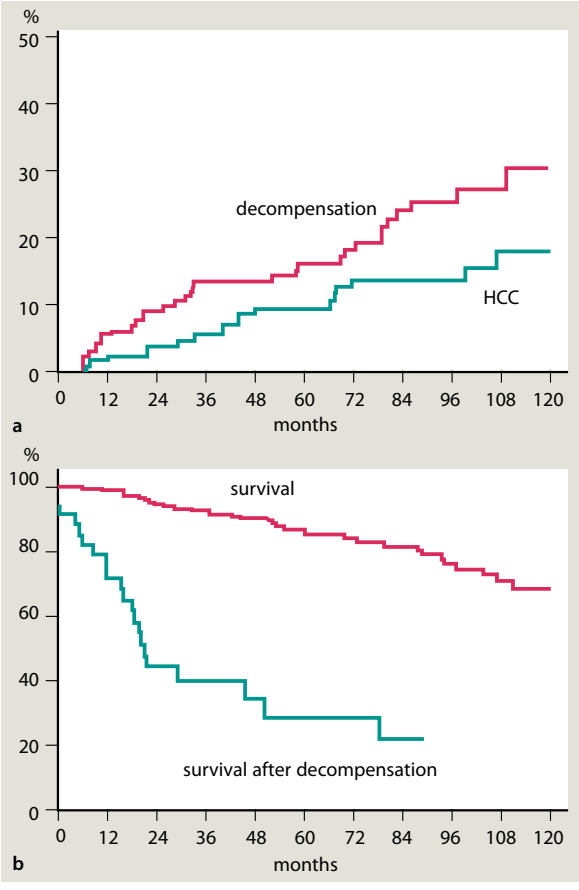


**Fig. 63.45** Natural history of chronic hepatitis B. (**a**) Cumulative incidence of hepatocellular carcinoma (HCC) and decompensation of liver disease. (**b**) Cumulative probability of survival and survival after the appearance of the first episode of decompensation (From [65])

### Chronic Hepatitis B

The natural course of hepatitis B infection is variable and is determined by many factors. Once established, chronic hepatitis B is a potentially serious disease with a severe long-term prognosis (Fig. 63.45). Twenty to 30% of chronically HBV infected patients progress to liver cirrhosis within 5–10 years, which, after decompensation has a cumulative 5 year survival probability of only approximately 30% [65].

Age at infection, mode of transmission, host and viral factors (ongoing viral replication, genotype), as well as cofactors, e.g. alcohol abuse and coinfection with other viruses, are important determinants of whether or not an acute HBV infection will become chronic. Persisting symptoms, failure of aminotransferase, bilirubin and γ-globulin serum levels to normalize within 6 months after onset of acute disease and the presence of HBsAg and HBeAg for more than 6 months are strong indicators for progression to chronic HBV infection. Risk of chronicity is low for transmission through sexual contact, intravenous drug use, acupuncture, and transfusion as compared to perinatal (vertical) transmission.

#### Age

The risk of chronicity varies greatly with the age at which the infection is acquired. Generally *the younger the age at infection, the higher the probability of chronicity*. Overall the chronicity rate in adults is approximately 5%. In immunocompetent, otherwise healthy young adults the prevalence of chronic disease

is less than 5%. Neonates and children who acquire the infection in the first year of life have a 90% risk of the infection becoming chronic. The presence of HBeAg and a high maternal HBV DNA level is associated with increased risk of chronic infection in the infant. Fewer than 10% of babies born to HBeAg-negative/anti HBe-positive mothers become persistently infected [65]. For children aged 1–5 years at infection, the risk is about 30%, and for children older than 5 years the risk is less than 5% [105].

### Immunosuppression

In immunosuppressed patients (hemodialysis, organ transplantation, cancer chemotherapy), in children with Down syndrome, in patients with leukemia, and in HIV coinfected homosexual men a chronic course of hepatitis B is observed 5 times as often as in HBV infected patients without these cofactors. Reactivation of an apparently healed hepatitis B with seroreversion of HBeAg is also observed more frequently in immunosuppressed individuals.

Viral factors, such as HBV genotype, baseline HBV DNA levels, duration of HBV replication, persistent HBV replication viral load, and coinfections also have an impact on the course and outcome of HBV infection.

### HBV Genotype

Growing evidence suggests that HBV genotype may affect HBeAg seroconversion, the presence of HBV variants, and the natural history of liver disease (Table 63.20). These effects seem to be influenced by geographical and ethnic factors. Due to the unique distribution of HBV genotypes in Asia and the West, natural history studies of HBV can only reliably compare genotype B to C or genotype A to D [245]. Recombination between HBV genotypes is also possible, but the clinical significance of recombinant strains remains unclear.

Patients infected with genotype B have a significantly lower prevalence of HBeAg at presentation (53% versus 69%, $p < 0.01$) and a significantly higher rate of spontaneous HBeAg seroconversion. Furthermore, HBeAg seroconversion occurs nearly 10 years earlier in patients with genotype B than in patients infected with genotype C [41]. Asian patients with genotype C infection are more likely to experience prolonged HBV replication, delayed HBeAg seroconversion, more rapid progression to cirrhosis. They also have a higher risk of development of HCC than patients infected with HBV genotype B [245]. The reasons for why genotype C is more aggressive than genotype B are unclear. The higher prevalence of detectable HBeAg and core promoter mutations in genotype C may, at least in part, account for the more aggressive disease [266]. Sustained biochemical remission, clearance of HBV DNA as well as the rate of HBsAg clearance are more frequent in infection with genotype A than D or F [154, 203]. However, few data comparing genotypes A and D are available, and no definite conclusions as to their impact on the natural course of HBV infection are presently possible. In Europe most patients with genotype A have chronic hepatitis, whereas most patients with genotype D have acute hepatitis.

**Table 63.20** Clinical and virologic differences between HBV genotypes B and C

| Features | Genotype B | Genotype C |
|---|---|---|
| **HBeAg positivity** | Less | More |
| **HBeAg seroconversion** | Earlier | Later |
| **Immunoclearance phase** | Shorter | Longer |
| **HBsAg clearance** | More | Less |
| **Histological activity** | Lower | Higher |
| **Serum HBV DNA level** | Lower | Higher |
| **Precore stop codon mutation** | More | Less |
| **Basal core promoter mutation** | Less | More |
| **Cirrhosis and HCC** | Less (Japan, China) | More |

Source: According to [154].

Despite the potential impact of HBV genotypes on outcome and treatment (see below), HBV genotyping has limited clinical application to the study of the natural history and treatment of HBV infection, and contrary to HCV genotyping testing for HBV genotypes remains a research tool for now.

### Viral Load

The viral burden also has an impact on the course of HBV infection. Overall, patients with a highly replicative chronic hepatitis B ($> 10^5$ copies/mL in HBeAg positive and $> 10^4$ copies/mL in HBeAg negative chronic HBV infection) seem to develop progressive

disease, decompensation and HCC at higher rates than patients with low replicative HBV infection [33]. However, other authors failed to define exact baseline HBV DNA levels as predictors of decompensation or of development of HCC [268].

## Coinfections

Cofactors, such as alcohol consumption, intravenous drug abuse and coinfection with other viruses appear to confer an increased risk of disease progression to liver cirrhosis and HCC and may have important ramifications on choice of antiviral treatment regimen [63, 146, 172]. HBV shares routes of transmission with other viruses, such as hepatitis C virus (HCV) and hepatitis D virus (HDV), and with systemic retroviral infections. It is therefore not surprising that coinfection with other viral pathogens is not uncommon [213].

**Coinfection of HBV and HDV.** There are two scenarios for HDV presentation. (1) Coinfection, i.e. transmission of HDV simultaneously with HBV, and (2) superinfection, i.e. HDV transmission in a patient already infected with HBV. HDV co- and superinfection increases the risk of fulminant hepatitis in acute hepatitis B. The genotype of the infecting HDV appears to play a major role in determining the severity of liver disease, with HDV genotype I being associated with more frequent fulminant hepatitis in acutely HBV infected individuals.

Chronic HBV/HDV coinfection has an unfavourable course compared to HBV monoinfection. Progression to liver cirrhosis is faster and liver cirrhosis develops 10–15 years earlier than in chronic hepatitis B alone. Once cirrhosis has supervened, the presence of HDV increases the risk for HCC, hepatic decompensation, and mortality by 3.2-, 2.2-, and 2.0-fold, respectively, compared with HBV monoinfected cirrhotic patients [63]. The mechanism of viral interference seen in HBV/HDV coinfection is unclear. The presence of HDV appears to suppress HBV replication. With suppression of HBV replication, it is likely that HDV is the major factor in liver damage as it is believed to be directly cytopathic.

**Coinfection of HBV and HCV.** The reported prevalence rates of HBV/HCV coinfection vary between 10% to 35% depending on the population studied. In coinfected patients the phenomenon of viral interference is observed with each virus affecting the replication of the other. Thus, coinfection with HCV may decrease the replicative activity of HBV, resulting in an increased prevalence of "silent HBV infection." In addition to reduced serum concentration of HBsAg, HBsAg production may be delayed in coinfected patients compared to patients infected with HBV alone. On the other hand, HBV may also inhibit HCV replication. Generally, however, it is assumed that HCV inhibits HBV replication more than vice versa. Despite this interaction, liver disease is usually more severe and the prognosis worse among patients with dual HBV and HCV infection than in those infected with a single virus. Thus, coinfection with HCV does appear to significantly alter the natural history of HBV related liver disease. Acute HCV superinfection in patients with chronic HBV infection is clinically severe during its acute phase and the long-term prognosis is much worse than that following active hepatitis B in terms of continuing hepatitis activity after HBsAg loss and the development of cirrhosis or HCC [148]. The rate of HCC per 100 person-years follow-up was 3.7 in patients with HCV alone, 2.0 in those positive for HBsAg alone, and 6.4 in patients with HCV/ HBV coinfection [35].

**Coinfection of HBV and HIV.** Up to 90% of patients infected with HIV have serologic markers of past HBV infection. The prevalence of chronic HBV infection was 7.6% among HBV unvaccinated HIV positive subjects [116]. With the impressive improvements in survival in HIV-infected patients, treatment of viral hepatitis has assumed greater importance.

In HIV/HBV coinfected individuals, diminished anti-HBV immune surveillance reduces spontaneous clearance rates for HBsAg and HBeAg compared with non-HIV infected patients. HIV/HBV coinfection is also associated with lower ALT levels and higher HBV DNA serum levels, indicating more active viral replication. Increasing HIV-induced immunosuppression also amplifies the risk of quiescent HBV.

Improvement of an HIV-infected patient's immune response with highly active antiretroviral therapy (HAART) may induce spontaneous HBeAg to anti HBe seroconversion. On the other hand, HAART-induced immune reconstitution may also be associated with a flare of acute clinical hepatitis, presumably due to increased recognition and destruction of HBV-infected hepatocytes.

**Coinfection of HBV and Parvovirus B19.** In Vietnamese patients HBV/human parvovirus B19 coinfection was reported to occur in 21% of HBV

infected individuals. Coinfected patients had a greater likelihood of progression to more severe HBV-associated liver disease. The impact of parvovirus B19-infection on HBV-associated pathogenesis is poorly understood [226].

## Spontaneous Resolution

### HBeAg Seroconversion

Spontaneous *HBeAg seroconversion* to anti-HBe is a crucial event in the natural history of chronic hepatitis B and a prerequisite for HBsAg seroconversion. It is most common during the immune active phase of the disease when ALT levels are increased and serum HBV DNA levels are falling to levels less than 20,000 IU/mL ($10^5$ copies/mL). In Central Europe and in the USA spontaneous HBeAg seroconversion *occurs in 8–15% of untreated patients per year* and usually portends a favorable prognosis with loss of HBV DNA, biochemical and histological improvement followed by clinical remission [65, 102]. A recent longitudinal study has reported that up to 90% of white ("caucasian") adults with chronic hepatitis B clear HBeAg within 10 years of follow-up, with an incidence rate of 18 per 100 person years [66]. Older age, higher ALT levels (> 5 × ULN), HBV genotypes B (versus C) and A (versus D) and ethnicity other than Asian are associated with higher rates of spontaneous seroconversion. Among Asian children, most of whom have normal ALT, spontaneous HBeAg seroconversion occurs at a very low rate, less than 2% during the first 3 years of age and 4–5% in children older than 3 years.

Spontaneous HBeAg seroconversion is sustained in approximately two thirds of patients. However, despite HBeAg seroconversion in some patients occult disease may persist (see above) [190]. Seroreversion (reappearance of HBeAg) may occur in some patients, while in others HBeAg remains undetectable but high serum levels of HBV DNA reappear accompanied by persistent or intermittent increases of ALT levels. These patients have a naturally occurring mutant form of HBV in the precore or core promoter region that abolishes or down-regulates HBeAg production.

### HBsAg Seroconversion

In contrast to HBeAg seroconversion, spontaneous HBsAg seroconversion occurs in only aproximately 1% of patients per year, although recent studies suggest that HBsAg seroclearance rates in asymptomatic carriers of high endemic areas may be higher during a long-term follow-up, amounting to approximately 40% after 25 years [43, 44]. Serological clearance of HBsAg is of paramount importance in the natural history of chronic hepatitis B as its development and the advent of anti HBs is generally referred to as resolution of HBV infection. However, in some patients occult HBV may persist, and in a minority of cases HCC may develop [1, 271].

## Hepatitis Flares

Hepatitis flares or acute exacerbations are particularly frequent in HBeAg positive patients and are defined as intermittent elevations of aminotransferase activity over 200 U/L or more than 5–10 times the upper limit of normal or more than twice the baseline value [147]. Hepatitis flares may be spontaneous (virus induced) or drug induced (particularly if the drug has immunomodulating effects), such as ALT flares during the second to third month of interferon (IFN)α therapy, after abrupt withdrawal of a short course of corticosteroid therapy, or during or after lamivudine therapy (see below).

Acute flares of chronic hepatitis B should be differentiated from superinfection with other hepatotropic viruses. As many as 20–30% of these acute exacerbations may be caused by superinfection with HDV, HCV, or HAV. Severe exacerbations occur with equal frequency in patients who are HBeAg positive and in those with antibodies against HBeAg, and there is no single cutoff HBV DNA value to differentiate whether patients with anti HBe will have inactive disease or continue to have exacerbations [42]. Genotype C and male sex are independent factors predictive of reactivation of hepatitis B [44].

Acute exacerbation of hepatitis B is the result of CTL-mediated immune response against HBV antigen(s) and its downstream apoptotic mechanisms. In addition to CTL induced destruction of HBV containing hepatocytes, cytokines secreted by CTL, such as IFNγ and TNFα inhibit HBV gene expression and viral replication. A hepatitis flare is therefore a frequent event within 3 months before spontaneous HBeAg seroconversion and occurs in approximately two thirds of spontaneous HBeAg seroconversions. However, less

than 25% of hepatitis flares are followed by spontaneous HBeAg/HBV DNA seroclearance within this short time span.

Histologically, hepatitis flares are characterized by marked lobular necroinflammatory activity that may lead to bridging hepatic necrosis. The cellular infiltrate at the site of necroinflammatory reaction consists mainly of cytotoxic $CD8^+$ T cells.

The clinical spectrum varies from asymptomatic to typical symptoms of acute hepatitis to hepatic decompensation and hepatic failure. Twenty-three to 38% of patients with chronic hepatitis B in Taiwan and Hong Kong experienced icteric flare-ups with decompensating liver function, and significantly increased mortality rates [23]. Generally however, symptoms of hepatitis flares are less severe than those of acute hepatitis or acute superinfections.

The laboratory parameters, in addition to elevated serum ALT levels, may show increases of bilirubin concentration and prolongation of prothrombin time. More severe acute exacerbations are associated with a rise in serum AFP levels, usually 1–2 weeks after the peak levels of ALT. AFP levels correlate with bridging hepatic necrosis and AFP levels > 100 ng/mL may be used as a marker of severe parenchymal necrosis.

The elevation of ALT levels is usually followed by a decrease in serum HBV DNA concentration and in HBeAg positive patients by seroclearance of HBeAg and appearance of HBe antibodies. Generally, *the more severe the hepatitis flare the greater the likelihood of spontaneous HBeAg seroconversion*. However, if repeated spontaneous flares of disease activity are not followed by HBV DNA clearance fibrosis can develop at a significant rate. In addition to seroconversion, acute flares of chronic hepatitis B may be associated with mutations in the HBV genome, and patients with acute flares of disease may have reappearance of IgM anti-HBc. Not all therapy-related hepatitis flares are followed by HBeAg seoconversion. There is some evidence to suggest that in addition to stimulation of cytotoxic cells, the activation of $Th_1$ immunity with induction of a $Th_1$ cytokine response with non-cytolytic HBV suppression is a prerequisite for a successful HBV clearance [228].

## Liver Fibrosis and Cirrhosis

Untreated chronic hepatitis B is a potentially serious disease that may progress to liver cirrhosis and hepatocellular carcinoma. The incidence of cirrhosis ranges from 2 to 5 per 100 person-years and the 5-year cumulative incidence of progression to cirrhosis ranges from 8% to 20%. A higher rate of cirrhosis occurs in patients with HBeAg-negative than HBeAg-positive chronic hepatitis B. Overall, approximately 20–30% of patients with high replicative chronic HBV infection will develop liver cirrhosis.

Among the various risk factors identified for the progression to cirrhosis and HCC, a high level of HBV replication has been identified as the most important factor [255]. The cumulative incidence of cirrhosis increased from 4.5% for patients with HBV DNA levels of less than 300 copies/mL to 36.2% for patients with HBV DNA levels of $10^6$ or more copies/mL [106]. In Asian patients HBV genotype C is associated with a higher risk of developing cirrhosis than genotype B [42]. Development of cirrhosis is associated with accumulation of complex HBV variants, which exhibit an altered phenotype combining enhanced replication with defects in protein expression. This phenotype results from major mutations in the core promoter and C gene but is considerably influenced by additional mutations throughout the genome [169].

Progression of chronic hepatitis B to cirrhosis is insidious and only a minority (24%) of patients are symptomatic. The 5-year cumulative incidence of hepatic decompensation is 16%, and the first episode of decompensation is usually (49%) caused by ascites [64]. Patients who have more advanced disease at presentation, men, and patients older than 40 years have a higher risk of decompensation. The 5-year survival of compensated HBV-cirrhosis is 80–86%. Once decompensation occurs, the prognosis is poor. The probability of survival ranges from 55% to 70% at 1 year and from 14% to 28% at 5 years (Fig. 63.45). Active HBV replication is a negative prognostic factor, whereas viral clearance and ALT normalization correlate with increased survival [49, 61, 64, 199].

## Hepatocellular Carcinoma

The risk of HCC in patients with chronic hepatitis B increases with time (Fig. 63.45). In the USA up to 5,000 people die each year from HBV associated cirrhosis and HCC [192]. The risk of developing HCC in chronic HBV infection is 100–200 times that in non-infected individuals, particularly if the infection was

acquired during childhood. Wthin the HBsAg-positive group, HBeAg-positive carriers have the highest risk of HCC, but even carriers with anti-HBe antibodies have a substantial risk of cancer [255]. As in cirrhosis, a high level of HBV replication has also been identified as the most important factor for the development of HCC [270]. The cumulative incidence of HCC was 1.3% in persons with serum HBV DNA levels of less than 300 copies/mL and increased to an incidence of 14.9% for those with HBV DNA levels of $10^6$ or more copies/mL. *Elevated serum HBV DNA above the cut off level ≥ $10^4$ copies/mL was a strong predictor of HCC independent of HBeAg, serum aminotransferase level, and liver cirrhosis* [33]. In Asia, genotype C HBV infection is associated with an increased risk of HCC [24]. In most patients HCC develops on the background of liver cirrhosis after a latency period of 25–30 years after infection. After perinatal infection the risk of HCC is 0.1–0.6% per year, even in noncirrhotic livers, and approximately 25 % of patients infected as neonates die prematurely from cirrhosis or liver cancer [139].

HCC usually arises in the setting of compensated cirrhosis, which may be clinically silent. The incidence of HCC in HBV-cirrhosis is 2–7% per year and is 3–6 times higher in males than in females. Older age, alcohol and concurrent infection with HCV or HDV also increase the risk of HCC [64]. However, not all patients with chronic HBV infection develop cirrhosis or HCC. Long-term follow-up studies of HBsAg-positive blood donors and asymptomatic chronic HBsAg carriers with normal ALT levels showed a favorable prognosis that did not differ from that of an uninfected control population [164, 241].

Given the risk of HCC, twice-a-year screening of chronically HBV infected patients, and especially those with cirrhosis, with measurement of alpha fetoprotein (excellent negative predictive value but positive predictive value ranges from 9–30%) and hepatic ultrasonography is warranted [80].

## Prevention

Patients with chronic HBV infection should be counseled regarding lifestyle modifications and prevention of transmission. Household members and sexual partners are at increased risk of HBV infection. Persons who are HBsAg-positive should have sexual contacts vaccinated, use condoms during sexual intercourse if their partner has not been vaccinated or is not naturally immune, not share toothbrushes or razors, cover open cuts and scratches, clean blood spills with detergent or bleach, and not donate blood, organs or sperms.

HBsAg-positive individuals can participate in all activities including contact sports, should not be excluded from daycare or school participation, should not be isolated from other children, can share food, utensils and kiss others [159].

HBV infected health care workers may rarely transmit the virus to patients. Therefore, HBV infected HBeAg-positive health care workers should not perform exposure prone procedures without prior counseling from an expert review panel, and notify prospective patients of their HBV status prior to procedures. Several European countries use a threshold level varying from $10^3$–$10^5$ copies/mL (200–20,000 IU/mL) to determine if HBsAg-positive health care workers are allowed to perform exposure prone procedures [15, 21, 87].

Pharmacologic prevention of HBV disease is achieved by

- Immune prophylaxis (passive and active immunization), and by
- Suppression of HBV-reactivation

### Passive Immunization

The anti-HBs titer of regular immune globulin (1:100 to 1:512 in radio-immunoassay) is not sufficient to prevent HBV infection. Therefore, passive immunization is carried out by administering hepatitis B immune globulin (HBIG) that has an anti-HBs titer of at least 1:100,000. HBIG is used nearly exclusively for *postexposure prophylaxis*.

*Indications for HBIG* (in non-immune individuals) are accidental needle stick injuries with contaminated needles, mucocutaneous contact with or ingestion of HBV-containing material, prevention of perinatal HBV transmission from a HBsAg-positive mother, and the prevention of endogenous reinfection after liver transplantation in an HBV carrier.

Postexposure prophylaxis with HBIG, 0.06 mL/kg body weight i.m. should be given as soon as possible (within the first 12 h, not later than 48 h; HBIG is unlikely to provide benefit if the time from exposure is

longer than 14 days) and as a rule be combined with the first dose of vaccine in a contralateral site, followed by the other two doses of vaccine according to the usual schedule (see below). The risk of becoming a chronic HBV carrier is reduced by approximately 70% in patients receiving only passive immunization, and by 90–95% in those receiving vaccination.

*Neonates of HBsAg-positive mothers* are given both HBIG (0.5 mL i.m.) and hepatitis B vaccine within 12 h of delivery at two different sites.Two additional doses of HBV vaccine are given after 1–2 and 6–12 months. HBIG and concurrent hepatitis B vaccine have been shown to be 95% efficacious in the prevention of perinatal transmission of HBV (except for maternal carriers with very high serum HBV DNA levels).

For the *prophylaxis of endogenous HBV reinfection after liver transplantation,* the patient receives HBIG 10,000 IU (200 mL) i.v. in the anhepatic phase and 2,000 IU (40 mL) i.v. daily for seven consecutive days. During the further course an anti-HBs level in serum of at least 100 IU/L has to be maintained over 6–12 months.

The administration of HBIG after transfusion of HBV-infected blood generally cannot prevent the development of a post-transfusion hepatitis, but usually reduces the frequency of clinically severe disease.

The administration of HBIG is well-tolerated. In immune individuals (documented by anti-HBs or anti-HBc) no postexposure prophylaxis is required. If there is any doubt about the patient's immune status, HBIG should be administered. In non-responders to vaccination, two doses of HBIG, 1 month apart, should be given.

### Active Immunization (Vaccination)

All vaccines currently available in industrialized countries are obtained by recombinant techniques using cloned HBV *S* gene expression. The indications for vaccination and the recommended vaccination schedule are reported in Tables 63.21 and 63.22. Prevaccination screening for anti-HBs is generally not recommended except for adults in high-risk groups. In health care and public safety workers postvaccination seroconversion to anti-HBs should be documented. Annual testing of hemodialysis patients is recommended since immunity wanes rapidly in these individuals who are at a high risk of continued exposure to HBV.

The seroconversion rate is age dependent. Healthy children up to 10 years old seroconvert in nearly 100% of cases. Adults up to 40 years old seroconvert in 95% and those older than 40 years in approximately 90% of cases. A successful vaccination provides nearly 100% protection against infection and disease with all HBV subtypes. Successful vaccination against hepatitis B also provides protection against HDV infection, since infection and replication with HDV is dependent on the presence of HBV. In HCV-infected persons the same seroconversion rates are obtained as in non HCV-infected individuals. Seroconversion rates are lower in smokers, the obese, the elderly, and in immunocompromised individuals (patients on dialysis, patients receiving immunosuppressive drugs, HIV infected persons) [144].These groups require higher and more frequent doses.

Anti-HBs titers ≥ 10 IU/mL are considered protective. Booster vaccination is recommended *before* the titer falls below this level. After complete vaccination

**Table 63.21** Individuals that should be vaccinated against HBV infection

- All newborns, children and adolescents
- Health care workers
- Public safety workers
- Men who have sex with men
- Intravenous drug users
- HIV-infected individuals (preferably with helper cells ≥ 400/μL)
- Promiscuous persons
- Sexual partners of HBsAg-positive persons
- Patients on dialysis
- Patients receiving blood components regularly (for example hemophiliacs)
- Persons traveling to endemic areas
- Persons with close contact with chronically HBV infected patients (family members)
- HCV-infected persons

**Table 63.22** Hepatitis B virus vaccination recommendations

| Group | Schedule (mo)[a] | Recombivax HB®[b] | Engerix-B®[b] |
|---|---|---|---|
| Children/ Adolescents[c] | 0, 1 and 6 | 5 μg (0.5 mL) | 10 μg (0.5 mL/1 mL) |
| Adults | 0, 1 and 6 | 10 μg (1 mL) | 20 μg (1 mL) |
| Adults on dialysis | 0, 1, 2 and 6 | 40 μg (1 mL) | 40 μg (2 mL) |

[a]Protective antibodies may be demonstrated already after the second injection in approximately 80–90% of healthy individuals
[b]Given intramuscularly into the deltoid muscle
[c]Children: 1–10 years, Adolescents: 11–19 years

(3 dose vaccine regimen) in children the anamnestic response is highly effective, and a booster dose is usually not necessary at least for up to 15–18 years after the primary vaccination [161, 264].

Approximately 5% of healthy, immunologically unremarkable individuals do not respond at all ("non-responder") to vaccination, or only with anti-HBs titers less than 10 IU/mL ("hyporesponder"). In these persons HLA and immunoregulatory cytokine gene polymorphisms may contribute to the variable immune response to recombinant HBV vaccines [246]. Three additional vaccine injections in approximately 3 month intervals will lead to the production of protective anti-HBs titers in approximately 60–75% of primarily non- and hyporesponding individuals. Those who do not respond to these additional vaccine doses must receive HBIG upon potential contact with HBV (see above).

Large HBV vaccination programs in endemic regions have revealed a 2–3% incidence of *vaccine escape mutants* resulting from alterations in the HBsAg protein (typically, the substitution of glycine for arginine at aa 145, which makes this epitope unlikely to bind to antibodies generated to wild-type HBsAg) [104].

### Suppression of HBV-Reactivation

HBV reactivation is a well-recognized complication of HBV-infection. It typically occurs in asymptomatic HBsAg carriers undergoing immunosuppressive therapy, for example after organ transplantation, treatment with TNFα-blockers (infliximab, adalimumab), chemotherapy of malignant lymphoma, and of HBV associated HCC [27, 59, 98, 177]. Patients receiving chemotherapy regimens that include glucocorticoids appear to have the highest rates of HBV reactivation, and HBV reactivation has been reported more than 1 year after completion of chemotherapy. Since this complication may be life threatening, physician awareness is essential, and *patients undergoing cytotoxic therapy should be checked routinely for HBV serologic markers and HBV DNA levels*. Prophylactic administration of a nucleoside or nucleotide analog during chemotherapy should be strongly considered. The available data show a four- to sevenfold decrease in the incidence and a reduction in the severity of chemotherapy-related HBV reactivation in patients who receive lamivudine prophylaxis [170]. HBV reactivation is more likely to occur in patients with high pre-chemotherapy HBV DNA after withdrawal of pre-emptive lamivudine. A more prolonged course of antiviral therapy may be necessary in these patients after completion of chemotherapy in order to reduce post-chemotherapy HBV reactivation [100]. Thus, all HBsAg carriers should receive lamivudine 100 mg p.o. qd, or a comparable anti-viral agent, as prophylaxis, starting 7 days prior to the initiation of immunosuppressive or chemotherapy until at least 1 year following its completion [98, 120, 202]. Preemptive lamivudine therapy should also be considered in HBV associated HCC patients with an HBV DNA level of more than $10^4$ copies/mL undergoing transarterial chemo-lipiodolization [206].

## *Treatment*

### Treatment of Acute Hepatitis B

In the vast majority of adult patients acute hepatitis B is a self-limited disease that results in viral elimination and complete healing. Fewer than 5% of immune-competent adult patients with acute hepatitis B progress to chronic hepatitis.

Compared to placebo, patients with acute hepatitis B treated for 4 weeks with lamivudine show a more rapid decline of serum HBV DNA levels. However, the significance of this rapid decrease in viral load is unknown and it is not accompanied by a significantly greater biochemical and clinical improvement compared to untreated patients. The administration of lamivudine to patients with acute hepatitis B may slow the development of protective anti-HBs titers [121]. Therefore, *antiviral treatment is not recommended in patients with uncomplicated acute viral hepatitis B*.

Fewer than 1% of patients with acute hepatitis B have a fulminant clinicial course with acute liver failure. In these few patients the administration of nucleos(t)ide analogs might be considered, although at present there are no data to support this approach.

There is no place for herbal drugs in the treatment of acute and chronic hepatitis B.

### Treatment of Chronic Hepatitis B

In the majority of patients chronic hepatitis B is a controllable but not curable disease. Direct antiviral agents

effectively suppress HBV replication, but have little impact on host immune response, as reflected by a low rate of HBeAg seroconversion and negligible HBsAg seroconversion (i.e. viral elimination). Thus, most patients treated with antiviral agents will need prolonged or possibly life-long therapy.

Antiviral treatment of chronic hepatitis B has undergone major changes in the last few years and new medications continue to be evaluated. There are no universally accepted guidelines for the treatment of chronic hepatitis B and the best treatment still has to be defined. The following paragraphs reflect current (summer 2009) treatment practices in this highly dynamic area.

Which patients should be treated? When to start treatment? How to treat? How to monitor treatment? When to end treatment? These are the key questions that have to be addressed in treating patients chronically infected with HBV and for which the answers are not readily at hand.

The natural history of chronic HBV infection is variable with patients whose prognosis does not differ substantially from that of the uninfected population and others who progress to cirrhosis and HCC. Selecting patients for therapy should be based on an integrated approach, taking into account the determinants of substantial risk of progressive liver disease, the immune status of the patient, virological markers, such as viral load and genotype, and the necroinflammatory activity and degree of fibrosis.

Interferons (IFNs) and nucleos(t)ide analogs are currently approved for the treatment of chronic HBV infection. Compared with the IFNs, oral nucleos(t)ide analogs have excellent safety and tolerability profiles that allow treatment for prolonged periods of time. The therapeutic responses to nucleoside/nucleotide analogs seem to be comparable among patients with different HBV genotypes, while HBV genotype may be an important predictor of response to interferon (see below) [46, 111, 154, 275].

The criteria for starting treatment are not clearly defined and guidelines vary among different countries. In daily clinical practice treatment often is begun in the immunotolerant phase, characterized by increased serum HBV DNA levels with low histological disease activity and normal ALT levels. These patients have very low chances of a sustained therapeutic response, but a high probability of developing drug resistance.

Serological and virological markers, rather than patient characteristics, are currently used as parameters for monitoring treatment. Serum HBV DNA is presently the best non-invasive marker for the assessment of antiviral drug efficacy and the detection of emerging drug-resistant HBV [175]. Quantitative assessment of covalently closed circular (ccc) DNA, the highly stable, integrated form of HBV DNA in the liver and serum, would be a more sensitive end point for antiviral treatment, but measurement is difficult and the technique is not yet widely available [248].

## Goals of Therapy

Responses to antiviral therapy for chronic hepatitis B should be categorized as clinical, biochemical, virological, or histological, and as on-therapy or sustained off-therapy [107, 150, 158, 159].

The *clinical goals* of therapy are improving quality of life and ultimately survival. These goals are achieved by preventing progression of chronic hepatitis to cirrhosis and HCC. In patients with established HBV cirrhosis, therapy aims at preventing decompensation, hepatic failure and HCC.

The *virological goals* of treatment are the seroconversion of HBeAg to antiHBe and/or the persistent reduction of viral load as determined by a significant fall in serum levels of HBV DNA (*partial therapeutic response)*. The ultimate virological goal is the complete eradication of HBV as evidenced by clearance of HBV DNA from serum (by sensitive PCR-based and molecular assays) and disappearance of HBV DNA from liver, clearance of HBsAg and the development of anti-HBs *(complete therapeutic response)*. Unfortunately a complete sustained therapeutic response is seldom achieved. In the rare patient who loses HBsAg or develops anti-HBs, therapy should be discontinued [76]. One should keep in mind, however, that serologic clearance of HBsAg does not always imply clearance of viremia. It may rarely be caused by a point mutation in the S gene during therapy, which results in detection failure. In such patients, further verification and follow-up using a sensitive HBV-DNA test is advised [97].

The goals of therapy vary according to the HBeAg status of the patient. In *HBeAg-positive patients* treatment aims for suppression of HBV DNA to undetectable levels on PCR-based assays (PCR-based and molecular assays can detect as little as 40 IU/mL [200 copies/mL] and 10 IU/mL [50 copies/mL], respectively), normalization of serum ALT levels, and sustained clearance of HBeAg with seroconversion to anti-HBe [114, 184]. HBeAg seroconversion is a prerequisite for HBsAg seroconversion. In *HBeAg-negative patients* the goal of treatment is suppression of HBV DNA to undetectable

levels and normalization of serum ALT levels. Long-term suppression of HBV DNA to undetectable levels is significantly correlated with histologic improvement, HBeAg seroconversion, stabilization of the clinical status, and improvement of clinical outcome.

### Indications

Selecting patients for antiviral therapy relies on a combination of biochemical (serum ALT levels), virological (serum HBV DNA levels), histological (necroinflammation and fibrosis) and clinical (compensated or decompensated liver disease) findings. HBV replication is reflected by the presence in serum of detectable HBeAg and by viral load (1 IU = 5 copies).

The *pretherapeutic serum ALT level is the strongest determinant for HBeAg seroconversion during therapy*, and should be considered in selecting patients for treatment. HBeAg seroconversion correlates highly with pretherapeutic serum ALT levels, the highest HBeAg seroconversion rates being observed in patients with pretreatment ALT levels geater than 5 times the upper limit of normal (ULN). Patients with serum ALT levels ≤ 2 × ULN have very low chances of HBeAg seroconversion [36].

There is no international consensus as to the cut-off level of HBV DNA at which treatment should be started. Based on the REVEAL studies that demonstrated that a viral load of $10^4$ copies/mL (2,000 IU/mL) predicts the risk of cirrhosis and the risk of HCC the German guidelines suggest to start treatment (in patients without marked fibrosis or cirrhosis), as soon as the viral load is > $10^4$ copies/mL (2 × $10^3$ IU/mL) and the serum ALT levels are > 2 × ULN or the histology score is > A1/F1, irrespective of the HBeAg status. When marked fibrosis or cirrhosis is present, treatment is started whenever HBV DNA is detectable, irrespective of the level of viral load [33, 47, 106]. The American guidelines take into consideration the HBeAg status (as long as no cirrhosis is present) and suggest a cutoff value for HBV-DNA of > $10^5$ copies/mL (> 20,000 IU/mL) in most cases [55, 159].

The US treatment guidelines are outlined in Table 63.23, the indications for therapy according to the German guidelines are shown in Fig. 63.46 (note that cutoff levels of HBV DNA differ).

Antiviral treatment is *not* recommended

- In patients with acute HBV infection.
- In patients who have received postexposure prophylaxis.
- In chronic HBV-carriers with normal serum ALT levels.
- In patients with serum ALT levels ≤ 2 × ULN or with low replicative disease or minimal to mild histologic disease on liver biopsy, unless they are undergoing chemotherapy or immunosuppression. These patients should be observed, monitoring regularly clinical signs and laboratory parameters.

## Drugs Used in the Treatment of Hepatitis B

The following drugs are currently licensed for the therapy of chronic hepatitis B

- Interferons
  Standard interferon
  Pegylated interferon
- Nucleoside and nucleotide analogs
  Lamivudine
  Adevofir Dipivoxil
  Entecavir
  Telbivudine
  Tenofovir

**Table 63.23** United States treatment guidelines for HBV infection

| HBeAg Status | HBV DNA IU/mL | ALT × ULN | Potential First-Line Therapy |
|---|---|---|---|
| Positive | > 20,000 | ≤ 2 | No treatment |
| Positive | > 20,000 | > 2 | Treat with IFN, pegIFN, or nucleoside analogue[a] |
| Negative | > 20,000 | > 2 | Treat with IFN, pegIFN, or nucleoside analogue[a] |
| Negative | > 2000 | 1 to > 2 | Consider liver biopsy to help in treatment decision |
| Negative | ≤ 2000 | ≤ 1 | Observe |
| Positive or negative | Approximately ≥ 10 to 100 | Cirrhosis with ≤ 1 to > 2 | See text; paragraph on special therapeutic problems. |
| Positive or negative | Approximately < 10 to 100 | Cirrhosis with ≤ 1 to > 2 | If compensated, observe: if decompensated refer for liver transplantation |

[a]Adefovir or entecavir suggested, because of high rate of resistance to lamivudine and telbivudine. *IFN* interferon, *pegIFN* pegylated interferon

Source: From [55]. With permission

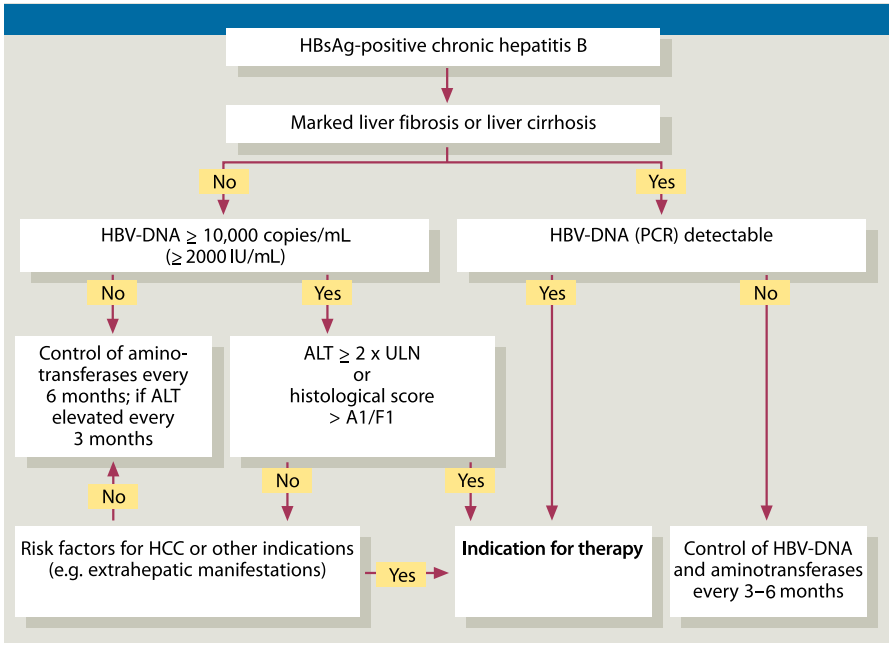


**Fig. 63.46** Indications for therapy of chronic hepatitis B (According to the German guidelines [47])

Many nucleos(t)ide analogs, such as emtricitabine, clevudine, famciclovir, ganciclovir, alamifovir are currently being studied, and probably will be approved for clinical use in the near future.

Direct antiviral agents, such as nucleoside and nucleotide analogs are well-tolerated and suppress viral replication effectively. However, they have little impact on the immune response, as reflected by a low rate of HBeAg seroconversion and negligible HBsAg seroconversion, and must be given for years (lifelong?). Their use is limited by the emergence of drug resistant mutants.

The advantage of immune modulatory drugs, such as the interferons is their finite duration of treatment and the absence of selection of resistant viral mutants. Although they elicit effective antiviral responses only in a minority of patients, in responders the antiviral effect usually is durable.

## Interferons

### Standard Interferon

Interferons (IFNs) are a heterogeneous family of glycoproteins that are produced by nucleated cells. Three main classes (α, β, γ) and many subclasses (16 with IFNα) are distinguished.

**Mechanisms of action.** Interferons have antiviral, antiproliferative and immunomodulatory properties (Table 63.24). The exact mechanisms of action are still poorly understood. IFNs bind to surface receptors on target cells and stimulate a series of complex intracellular reactions. Primarily, however, they induce the

**Table 63.24** Actions of interferons

- **Increased expression of membrane proteins**
  - HLA-class I and II
  - Beta-2-microglobulin
  - Fc-receptor
- **Antiviral effects**
  - Decreased cellular viral uptake
  - Inhibition of intracellular viral processing
  - Decreaed synthesis of (viral) mRNA
  - Decreased protein synthesis
- **Antineoplastic/antiproliferative effects**
  - Inhibition of cell division (arrest of G1/G0-S-transition)
  - Decreased oncogene expression
  - Direct cytotoxicity
- **Immunomodulatory effects**
  - Cytokine induction
  - Complement induction (C2 and B)
  - Activation of macrophages, NK-cells and cytotoxic T-cells

synthesis of various proteins and enzyme systems, such as 2', 5'-oligoadenylate synthetase, that degrade viral RNA. The intrahepatic $CD8^+$ T-lymphocyte, but not the $CD4^+$ T-lymphocyte or NK/NKT-cell response, is important for HBV clearance during IFNα therapy, and the antiviral effect may be mediated by both cytolytic and non-cytolytic mechanisms [222].

**Pharmacology**. The bioavailability after subcutaneous administration is 90%. Peak serum values are reached after approximately 6–8 h for IFNα2a and 3–12 h for IFNα2b. The half-life is 5 h for IFNα2a and 3 h for IFNα2b. Elimination is primarily renal by glomerular filtration, tubular resorption and tubular degradation.

**Interactions.** IFNs inhibit cytochrome P4501A2. They can enhance the unwanted effects of ACE-inhibitors, in particular the development of granulocytopenia and aggravate the anticoagulatory actions of warfarin and phenprocoumon.

**Adverse reactions.** The multiple potential side effects of IFNs are summarized in Table 63.25 Acute side effects, particularly flu-like symptoms, begin 1–4 h after the first IFN injection and gradually diminish after the 3rd or 4th application of IFN. These side effects are usually well-controlled by administering IFN at bedtime combined with paracetamol 1 g p.o.

Thrombo- and granulocytopenia appear already after one week and persist during the entire duration of IFN-therapy. After discontinuation of IFN platelets and leukocytes normalize again. Patients with IFN-induced leukopenia up to approximately 2500–3000/μL do not appear to have an increased incidence of infections. Anemia starts to appear after 6–10 days of IFN treatment and reaches its peak level after approximately 14 days. Despite continuation of IFN-therapy hemoglobin values return to normal in most cases. A dose reduction due to untoward side effects is necessary in 5–20% of patients treated with IFN, while withdrawal of therapy is required in only 4% of patients.

As opposed to nucleos(t)ide analogs, HBV resistance to IFN does not develop and therapy with IFN may be stopped abruptly, without the risk of an acute hepatitis flare.

**Contraindications**. IFN is contraindicated in the conditions listed in Table 63.26.

**Dosing**. IFNα may be applied daily or three times a week. The most effective dosing schedule is either 5–6 MU s.c. qd or 9–10 MU s.c. tiw.

**Duration of treatment.** IFN treatment is usually performed for 3–6 months. HBeAg-negative patients may require 1–2 years of therapy.

### Pegylated Interferon

(See also Section 63.3 "Treatment of Chronic Hepatitis C".)

**Dosing.** PegIFNα-2a is given once weekly 180 μg s.c. (1 or 0.5 mL prefilled syringe). At the time of writing PegIFNα-2b is not yet approved for the treatment of chronic hepatitis B.

**Duration of treatment.** Forty-eight weeks, although a lower dose and/or shorter duration of therapy may also be effective. HBeAg-negative patients may require 1–2 years of therapy.

**Adverse reactions.** As with standard IFN the most common dose dependent side effects of pegIFN are flu-like symptoms. These occur mostly during the initial phase of therapy, subside during the course of IFN treatment and do not require the pegIFN dose to be reduced. In case of severe side effects, e.g. marked hematologic changes, psychiatric problems, dose reduction or discontinuing therapy should be considered (see also Section 63.3 "Treatment of Chronic Hepatitis C"). Dose reductions, mostly because of neutropenia, may be necessary in up to 50% of patients. Low neutrophil count at baseline and cirrhosis are independent predictors of dose reduction or therapy discontinuation [238].

**Results of treatment of standard and pegylated interferons**. *Standard IFNα* was the first treatment approved for chronic HBV infection. In 1993 a meta-analysis confirmed the efficacy of IFN compared to placebo in chronic HBV infection [252]. Treatment with IFN led to more frequent loss of viral replication markers (HBeAg seroconversion: 33% versus 12%), more frequent loss of HBV DNA (37% versus 17%), and more frequent loss of HBsAg (7.8% versus 1.8%) within 12 months after ending IFN therapy.

In approximately 80% of patients showing a favourable response to IFN treatment, the sustained response rates are durable. HBeAg reactivation (relapse) occurs in only 13% of responders. In HBeAg negative patients relapse is common after discontinuation of treatment. Prolonged treatment courses are more effective in obtaining sustained response. The abrupt discontinuation of IFN treatment is not associated with the risk of hepatitis flare (in contrast to nucleos(t)ide analogs).

IFN-induced serological clearance of HBeAg and HBV is associated with improvement of liver histology and of clinical outcome. *IFN therapy in HBeAg positive chronic hepatitis and in HBeAg positive compensated cirrhosis increases the rate of HBeAg seroconversion, significantly lowers the incidence of decompensation of*

**Table 63.25** Adverse reactions of interferons

**Local inflammatory reaction** at the site of application (30%)[a]

**Flu-like symptoms (90%)**
- Lassitude, malaise
- Fever
- Myalgias
- Arthralgias
- Headaches

**Cardiovascular (10%)**
- Chest pain
- Edema
- Hypertension
- Hypotension
- Heart failure
- Arrhythmias (primarily supraventricular tachycardia)
- Cyanosis

**Psychiatric-neurologic**
- Depression, suicidal tendencies (15%)
- Fatigue (90%)
- Headache (50%)
- Dizziness (20%)
- Irritability (15%)
- Insomnia (15%)
- Confusion (10%)
- Hallucinatory psychosis
- Paresthesias (7%)
- Epileptic seizures
- Tinnitus, hearing loss
- Peripheral neuropathy
- Cramps of calf muscles

**Dermatologic**
- Maculo-papular rash (7–18%)
- Alopecia (20%)
- Pruritus (13%)
- Diffuse erythema
- Urticaria

**Endocrine and metabolic**
- Hypocalcemia (10–50%)
- Hyperglycemia (33–39%)
- Hyperphosphatemia (2%)
- Hyponatremia (SIADH[b]) (< 1%)
- Hypertriglyceridemia (< 1%)

**Gastrointestinal and hepatic**
- Anorexia (30–70%)
- Nausea (30–50%)
- Vomiting (generally mild; 10–30%)
- Diarrhea (22–34%)
- Elevation of AST (80%)
- Elevation of bilirubin (30%)
- Elevation of alkaline phosphatase (48%)
- Dysgeusia to loss of taste (13%)
- Pancreatitis (< 5%)
- Xerostomia
- Abdominal cramps
- Flatulence

**Hematologic**
- Bone marrow suppression (30–70%)
- Granulocytopenia (30–70%)
- Thrombocytopenia (22–70%)
- Anemia (25–65%)

**Immunologic**
- Exacerbation of autoimmune diseases, e.g. thyroiditis
- Formation anti IFN antibodies

**Urogenital**
- Proteinuria (15–25%)
- Interstitial nephritis, nephrotic syndrome
- Erectile dysfunction (6%)
- Irregularities of menstrual bleeding

**Pulmonary**
- Cough (27%)
- Oropharyngeal irritation (14%)
- Dyspnea (7%)
- Bronchiolitis obliterans organizing pneumonia (cryptogenic organizing pneumonia)
- Interstitial pneumonia

**Ocular**
- Retinopathy
- Subconjunctival and retinal hemorrhages
- Loss of vision
- Conjunctivitis

**Ear, Nose and Throat**
- Epistaxis (4%)
- Rhinitis (3%)

[a]The numbers in parenthesis relate to the frequency of side effects
[b]SIADH syndrome of inappropriate ADH secretion

*cirrhosis and progression to HCC, and prolongs survival* [62, 151–153, 179, 237]. Thus, responders to IFN have an improved long-term prognosis with a significant reduction of liver-related mortality. However, IFN therapy lacks efficacy in approximately 40–60% of patients (non-responders). In contrast to chronic hepatitis C adding ribavirin to IFN does not increase the efficacy of IFN in patients with chronic hepatitis B [155].

The therapeutic response to standard IFN is modulated by a variety of factors and influenced by genetic polymorphisms in IFN metabolic pathways [119]. HBV genotypes are independent predictors of IFN responsiveness. Sustained response rates to standard IFN are better in patients with genotype A than those

**Table 63.26** Contraindications for interferons

- Decompensated liver cirrhosis (Child-Pugh B or C)
- Marked thrombo- (< 50,000/μl) and/or leukopenia (< 2000/μL)
- Cerebral seizures
- Depression
- Pregnancy
- Immunosuppression (AIDS, organ transplantation, drugs)
- Autoimmune diseases (e.g. thyroiditis, autoimmune hepatitis)
- Heart diseases (heart failure, coronary heart disease, especially tachycardic arrhythmias)
- Marked renal insufficiency
- Severe bacterial infections

**Table 63.27** Predicitive factors for response to interferon therapy

**Positive determinants of response** (response rate 40%; in 80% of responders response is durable)

- Short duration of chronic hepatitis B
- Infection acquired in adult age
- High pre-treatment serum ALT levels (> 5 × ULN)
- Low viral load
- Active liver disease
- Female gender
- HBV genotype A
- White ("Caucasian")

**Negative determinants of response**

- Long duration of chronic hepatitis B
- Perinatally acquired HBV infection
- Advanced cirrhosis (Child-Pugh stage B or C)
- High viral load
- HBeAg negative chronic hepatitis
- Low ALT levels (≤ 2 × ULN)
- Male gender
- HBV genotype D
- Asian extraction
- Immunosuppression (e.g. HIV infection)
- Coinfection with HDV or HCV

with D, and with genotype B better than those with C [57, 275]. In a multicenter study, the overall response rates according to HBV genotype were: genotype A, 47%; genotype B, 44%; genotype C, 28%; and genotype D, 25% [111].

In order to obtain optimal treatment results, patients receiving IFN therapy must be carefully selected. *IFN is most likely to benefit patients with replicative infection and active liver disease*. HBsAg positive, HBeAg negative, HBV DNA negative patients with normal serum ALT values are carriers with no evidence of active virus replication or ongoing liver damage and should not be treated. HBV DNA positive patients with decompensated cirrhosis carry a high risk of IFN-induced serious infections and hepatic failure and therefore should not receive IFN. Thirty to 50% of patients treated with IFN exhibit a transient rise in serum aminotransferase levels. This flare should not be misinterpreted for relapse of hepatitis and erroneously lead to discontinuation of IFN therapy. Instead, IFN treatment should be continued, since the rise in aminotransferases in this case is evidence of IFN induced stimulation of immune response with consequent lysis of virally infected hepatocytes.

In the Mediterranean Basin, Middle and Far East about 20% of HBsAg carriers with antibodies against HBeAg have detectable serum levels of HBV DNA and necroinflammation on liver biopsy. These anti HBe positive patients with chronic HBV infection should receive IFN [14].

Table 63.27 summarizes the predictive factors for response to IFN therapy.

The major limitations of immmunomodulatory therapy with IFN, in addition to its lack of efficacy in 40–60% of patients, are the numerous contraindications, the profile of untoward effects, and the fact that it cannot be given to patients with decompensated liver cirrhosis.

In 2003 *pegylated interferon* (pegIFN) has been demonstrated to be effective in patients with chronic hepatitis B [46]. Both pegIFNα-2a and pegIFNα-2b are safe and effective in HBeAg positive and HBeAg negative chronic hepatitis B [16, 17, 46, 111, 137, 166]. Non-responders to standard IFN or lamivudine may be successfully treated with pegIFN [68]. Sustained HBeAg seroconversion 6 months after the end of 48 weeks of therapy with pegIFN is seen in approximately 30% of patients [137]. The factors predictive of a response to pegIFN correspond to those reported in Table 63.27 for standard IFN [11]. Quantitative HBeAg determination may be a useful adjunctive measurement for predicting HBeAg seroconversion in patients treated with pegIFN [73].

Patients with HBV genotype A have higher HBeAg seroconversion rates than those with genotype D, and those with genotype B higher than those with genotype C following treatment with pegIFN [46]. HBsAg seroconversion was observed in 7% of patients infected with HBV genotype A after 1 year of pegIFN therapy [69]. However, taken together, data regarding the reponse of various HBV genotypes to pegIFN are limited and conflicting, and whether HBV genotypes (as in treatment

with standard IFN) correlate with the response to pegIFN therapy awaits further examination [154].

There are some some data to suggest that combination therapy with pegIFN and adefovir may lead to marked decreases in serum HBV DNA and in intrahepatic cccDNA levels [253]. The evidence, however, still is too weak to recommend this regimen.

Virus induced flares, which occur after an increase in HBV DNA level and most probably are indicative for increased expression of viral antigens, do not lead to treatment response. In contrast, host induced flares, which are followed by a decrease in HBV DNA levels, are associated with treatment response [67].

Discontinuing pegIFN is generally necessary in less than 10% of patients, and the most frequent reasons for early discontinuation of therapy are psychiatric side-effects (depression, psychosis) and flu-like symptoms.

## Nucleosides and Nucleotides

### Lamivudine

Lamivudine (LAM), a nucleoside analog, the (−) enantiomer of 2',3'-dideoxy 3'-thiacytidine (3TC), originally used for the treatment of patients with HIV infection, was the first oral antiviral agent licensed for the treatment of chronic hepatitis B.

**Mechanism of action**. Lamivudine is phosphorylated to the triphosphate (3TC-TP) which competes with dCTP for incorporation into growing DNA chains, causing chain termination. LAM inhibits the reverse transcriptase action of HBV DNA polymerase thus inhibiting viral replication. LAM monophosphate is incorporated by HBV DNA polymerase into the viral DNA, resulting in DNA chain termination.

Lamivudine also may exert immunologic effects and can reverse the T cell hyporesponsiveness to HBV, restoring antiviral T cell activity [10]. This immunologic effect, however, is probably of subordinate importance compared to its direct antiviral activity.

**Pharmacology**. After oral application LAM is well absorbed. Ingestion of food does not affect the AUC. Protein binding is < 36%, the half-life in adults is 5–7 h. LAM is eliminated by the kidneys and is found unchanged in the urine.

**Interactions**. Trimethoprim/Sulfamethoxazole reduces the renal clearance of LAM. Concomitant use of ribavirin and nucleoside analogs may increase the risk of developing hepatic decompensation or other signs of mitochondrial toxicity, including pancreatitis or lactic acidosis.

**Adverse reactions**. Lamivudine 100 mg once daily is very well tolerated, the side effects being comparable to placebo. Long term treatment for up to 6 years has an excellent safety profile in patients with compensated liver disease [157]. Pancreatitis, lactic acidosis and neuropathy seen in lamivudine treated HIV patients are extremely rare events in lamivudine treated patients with chronic hepatitis B. This is probably related to the higher lamivudine doses administered in HIV infected patients. In children lamivudine may impair glucose metabolism as determined by the oral glucose tolerance test [58].

*Upon abrupt discontinuation of lamivudine acute exacerbation of hepatitis B may occur*. Flares of hepatitis during therapy should not be confused with side effects of lamivudine (check for lamivudine resistant mutants).

**Contraindications**. Hypersensitivity to lamivudine or any component of the formulation. Low dose (100 mg daily) lamivudine monotherapy of chronic HBV infection in an HIV infected patient is contraindicated, since it will lead to rapid development of HIV lamivudine resistance.

**Dosing**. 100 mg p.o. qd. The dose of lamivudine should be increased to 150 mg twice daily in patients who have HIV coinfection to prevent the development of HIV resistance. Dose adjustment is required in renal insufficiency (Table 63.28).

**Duration of treatment**. In *HBeAg positive patients* duration of therapy usually is 1 year. If HBeAg seroconversion does not occur, therapy should be extended, monitoring for the emergence of drug resistant mutants. For almost all patients with *HBeAg negative chronic hepatitis B* a year of therapy is insufficient. In the absence of emergence of lamivudine resistant mutants, treatment should be continued for several years, regularly monitoring serum ALT levels and HBV DNA concentration, and the appearance of viral resistance,

**Table 63.28** Dose adjustment of lamivudine to renal function (creatinine clearance)

| ClCr (mL/minute) | Lamivudine-initial dose (mg p.o. qd) | Lamivudine-subsequent doses (mg p.o. qd) |
|---|---|---|
| ≥ 50 | 100 | 100 |
| 30–49 | 100 | 50 |
| 15–29 | 100 | 25 |
| 5–14 | 35 | 15 |
| < 5 | 35 | 10 |

in which case treatment must be modified (see below). There are no guidelines regarding the intervals of follow up examinations. It seems reasonable to monitor ALT every 4–6 weeks for the first 6 months of therapy, every 3 months during the following 6 months and thereafter once or twice yearly. HBV DNA levels should be measured 12 and 24 weeks after the start of antiviral therapy and thereafter every 3–6 months. HBeAg and anti-HBe (in HBeAg positive patients) should be determined 12 months after initiating therapy, thereafter patients should be assessed every 6 months for HBeAg seroconversion.

Discontinuation of lamivudine therapy should be considered 6 months after HBeAg seroconversion has been confirmed on repeated testing. If lamivudine therapy is discontinued too soon, HBeAg may recur (seroreversion).

In HBeAg negative patients, however, relapse after discontinuation is common even after sustained suppression of serum HBV DNA levels to undetectable levels.

**Results of treatment**. LAM leads to a significant reduction of serum HBV DNA and serum cccDNA levels, to increased rates of HBeAg loss, HBeAg seroconversion and ALT normalization, and to a decrease in necroinflammatory activity and fibrogenesis [52–54, 124, 194, 267]. Continuous treatment with LAM delays clinical progression in patients with chronic hepatitis B and advanced fibrosis or cirrhosis by significantly reducing the incidence of hepatic decompensation and the risk of HCC [149]. In IFN nonresponders lamivudine maintains its antiviral activity and is as effective as in previously treatment-naïve patients [207].

In *HBeAg positive patients* at the end of 1 year treatment, LAM leads to normalization of serum ALT levels in 50–70% of patients, HBeAg loss is observed in approximately 30% with HBeAg seroconversion (appearance of antiHBe) occurring in 16% of patients [52].

In *HBeAg negative patients* HBV DNA loss and ALT normalization are seen in 65–96%, and histologic improvement is observed in approximately 60% of patients after 12 months of therapy. After discontinuing treatment, relapse occurs in 48–90% of patients. Only 40% of patients achieve a virological response after 4 years of therapy due to the emergence of LAM-resistant mutants [88, 200, 223].

In contrast to IFNs, *lamivudine may also be administered to patients with decompensated cirrhosis* (Child-Pugh stage B or C) irrespective of the patient's HBeAg status. *Patients with decompensated cirrhosis with detectable HBV DNA in serum should be placed on antiviral therapy* irrespective of the HBeAg status. In 60–70% of patients with decompensated cirrhosis lamivudine treatment leads to improvement in liver function [92, 163]. Despite these beneficial effects, it is questionable whether or not lamivudine treatment reduces the need for liver transplantation in patients with clinically far advanced, decompensated HBV cirrhosis.

The data on the efficacy of lamivudine therapy in patients with hepatic decompensation due to severe *acute exacerbations of chronic hepatitis B* are conflicting. It appears that lamivudine may be beneficial, provided therapy is begun early enough, before serum bilirubin level rises markedly > 20 mg/dL [37, 231].

Important *predictors of response* to lamivudine therapy are the histologic activity of disease, the pretreatment ALT levels, the magnitude of decline in HBV DNA concentration, the duration of therapy, the age of the patient and HBV genotype. High levels of necroinflammation in liver biopsy are associated with a higher probability of initial virological and biochemical response. Rates of HBeAg seroconversion correlate with the pretherapeutic serum ALT levels [36]. High baseline ALT levels (> 5 × ULN) are associated with seroconversion rates of approximately 42%, whereas in patients with ALT levels < 2 × ULN seroconversion rates range between 2% and 7% [186].

The HBeAg seroconversion rate is also determined by the magnitude of the initial viral load decline and by the duration of therapy. A pronounced initial response, characterized by a decrease in serum HBV DNA levels at week 12 of therapy by at least 1 $\log_{10}$ copies/mL compared with the baseline value has been reported to be associated with increased response rates [201]. Recent data suggest that even earlier measurements of HBV DNA may predict the long-term outcome of lamivudine therapy. A fall of HBV DNA concentration to levels of less than 4 log copies/mL (2,000 IU/mL) at week 4 of lamivudine treatment predicts a favorable outcome, while the addition of or switch to an alternative antiviral agent should be considered for patients who fail to achieve this early target [269]. Continuing lamivudine treatment for 3–5 years may lead to an increase in HBeAg seroconversion in 30–50% of patients. However, prolonged therapy is limited by the development of lamivudine resistance in a progressively increasing number of patients. Thus,

the potential benefit of extending treatment beyond 1 year may be offset by the selection of resistant mutants, negating the initial benefits of therapy. In addition, HBV genotype and patient age seem to be important factors in determining sustained HBeAg response. Patients with genotype A and B and those younger than 36 years have higher HBeAg seroconversion rates than patients with genotype C and D [38, 111].

The *durability of HBeAg seroconversion* to anti HBe is sustained in only 30–80% of cases after lamivudine is stopped [52, 150]. The sustained response rate following lamivudine treatment is significantly lower than that following IFN. The durability of response is particularly low in patients with HBV genotype C infection, in older patients and if treatment is maintained for less than 4–8 months after HBeAg seroconversion [38, 140, 216, 236]. Lamivudine retreatment for reappearance of hepatitis B markers can achieve resumption of viral suppression [53].

The pretherapeutic viral load is a predictor of *viral breakthrough* during treatment. Patients with baseline serum HBV DNA concentrations of greater than $10^6$ copies/mL are more likely to develop virologic breakthrough because of drug-resistant mutations compared to patients with low replicative chronic HBV infection [163]. There is also a direct correlation between the level of HBV DNA reduction achieved during nucleos(t)ide treatment and the probability of development of drug resistance, i.e. *a rapid and profound viral suppression is associated with a lower probability of drug resistance and viral breakthrough*.

The *clinical outcome* is related to the virological response. Loss of virological response or treatment withdrawal leads to clinical deterioration in approximately 30% patients after 18 months [78]. HBeAg negative cirrhotic patients with a sustained decrease of serum HBV DNA to undetectable levels by PCR are significantly less likely than those with viral breakthrough to develop decompensated cirrhosis or HCC. Maintaining virological response leads to a longer survival in patients with Child-Pugh stage A cirrhosis compared to patients with virological breakthrough [51]. Overall, in patients who do not develop resistant viral mutants long-term lamivudine therapy significantly improves survival and reduces the risk of major complications of chronic hepatitis B and cirrhosis [182].

*Abrupt discontinuation of treatment* should be avoided, since it can provoke necroinflammatory flares and in rare cases fatal acute liver failure. 90% of these flares occur withing 6 months after stopping lamivudine therapy. In many patients on lamivudine the appearance of YMDD mutants is heralded by hepatitis flares. Such flares may be more severe than those observed during the natural course of wild-type HBV chronic infection and may be associated with hepatic decompensation. Although such hepatitis flares may be followed by HBeAg seroconversion and HBV seroclearance, new and distinct mutant HBV may also be selected and other hepatitis exacerbations may be elicited.

### Adefovir Dipivoxil

Adefovir dipivoxil (ADV) is an acyclic nucleotide (adenosine) analog.

**Mechanism of action**. ADV inhibits RNA-dependent HBV DNA polymerase resulting in inhibition of viral replication.

**Pharmacology**. Adefovir is a prodrug that is rapidly converted in the intestine to its active metabolite ADV. Bioavailability after oral application is 59%, protein binding is ≤ 4%. Peak concentrations in plasma are reached 1.7 h after ingestion. Half-life is 7.5 h. ADV is excreted by the kidney with 45% recovered in urine as active metabolite within 24 h.

**Interactions**. Aminoglycosides, vancomycin, NSAID and other potentially nephrotoxic medications may increase the risk of renal damage due to ADV. Concomitant use of ribavirin and nucleoside analogs may increase the risk of mitochondrial toxicity and result in hepatic decompensation, pancreatitis or lactic acidosis.

**Adverse reactions**. ADV is well-tolerated and for a majority of adverse reactions the incidence is similar or even less than that observed in patients treated with placebo. The most common adverse events are asthenia, headache, and abdominal pain. An increase in serum ALT levels and the occurrence of hematuria (11% grade ≥ 3) are seen especially in patients with daily doses greater than 10 mg. The most serious side effect is renal dysfunction (rise in serum creatinine, glycosuria, renal insufficiency), which occurs in fewer than 10% of patients. In liver transplant patients with baseline renal dysfunction, prevalence of increased serum creatinine has been observed to be as high as 32–53%. However, it is doubtful whether all these changes in the liver transplant patient can be attributed to ADV. Upon abrupt discontinuation acute exacerbation

of hepatitis B may occur.

**Contraindications**. Hypersensitivity to ADV or any component of the formulation.

**Dosing**. 10 mg p.o. qd. In renal insufficiency the dose has to be adjusted according to the creatinine clearance (ClCr):

ClCr 20–49 mL/min: 10 mg every 48 h
ClCr 10–19 mL/min: 10 mg every 72 h
Hemodialysis: 10 mg every 7 days (following dialysis)

**Results and duration of treatment.** ADV monotherapy, 10 mg daily for 48 weeks, is safe and effectively suppresses HBV replication in patients with HBeAg positive and HBeAg negative chronic hepatitis B [89, 90, 165, 247, 274]. Compared to placebo ADV leads to a significant decrease in the necroinflammatory activity, to improvement of fibrosis, to significant reduction in serum HBV DNA levels, loss of HBeAg and increased HBeAg seroconversion rates in HBeAg positive patients, and to normalization of serum ALT values after 48 weeks of therapy [89, 90, 165, 274].

The initial drop of HBV DNA titer in serum at week 12 of ADV therapy appears to be predictive of subsequent HBeAg seroconversion. The ADV induced decrease in serum HBV DNA levels seems to be independent of HBV genotype [249]. Although the initial antiviral effect of ADV seems to be slower and the degree of HBV DNA reduction less than with LAM and entecavir, its antiviral effect increases over time and in the majority of patients HBeAg seroconversion is durable. Loss of HBsAg with appearance of antiHBs during ADV therapy is very rare (1.6%).

The optimal duration of therapy is unknown, but higher rates of HBeAg seroconversion (up to 43% after 3 years) can be achieved by continuing treatment. Extending treatment for 4–5 years is associated with maintained virus suppression, ALT normalization, and improved liver histology in 65 to 80% of patients. As with other nucleos(t)ide analogs, abrupt discontinuation of treatment should be avoided. In up to 25% of patients a flare up of hepatitis (ALT > 5–10 × ULN) will occur within 3 months after abrupt stop of ADV treatment.

In *HBeAg positive patients* treatment with ADV beyond 48 weeks is well-tolerated and produces long-term virological, biochemical, serological, and histological improvement [168]. Therapy may be stopped 6 months after HBeAg seroconversion has been achieved ("consolidation phase").

**Table 63.29** Comparison between interferon α, lamivudine und adefovir dipivoxil in patients with HBeAg positive chronic hepatitis B

| Parameter | Interferon (untreated) | Lamivudine (Placebo) | Adefovir Dipivoxil (Placebo) |
|---|---|---|---|
| | 12–24 weeks | 52 weeks | 48 weeks |
| **HBV DNA loss** (%)[a] | 37 (17) | 44 (16) | 21 (0) |
| **HBV DNA reduction** ($\log_{10}$) | No data | No data | 3,52 log (0,55) |
| **HBeAg loss** (%) | 33 (12) | 32 (11) | 24 (11), 44 after 72 weeks |
| **HBeAg seroconversion** (%) | 18[b] | 16–18 (4–6), 50 after 5 years | 12 (6), 23 after 72 weeks |
| **HBsAg loss** (%) | 11–25 after 5 years (in white patients) | Not enough data | Not enough data |
| **ALT normalization** (%) | 23[b] | 41–72 (7–24) | 48 (16) |
| **Durability of response after HBeAg seroconversion** (%) | 80–90 after 4–8 years | 77 after 3 years | No data |
| **Emergence of drug resistant mutants** | No | Yes | Yes (less often than lamivudine) |
| **Use in decompensated liver cirrhosis** | Contraindication | Possible | Possible |
| **Efficacy with normal ALT levels** | No | No | Probably no |
| **Efficacy in precore mutants** | Yes | Yes | Yes |
| **Efficacy with high viral load** | Less effective | Effective | Effective |
| **Abrupt discontinuation** | Possible without fear of acute exacerbation of hepatitis | Risk of acute exacerbation of HBeAg-positive chronic hepatitis | Risk of acute exacerbation of HBeAg-positive chronic hepatitis |

[a]Interferon and lamivudine, hybridization assay (lower limit of detection $10^5$ copies/mL); Adefovir dipivoxil PCR (lower limit of detection 400 copies/mL)
[b] Difference between treated and untreated

In patients with no HBeAg seroconversion but with satisfactory viral suppression (HBV DNA undetectable) ADV therapy should be extended for 3 to 4 (to 5) years. Monitoring renal function and the possible emergence of drug resistant mutants is mandatory.

Compared to HBeAg-positive patients it is difficult to establish when therapy can safely be discontinued in *HBeAg-negative patients*. If ADV treatment is stopped after 1 year, more than 90% of HBeAg negative patients will have a virological and biochemical relapse. Therefore, treatment of HBeAg negative patients should continue as long as HBV DNA suppression is achieved. Treatment for up to 5 years is well-tolerated and produces significant, increasing improvement in necroinflammation and hepatic fibrosis, durable suppression of HBV replication and normalization of ALT in 67–83% of patients [91].

In Table 63.29 important parameters of IFN, LAM and ADV effects in patients with chronic hepatitis B are summarized

### Entecavir

Entecavir (ENT) is a guanosine nucleoside analog with potent activity against HBV. The corresponding equivalent antiviral dose is approximately 20–100 times lower than that for adefovir dipivoxil and 200–1,000 times lower than the dose for lamivudine.

**Mechanism of action**. ENT is intracellularly phosphorylated to guanosine triphosphate, the active form, which competes with the natural substrate thereby suppressing effectively the activity of HBV polymerase (reverse transcriptase). ENT suppresses the activity of HBV polymerase at three levels: base priming, reverse transcription of the negative strand from the pregenomic messenger RNA and the synthesis of the positive strand of HBV DNA.

**Pharmacology**. The oral bioavailability of the solution is 100%. Solution and tablet can be used interchangeably. Food delays oral absorption. Peak levels in plasma are reached 0.5–1.5 h after oral administration. Protein binding is 13%. ENT undergoes minor hepatic conjugation with glucuronide and sulphate and does not inhibit or induce the cytochrome P450 enzyme system. The half-life is 5–6 days (risk of accumulation). Entecavir is predominantly excreted by the kidneys with 60–70% of the dose recovered as unchanged drug in the urine.

**Interactions**. Ganciclovir and valganciclovir may increase the adverse effects of entecavir and other nucleoside reverse transcriptase inhibitors. Concomitant use of ribavirin and nucleoside analogs may increase the risk of hepatic decompensation or other signs of mitochondrial toxicity, including pancreatitis or lactic acidosis.

**Adverse reactions**. ENT is well tolerated and the safety profile is similar to lamivudine. In approximately 10% of patients elevations of ALT are observed. In most patients mild-to-moderate adverse reactions including headache, upper respiratory tract infection, cough, nasopharyngitis, fatigue and upper abdominal pain are observed. However, the drug has to be discontinued because of side effects in only 1% of patients [215]. Upon abrupt discontinuation acute exacerbation of hepatitis B may occur.

**Contraindications**. Hypersensitivity to ENT or any component of the formulation.

**Dosing**. 0.5 mg p.o. qd on an empty stomach, i.e. at least 2 h before or after a meal. In lamivudine-resistant patients: 1 mg p.o. qd. Dose adjustment is required in renal impairment:

ClCr 30–49 mL/min: 50% of usual dose
ClCr 10–29 mL/min: 30% of usual dose
ClCr < 10 mL/min (including dialysis): 10% of usual dose (after hemodialysis).

**Results and duration of treatment.** Entecavir seems to be the most potent inhibitor of HBV replication, with a mean of 6.8 log10 and 5.0 log10 reduction in HBV DNA levels at 1 year in HBeAg-positive and HBeAg-negative patients, respectively.

ENT 0.5 mg daily is superior to LAM 100 mg daily with regard to rates of histologic improvement, mean reduction in serum HBV DNA levels, undetectable serum HBV DNA levels (determined by PCR) and normalization of serum ALT levels in chronic hepatitis B patients irrespective of the HBeAg status. HBeAg seroconversion rates following short-term treatment do not exceed those achieved by lamivudine. At week 48 the rate of HBeAg-seroconversion is similarly low in entecavir and lamivudine treated HBeAg-positive patients, 21% and 18%, respectively [31, 128, 129].

The optimal duration of treatment is unknown. Entecavir treatment through 96 weeks results in continued benefit for patients with HBeAg-positive chronic hepatitis B, and long-term treatment is probably required to obtain and sustain virological, histological and clinical benefits [83]. No evidence of viral resistance

to entecavir is to be expected during a relatively short treatment period of 48–52 weeks. The emergence of entecavir resistance over a 2-year treatment period in previously nucleoside naïve patients is also rare, occurring mostly in patients with lamivudine resistant HBV variants [45]. Long-term treatment with entecavir will probably lead to the development of appreciable viral resistance albeit at a reduced rate compared to lamivudine. Entecavir resistant strains are sensitive to adefovir.

### Telbivudine

Telbivudine is a synthetic thymidine nucleoside analog with strong activity against HBV.

**Mechanism of action**. Telbivudine inhibits HBV DNA polymerase. It is phosphorylated by cellular kinases to the active triphosphate form. Incorporation of telbivudine 5'-triphosphate into viral DNA causes DNA chain termination, which results in inhibition of HBV replication.

**Pharmacology**. Absorption is not affected when administered with food. Peak serum concentrations are achieved 1–4 h after an oral dose. Telbivudine is neither a substrate nor an inhibitor of the cytochrome P450 enzyme system. It is eliminated primarily by renal excretion of unchanged drug. The terminal elimination half-life is 40–49 h.

**Interactions**. Drugs that alter renal function may alter plasma concentrations of telbivudine.

**Adverse reactions**. Abdominal pain, arthralgia, back pain, cough, nausea, vomiting, loose stools, influenza-like symptoms, and insomnia may occur. Occasionally a peripheral neuropathy was observed in patients treated with telbivudine. The risk of peripheral neuropathy is increased when telbivudine is combined with pegylated interferon.

**Contraindications**. Sensitivity to telbivudine or to any component of the product.

**Dosing.** 600 mg p.o. qd with or without food. In patients with renal impairment:

ClCr ≥ 50 mL/min: no dosage adjustment is necessary.
ClCr 30–49 mL/min: 600 mg p.o. once every 48 h.
ClCr < 30 mL/min (not requiring dialysis): 600 mg p.o. once every 72 h.
ClCr < 30 mL/min (requiring dialysis): 600 mg p.o. once every 96 h (to be administered after hemodialysis).

**Results and duration of treatment**. Telbivudine is one of the most potent antiviral agents for HBV currently available, and there is evidence for marked dose-related antiviral activity [126]. Data from phase 2 trials indicate that telbivudine is more effective than lamivudine with improved tolerability [265]. Among patients with HBeAg-positive chronic hepatitis B, the rates of therapeutic and histologic response at 1 year are significantly higher in patients treated with telbivudine than in patients treated with lamivudine. In both the HBeAg-negative and the HBeAg-positive groups, telbivudine demonstrates greater HBV DNA suppression with less resistance than does lamivudine [96, 125]. The efficacy of telbivudine for HBV strains resistant to either lamivudine or adefovir has not been clearly defined.

There are presently no large scale studies investigating the optimal duration of treatment with telbivudine. However, it can be deduced from the experience with other nucleoside analogs that long-term treatment will be optimal, provided the drug is tolerated and no viral resistance develops.

### Tenofovir, Emtricitabine, Clevudine

Tenofovir, emtricitabine and clevudine are also potent anti HBV drugs, although the latter two have not yet been approved by the FDA for the treatment of chronic hepatitis B.

**Tenofovir**. Tenofovir is an acyclic nucleotide analog of adenosine monophosphate. It is available as a prodrug, tenofovir disoproxil fumarate (TDF).

Tenofovir inhibits viral reverse transcriptase and acts as a DNA chain terminator. After uptake by cells TDF undergoes phosphorylation to its active metabolite TDF diphosphate that inhibits RNA- and DNA-directed reverse transcriptase and causes premature DNA termination.

Oral bioavailability of tenofovir from TDF 300 mg is 25% in the fasted state. Administration of TDF following a high-fat meal (roughly 700–1,000 kcal containing 40–50% fat) increases the oral bioavailability. Protein binding of tenofovir is negligible. The intracellular half-life ranges between 15–50 h. Following a single, orally administered dose, the terminal

elimination half-life of tenofovir is approximately 17 h. Neither TDF nor tenofovir are substrates of cytochrome P450 enzymes. TDF is eliminated by a combination of glomerular filtration and active renal tubular secretion. Seventy to 80% of the dose is recovered in the urine as unchanged drug.

The usual daily dose is 300 mg p.o. with or without food, but recent data show that low-dose TDF monotherapy (75 mg qd) can also control HBV viremia for an extended period of time without the emergence of viral resistance [50]. TDF at a daily dose of 300 mg has superior antiviral efficacy with a similar safety profile as compared with ADV at a daily dose of 10 mg [168a].

TDF is effective against LAM-resistant HBV. In patients with LAM-resistant hepatitis B, treatment with tenofovir is well-tolerated and results in significant virological, serological, and biochemical improvements on par with those seen with high-dose ADV (30 mg/day) therapy, without the complication of renal toxicity (see below) [123]. TDF may be particularly useful as part of combination therapy in HBV-HIV coinfected LAM resistant patients [6, 7, 189]. If treatment for HIV infection with tenofovir is discontinued in a patient co-infected with HBV, the patient should be closely monitored for several months for signs and symptoms of worsening hepatitis infection.

Tenfovir is also effective in HBV patients with virologic breakthrough or suboptimal response to ADV in the absence of ADV-resistant mutations [221]. Due to its antiviral efficacy, its safety profile and the lack of resistant mutations at least up to week 48 of treatment TDF has the potential to be used as a first-line drug in patients who have not received treatment.

**Emtricitabine.** Emtricitabine (also referred to as FTC) is a nucleoside analog of cytosine. It is phosphorylated by cellular enzymes to emtricitabine 5'-triphosphate, inhibits viral reverse transcriptase and acts as a chain terminator of nascent viral DNA.

The optimal dose appears to be 200 mg po qd. The mean absolute bioavailability after an oral dose is 93% and peak plasma concentrations occur at 1–2 h post-dose. Its plasma half-life is approximately 10 h, but the metabolite, emtricitabine-triphosphate, has an intracellular half-life of approximately 39 h. Food has no clinically significant effect on emtricitabine's systemic exposure.

The drug does not undergo hepatic enzyme metabolism, and is excreted renally.

Emtricitabine 200 mg once daily is well tolerated and demonstrates a potent antiviral response for up to 2 years in patients with chronic hepatitis B infection [82]. Safety and efficacy of treatment have not yet been established in patients co-infected with HBV and HIV.

**Clevudine.** Clevudine is a new pyrimidine analog with potent anti-HBV activity in vitro.

A dose-escalation study evaluated clevudine at 10, 50, 100, and 200 mg once daily for 28 days. The drug was well tolerated, with no dose-limiting toxicities [167] A dose of 30 mg p.o. qd shows potent and sustained antiviral efficacy in patients with HBeAg-positive and HBeAg-negative chronic hepatitis B [259, 260]. Importantly, the suppression of antiviral activity is maintained at 12 and 24 weeks post treatment [101].

## Combination Therapy

The rationale for antiviral combination therapy is to increase efficacy of treatment by additive or synergistic antiviral effects and to delay, reduce or prevent the selection of drug resistant mutants.

Significantly higher rates of sustained viral response (HBV DNA nondetectable 6 months after end of treatment) have been reported with pegIFNα than with lamivudine. In HBeAg-positive patients the post-therapeutic rates of HBeAg seroconversion with pegIFNα compared to lamivudine were 32% and 19%, respectively. The combination of pegIFNα with lamivudine did not yield better results than monotherapy with pegIFNα [137]. In patients with chronic HBeAg-negative hepatitis the rate of undetectable HBV DNA 24 weeks after discontinuation of therapy with pegIFNα and lamivudine was 19% and 7%, respectively. Again the combination of pegIFNα with lamivudine did not improve the results of monotherapy with pegIFNα [166]. The response rates may vary by HBV genotype (A 47%, B 44%, C 28%, D 25%), but there is no evidence that the combination with lamivudine confers additional benefit to monotherapy with pegIFNα [111, 207, 220, 239]. Thus, *adding lamivudine to pegIFN does not improve the results of pegIFN monotherapy*. There are indications, however, that combination therapy may delay the selection of lamivudine-resistant variants and may reduce viral breakthrough during long-term lamivudine therapy [109, 111, 180]. At present the combined use of pegIFNα and oral antivirals is not recommended in clinical practice outside of clinical trials.

Adefovir dipivoxil may be combined with lamivudine after the emergence of lamivudine resistant mutants. Combination of lamivudine and ADV in treatment naïve patients is not associated with an antiviral, biochemical or serologic benefit compared to monotherapy. The seroconversion rate achieved by combination therapy is not better than that by LAM monotherapy [219]. However, the emergence of lamivudine resistant mutants and the rates of viral breakthrough are higher in patients receiving lamivudine monotherapy.

The combination of ADV plus emtricitabine resulted in more potent suppression of HBV DNA over 96 weeks of therapy. More patients in the combination group had normalization of ALT and HBV DNA < 300 copies/mL at week 96 when compared with the ADV monotherapy group [103]. Further studies are required before ADV/FTC combination therapy can be recommended.

The combination of lamivudine and famciclovir is effective in suppressing HBV replication [210]. However, there is no information yet whether both drugs combined reduce or delay the emergence of resistant viral mutants.

Telbivudine exhibits a significantly greater virological and biochemical response than lamivudine. The efficacy of a combination of both antivirals is similar to that of telbivudine alone [127].

The strategy of sequential, staggered therapy, using an antiviral initially to decrease HBV DNA levels before adding pegIFNα is appealing. Preliminary results suggest that the sustained viral response rates are higher and the relapse rates after discontinuation therapy might be lower than after lamivudine monotherapy [25, 26, 205, 206].

Another novel approach for treating patients with chronic hepatitis B aims at combining the antiviral effect of lamivudine with the immune modulator activity of vaccine therapy. First results in a small group of patients were encouraging with HBeAg seroconversion rates of 56% and no viral breakthrough after 1 year of combination therapy [95].

## Special Therapeutic Problems

### Liver Cirrhosis

Patients with Child's class A cirrhosis may benefit from treatment with IFN-α or pegIFN, thereby reducing the rate of decompensation and obviating or delaying the need for liver transplant. Patients with Child's class B or C cirrhosis have minimal benefit from IFN therapy, and IFN induced ALT flares may ignite necroinflammatory activity, precipitate hepatic decompensation and potentially cause liver failure. In addition IFNs may deteriorate the already existing leukopenia and thrombocytopenia, and increase the risk of bacterial infections (e.g. spontaneous bacterial peritonitis) or of bleeding complications. IFNs are contraindicated in decompensated cirrhosis.

Lamivudine is well tolerated in patients with decompensated liver cirrhosis and can stabilize or improve liver function. ADV has not been evaluated in patients with decompensated liver cirrhosis. Studies with entecavir are ongoing.

Both entecavir and adefovir are effective in patients with compensated HBV cirrhosis. In patients with HBV cirrhosis with previous lamivudine resistance, "adefovir salvage" appears more effective and less expensive than "entecavir salvage" [113].

### Coinfection with Other Viruses

The primary endpoint of treatment in patients with *HBV/HDV* coinfection is the suppression of HDV replication. Currently, the only approved treatment of chronic hepatitis D is IFN-α. 9 MU 3 times a week (or pegIFN once weekly) for 1 year is recommended. The addition of ribavirin to pegIFN does not improve outcome. Lamivudine is ineffective in inhibiting HDV replication.

Due to limited information firm recommendations on treatment of *HBV/HCV* coinfected patients cannot be made presently [159]. In one study the combination therapy with pegIFN-a2b and ribavirin was highly effective in inducing a virological response concerning HCV in patients with HBV/HCV coinfection. However, HBV replication may increase after the clearance of HCV and thus both viruses must be closely monitored even in patients with initially undetectable HBV-DNA [193].

The decision to treat HBV infection in *HBV/HIV* coinfected patients should be based on whether or not HIV treatment is ongoing or planned. HBV infection should be treated with pegIFNα, adefovir or entecavir in patients in whom HAART is not planned in the near future. Monotherapy with lamivudine should be

avoided in these patients, since it leads to selection of HIV resistant mutants. If HAART is planned, a regimen containing drugs active against both viruses should be used. Lamivudine, emtricitabine, entecavir and tenofovir have activity against both HIV and HBV. Lamivudine plus tenofovir or emtricitabine plus tenofovir are preferred. ADV has negligible activity against HIV. The rate of HBV resistance to lamivudine in HBV/HIV coinfected patients is high, reaching 90% at 4 years. In patients resistant to lamivudine adding tenofovir is the preferred option. Patients who are already on HAART that does not include a drug active against HBV may be treated with pegIFNα, adefovir or entecavir [159].

### HBV-Induced Glomerulonephritis

HBV-induced glomerulonephritis, with the exception of membranous nephropathy only rarely remits spontaneously. Corticosteroids and cytotoxic agents for HBV-induced glomerular disease are of little benefit, and may increase viral persistence and replication. Uncontrolled data suggest that IFNα (5 MU s.c. qd for 16 weeks) is beneficial, and patients who show HBeAg clearance will also have an improvement in their renal function. Treatment with oral antivirals, for example lamivudine and entecavir, (dose should be adjusted for level of kidney function; adefovir should probably be avoided because of potential nephrotoxicity) that is associated with diseappearance of HBV DNA also will result in a reduction in proteinuria. The optimal choice of an agent and duration of therapy in HBV-induced glomerulonephritis are unclear.

### Children

The response rates to IFNα of children in central Europe and in the USA with active HBV infection and elevated aminotransferases correspond to those of adult patients. However, in Asia most children acquire HBV infection perinatally, and have normal ALT levels. Fewer than 10% of these children respond to IFNα by clearing HBeAg.

Lamivudine is safe and effective in children. With increasing duration of treatment, however, the number of drug resistant mutants increases up to 64% of patients after 3 years of treatment [214].

### Asian Patients

Asian patients usually respond poorly to IFNs and oral antivirals [140, 141, 216]. The rates of durable responses are lower, and relapses more frequent. This probably is due to a higher percentage of immunotolerant patients with perinatally acquired HBV infection. A frequent profile, found particularly in Asian children infected perinatally consists of HBeAg positivity, elevated serum levels of HBV DNA and normal ALT values. The response rate in this group of patients is less than 10% and treatment is not warranted. However, the response in patients with elevated ALT is similar to that in Caucasian patients. Therefore, Asian patients with elevated ALT values should be offered IFN treatment.

### Liver Transplantation (see Chapter 103)

The precore stop mutation does not appear to influence the posttransplantation outcome, and no significant difference in posttransplantation HBV recurrence (47–57%), graft failure (20–23%), or death are observed in patients harboring HBV with or without precore stop mutants.

Surface gene mutations have been noted in patients after liver transplantation who exhibit HBV recurrence despite receiving hepatitis B immune globulin (immune escape mutant).

**Fibrosing cholestatic hepatitis (FCS)**. FCS is a severe, rapidly progressive variant of chronic hepatitis B and C in the transplanted liver. It is due to HBV reactivation during immunosuppressive therapy and "flooding" of the transplanted hepatocytes with HBsAg. The disease is characterized histologically by the absence of a marked necroiinflammatory reaction. Instead portal fibrosis with periportal fibrous septa extending into the liver lobule stand out (see Fig.63.36). In addition, extensive ductular reaction, hepatocyte ballooning and bilirubinostasis in hepatocytes, and widespread HBcAg-immunoreactivity of hepatocyte nuclei are observed (see Chapter 63.2). It has been postulated that hepatocellular alterations in FCS, in contrast to the usual pathogenesis of HBV infection, are caused by a direct cytopathic effect of HBV on hepatocytes [71]. FCS rapidly progresses to liver failure without specific therapy.

## Nonresponders and Drug Resistance

**Nonresponders**. Long-term benefit of IFN therapy is unsatisfactory in approximately 50% of patients with chronic HBV infection, be it because of a primary non-response to IFNs or because of a relapse after an initial response. The benefit of a second course of IFN has not been consistently demonstrated. It appears that retreatment is more successful in patients who relapse after an initial response to IFN than in primary IFN nonresponders. Retreatment with a higher IFN dose may lead to seroconversion in approximately 30% of patients who initially responded to IFN, but then relapsed. Retreatment of primary nonresponders with IFN alone is associated with a very low rate of sustained response, and is not warranted.

Response of IFNα nonresponders to oral antivirals is similar to treatment of naïve patients [207]. Limited data suggest that retreatment with a combination of IFN and an oral antiviral is not more effective compared to retreatment with antiviral monotherapy, e.g. lamivudine.

**Drug Resistance**. The development of resistance is determined by an interplay of viral, host, and drug characteristics. The reverse transcriptase activity of HBV DNA polymerase gene lacks a proofreading capability. Therefore, HBV exhibits a mutation rate more than tenfold higher than other DNA viruses, and a large number of mutant strains of HBV are generated daily. Drug-resistant mutant strains of HBV containing point mutations of the polymerase gene usually emerge after several months to years of oral antiviral therapy [81]. Development of specific point mutations also may confer cross-resistance to other antiviral drugs. Patients treated with IFNs do not develop drug resistant HBV strains.

When using oral antiviral agents in clinical practice, an awareness of the incidence, clinical manifestations and drugs with cross resistance to mutant HBV is necessary. The detection of point mutations in the HBV polymerase gene that confer enhanced replication of mutant HBV strains in the presence of the drug in *in vitro* assays defines *genotypic resistance. Phenotypic resistance* is defined by the redetection of HBV DNA on two consecutive occasions in a compliant patient who had previously suppressed HBV DNA. Phenotypic resistance is associated with viral rebound and with serum ALT elevations in many patients. Genotypic resistance is usually followed after several weeks to months by *virological breakthrough*, i.e. a rise in serum HBV-DNA levels of 1 log10 copies/mL compared to the lowest value during therapy. Virological breakthrough is followed by a rise of serum ALT levels and a worsening of liver histology after a period of several weeks (*clinical breakthrough*) [72, 76, 160]. Resistant HBV can be detected either phenotypically with an increase in serum HBV DNA levels in a previously suppressed patient or by a confirmatory assay (currently, the most sensitive confirmatory test for genotypic resistance is the lineprobe assay).

**Table 63.30** Suggested management of patients with drug resistant HBV mutants[a]

| Resistance to | Action |
|---|---|
| **Lamivudine**[b] | Add adefovir<br>or<br>Add or switch to tenofovir<br>or<br>Switch to entecavir |
| **Adefovir Dipivoxil** | Add lamivudine<br>or<br>Add telbivudine or<br>or<br>Add entecavir |
| **Entecavir** | Add adefovir<br>or<br>Add tenofovir |
| **Telbivudine** | Add adefovir<br>or<br>Add tenofovir<br>or<br>Switch to entecavir |
| **Tenofovir** | Add entecavir<br>or<br>Add telbivudine<br>or<br>Add lamivudine |

[a]Not all suggestions are based on controlled studies
[b]Telbivudine and emtricitabine show cross-resistance with lamivudine resistant mutant HBV
Source: Adapted from [47, 76]

The clinical consequences following the emergence of drug resistant HBV range from asymptomatic viremia to serum ALT flares, progressive liver disease with worsening liver histology, hepatic decompensation with liver failure and rare instances of death [149]. Cirrhotic patients developing HBV drug resistance are significantly more likely to develop HCC on long-term follow-up [4].

Table 63.30 summarizes the suggested management of patients with drug resistant HBV mutants.

***Resistance to Lamivudine***. Lamivudine therapy may be limited by the emergence of drug resistant mutants.

The development of drug resistance leads to HBV DNA breakthrough, compromises therapeutic success as determined by liver histology, reduction in HBV DNA levels and HBeAg seroconversion rates, and ultimately accelerates disease progression [53, 54, 149]. The amount of ccc DNA in the serum is reported to be higher in patients who develop YMDD mutants than in those without mutants [93].

The most important mutation conferring lamivudine resistance involves the substitution of methionine by valine or isoleucine in domain C (tyrosin-methionine-aspartate-aspartate (YMDD) motif; rtM204V/I) of the HBV reverse transcriptase ("escape mutants"). The probability of YMDD mutations after 1 year of therapy is related to the magnitude of early viral suppression. The more effective the viral suppression at weeks 12 and 24 after the onset of treatment, the lesser the chance of developing drug resistant mutants. A satisfactory response is characterized by a decrease in viral load at week 12 of therapy by at least 1 $\log_{10}$ copies/mL, and at week 24 by 2 $\log_{10}$ copies/mL compared with the baseline value. Patients with primary treatment failure, i.e. those who fail to achieve a 2 $\log_{10}$ decline in HBV DNA after 24 weeks of treatment seem to be at greater risk of developing drug-resistant HBV. Patients with HBV DNA levels of more than $10^3$ copies/mL after 6 months of lamivudine therapy have a 63% chance of subsequently developing lamivudine resistant YMDD variants [263]. *After 1, 2, 3, 4 and 5 years of treatment, lamivudine resistant mutants emerge in 16%, 36%, 56%, 75% and approximately 80% of treatment naïve patients, respectively* [76].

Further determinants for the occurrence of lamivudine-resistant HBV mutants during therapy are HBeAg status, HBV DNA and ALT levels. Patients with HBeAg seropositivity, high HBV DNA levels and low pretherapeutic serum ALT levels (< 5 × ULN) have a significantly increased incidence of lamivudine resistance [29]. Recently serum HBV RNA (indicating active transcription and virus particle formation) was reported to be a predictor of early emergence of the YMDD mutant in patients treated with lamivudine [93]. Immunosuppressed patients are at particular risk of developing drug resistance.

The association of increased body mass index with lamivudine resistance may be caused by inadequate drug dosing or the adverse impact of fatty liver disease. There are no convincing data that the presence of pre-core/core-promoter variants or a particular HBV genotype predisposes a patient to lamivudine resistance [2].

The initial assumption that lamivudine resistant mutant viruses are less pathogenic than the HBV wild-type cannot be corroborated. During longer treatment periods lamivudine resistant mutants adapt and facilitate the selection of increasingly more pathogenic viruses.

*Management of patients with lamivudine resistant hepatitis B virus*. Lamivudine resistant HBV remains susceptible to adefovir, entecavir and tenofovir (Table 63.30). Telbivudine and emtricitabine show cross resistance with lamivudine resistant mutant HBV.

*Adefovir* suppresses HBV replication of lamivudine resistant HBV mutants, is well-tolerated, and is associated with virological and biochemical improvement [7, 184, 187, 188, 254, 277]. Factors associated with virologic response to ADV in lamivudine resistant patients are female gender, HBeAg-negative status, low baseline serum HBV-DNA levels, and genotype D HBV [18]. Upon emergence of genotypic viral resistance lamivudine therapy should not be discontinued. Instead, adefovir should be *added* to lamivudine [32, 133, 188]. "Add-on-therapy" is more effective in maintaining long-term biochemical and virological remission than switching-to adefovir monotherapy, and patients with lamivudine resistant hepatitis B are unlikely to develop genotypic resistance to adefovir [79, 134, 198, 256]. However, despite combined treatment with adefovir and lamivudine the emergence of adefovir resistance in lamivudine resistant patients may present early [257]. A daily dose of 20 mg is safe and seems to be superior to the regular dose of ADV of 10 mg p.o. qd [94]. Add-on salvage therapy with adefovir has also been shown to be cost-effective [112]. Sequential monotherapy is associated with an increased rate of drug resistance to adevofir and should be avoided [75, 258].

*Tenofovir* is an effective alternative to adefovir for the treatment of patients with lamivudine resistant HBV infection [233, 244]. Tenofovir is superior to adefovir and entecavir in the treatment of lamivudine resistant HBV. The 300 mg dose of tenofovir provides significantly higher concentrations than the minimal inhibitory concentration for HBV replication. Therefore, it might be assumed that the incidence of drug resistance with tenofovir will be lower than with other agents. A final assessment, however, is not yet possible and large-scale trials are still ongoing.

*Entecavir* has potent antiviral activity against lamivudine-resistant HBV and may be given to patients with known lamivudine resistance or who develop resistance during lamivudine therapy. The proportion of patients with undetectable HBV DNA by PCR and normalization of ALT levels increases with continued entecavir treatment. Through 96 weeks of treatment, entecavir resulted in continued clinical benefit in lamivudine-refractory HBeAg-positive chronic hepatitis B patients with a safety profile comparable to lamivudine [212]. However, the optimal duration of treatment is not known and up to 10% of lamivudine resistant patients may develop genotypic resistance to entecavir at 2 years of treatment.

In contrast to adevofir ("add-on"), the lamivudine resistant patient should be switched to entecavir; i.e., lamivudine should be discontinued. The adult dose of entecavir in lamivudine resistant patients is 1 mg daily compared to 0.5 mg daily in nucleoside naive patients. Although entecavir is beneficial in lamivudine resistant patients, its antiviral activity is substantially lower compared to wild type HBV in previously untreated patients even if the higher dose of 1 mg daily is given [30, 211].

Data on the impact of *pegIFN* on chronic hepatitis B resistant to lamivudine are controversial. They range from only marginal efficiency of pegIFNα to response rates of approximately 30% [68, 143].

***Resistance to Adefovir Dipivoxil***. Resistance to adefovir does occur albeit at a slower rate compared to lamivudine. Mutations known to confer resistance to ADV include an asparagine-to-threonine change in domain D (rtN236T) and an alanine-to-valine or -threonine change in domain B (rtA181V/T) [5]. Resistance is usually not detected during the first 48 weeks of treatment. Thereafter the cumulative probability of adefovir resistant mutations increases. *After 3 and 5 years of treatment resistance rates amount to 11% and 29%, respectively*. However, the rate of documented adefovir resistance depends on the sensitivity of the investigational techniques applied and studies using more sensitive techniques to detect antiviral-resistant mutants reported rates of adefovir resistance of 19 to 32% at 48 weeks and 58% at 96 weeks [75, 142]. Adefovir resistance can be associated with significant viral rebound and fatal hepatic decompensation [74]. Risk factors for the development of adefovir resistance have not been well-defined, although it seems reasonable to anticipate that they probably will correspond to those described for lamivudine. Patients who have lamivudine-resistant HBV seem to be at increased risk of adefovir resistance when adefovir is used alone (see above).

Rare cases of variant hepatitis B virus with primary resistance to adefovir have also been described [208].

*Management of adefovir resistant HBV*. Adefovir resistant HBV remains sensitive to lamivudine, telbivudine, entecavir, tenofovir, and emtricitabine in vitro [74]. However, clinical experience with salvage therapy in ADV resistant patients is very limited. It is important to distinguish primary treatment failure with adefovir (i.e., lack of a 2 log10 decline in HBV DNA) that occurs in 25% of treatment-naive patients at 1 year versus the emergence of adefovir-resistant HBV, because management recommendations differ. Upon emergence of ADV-resistant HBV, treatment should be continued by adding either lamivudine, telbivudine or entecavir. Some concern has been voiced for the potential emergence of mutant strains of HBV with resistance to both drugs upon adding lamivudine to adefovir in ADV resistant patients [12, 13]. In patients with primary treatment failure a combination of tenofovir (300 mg daily) and emtricitabine (200 mg daily) resulted in undetectable HBV viral DNA levels. Thus, tenofovir, in combination with emtricitabine, may be an alternative treatment for those with detectable HBV DNA on adefovir [204].

***Resistance to Entecavir***. Resistance to entecavir is rare compared to lamivudine. No cases of genotypic resistance were observed in treatment-naive patients after 1 year [45]. The specific point mutations that confer resistance to entecavir include the rtI169T, rtT184G, rtS202I, and rtM250V mutants in the presence of a lamivudine-resistant strain of HBV. Entecavir monotherapy does not lead to the selection of these mutants of HBV unless there is a subpopulation of HBV quasispecies in the host that are already resistant to lamivudine. Thus, resistance to entecavir requires the presence of preexisting lamivudine-resistant strains of HBV along with the emergence of additional mutations [76]. Based on in vitro data, entecavir-resistant HBV is also resistant to lamivudine, emtricitabine, and telbivudine, but should remain susceptible to adefovir and tenofovir.

***Resistance to Telbivudine***. The rtM204I (domain C) mutation confers cross-resistance to lamivudine as well as to telbivudine. Resistance to telbivudine is 5% at 1 year in telbivudine monotherapy and 10% in combination with lamivudine [127].

***Resistance to Emtricitabine***. The rates of development of resistant mutants are similar in emtricitabine and lamivudine. Resistance is conferred by the rtM204V/I mutation with or without the accompanying rtL180M mutation. Emtricitabine-resistant strains of HBV retain susceptibility to adefovir, tenofovir, and entecavir, but are cross resistant with lamivudine and telbivudine.

Chronic hepatitis B, once a purely diagnostic field, has become now a highly dynamic and complex clinical and research subject with rapidly emerging new therapies. New nucleos(t)ide analogs are already beginning to replace the first generation drugs, such as lamivudine and adefovir. The aim of the preceding discussion was to lay the foundation for a rational decision making process in patients with chronic hepatitis B. The indication for antiviral therapy should take into careful consideration the patient's wish, contraindications and accompanying diseases, the urgency of treatment and the chances of HBV elimination. As a rule, there is no need for ad hoc therapeutic decisions and the clinician has enough time to observe the patient and the course of hepatic and viral parameters before deciding on the appropriate therapeutic approach (with the exception of patients with decompensated HBV-cirrhosis). Antiviral treatment should not be offered to patients who have persistently normal ALT levels or mild disease on liver biopsy unless they are undergoing chemotherapy or immunsuppression. In patients who are undergoing antiviral treatment careful monitoring and surveillance for early recognition of the emergence of drug resistant HBV (except in IFN treated patients) is mandatory. The awareness for the potential cross-resistance among the available oral antiviral agents is important when performing salvage or combination therapy.

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## Hepatitis C

The virus causing non-A, non-B-hepatitis was identified as an RNA virus in 1989 and named hepatitis C virus (HCV).

### *Epidemiology*

Chronic hepatitis C represents one of the biggest healthcare problems worldwide. Approximately 170 million people had contact and 130 million people are chronically infected with HCV, which corresponds to a worldwide prevalence of chronic hepatitis C of approximately 3%. It is the most common cause of hepatocellular carcinoma (HCC) in Europe, and the majority of liver transplants performed in Europe and in the United States are for chronic hepatitis C. Hepatitis C is an important cause of premature mortality, especially in persons aged 55–64 years [253].

Epidemiological parameters (prevalence, incidence, disease transmission patterns and genotype distribution) have changed substantially in Western Countries over the last 15 years. Increased blood transfusion safety, improvement of healthcare conditions, expansion of intravenous drug use and immigration to Europe and to the USA from endemic areas are the main factors responsible for this change.

The prevalence rate of chronic HCV infection in Europe shows a north-south gradient. Prevalence in Scandinavia is 0.1–0.2%, in Germany 0.6% (figure probably too low due to under-reporting), while in Southern Europe prevalence rates of 2.5%–3.5% are registered. In isolated areas in Greece and Southern Italy prevalence rates of 7–20% have been reported [52].

The overall incidence in the USA in 2006 was estimated to be 0.3 per 100,000 [248]. The prevalence of antibodies to HCV in the US is approximately 1.6% (equating to about 4.1 million anti-HCV positive persons), while the prevalence of positive HCV RNA is 1.3% [5]. The region with the highest prevalence of HCV worldwide is the Middle East and Africa, particularly Egypt, with prevalence rates up to 20% [113].

In the US HCV infection is more prevalent among African Americans and Hispanics than among non-Hispanic Whites. The course of chronic hepatitis C in Latinos is more aggressive, with higher risk of

developing cirrhosis than any other ethnic group or race. Both African Americans and Latinos also have inferior treatment responses, compared to those of non-Hispanic whites. The causes of this more aggressive progression are complex, and, in addition to genetic factors, decreased efficacy of treatment is probably related to the metabolic syndrome, insulin resistance, and hepatic steatosis [173, 196, 241].

The distribution of HCV-genotypes and subtypes (see Section 63.1) varies worldwide. In Germany and in the USA genotype 1b is most frequent, responsible for approximately 50% of HCV infections, followed by genotype 1a (approximately 20%) and 3a (approximately 15%). HCV genotype 4 is the most common variant of HCV in the Middle East and Africa. In Egypt more than 90% of infections are due to genotype 4. Recent studies suggest that HCV-4 infection is spreading beyond its strongholds in Africa and the Middle East to Western Europe [113].

## Transmission

Even before HCV was discovered, approximately 90% of cases of posttransfusion hepatitis were classified as non-A, non-B-hepatitis, suggesting parenteral transmission as an important route of acquiring hepatitis C. In the 1990s risk groups with a high prevalence of HCV infection were hemophiliacs (70–90%), injection drug users (50–90%), HIV-infected patients (90%), alcoholics (30%), and patients on chronic hemodialysis (5–30%) [143]. Coinfections of HCV with HBV or HIV were particularly prevalent among hemophiliacs or injection drug users.

Currently the most important sources of infection for persons with hepatitis C in Western countries are injection drug use (60%), sexual (15%), nosocomial, health-care work, perinatal (5%), and unknown (10–20%). The risk of nosocomial spread of HCV is emphasized by a recent outbreak of HCV infection during sclerotherapy of varicose veins [41].

*Blood transfusion* was a major risk factor for HCV infection in the past. The incidence of post transfusion hepatitis C has declined sharply after the introduction of reliable and sensitive HCV tests. The risk of acquiring the infection through infected blood stored for transfusion nowadays is approximately 1:120,000 units, which is lower than the prevalence of HCV in the general population. New tests detecting minute amounts of HCV genetic material will further decrease the risk of transfusion-transmitted HCV to a range between 1:500,000 and 1:1,000,000 units.

Forty to 60% of cases of HCV transmission occur by contaminated needles in injection drug users, in persons receiving tattoos or by accidental needle stick injury (especially healthcare workers, manifestation rate 5.4%). Healthcare workers adhering to general hygienic measures are not at an increased risk of acquiring HCV infection.

Only in approximately 5–10% of cases transmission is *sexual, perinatal* or via *breast milk*. The risk of sexual transmission is much lower than for HBV. It probably depends on the number of sexual intercourses and the degree of HCV viremia. HCV antibodies occur in approximately 2% of the spouses [228]. Vertical perinatal HCV transmission occurs in approximately 5% of infants born to anti HCV positive mothers. It increases to up to 40% in HCV/HIV coinfection.

No risk factors for HCV infection can be identified in up to 45% of cases ("community acquired"/"sporadic" HCV infection). HCV infection in endemic countries has a strong familial component (clustering of HCV cases) explained, at least partly, by specific modes of intrafamilial viral transmission and supporting the role of host genes predisposing to HCV infection [181].

A rare, but important route of transmitting HCV is by *infected organs* during transplantation (liver, kidney, heart, bone marrow). A chronic HCV infection develops in approximately 50% of these initially HCV negative transplant recipients. Infection of an originally HCV negative donor liver by persistent HCV in the extrahepatic tissues of the recipient has also to be kept in mind.

A recent study from Spain suggests that hospital admission per se is a risk factor for acquiring HCV infection [141].

The *incubation period* of acute hepatitis C in symptomatic patients is between 2–12 weeks [169].

## Pathogenesis

HCV-induced liver injury is primarily mediated by immunologic mechanisms rather than by direct HCV cytotoxicity. HCV is taken up by hepatocytes with the help of the low-density lipoprotein receptor, is

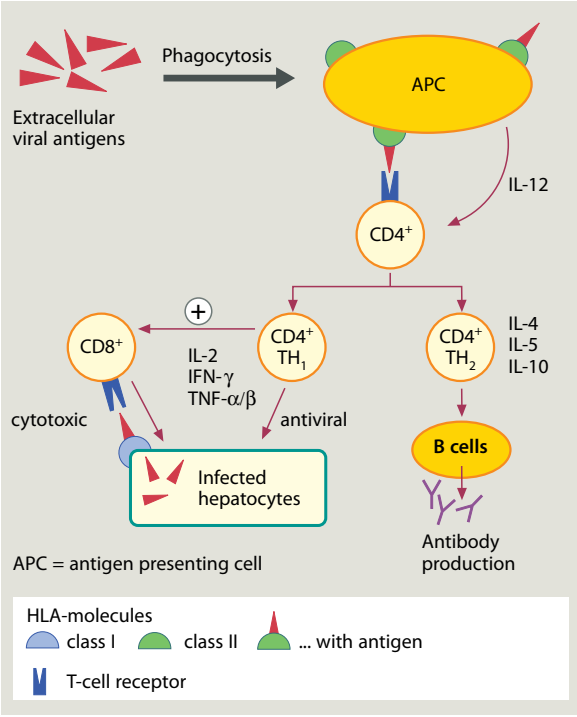


**Fig. 63.47** Immune pathogenesis of hepatitis C. See text

processed intracellularly and is actively secreted by infected cells through a Golgi-dependent mechanism while bound to very low density lipoprotein (Fig. 63.12) [156]. Processed viral peptides are expressed on the surface of hepatocytes in association with HLA-class I molecules where they are recognized by the T cell receptor of HLA-class I restricted cytotoxic $CD8^+$ T-cells (CTL) (Fig. 63.47). Virus-specific CTL induce increased hepatocyte loss by apoptosis (via FAS ligand) and play an important role in viral clearance, i.e. in the outcome of acute HCV infection [122]. In addition to direct virus specific cytotoxicity, antibody mediated cytotoxicity (ADCC) may play a role in hepatocyte death. The HCV envelope E2 protein expressed on the hepatocyte surface has been shown to be able to mediate ADCC [161a].

While a prerequisite for CTL action is their direct interaction with the infected cell, virus specific $CD4^+$ T-helper cells (Th1 and Th2) do not require a direct contact with HCV infected hepatocytes in order to unfold their activities. They recognize antigen-presenting cells that have taken up, processed and expressed viral antigens in association with HLA class II molecules on their surface. This reaction not necessarily has to take place in the liver, but may also occur in extrahepatic sites, for example the lymph nodes. The ability of HCV to infect, replicate in, and produce progeny virus in perihepatic lymph nodes and peripheral blood mononuclear cells is interesting in this regard [30, 170]. The main antigen presenting cells (APC) are macrophages, dendritic cells and Kupffer cells. The interaction of T-helper cells with APC leads to the release of cytokines that both stimulate T-cell proliferation and increase expression of HLA-molecules on the hepatocyte surface resulting in a reduced viral replication. Based on their cytokine profile various types of $CD4^+$ T-helper cells are differentiated. Th1-cells produce interleukin (IL)-2, interferon (IFN) γ, tumor necrosis factor (TNF) α and β and stimulate cell-mediated immune reactions. Th2-cells secrete IL-4, -5 and -10 and primarily control the humoral immune response. Therefore, a strong Th-immune response and a vigorous CTL-response are always linked. *Patients in whom acute hepatitis C resolves completely have a stabile and strong Th1-immune response that in cooperation with CTL leads to complete HCV clearance*. Notably a strong IFNγ response is associated with efficient viral clearance that primarily results from antigen-driven selection/survival of HCV-specific T cells expressing high-avidity T-cell receptors [165].

The development of chronic HCV infection is often associated with impaired innate and adaptive immune responses. Broadly reactive neutralizing antibodies and multispecific T-cell responses are generated during chronic HCV infection but are too weak to clear the virus. Despite the presence of intrahepatic virus-specific CTL, HCV persists, apparently due to an ineffective antiviral T-cell response [164, 226]. The precise mechanisms underlying the failure of intrahepatic HCV-specific CTL to control the virus during persistent infection are not fully understood. Deranged ability to secrete IFNγ and impaired cooperation with T-helper cells may contribute to this failure [223]. In addition, HCV itself has been shown to inhibit IFNα secretion by primary hepatocytes and human hepatic cell lines in vitro. Silencing IFNα gene expression results in increased HCV replicon expression [265].

Continued generation of *HCV escape mutants* due to selection pressure from both humoral and cellular immunity represents another mechanism of HCV persistence [244].

Non-immunologic mechanisms probably also contribute to the pathogenesis of hepatocyte injury in HCV

infection. HCV has been shown to induce the accumulation of autophagosomes in hepatocytes by stimulating the unfolded protein response without enhancing autophagic protein degradation, thereby perturbating the normal autophagic response [221]. Furthermore, HCV may limit hepatocyte regeneration by causing hepatocyte arrest in the G1 phase, thereby impairing hepatocellular function [140].

*Steatosis* and factors related to the metabolic syndrome, such as insulin resistance, also appear to play a role in the pathogenesis of chronic hepatitis C. The prevalence of steatosis in chronic hepatitis C is approximately 40–55%, about twice that in chronic hepatitis B [134, 233]. The pathogenesis, prevalence and the severity of steatosis varies according to the infecting HCV genotype. Steatosis is more frequent and usually more severe in patients with HCV genotype 3 infection and experimental evidence suggests that HCV core protein components are capable of inducing triglyceride accumulation [1]. Virus induced dysfunction of liver microsomal triglyceride transfer protein may interfere with very low density lipoprotein assembly, thus enhancing intracellular lipid accumulation (see also Chapter 89) [154]. In addition, it has been shown recently that specific amino acid residues of the HCV-3 core protein directly up-regulate fatty acid synthase expression and thus alter the cellular lipid profile [103, 255].

The severity of steatosis in genotype 3 infection correlates with the level of HCV RNA in serum and the liver and steatosis is significantly reduced or even disappears in patients with sustained virological response to antiviral therapy [124, 162, 186]. While HCV genotype 3 has a direct steatogenic effect, lipid accumulation in patients with genotype non-3 HCV infection is mostly due to metabolic and toxic factors, such as obesity, insulin resistance and alcohol abuse [28, 158, 234, 258].

Steatosis not only is a histologic characteristic of HCV infection, but also appears to play an important role in the pathogenesis of disease progression. Most studies have shown a correlation between presence and severity of steatosis and stage of fibrosis [57, 80, 127, 199]. Steatosis in chronic hepatitis C may contribute to the progression of liver injury via upregulation of mediators in hepatocyte apoptosis, such as Fas, TNF-R1 and active caspase-3, and by activating NF-κB [95, 247]. Evidence is accumulating, however, that it is not the fat per se, but rather the pathogenetic mechanisms underlying steatosis that are the pacemaker of accelerated fibrogenesis in chronic hepatitis C. Thus, the level of insulin resistance has been shown to be associated with the stage of fibrosis, and insulin resistance has been reported to be an independent predictor of advanced fibrosis in chronic HCV infection [22, 49, 58, 90, 158].

In addition, steatosis negatively influences the rate of response to antiviral treatment. Steatosis and factors related to the metabolic syndrome, particularly obesity and insulin resistance are associated with a decreased efficacy of interferon-based therapies, which may partly be overcome by adjusting the dose to the weight of the patient (see below) [31, 35, 83, 232].

Hepatic fibrosis in chronic hepatitis C infection may also be advanced by the interaction between HCV and stellate cells. HCV E2 glycoprotein has been shown to bind to the cell surface of human hepatic stellate cells and to up-regulate matrix metalloproteinase-2 [148]. HCV nonstructural genes may also directly induce profibrogenic mediators, such as transforming growth factor $\beta_1$ in infected hepatocytes [211]. These findings would explain the frequent observation of progressive liver fibrosis despite a low level of inflammation.

The role of iron overload as a cofactor in the progression of chronic hepatitis is controversial. Most studies support a role of HFE mutations with consequent iron loading as risk factors for fibrogenesis and disease progression in chronic hepatitis C [72, 73, 235]. Heterozygosis for β-globin mutations has recently been described as a novel risk factor for both hepatic iron accumulation and progression of fibrosis in patients with chronic HCV infection [209]. Other investigators, however, failed to document iron overload in chronically HCV-infected patients and were unable to demonstrate a relation between hepatic iron content and the liver damage process in HCV infection [220].

## Clinical Manifestations and Diagnosis

The diagnosis of HCV infection is based on clinical manifestations, but above all on laboratory tests. Imaging techniques, such as ultrasound, CT and MRI play a role in excluding other liver disorders and in the diagnosis of architectural changes leading to cirrhosis. Liver biopsy is still the gold standard in grading the necroinflammatory reaction and in staging fibrosis.

However, noninvasive techniques, such as transient elastography are becoming more important in assessing fibrosis and cirrhosis.

For serologic diagnosis see Section 63.1; for histopathology see Section 63.2.

### Acute Hepatitis C

Most patients with acute hepatitis C are asymptomatic. Therefore, detection of HCV infection and diagnosis are difficult [17, 206]. Symptoms develop in approximately 20–30% of patients. The incubation period ranges from 2 to 12 weeks [169]. The most common symptoms are fatigue, jaundice, dyspepsia with vague abdominal pain and flulike complaints.

As an indication of hepatic injury, serum ALT levels rise up to approximately 500 IU/L 4–12 weeks after viral exposure. After exposure to HCV, there is a window of 1–3 weeks before serum HCV RNA can be detected. Detection of anti HCV IgM in the early phase of the illness may help in the diagnosis of acute hepatitis C [202].

Proposed criteria for the diagnosis of acute hepatitis C infection are listed in Table 63.31.

### Chronic Hepatitis C

Patients with chronic hepatitis C are either asymptomatic or complain of nonspecific symptoms, such as fatigue, right upper quadrant pain, nausea, anorexia, dyspepsia or diffuse muscle aches. Although usually mild and nonspecific, these symptoms often markedly impair the quality of life [224].

Mild to moderate fluctuating elevations of serum ALT levels between 50–500 IU/L are characteristic. ALT flares with an increase in serum levels up to ten times the ULN occur in approximately 20% of patients. On the other hand, approximately 25–30% of patients have persistently normal ALT activity (PNALT), despite ongoing liver damage [2, 262]. PNALT has been defined as at least two ALT measurements within the normal range taken during a period of at least 6 months. However, levels fluctuate, therefore more determinations and longer time intervals might be more meaningful. Currently, no strict consensus exists on the definition of ALT flare or of PNALT.

Gender, viral factors (genotype, HCV-RNA titer) and indicators of metabolic syndrome (body mass index, blood pressure, blood glucose, cholesterol and triglyceride concentration) affect ALT levels in patients with chronic hepatitis C [187]. Once cirrhosis develops, aminotransferases are only mildly elevated (usually < 100 IU/L) and AST levels are higher than ALT levels. Among patients with advanced chronic hepatitis C, mild elevations of serum $\alpha_1$-fetoprotein values occur in 17% of cases, even in the absence of HCC [46].

Increased serum γGT levels in patients with chronic hepatitis C are associated with liver steatosis and fibrosis, and indicate more advanced liver disease rather than reflecting cholestasis [9].

Hyperferritinemia is encountered in 20–27% and is associated with liver iron deposits in 39–46% of cases of chronic hepatitis C. Hepatic iron deposits are usually associated with a more advanced stage of fibrosis [81, 212].

The extent of apoptotic activity, steatosis and of fibrotic liver injury in patients with chronic hepatitis C has been reported to be mirrored by caspase activity in serum [8, 213]. Determinations of serum caspase levels, however, have not yet been adopted into clinical practice.

Retinol-binding protein 4 (RBP4) is an adipocytokine associated with insulin resistance. It has been shown recently that serum RBP4 levels were independently linked to steatosis in patients with chronic HCV genotype 1 infection [177].

Chronic hepatitis C is often associated with autoimmune reactions. Various autoantibodies (ANA [32%], SMA [11%], LKM-1 [5%], thyroid antibodies

**Table 63.31** Proposed criteria for diagnosis of acute hepatitis C infection

| | |
|---|---|
| **Primary criteria** | Presence of HCV RNA in serum of a previously HCV-negative patient. |
| | Seroconversion from anti HCV-negative to anti HCV-positive. |
| **Secondary criteria** | Elevated aminotransferase levels ≤ 10–20 times the ULN. |
| | Known or suspected exposure to HCV within the preceding 6 months. |
| | All other causes of acute liver damage excluded. |
| **Additional considerations** | Sudden onset of liver disease. |
| | Repetition of recombinant immunoblot assay testing to demonstrate eventual increase in the number of reactive proteins. |

Source: From [206]. With permission

[21%] or rheumatoid factor [29%], antibodies against basal membranes, thymic cells and laminin A and C) occur in up to 65% of patients with chronic HCV infection. Their serum titers are usually low and, in contrast to autoimmune hepatitis, they represent nonspecific immune phenomena. Their prevalence rises with increasing age, especially in women, and is not influenced by interferon therapy.

Anti LKM-1 autoantibodies are considered specific markers of type 2 autoimmune hepatitis (see Chapters 36 and 72), but are also found in 5% of patients with chronic hepatitis C. They are directed against a 33 amino acid sequence of cytochrome P4502D6 (CYP2D6). This conformational epitope on CYP2D6 shares a sequence homology with the HCV NS3 and NS5a proteins. Cross-reactivity due to molecular mimicry could explain the presence of anti-LKM1 in patients chronically infected with HCV [139].

Antibodies against cytochrome P4502E1 occur in 40% of patients with chronic hepatitis C [242]. Recently, a subset of HCV patients with positive AMA have been described who presented with a broad spectrum of clinical features, including liver, autoimmune and neoplasic manifestations. Two-thirds of these patients presented an associated systemic autoimmune disease, mainly Sjögren's syndrome or systemic sclerosis, together with a high frequency of multiple autoantibodies and an increased prevalence of cirrhosis and neoplasia [191].

*The hallmark of progression of chronic hepatitis C is fibrosis*. Various panels of non-invasive serum markers to differentiate mild from moderate-to-advanced liver fibrosis in chronic hepatitis C patients have been devised [29, 66, 82, 118, 128, 172, 237]. Most of them, however, are unreliable and cumbersome to calculate. They render liver biopsy unnecessary in only a minority of patients and have not gained wide acceptance in clinical practice [125]. Measurements of expression profile of genes involved in extracellular matrix turnover are emerging as new techniques in determining hepatic fibrosis, but they have not yet been adopted into clinical practice [6].

*Transient elastography* (see also Chapter 28) reflects "elasticity of tissue" and is increasingly used as a non-invasive technique to determine "liver stiffness", i.e. fibrosis. It is suitable for the diagnosis of advanced fibrosis/cirrhosis but cannot reliably discriminate lower grades of fibrosis [4, 71, 115, 266].

*Liver biopsy* still is the method of choice to evaluate liver fibrosis.

## Extrahepatic Manifestations of Chronic Hepatitis C

There is a growing body of evidence to suggest that HCV can replicate in extrahepatic tissues and cells, including peripheral blood mononuclear cells, dendritic cells, granulocytes, B lymphocytes, and monocytes/macrophages [17]. Extrahepatic manifestations in chronic HCV infection are quite common. The association of HCV infection with autoimmune and hematologic processes is well documented. The sialotropism of HCV may explain the association with Sjögren's syndrome, and its lymphotropism links the virus to cryoglobulinemia, autoimmune cytopenias, and lymphoma [190]. In a large prospective cohort study 74% of patients chronically infected with HCV had a least one clinical extrahepatic manifestation. The most common were mixed cryoglobulinemia (40%), arthritis and arthralgia (23%), peripheral neuropathy and paresthesia (17%), myalgia (15%), pruritus (15%), and sicca syndrome (11%) [24]. Furthermore, an association of HCV infection with polyarteritis nodosa, glomerulonephritis, porphyria cutanea tarda, cutaneous vasculitis, lichen planus, erythema nodosum, erythema exsudativum multiforme, urticaria, immune thyroid disorders, corneal ulcers, diabetes mellitus, malignant non-Hodgkin's lymphomas, idiopathic thrombocytopenia and idiopathic pulmonary fibrosis has been demonstrated [189]. The association between some of these disorders and HCV may not be direct, but rather mediated by cryoglobulins.

### Mixed Cryoglobulinemia

Cryoglobulins are serum immunoglobulins that precipitate reversibly upon cooling. This reaction results in an increased blood viscosity, in red blood cell clumping, and in functional impairment of platelets ultimately leading to circulatory disturbances. Three types of cryoglobulinemia are differentiated. In *mixed cryoglobulinemia* (Type II) generation of immune complexes and cryoprecipitation involves polyclonal IgG- and mono- or polyclonal IgM-antibodies. Rheumatoid factor activity (monoclonal IgM-antibody against IgG) commonly is present.

The pathogenetic mechanisms of HCV-associated mixed cryoglobulinemia are unclear. Immune complexes consist of HCV-RNA, anti-HCV antibodies and IgM rheumatoid factor. The concentration of HCV-RNA in cryoprecipitates may be 10–1,000 times higher

than the serum HCV-RNA level. Cryoglobulinemia more often occurs in patients with long-standing HCV infection, high serum γ-globulin levels and liver cirrhosis. An association with a specific HCV genotype cannot be demonstrated.

Cryoglobulins are more common in women. The overall prevalence of mixed cryoglobulins in various liver diseases is 41%, with the highest values being observed in chronic hepatitis C (47–54%) compared to 15% in chronic HBV infection. HCV-RNA may be demonstrated in 42–95% of patients presenting clinically with cryoglobulinemia. Cryoglobulinemia is symptomatic in about 1% of HCV infected patients. Clinical manifestations include signs and symptoms of vasculitis with Raynaud-type symptoms, erythemas, palpable purpura and hyperpigmented skin (Fig. 63.48). Synovitis, arthralgias and arthritis, peripheral neuropathies, marked fatigue, membranoproliferative glomerulonephritis, renal failure and hepatic involvement may occur. The development of renal injury is associated with a severe prognosis.

The impact of cryoglobulins on the natural history of liver disease is not clear. While there appears to be an independent association between cryoglobulinemia and steatosis as well as advanced fibrosis, a recent report failed to identify an influence of cryoglobulins on the clinical course of hepatitis C [200, 243].

The overall risk of non-Hodgkin's lymphoma in patients with HCV related cryoglobulinemic syndrome is about 35 times higher than in the general population [157].

HCV-associated mixed cryoglobulinemia usually responds to treatment with pegylated interferon and ribavirin [147].

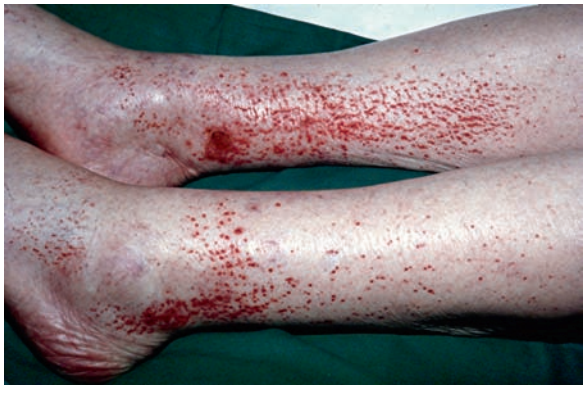

**Fig. 63.48** Mixed cryogobulinemic purpura in a patient with chronic hepatitis C

### Glomerulonephritis

Membranoproliferative glomerulonephritis (MPGN) is the most common renal manifestation of HCV infection, representing 70–80% of HCV-associated glomerulonephritis. HCV-associated MPGN may be cryoglobulin related and non-cryoglobulin related. The prevalence of HCV infection in MPGN is 10–20% in the United States [17]. Most of the remaining HCV-associated glomerulonephritides fall upon membranous glomerulonephritis (MGN).

MPGN and MGN are rare immune complex diseases that are characterized clinically by hematuria, proteinuria and renal dysfunction. Only 15% progress to end-stage renal disease. HCV glomerulonephritis is rare in children and most often develops in infected adults in their 40s and 50s after a long history of HCV infection. Many patients have symptoms of cryoglobulinemia [111].

In *HCV-associated MPGN,* immune complexes with cryoglobulin-like ultrastructure are deposited in the mesangium and in the subendothelial space of glomerular capillaries. These complexes consist of HCV-RNA, anti-HCV IgG, IgM (rheumatoid factor) and complement component C3. HCV replication within renal epithelia is not known to occur. Serum complement factors C3, C4, CH50 are diminished, the rheumatoid factor often is positive and commonly cryoglobulins and circulating cryoprecipitates are demonstrable.

In contrast, patients with *HCV-associated MGN* have normal serum complement levels and no circulating rheumatoid factor or cryoglobulins. HCV-core antigen, however, may be demonstrated immunohistochemically in the glomeruli.

Treatment with plasmapheresis (removes cryoglobulins), corticosteroids, cytotoxic drugs/rituximab and pegylated interferon/ribavirin has proven to be effective in some patients.

### Polyarteritis Nodosa

Polyarteritis nodosa (PAN) may be associated with many viral diseases (HAV, HBV, HCV, HIV, Varicella zoster, CMV, EBV, parvovirus, human T-cell leukemia virus type I). Anti-HCV antibodies may be demonstrated in 20% of patients with PAN, while HCV-RNA is demonstrable in only 5% of patients. The pathogenesis is characterized by the deposition of immune complexes

of HCV antigens and cryoglobulins (?) in the vessel walls. The consequent binding and activation of complement results in a narrowing of the vessel lumen and leads to ischemic tissue injury.

### Porphyria Cutanea Tarda

The association of porphyria cutanea tarda (PCT) with chronic liver disease, especially of alcoholic origin, is well known. The disease is characterized by a defect in uroporphyrinogen-III-decarboxylase (see Chapter 84). Symptoms include fragile skin with blister formation, cutaneous bleeding tendency, de- and hyperpigmentation, and hirsutism. Patients with PCT have an increased incidence of liver disease. Extrinsic factors, such as excessive sun exposure, ethanol, estrogens, and iron trigger disease symptoms.

The prevalence of markers of HCV infection in patients with PCT exhibits geographical variations. While the prevalence of HCV in patients with PCT in the mediterranean area (Spain, Italy) amounts to 70–80%, HCV prevalence in central and northern Europe is less than 10% [56]. Overall, HCV prevalence in patients with PCT is approximately 50%, much higher than that reported in the general population [77]. Although this strong association suggests a possible etiopathogenic role of HCV in PCT this has not been substantiated yet. It appears that HCV-induced liver injury contributes to the manifestation of PCT symptoms in persons with an inborn deficiency of uroporphyrinogen-III-decarboxylase.

### Sjögren's Syndrome

Sjögren's syndrome (SS) is characterized by a diminished secretory activity of salivary and lacrimal glands (sicca syndrome). Sicca syndrome secondary to liver disease is often associated with primary biliary cirrhosis and chronic HCV infection. In addition to reduced salivation and lacrimation approximately 60% of patients present with a systemic process with diverse extraglandular manifestations, with articular involvement (44%), vasculitis (20%), and neuropathy (16%) being the most frequent features observed. The main immunologic features are antinuclear antibodies (65%), hypocomplementemia (51%), and cryoglobulinemia (50%). Only approximately 25% of patients with SS secondary to HCV infection have positive anti-Ro/SS-A and/or anti-La/SS-B antibodies [191]. These antibodies react with salivary and lacrimal gland epithelia.

### Sarcoidosis

Sarcoidosis is a systemic disease that may be associated with HCV infection. It is triggered (initial manifestation or reactivation) by interferon in 75%, and is unrelated to interferon treatment in 25% of cases. Two thirds of patients who develop interferon associated sarcoidosis do so during the first 6 months after starting therapy [192].

### Thyroid Disorders

Approximately 10% of HCV infected persons (predominantly women) have thyroid antibodies already prior to interferon therapy. On the other hand antibodies to HCV are more frequent in patients with thyroiditis compared to the general population. Five percent of patients with chronic hepatitis C have a history of thyroid disorder. It is unclear if there is a direct pathogenetic link between HCV infection and thyroid dysfunction, mainly autoimmune hypothyroidism (Hashimoto's thyroiditis).

Patients receiving interferon therapy for hepatitis C may develop thyroid dysfunction. Up to 15% of patients with hepatitis C receiving interferon develop clinical thyroid disease, in up to 40% with thyroid antibodies. Patients seropositive for LKM1 antibodies seem to be particularly susceptible to developing autoimmune thyroid disease during interferon treatment [135, 160]. Interferon induced thyroiditis (autoimmune type and non-autoimmune type) may be a major clinical problem resulting in discontinuation of interferon therapy. Non-autoimmune interferon induced thyroiditis can manifest as destructive thyroiditis or as hypothyroidism with negative thyroid antibodies.

### Dermatologic Disorders

Dermatologic disorders associated with chronic HCV infection encompass the cutaneous manifestations of

mixed cryoglobulinemia, porphyria cutanea tarda, lichen planus, erythema exsudativum multiforme, erythema nodosum, cutaneous vasculitis, urticaria, Behcet's disease and vitiligo [13].

Many dermatologic manifestations are due to deposits of cryoglobulin immune complexes in the vessel walls. Cutaneous vasculitis manifests clinically with palpable purpura, predominantly of lower extremities (Fig. 63.48).

The occurrence of generalized lichen planus in patients with chronic hepatitis C is well known. Lichen planus may also be triggered by interferon therapy [161]. The causal association of oral lichen planus with HCV infection recently has been put in doubt in an Italian case-control study and it has been suggested that the concomitant occurrence of both disorders may be due mainly to the frequency of each disease in the population [52].

### Metabolic Manifestations

HCV infection is associated with the metabolic syndrome, and compared to the general population patients with HCV infection have a higher prevalence of type 2 diabetes mellitus. Chronic infection with HCV can induce insulin resistance in a genotype-dependent fashion, thus contributing to steatosis and progression of fibrosis (see paragraph on "Pathogenesis") [94, 214, 257]. Possibly there is a direct correlation between the viral load and the presence of insulin resistance in HCV patients [93].

The metabolic effects of HCV also have a negative impact on the response of HCV to antiviral treatment. On the other hand, clearance of HCV improves insulin resistance, β-cell function and parameters of glucose metabolism [114, 183, 198].

### Hematologic-Oncologic Disorders

HCV has been demonstrated to occur in bone marrow cells, lymphocytes and peripheral blood mononuclear cells. Among the hematological disorders associated with HCV infection are idopathic thrombocytopenia, anemia, lymphadenopathy and malignant non-Hodgkin's lymphomas.

**Immune thrombocytopenic purpura (ITP).** Patients with ITP show markers of HCV infection in approximately 10% of cases, and up to 80% of patients with chronic hepatitis C have platelet antibodies, in comparison to approximately 45% of those with hepatitis B. HCV-RNA may be demonstrated within the platelets of some patients with HCV-associated ITP. *Eltrombopag* is an orally active thrombopoietin-receptor agonist that stimulates thrombopoiesis. It has been shown to increase platelet counts in patients with thrombocytopenia due to HCV-related cirrhosis [151]. However, at the time of writing it has not yet been approved for clinical use in cirrhosis induced thrombocytopenia. *Recombinant interleukin-11* (rIL-11), which has both thrombopoietic and anti-inflammatory properties, was recently evaluated in 12 patients with HCV associated ITP. At high dose (50 μg/kg daily) mean platelet counts rose from initial $54 \times 10^9$/L to $103 \times 10^9$/L and at low dose (15–35 μg/kg tiw) from an initial $51 \times 10^9$/L to $74 \times 10^9$/L. Side effects of rIL-11 were common and troublesome, but could be reduced by lowering the dose [68].

**Anemia.** Hemoglobin concentrations in patients with chronic HCV infection decrease mainly as a result of ribavirin-induced hemolysis. Although ribavirin-associated anemia can be reversed by dose reduction or discontinuation, this approach compromises outcomes by significantly decreasing sustained viral response rates. Recombinant human erythropoietin has been used with varying success to manage ribavirin-associated anemia. Viramidine, a liver-targeting prodrug of ribavirin, has the potential to maintain the virologic efficacy of ribavirin while decreasing the risk of hemolytic anemia in patients with chronic hepatitis C [150].

*Aplastic anemia*, usually severe and irreversible may occur several weeks or months following acute viral hepatitis C.

**Lymphadenopathy**. Enlarged hepatic hilar lymph nodes occur in up to 40% of patients with chronic hepatitis C.

**Non-Hodgkin's lymphoma (NHL).** The prevalence of NHL is increased among patients with chronic HCV infection. HCV infection confers a 20–30% increased risk of NHL overall, and a threefold higher risk of Waldenström's macroglobulinemia, a low-grade lymphoma [76].

The prevalence of HCV infection in NHL ranges between 7.4% and 37.0%. The spectrum of HCV induced lymphomas includes the intermediate to high-grade lymphoma, and the more common indolent, low-grade lymphoma, preceded by long-standing

symptomatic mixed cryoglobulinemia Type II (diffuse large B-cell lymphoma, marginal zone lymphoma, and lymphoplasmacytic lymphoma) [42, 146, 166]. HCV has also been ascribed a role in the development of gastrointestinal MALT lymphoma [130].

HCV has oncogenic potential, but the intimate pathogenetic mechanism involved in HCV-related tumorigenesis remains unknown.

Regression of HCV associated low-grade NHL after treatment with pegylated interferon plus ribavirin has been reported [145]. Viral reactivation may occur during chemotherapy and complicate the underlying liver disease.

In addition to NHL a Swedish study also showed a significantly increased prevalence of *malignant melanoma* in patients with HCV infection [50].

### Nervous System and Psychiatric Disorders

**Neuropathy.** Neurologic manifestations in HCV-infected patients occur predominantly in the peripheral nervous system. The prevalence of peripheral nerve involvement in patients with mixed cryoglobulinemia ranges between 20% and 35%. Clinical manifestations result from immune complex deposits within the vasa nervorum of the peripheral nerves leading to vasculitis. Eighty percent of cases of HCV-associated peripheral neuropathy manifest as a very painful *symmetrical distal polyneuropathy* that involves the legs [25]. *Mononeuritis multiplex* occurs more rarely.

In addition to polyneuropathy cerebral infarcts, cerebral nerve palsies and a poyradiculitis manifesting in the form of a Guillain-Barré syndrome have been described in chronic HCV infection. Electrophysiologic investigations confirm axonal degeneration, and histology shows a vasculitis.

The response to antiviral therapy and plasmapheresis is discouraging.

**Psychiatric disorders.** There is growing evidence that HCV may have a direct effect on neuropsychiatric function [129, 249]. HCV involvement of the central nervous system may manifest as fatigue, depression and result in a markedly diminished quality of life [37]. Depression also is a major side effect of interferon treatment and may lead to discontinuing therapy.

The 5-hydroxytryptamine receptor type 3 antagonist ondansetron has been reported to have a significant positive effect on fatigue in chronic hepatitis C [179]. Citalopram treatment is highly effective in HCV patients with interferon-induced depression [120].

### Others

**Arthritis** and **arthralgias** are often associated with cryoglobulinemia of viral hepatitis. The condition is probably mediated by immune complexes.

**Gallbladder disease**. Chronic hepatitis C was strongly associated with gallbladder disease (gallstones or cholecystectomy) among men but not women in the United States in a study of more the 13,000 persons [15].

**Cardiovascular disease**. Of all HIV-infected individuals in the United States, approximately 15–30% are co-infected with HCV. It has been suggested that hepatitis C may be independently associated with cardiovascular disease in HIV-infected individuals. HCV/HIV coinfection was associated with a > 4.5-fold increased prevalence of cardiovascular disease after controlling for age and other confounders [69].

**Lung disease**. There are case reports and small series suggesting an association between *idiopathic pulmonary fibrosis* and chronic hepatitis C. HCV-RNA has been demonstrated in bronchoalveolar lavage in some of these patients [3, 19, 100].

**Sexual dysfunction.** Sexual dysfunction as assessed by a questionaire evaluating sexual drive, erection, ejaculation, sexual problem assessment, and overall sexual satisfaction is highly prevalent among men with chronic hepatitis C, and is independent of depression [38]. It is not surprising that this contributes to the HCV-associated reduction in quality of life.

## Differential Diagnosis

The discussion on the differential diagnosis of hepatitis B pertains also to hepatitis C (see Section 63.3 "Hepatitis B"). Chronic HCV infection associated with anti-LKM antibodies must be distinguished from autoimmune hepatitis (see Chapter 72).

## Natural Course and Prognosis

The natural course of hepatitis C is influenced by a number of host factors (age, gender, immune status,

genetic susceptibility, alcohol use, smoking, hepatotoxic drugs, accompanying diseases), by coinfections with other hepatotropic viruses, and by viral factors (HCV genotype, viral load). In patients with acute hepatitis C who successfully clear the virus, HCV-RNA concentrations already decline prior to the rise in serum aminotransferase levels. Patients with icteric acute hepatitis C seem to have a better prognosis and do eliminate the virus more often than anicteric patients. Host related factors, in particular female sex and cell mediated immunity (HCV specific T cell response, i.e. virus-specific CD4 function and maturation of antiviral memory CD8 response), play an important role in the spontaneous clearance of HCV [236]. A negative HCV-RNA test and broad cell mediated immunity within the first month after onset of the symptoms represent efficacious predictors of viral clearance [222]. Alcohol and HIV coinfections are negatively associated with viral clearance [178]. HCV genotype 1b infection signals an unfavorable prognosis with a chronic course.

There is recent evidence that even after serological eradication of HCV (HCV-RNA negative, HCV antibodies positive) the virus may persist at low levels and affect the natural history and liver histology. Nonviremic HCV antibody-positive patients may present fibrosis and inflammatory activity similar to viremic cases. The presence of a $CD8^+$ rich inflammatory infiltrate suggests an ongoing immune response in the liver, supporting the view that HCV may persist in the liver in the majority of HCV RNA-negative cases [91].

*Fulminant hepatitis* occurs in less than 1% of cases of acute hepatitis C and may be more common in patients with underlying chronic hepatitis B virus infection, or be associated with hepatitis A superinfection in patients with chronic hepatitis C [32, 240].

HVC infection results in a high frequency (approximately 80%) of chronic disease. Twenty to 30% of patients with chronic hepatitis C insiduously progress to *cirrhosis* (Fig. 63.49). The most important process on the road to cirrhosis is progressive fibrosis. Duration of infection is the most consistent factor significantly associated with progression of fibrosis. It is important to realize that the *progression of fibrosis is not linear*, and requires evaluation over long follow-up periods. The majority of fibrosis progression occurs in patients aged 50 years or older [75, 185, 231]. In a large meta-analysis of 111 studies of 33,121 individuals with chronic HCV infection the estimated prevalence of cirrhosis at 20 years after the infection was 16% [231].

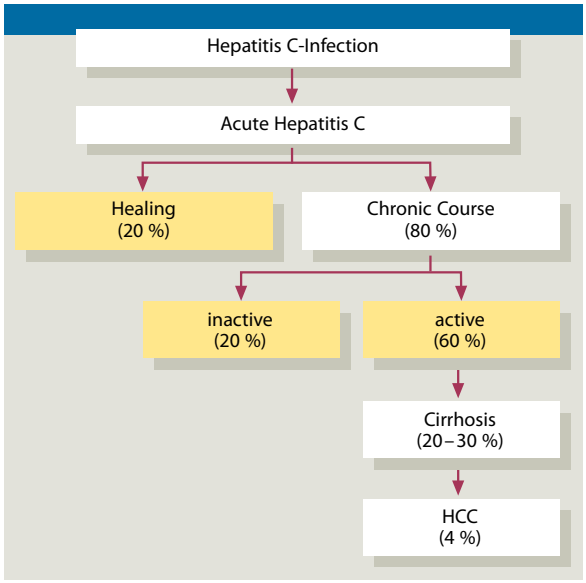


**Fig. 63.49** Natural course of hepatitis C

Host factors predictive of disease progression are (1) acquisition of HCV infection after the age of 40–55, (2) a high body mass index and hepatic steatosis, (3) even a relatively low alcohol intake (30–50 g/day in men and 20–50 g/day in women), justifying the recommendation that alcohol intake should be avoided in all patients with chronic HCV infection, and (4) smoking (nicotine and cannabis) [45, 54, 87–89, 96, 101, 127, 176]. Progression of liver fibrosis in women may be affected by hormonal factors. Menopause appears to be associated with accelerated liver fibrosis progression. Since estrogens may have an antifibrotic effect, progression of fibrosis possibly may be delayed or even prevented by hormone replacement therapy [48].

*Hepatocellular carcinoma* may develop on the background of liver cirrhosis after a mean latency period of 20–25 years. HCV accounts for approximately one third of HCC cases in the United States. The risk of developing HCC once cirrhosis has developed is up to 3% per year. In contrast to HBV infection, HCV associated HCC occurs almost exclusively in patients with cirrhosis [39]. Overweight, diabetes mellitus, chronic HBV coinfection and HCV genotype 1b are associated with an increased risk of HCC occurrence in patients with HCV-related cirrhosis [20, 99, 167, 239].

Thus, the natural course of chronic hepatitis C implies on one hand that in most patients chronic hepatitis C has a relatively benign clinical course over years; on the other hand persons infected for years or decades are diagnosed not until advanced liver disease with its complications becomes manifest. The serum levels of aminotransferases do not mirror viral replication in the liver, and in immunosuppressed patients low serum aminotransferases are associated with high HCV-RNA concentrations.

Survival of patients with chronic hepatitis C is generally not impaired until cirrhosis has developed. The 3, 5, and 10-year survival rates in 384 patients with compensated cirrhosis were 96%, 91%, and 79%, respectively [60].

In a more recent study a cohort of 214 patients with compensated cirrhosis due to HCV were followed for 17 years. HCC developed in 68 (32%), ascites in 50 (23%), jaundice in 36 (17%), upper gastrointestinal bleeding in 13 (6%), and encephalopathy in 2 (1%), with annual incidence rates of 3.9%, 2.9%, 2.0%, 0.7%, and 0.1%, respectively. HCC was the main cause of death (44%) and the first complication to develop in 58 (27%) patients, followed by ascites in 29 (14%), jaundice in 20 (9%), and upper gastrointestinal bleeding in 3 (1%). The annual mortality rate was 4.0% [204].

## Chronic Hepatitis C with Persistently Normal Aminotransferases

Patients with PNALT usually have a higher viral load but a more favorable histology with a slower rate of fibrosis progression when compared with patients with persistently elevated ALT levels [2, 70, 215]. The potential for progression to advanced liver disease, however, still exists [70]. Spontaneous resolution of HCV infection in patients with PNALT may occur, but is a very rare event, and on long-term follow-up 30% of such carriers become candidates for antiviral therapy within 5 years [33, 168, 260].

## Hepatitis C and Pregnancy

Women chronically infected with HCV can have an uneventful pregnancy without worsening of liver disease or other adverse effects on the mother or fetus. Spontaneous improvement of chronic hepatitis C during pregnancy has been reported, but there is evidence of deterioration of hepatitis C after delivery [65]. Vertical transmission of HCV is much less efficient than for HBV, occurring in approximately 5–10% of infants born to HCV-RNA positive mothers.

## Hepatitis C in Children

Chronic hepatitis C in children usually is associated with lower ALT and HCV-RNA levels in serum combined with only mild histopathological alterations. Children appear to have a relatively decreased risk of disease progression. Only a few children, however, clear viremia spontaneously (most likely infected with genotype 3) and persistent viral replication may lead to end-stage liver disease in a small subgroup characterized by perinatal exposure, maternal drug use, and infection with HCV genotype 1a [18].

## Coinfection with HBV

Coinfection with HBV usually is associated with a more severe course with a high risk of progression to end-stage liver disease and development of HCC. Interferon therapy might be less effective in preventing HCC among patients with chronic hepatitis C who are anti-HBc-positive than in those with chronic hepatitis C who are anti-HBc-negative [99]. One should also be aware of occult HBV infection present in patients with chronic HCV liver disease, i.e. many coinfected patients lack serologic markers of HBV infection. In one study HBV-DNA sequences were detected in the liver in one third of patients despite the absence of HBsAg, and patients with HCV were significantly more likely to be positive for HBV-DNA than controls (33% versus 14%) [23]. HCV infection, however, may also inhibit HBV replication and result in seroconversion with loss of HBsAg in some coinfected patients.

## Coinfection with GBV-C/HGV

A concomitant GBV-C/HGV infection does not appear to impact the natural course of HCV infection.

### Coinfection with HIV

**Effect of HIV on chronic hepatitis C.** In the pre-HAART era, coinfection of HCV patients with HIV was a significant factor for accelerated progression of chronic hepatitis C to cirrhosis, but was not associated with the development of HCC (probably because most patients succumbed to AIDS prior to the appearance of HCC) [119]. HIV coinfection reduced considerably the survival of patients with HCV-related end stage liver disease independently of other markers of poor prognosis [180]. The introduction of HAART has changed this scenario dramatically. *HCV infection in successfully treated HIV patients has a course similar to that in HIV negative patients*. Once immunodeficiency is advanced with T helper cells < 100/μL HCV infection may run a rapidly progressive, occasionally cholestatic course and patients may die from liver related complications (bleeding, hepatic insufficiency).

When interpreting elevated liver enzymes in HIV/HCV coinfected patients, one should also be aware of the hepatotoxic potential of antiretroviral drugs (see Chapter 71).

**Effect of HCV on HIV infection.** HCV coinfection does not seem to affect adversely the course of HIV infection. In a recent epidemiological study coinfection with HCV was even associated with a significant decrease in the mortality of HIV-infected patients [51].

### $\alpha_1$-Antitrypsin Deficiency

The concomitant occurrence of $\alpha_1$-antitrypsin deficiency and HCV infection results in an increased risk of cirrhosis and a shortened life expectancy.

### Porphyria Cutanea Tarda

Patients with porphyria cutanea tarda and HCV infection have an accelerated progression of liver disease.

### Diabetes Mellitus

Type 2 diabetes mellitus and the metabolic syndrome have profound effects on HCV infection (see above). Diabetic patients with HCV cirrhosis have been reported to have a more severe hepatic encephalopathy compared to patients without diabetes mellitus [218]. For patients with chronic hepatitis C and advanced cirrhosis, diabetes mellitus increases the risk of developing HCC [167, 239].

## *Prevention*

Currently immune prophylaxis, neither active nor passive is available for hepatitis C. Prevention of hepatitis C relies on identifying and counselling uninfected persons at risk for hepatitis C (e.g., injection-drug users) regarding ways to protect themselves from infection and on identifying and preventing transmission of HCV in healthcare settings.

### Approach to Accidental Exposure (Needle Stick Injury)

There are no guidelines as how to proceed after accidental needle stick exposure to HCV infected material. The approach practiced at the author's institution is outlined here.

After accidental injury with HCV infected blood immediate cleaning and disinfection of injured skin seems reasonable, although not proven by studies. Thereafter the potentially HCV positive person ("blood donor") and the injured person (in order to document HCV negativity) are tested immediately for the presence of HCV antibodies and HCV RNA, and aminotransferase levels in serum are determined in both. The accident should be documented carefully and the risk of transmission (depth of the wound, needle type, clinical and virological state of the potentially contagious person) should be estimated.

During the first 2 months after exposure aminotransferases are determined in intervals of 2 weeks, thereafter once monthly for 4 months. During this period careful attention is to be given to the appearance of nonspecific symptoms of viral hepatitis. Measuring HCV RNA in serum 2, 4 and 12 weeks after the injury allows one to determine whether or not an infection has occurred. After 3 and 6 months, testing for anti HCV (in addition to measuring aminotransferases) is recommended. If aminotransferases rise, measurement of HCV antibodies and HCV RNA in serum should occur in any case.

Postexposure prophylaxis with immunoglobulin or the immediate administration of interferon is not indicated. Only if acute infection is documented treatment with standard or pegylated interferon should be started (see below).

## *Therapy*

The goal of treatment is sustained clearance of HCV. In the case of acute hepatitis C chronicity is prevented, in chronic hepatitis C sustained elimination of HCV is followed by disappearance of necroinflammatory activity and prevention of the development of cirrhosis and HCC.

### Therapy of Acute Hepatitis C

There are no established treatment guidelines for acute hepatitis C. Interferon α (IFN) and more recently pegylated interferon α (pegIFN) monotherapy have been shown to be effective in patients with acute hepatitis C. Treatment regimens used are

IFN 2a/2b 5 MU s.c. daily for 4 weeks followed by 5 MU s.c. three times weekly for 20 more weeks (total duration of therapy 24 weeks)

or

pegIFN α-2a (180 µg once weekly) or pegIFN α-2b (1.5 µg/kg once weekly) for 12–24 weeks.

If treatment is started within 8 to 12 weeks after the onset of symptoms of acute hepatitis or the appearance of HCV RNA, end-of-treatment and sustained virological response rates in patients who strictly adhere to the treatment regimen are > 90% and approximately 90%, respectively [44, 108, 112, 206, 250, 251].

There is some controversy as to when to start antiviral therapy. In view of the high rate (up to 68%) of spontaneous viral clearance within 12 weeks after the onset of symptomatic disease some authors advocate treating only those patients who remain HCV RNA positive for more than 3 months after the onset of disease [74, 131, 205]. Others, however, recommend early initiation of treatment within 8–12 weeks after the appearance of HCV RNA [44, 112, 251]. Considering recent data that suggest that even non-viremic patients with HCV antibodies may have persistent hepatic HCV infection the author prefers the early approach to therapy [91].

Taking into account the excellent sustained viral responses of IFN or pegIFN monotherapy there is no compelling evidence to include ribavirin in the treatment of acute hepatitis C.

### Therapy of Chronic Hepatitis C

IFN combined with ribavirin was introduced as the standard treatment of chronic hepatitis C in 1999. In November 2000 standard IFN has been replaced by pegIFN in chronic HCV infection [26, 40, 86, 132, 138, 149, 184, 259]. Current developments are characterized by attempts to predict early the viral response to therapy in the individual patient, thus allowing for the indvidualization of treatment. In addition, new substances are being developed.

#### Definitions of Response to Treatment

Patients responding to therapy usually show normalization of elevated aminotransferase levels within 4–6 weeks (*biochemical response*) with HCV RNA becoming non-detectable (*virologic response*). The *end-of-treatment response* describes results of HCV RNA testing at the end of treatment, while *sustained viral response* is defined by negative HCV RNA status ≥ 6 months after the end of therapy. If initially normalized aminotransferases rise again after the end of treatment, *biochemical relapse* is said to be present, while renewed HCV RNA positivity characterizes *virologicl relapse. Breakthrough phenomenon* describes the renewed elevation of initially normalized aminotransferase levels (*biochemical breakthrough*) or the reappearance of initially nondetectable HCV-RNA (*virologic breakthrough*) during therapy. If aminotransferase levels do not normalize and HCV-RNA remains detectable during treatment the patient is a *nonresponder*. The definition of terms based on HCV-RNA during therapy is summarized in Table 63.32.

#### Drugs used in the Treatment of Chronic Hepatitis C

**Interferons.** For actions, pharmacology and side effects of interferons see this Chapter, Section on "Hepatitis B".

**Table 63.32** Definition of terms based on HCV-RNA during therapy

| Term | HCV-RNA |
|---|---|
| **Non-response** | Positive at all times during therapy |
| **Relapse** | Negative during therapy, positive after end of treatment |
| **Rapid Virologic Response** | Negative (< 10–30 IU/mL) after 4 weeks of treatment |
| **Complete Early Virologic Response** | Negative (< 10–30 IU/mL) after 12 weeks of therapy |
| **Partial Early Virologic Response** | ≥ 2-log reduction after 12 weeks of therapy |
| **Sustained Viral Response** | Negative 24 weeks after end of therapy |

***Standard IFN*** is not used anymore in the therapy of chronic hepatitis C.

Coupling polyethyleneglycol to IFNα (pegylation) results in the formation of ***pegylated interferons*****.** They have improved pharmacokinetic and pharmacodynamic characteristics compared to standard IFN, and are therefore easier to use. Pegylation of IFNα leads to an increased solubility in water, to a reduced antigenicity and to a prolonged half life and duration of action due to slowed renal clearance [78].

Currently 2 pegylated interferons (peg-interferon α-2b [12 kD]) and peg-interferon α-2a [40 kD]) are available for treatment of chronic hepatitis C. PegIFN α-2b is administered at a weight-based dose of 1–1.5 μg/kg s.c. once weekly while pegIFN α-2a is given at a flat dose of 180 μg s.c. once weekly. In patients infected with HCV genotype 1 ( probably also with others genotypes ) the rates of sustained virologic response do not differ significantly between the two peginterferon – ribavirin regimes ( see below ) [151a].

***Consensus interferon*** (interferon-alfacon-1) combines the most frequent amino acid sequences of naturally occuring IFNs-α to one "consensus protein". Its biological activity in vitro is approximately ten times higher than that of IFN α-2a or 2b. Response rates to consensus IFN are comparable to those of standard IFN [85]. The vast majority of specialized centers do not use consensus interferon in patients with HCV infection.

***Albinterferon α-2b*** **i**s a novel recombinant 85.7-kDa protein consisting of IFN α-2b genetically fused to human albumin. It has been shown to retain the antiviral properties of IFNα. Due to its longer half life it can be administered with an improved dosing schedule, once every 2 or 4 weeks, at a dose of 900 μg or 1,200 μg s.c., respectively, offering comparable efficacy to pegIFN α-2a (at the time of writing albinterferon α-2b has not yet been approved for clinical use) [7, 264].

**Ribavirin**. Ribavirin is a purine nucleoside analog that inhibits the replication of a wide range of RNA and DNA viruses. In patients with chronic hepatitis C it is administered orally, adapted to the patient's weight (< 75 kg: 500 mg bid; ≥ 75 kg: 600 mg bid). In obese patients weighing 105–125 kg its dose should be increased to 1,400 mg/day.

**Amantadine**. Most authors agree that adding amantadine to pegIFN/ribavirin combination therapy (triple therapy) does not improve significantly virologic response rates in patients with chronic hepatitis C [43, 62, 171, 246].

## Combination Treatment

The treatment of chronic hepatitis C is outlined in Table 63.33. Patients with genotype 1 or 4 infections are treated for 48 weeks. Treatment duration of 24 weeks compared with 48 weeks does not impair sustained viral response rates in patients with HCV genotype 2 or 3 infection. Thus, treatment for 24 weeks with pegIFN and ribavirin is sufficient in HCV genotype 2 or 3 infected patients [225, 261]. In contrast to genotype 1 or 4, in patients with HCV genotype 2 or 3 infection,

**Table 63.33** Standard treatment of chronic hepatitis C with pegylated interferon and ribavirin

| Genotype | (1) PegIFN α-2a<br>(2) PegIFN α-2b | Ribavirin[a] | Duration of Therapy[b] |
|---|---|---|---|
| 1 or 4[c] | (1) 180 μg s.c. qw | ≥ 75 kg: 600 mg bid | 48 weeks |
| | (2) 1–1.5 μg/kg s.c. qw | < 75 kg: 500 mg bid[c] | |
| 2 or 3[d] | (1) 180 μg s.c. qw<br>(2) 1–1.5 μg/kg s.c. qw | 400 mg bid | 24 weeks |

[a]Ribavirin dose should be adjusted to 1,400 mg/day in obese patients (105–125 kg) with HCV genotype 1 infection

[b]HCV-RNA should be tested already 4 weeks after the start of therapy in order to recognize rapid viral responders

[c]In patients with HCV genotype 1 or 4 infection, quantification of the viral load is mandatory at baseline and during therapy

[d]Patients with HCV genotype 2 or 3 infection are sufficiently treated with combination therapy for 24 weeks regardless of baseline viral load

flat doses of 800 mg ribavirin daily are proven to be sufficient.

A treatment algorithm for chronic hepatitis C is outlined in Fig. 63.51 (for individualizing treatment see below). In order to avoid unnecessary therapy (costs, side effects), *stopping rules* must be taken into account. Response to antiviral therapy should be assessed after 12 weeks of treatment. *Therapy should be stopped in patients who do not show at least a 2 log decline of baseline viral load after 12 weeks of treatment*, since these patients with all likelihood will not attain a sustained viral response with continued therapy. This stopping rule, however, is not absolute as treatment decisions always must be individualized (see paragraph on "Nonresponders").

**Side effects of and contraindications for combination therapy.** The numerous potential adverse effects of and contraindications for IFNs are listed in Tables 63.25 and 63.26, respectively. The contraindications for ribavirin treatment are reported in Table 63.34. The time course of the major side effects occuring during combination therapy is depicted in Fig. 63.50.

*Flu-like symptoms* regularly occur during the first weeks of treatment and then subside. They are usually well controlled by paracetamol 1 g p.o. prior to the application of pegIFN.

Systemic ribavirin causes dose-related reversible *hemolytic anemia* and bone marrow suppression. Anemia is usually mild and only about 10% of patients have a hemoglobin level of less than 10 g/dL. Occasionally, however, anemia may be so severe as to necessitate discontinuation of treatment. For patients with no cardiac disease who experience significant anemia during therapy dose reduction of ribavirin to 600 mg/day is recommended for Hb levels of 8.5–10 g/dL. If Hb falls to less than 8.5 g/dL, ribavirin should be discontinued. If a patient with stable cadiac disease experiences a > 2 g/dL Hb drop in any 4-week period, the dose of ribavirin should be reduced by 600 mg/day. Ribavirin should be discontinued permanently if patients have a Hb level < 12 g/dL 4 weeks at the reduced dose of ribavirin [227].

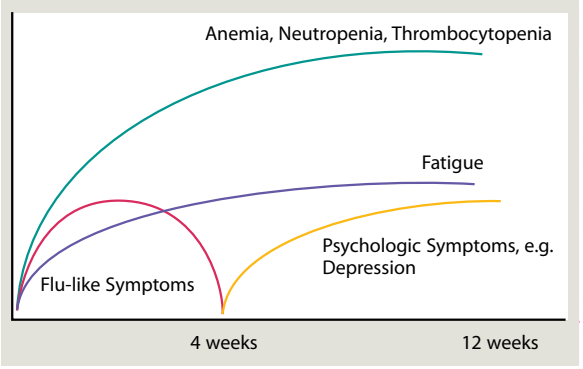


**Fig. 63.50** Major side effects of interferon therapy in relation to duration of treatment

**Table 63.34** Contraindications for treatment with ribavirin

- Severe hepatic dysfunction, decompensated liver cirrhosis
- Anemia (Hb < 10 mg/dL)
- Hemoglobinopathies (e.g. thalassemia, sickle cell anemia)
- Severe cardiac diseases (e.g. coronary heart disease, myocardial infarction)
- Pregnancy or inadequate contraception
- Breastfeeding
- Allergies
- Renal failure (creatinine clearance < 50 mL/min)
- Ongoing alcohol or substance abuse
- Poor compliance

Ribavirin alternatives with a lower risk for hemolytic anemia are therefore desirable. *Viramidine* is the amidine prodrug of ribavirin, which is taken up preferentially by hepatocytes during first-pass metabolism and has less drug available for entry into erythrocytes.

Erythropoetin (epoetin alpha) 40,000–60,000 units s.c. once weekly leads to an increase in Hb levels of 2.3–2.8 g/dL. It may, however, worsen pegIFN induced thrombocytopenia [92]. Erythropoetin treatment of anemia in HCV infected patients has not been approved by the FDA.

Due to its *teratogenic* and *mutagenic potential* pregnancy and breastfeeding are contraindications for ribavirin therapy. Women in childbearing age treated with ribavirin should be advised to practice effective anticonception that should continue up to 4–6 months after end of treatment. Men treated with ribavirin should not procreate during and up to 7 months after end of ribavirin therapy.

Over the course of pegIFN treatment, nearly 50% of subjects experience a grade 3 or 4 *neutropenia*. 4–5% of patients experience an absolute neutrophil count less than $0.5 \times 10^9$/L (500/mm$^3$) (grade 4 neutropenia).

With neutropenia of $0.5–1.5 \times 10^9$/L the dose of pegIFN should be reduced by 50% with an absolute neutrophil count of $< 0.5 \times 10^9$/L pegIFN should be discontinued. Human granulocyte colony-stimulating

factor (G-CSF) (filgrastim) 300 µg s.c. biw–tiw should be considered.

With *thrombocytopenia* < 50 × $10^9$/L the dose of pegIFN should be reduced by 50%. With platelet numbers < 25 × $10^9$/L pegIFN should be discontinued. Eltrombopag is promissing but not yet approved for clinical use in IFN-indnced thrombocytopenia [151]. The only product approved by the FDA for the management of cancer chemotherapy-related thrombocytopenia is interleukin-11 (oprelvekin). Recombinant interleukin-11 appeared useful in the treatment of HCV associated immune thrombocytpenic purpura (see above) [67]. No data are available on interleukin-11 in ribavirin induced thrombocytpenia.

*Depression* may be severe and a cause for poor compliance and discontinuation of therapy. Antidepressants, such as citalopram (20 mg/day), which may also be used prophylactically, are effective in treating IFN induced depression and restoring compliance [120, 210].

## Results of Treatment

Treatment results have improved constantly over the last years and overall sustained viral response rates (SVR) after treatment for 48 weeks currently are approximately 50% in patients with HCV genotype 1 or 4 infection, and around 80% for those with genotype 2 or 3 infection.

Factors associated with high SVR are

- Non-1 genotype
- Baseline viral load ≤ 2 × $10^6$ copies/mL
- Weight ≤ 75 kg
- Age ≤ 40 years
- Non–African American
- No or minimal fibrosis
- Body mass index < 30 kg/$m^2$
- Absence of steatosis or metabolic syndrome
- Female sex [254]

IFN-based therapies are conducted over longer periods of time and are associated with potentially serious adverse effects. Thus, patient motivation and guidance are essential in initiating and maintaining treatment and key to the success of therapy. Adherence to the treatment protocol is essential in achieving optimal results.

Ninety percent of patients who achieve an SVR remain HCV-RNA undetectable at a mean of 4.1 years of follow-up and SVR is durable up to 18 years after treatment cessation [144]. *Clinical relapse is extremely rare in patients who achieve an SVR and these patients may be deemed clinically cured of chronic HCV*. Elevated liver enzymes normalize, quality of life improves, the risk of cirrhosis and HCC diminishes [14, 98, 238]. Although achieving SVR is associated with a lowering of long-term liver related mortality, residual HCV-RNA may persist at very low levels in the liver, the serum and peripheral lymphoid cells, and the risk of complications and development of HCC is not completely abolished. Therefore, careful long-term observation of these patients (especially elderly male HCV cirrhotics) even after achieving SVR is advisable [21, 117, 188].

The beneficial effects of pegIFN/ribavirin therapy on liver histology are closely related to virologic response [27]. Successful treatment may result in regression of fibrosis. Full-blown cirrhosis, however, is irreversible, despite claims to the contrary by some [144].

## Individualizing Treatment

The response to pegIFN/ribavirin treatment is influenced by patient and virus related factors. Current attempts try to *predict response to therapy* in order to be able to tailor treatment to the needs of the individual patient [12].

The major *patient related factors* associated with impaired response to combination therapy are

- Advanced fibrosis or cirrhosis (SVR in 8–44% of patients), ethnic factors (African Americans have less favorable response to treatment [19–26%] than whites)
- Male gender
- Older age
- Obesity
- Metabolic syndrome (insulin resistance)
- Elevated pretreatment γGT levels (probably a surrogate marker for hepatic steatosis, fibrosis and intrahepatic expression of TNF α) [53, 84, 102, 197, 219, 229]

The major *viral factors* associated with impaired response are

- HCV genotype 1 and
- High viral load.

Individualizing treatment based on patient related factors is difficult. Weight-based ribavirin, i.e. increasing the dose to 1,400 mg/day in obese patients weighing 105–125 kg has been shown to be more effective than flat-dose ribavirin [106]. However, even with weight-based dosing, response rates in African Americans are lower than reported in other ethnic groups [107].

Since patient related factors are unreliable in predicting the individual response to treatment, viral factors are being increasingly analyzed. *Viral kinetic analysis*, i.e. quantification of HCV-RNA during early therapy is becoming increasingly important in individual treatment decisions. Viral kinetic analysis can be used as an early predictive marker for SVR and on the other hand may allow for early detection of nonresponders and early treatment discontinuation [175].

HCV-RNA reduction during reduction antiviral therapy typically shows a biphasic pattern with a rapid first phase during the initial 24–48 h and a less rapid second phase of viral decline thereafter [153, 163]. The more rapidly HCV-RNA declines during therapy the higher is the chance to achieve SVR. Identifying patients with a *rapid virologic response* (RVR), i.e. non-detectable HCV-RNA by PCR after 4 weeks of treatment allows for a shortened treatment without compromising SVR rates [61]. Thus, irrespective of genotype *HCV RNA should be tested 4 weeks after initiation of therapy in order to recognize RVR*.

**HCV genotype 1 and 4**. Shortening of treatment from 48 to 24 weeks in HCV genotype 1 patients with a low baseline viral load (< 600,000–800,000 IU/mL) and a RVR does not impair SVR. In patients with genotype 4 achieving RVR, 24 weeks of treatment are sufficient irrespective of the baseline viral load [36, 64, 110, 136, 137, 245, 256, 263].

On the other hand, extending the treatment duration from 48 weeks to 72 weeks in HCV genotype 1 infected patients who fail to achieve a RVR significantly improves SVR rates. Treatment extension does not seem to increase the rate of side effects induced dose reduction or therapy discontinuation [174, 203].

**HCV genotype 2 and 3**. Shortening of treatment from 24 to 16 weeks in patients with low pretreatment viremia (≤ 400,000 IU/mL) in patients with RVR does not impair SVR [36, 136, 137, 217]. When administered for 24 weeks with pegIFN, ribavirin doses of 400 and 800 mg/day produce equivalent outcomes in patients infected with HCV genotype 3 [63].

An individualized genotype-specific treatment algorithm of chronic hepatitis C is outlined in Fig. 63.51.

### New Approaches to Treatment

Despite the improvements achieved over the last years, treatment of chronic hepatitis C still is unsatisfactory. Intense research efforts to develop new antiviral drugs are ongoing. Among them are *serine protease inhibitors* (telaprevir, boceprevir), *polymerase inhibitors*, *NS5A inhibitors*, *cyclophilin inhibitors* and antiapoptotic *caspase inhibitors* [123, 182]. Some of these emerging drugs are currently tested in phase 2 studies.

Serine protease inhibitors intensify the action of standard combination therapy and telaprevir has an increased antiviral effect when combined with pegIFN [68, 116, 207]. The combination of telaprevir, pegIFN, and ribavirin is generally well tolerated [126].

The problem of protease inhibitors is the emergence of viral resistance.

*Long-acting interferons* currently tested in phase 2 studies are *locteron, interferon-omega*, and an *oral interferon (belerofon). Albinterferon α-2b* (see above) is already tested in phase 3 studies

## Therapeutic Problems in Special Patient Populations

### Patients with Persistently Normal Aminotransferase Levels (PNALT)

Most patients with PNALT have a slow rate of fibrosis progression and a favorable natural history of HCV infection. However, the potential for progression to advanced liver disease persists and 10% of the PNALT patients have stage 3 bridging fibrosis on liver biopsy [70]. Patients with PNALT treated with pegIFN/ribavirin have a rate of SVR comparable to that in patients with elevated ALT and should not be excluded from treatment on the basis of ALT alone [104, 260]. The decision to initiate combination therapy should be individualized based on the severity of liver disease by liver biopsy.

### Nonresponders and Relapsers

The lack of response of chronic hepatitis C to current antiviral therapy may have many reasons which have been discussed above. It may be treatment-related (drug efficacy, dosing, side effects), host-related (adherence to treatment protocol, alcohol use, accompanying

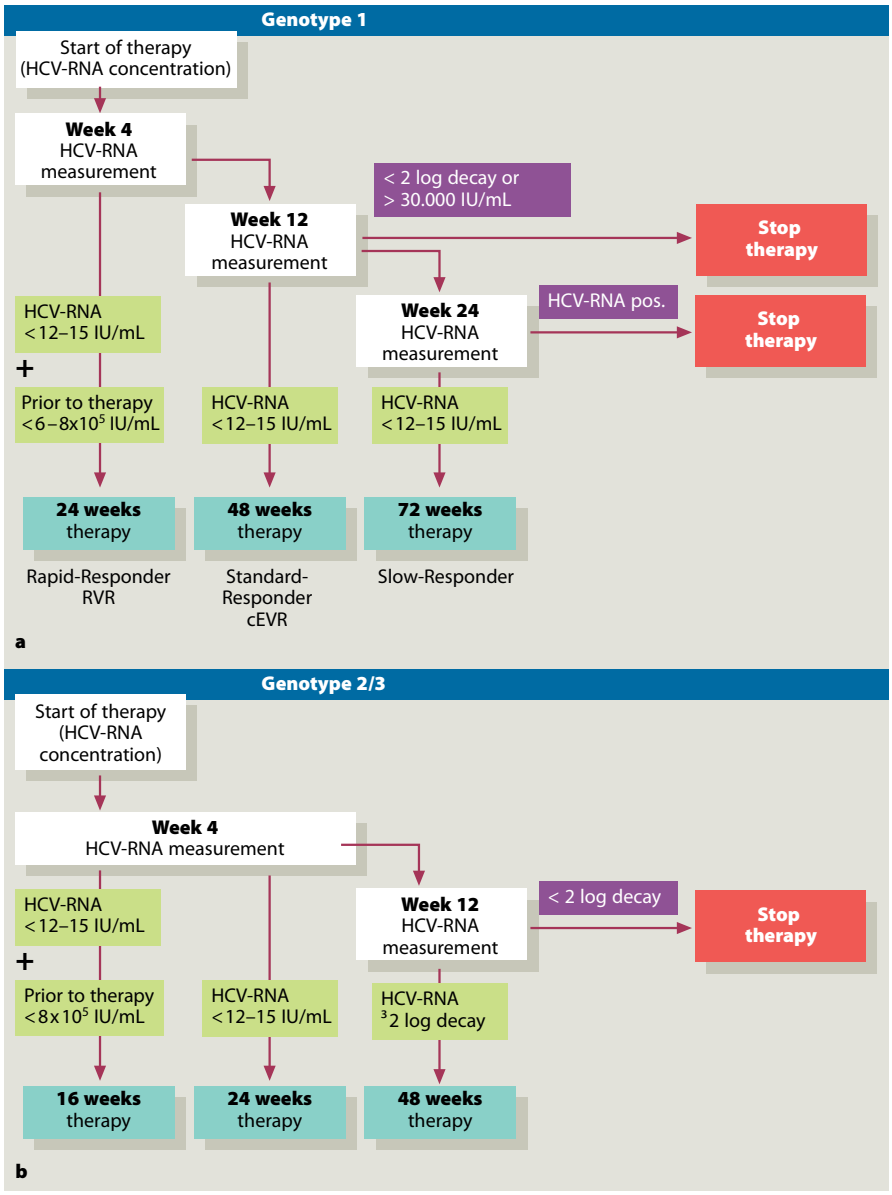


**Fig. 63.51** Genotype-specific and individualized treatment algorithms for chronic hepatitis C (according to the 2009 guideline of the German, Austrian and Swiss Society for Gastroenterology; publication in print). (**a**) Treatment algorithm in genotype 1 HCV-infection. (1) HCV-RNA not detectable with a highly sensitive assay (< 12–15 IU/mL or < 50 IU/mL, depending on the assay used). (2) Cut-off value for initial viral load prior to therapy 600,000 IU/mL for pegIFN alpha-2b and 800,000 IU/mL for pegIFN alpha-2a. Treatment should possibly not be shortened in patients with negative predictive factors, such as advanced fibrosis or cirrhosis, metabolic syndrome, insulin resistance, hepatic steatosis. No data available in patients with persistently normal aminotransferase levels. (**b**) Treatment algorithm in genotype 2/3 HCV-infection. (1) HCV-RNA not detectable with a highly sensitive assay (< 12–15 IU/mL or < 50 IU/mL, depending on the assay used). (2) Treatment should not be shortened in patients with advanced fibrosis or cirrhosis. Negative predictive factors, such as nonalcoholic fatty liver disease and low ALT levels prior to therapy should be taken into consideration. No data available in patients with persistently normal aminotransferase levels. (3) If HCV-RNA still is detectable (lower limit of detection < 12–15 IU/mL) at week 24 therapy should be stopped. (4) Duration of treatment (36, 48 or 72 weeks) in slow responders is not precisely defined, and is currently investigated in prospective studies. Complete early virologic response (cEVR)

diseases, advanced liver disease), related to viral factors (genotype 1/4 vs. 2/3, viral coinfections) and ethnic factors (response rates in African Americans is less than half the rate seen in non-Hispanic whites).

There are no accurate tests to screen for antiviral nonresponse prior to initiating therapy, but results of HCV-RNA kinetics at 4 and 12 weeks after the start of therapy are important to recognize difficult-to-treat patients.

The options to treat nonresponders and relapsers are limited. They can be retreated with initial combination therapy for longer periods (for example 72 weeks), or with higher doses (for example doubling the dose of pegIFN) [105, 121, 201, 230].

If treatment with currently available drugs does not result in SVR the only remaining option is to wait for new drugs. Protease inhibitors combined with pegIFN exhibit antiviral activity and offer a potential new therapeutic option for this hard-to-treat, nonresponder patient population [208]. Long-term maintenance therapy with pegIFN does not reduce the rate of disease progression in patients with chronic hepatitis C and advanced fibrosis, with or without cirrhosis, who did not respond to initial treatment with pegIFN and ribavirin [46a].

### Liver Cirrhosis

Patients with HCV related cirrhosis respond poorly to pegIFN/ribavirin therapy and frequently relapse. Decompensated cirrhosis is a contraindication for IFNs, although in decompensated cirrhotics, HCV clearance may be life-saving [97]. Achievement of SVR in patients with cirrhosis after pegIFN/ribavirin therapy is associated with a reduction of liver-related mortality lowering both the risk of complications and HCC development. Irrespective of SVR achievement, all patients should continue surveillance because the risk of occurrence of HCC is not entirely avoided [21, 47]. Prolonged (2 years) standard IFN monotherapy has little or no impact on complication-free survival in patients with compensated HCV cirrhosis [59].

### Recurrent Disease after Liver Transplantation

Nineteen studies (611 patients) evaluating antiviral therapy with pegIFN in combination with ribavirin for the management of recurrent hepatitis C after liver transplantation were reviewed recently. The mean rate of SVR was 30.2%. Dose reduction and discontinuation of treatment were common in this patient population. The lack of an early virologic response at 3 months of therapy was the most frequent predictive factor of nonresponse [11] (see also Chapter 103).

### End-Stage Renal Disease

Patients with end-stage renal disease continue to have a high prevalence of HCV infection and are generally difficult to treat. Administration of ribavirin is restricted because of anemia which is universal in this patient population. Furthermore, ribavirin accumulates and cannot be removed by dialysis. Despite these caveats cautious use of reduced doses of ribavirin is possible with close monitoring of hematocrit levels and additional measures to enhance compensatory erythropoiesis, such as erythropetin [142].

IFNα monotherapy in dialysis patients shows a high frequency of adverse effects. The SVR, however, is relatively high (34%) in patients who complete treatment [195]. PegIFN once weekly provides more effective and safer therapy than standard IFNα three times a week for treatment-naïve dialysis patients with chronic hepatitis C [133]. As shown recently in a large cohort of patients HCV infected patients on hemodialysis can be treated successfully with pegIFN plus ribavirin with many patients attaining SVR [10, 194].

HCV infected renal transplant recipients have diminished patient and graft survival rates compared with uninfected controls. SVR achieved in patients on hemodialysis has been durable even after subsequent renal transplantation. However, IFN-based therapy of hepatitis C has poor tolerance and safety after renal transplant [55, 142].

### Children

Combination treatment of IFNα or pegIFN with ribavirin is effective and well tolerated in children and adolescents with chronic hepatitis C. Weekly dosing of pegIFN is a considerable advance for this age group [79, 252].

### Ethnic Groups

As in chronic hepatitis B, ethnicity is also a factor affecting response to antiviral treatment in patients with chronic HCV infection. *Asians* are more likely to achieve an SVR to treatment with pegIFN and ribavirin than are whites [155]. *African Americans* seem to have a global defect in their ability to eradicate HCV infection following antiviral combination treatment which transcends across all genotypes. They have significantly lower response rates to treatment than non-Hispanic white patients [34, 159, 216].

### Intravenous Drug Users

HCV treatment should be offered to intravenous drug users (IDU) who are in naltrexone or methadone programs and have a good compliance. The results of antiviral therapy are comparable to non-IDU patients with overall SVR rates of 62% [109].

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## Hepatitis D

In 1977 Rizzetto discovered by immunofluorescence a previously unrecognized nuclear antigen that was subsequently shown to be a novel human pathogen, hepatitis delta virus (HDV) [12]. HDV is a defective virus, a subviral RNA satellite whose replication cycle depends on envelope proteins provided by HBV. Thus, HBV is the natural helper virus of HDV and HDV infection only occurs in association with an acute or persistent HBV infection [6].

### *Epidemiology*

HDV is distributed worldwide. The highest prevalence rates of approximately 20% are found in the Mediterranean area and in the Middle East, where HDV is endemic. Despite the decrease of its prevalence in Turkey, delta hepatitis remains a significant health problem in parts of the country with low socio-economic levels [4]. Surprisingly HDV is relatively rare in the Far East, where HBV is endemic.

HDV displays genetic heterogeneity and circulates within a single infected host, as a mixture of different, albeit closely related genotypes (quasispecies). Three major genotypes are distinguished, that differ in their global distribution. Genotype I is the most common worldwide and is most predominant in the United States, Europe and the Middle East. Genotype II is found predominantly in the Far East, whereas genotype III has been identified in northern South America, where it is associated with outbreaks of severe and fulminant hepatitis and linked with the coinfecting HBV genotype F [1].

Approximately 5% of the global HBsAg carriers, or 15 million people worldwide, have been estimated to be coinfected with HDV [13]. In Central Europe 1–2% of HBsAg carriers are anti-HDV positive. Markedly higher prevalence rates are found in intravenous drug users (38%), in men who have sex with men, and in patients with hemophilia (50%). With improvement in public health standards, increased HBV vaccination rates, and measures to reduce HIV spread, the prevalence of HDV has fallen considerably over the past 20 years.

HDV infection occurs either as a *coinfection*, i.e. simultaneously with HBV infection, or as a *superinfection* of a chronic HBsAg carrier. In superinfection the incubation period is 15–45 days. During the incubation period viremia and infectiosity are high, whereas in the acute and chronic phase both are somewhat lower. Like HBV, HDV is transmitted parenterally.

HDV-containing material is extraordinarily infectious. Minimal doses of infectious serum, diluted up to $10^{-12}$, may cause a hepatitis in HBsAg positive chimpanzees. Familial clustering of HDV infections by the inapparent parenteral route emphasizes the importance of these experimental findings. In endemic areas HDV is transmitted primarily through sexual contact, whereas in non endemic regions (USA, Northern Europe) HDV infection is mainly confined to intravenous drug users. As a result of universal blood screening for HBsAg and HBV vaccination HDV infection has virtually disappeared in polytransfused patients and hemophiliacs in Western countries. Epidemics due to the introduction of the virus by migrants have been described.

### *Pathogenesis*

HBV and HDV bind to the same cellular receptor and require a highly conserved preS1-sequence within the L-protein [5]. Once inside the cell, however, HDV can replicate its genome in the absence of HBV gene products and the spread of HDV does not depend on active replication of HBV.

It is assumed that liver injury in HBV/HDV coinfection is immune mediated. *In vitro* experiments with HBV infected cells, however, also document a direct cytopathic effect of HDV. The large hepatitis delta antigen may induce liver fibrosis through the regulation of transforming growth factor-β- induced signal transduction. This regulation of transforming growth factor-β-mediated signalling is accomplished by isoprenylation of the large hepatitis delta antigen [3]. Intracellular HDV suppresses replication of HBV, so that it is not surprising that HDV positive patients not infrequently are HBsAg negative with very low levels of serum HBV-DNA. With suppression of HBV replication, it is likely that HDV becomes the major pathogenic factor in chronic liver damage. In chronic HBV carriers no efficient immune response against HDV develops, and antibodies against HDV are not protective [8].

## Clinical Manifestations

Acute hepatitis caused by *coinfection* with HBV and HDV cannot be distinguished from HBV monoinfection based solely on clinical findings and routine laboratory parameters, such as liver enzymes and bilirubin. However, the clinical intensity of the disease might be more pronounced in HBV/HDV coinfected patients. The duration of HDV infection is determined by the duration of HBV infection. Complete healing of acute hepatitis B, leads to resolution of simultaneous hepatitis delta infection.

HDV *superinfection* of chronic hepatitis B manifests clinically as an acute hepatitic flare. On liver histology a high necroinflammatory activity prevails and the further clinical course is characterized by worsening of chronic hepatitis B.

Despite low levels of HBV replication, HDV can establish *infection in the transplanted liver* within a few days after liver transplantation. This type of HDV infection remains latent and is associated with no signs of liver disease. However, if HBV replication intensifies, the latent HDV infection is rapidly transformed into a florid hepatitis [6].

## Diagnosis

(See Section 63.1)

The diagnosis of HDV infection by demonstrating HDV-RNA in serum by PCR is presently the most reliable diagnostic method. The technique is very sensitive and PCR can detect 10–100 copies of the viral genome in serum. In the early phase of acute HDV infection HDV-RNA is present in serum, before antibody seroconversion occurs.

The presence of high titers of IgM anti-HDV is indirect evidence of HDV infection. The presence of IgM anti-HBc suggests simultaneous acute HBV/HDV coinfection and can be used to distinguish coinfection from superinfection, in which case the test for IgM anti-HBc is negative. *Chronic HDV infection is defined by the persistence of HDV-RNA in serum for more than 6 months after infection*. As a rule, patients with chronic HDV infection usually maintain high titers of IgM anti-HDV as well as high titers of IgG anti-HDV, although the IgM are monomeric and not pentameric as in primary infection [9]. Interestingly, in superinfection HDV may suppress the replication of HBV through virus interference, so that in the chronic phase of HBV/HDV infection HBV may be undetectable.

In 10% of patients with chronic hepatitis D, antibodies against hepatic and renal microsomal antigens (LKM) are present in serum. In contrast to LKM antibodies present in patients with autoimmune hepatitis and in some patients with chronic hepatitis C, the LKM antibodies found in HDV infection are LKM type 1 and not type 3.

## Natural Course and Prognosis

Infection with HDV occurs either simultaneously with HBV infection or as superinfection of a chronic HBsAg carrier. In both cases HDV may modify the natural course of HBV infection, generally worsening liver disease. Since HDV completely depends on the help from HBV (production of HBsAg), the duration of HDV infection is determined by the duration of HBV infection.

In the vast majority of cases a *simultaneous HBV/HDV infection* runs essentially the same course as an acute hepatitis B and has a comparable prognosis. Chronic infections occur in fewer than 5% of patients. However, HDV infection may worsen acute disease and increase the risk of fulminant hepatitis to 2% of cases and in intravenous drug users to even 5%.

By contrast, more than 90% of HBsAg carriers with *HDV-superinfection* develop progressive disease. HDV-superinfection may accelerate the course of a chronic hepatitis B and transform asymptomatic or mild forms of chronic HBV infection into a severe disease with high necroinflammatory activity. Acute exacerbations and more frequent fulminant courses worsen the prognosis of hepatitis B and are associated with a higher mortality rate compared to hepatitis B alone. In HDV-superinfection, especially with genotype 1, chronic disease with faster progression to cirrhosis and increased risk of developing hepatocellular carcinoma is more frequent than in chronic HBV monoinfection.

Patients with mild chronic hepatitis D have a 5-year probability of survival of 100%, and those with compensated HDV cirrhosis of 81%. Once decompensation supervenes the 5-year survival falls to 49% [14].

*Fulminant courses* have mortality rates of up to 80%. In one third of cases they are due to simultaneous

HBV/HDV infection, and in the remaining two thirds they are caused by superinfection of a chronic HBsAg carrier. Genotype I seems to be more pathogenic than genotypes II and III, causing fulminant disease more frequently. In certain geographical regions, e.g. Eastern Mediterranean and Asia, HDV disease may run a mild course and in some patients an asymptomatic HDV infection ("HDV carrier state") is observed. Overall in chronic HBV/HDV infection older age, HDV genotype 1, and HBV genotype C seem to correlate with adverse outcomes [15].

Additional coinfection of the HBV/HDV infected patient with HIV further worsens liver disease. In patients with HIV coinfection the suppressive effect of HDV on HBV replication is reduced. Persistence or reactivation of HBV and HDV is frequently observed in patients with AIDS.

## *Prevention*

Formalin inactivates HDV. A passive immunization preventing superinfection of HBsAg carriers with HDV is not available. Persons with chronic HBV infection should be advised to diligently observe exposure prophylaxis including avoidance of unprotected intimate contacts with HDV infected individuals. In view of the absolute dependence of HDV on HBV coinfection, *active immunization against hepatitis B is the most effective measure against HDV infection*. Immunity against HBV infection quasi deprives HDV from its culture medium.

## *Therapy*

Chronic delta virus hepatitis is difficult to treat and the results of drug treatment are disappointing with rare sustained responses (clearance of serum HBsAg and seroconversion to anti-HBs) and frequent relapses after cessation of treatment. Thus far, only interferons have been shown to induce a biochemical and virologic response in HDV-infected patients, but the data available are limited and are based on small trials. In pilot studies with small number of patients 48 weeks treatment with IFN-α (9–10 MU s.c. three times a week) or pegIFN-α-2b (1.5 μg/kg per week) led to improvement of liver histology and sustained loss of HDV RNA in 20–43% of patients. The addition of ribavirin had no effect on the viral clearance rate [2, 7, 11]. Two years of treatment with IFN-α does not appear to increase sustained response rates over 1 year treatment [16]. The decrease and disappearance of IgM anti-HDV may predict impending resolution of chronic HDV disease. However, although disease of a short-standing may respond better to therapy, clear predictors of response are still unidentified [10].

Available evidence does not support the use of deoxynucleotide analogs. Famciclovir has no effect on disease activity and HDV-RNA levels. Twelve or 24-month lamivudine treatment does not significantly affect biochemical, virological or histological parameters. In the case of unsuccessful treatment of delta hepatitis with IFN, chronic hepatitis B with persistent HBV replication should be treated with nucleos(t)ide analogs.

Antisense oligonucleotides and prenylation inhibitors hold promise as therapeutic agents of the future.

Patients with end-stage liver disease due to HDV superinfection and those with acute fulminant hepatitis D should be evaluated for orthotopic liver transplantation. The risk of reinfection is lower for HDV than for HBV under long-term administration of hyperimmune serum against HBsAg. However, despite low levels of HBV expression HDV may reappear in the liver of the immunosuppressed post-transplant patient. This serologic profile is associated with a latent and relatively mild disease.

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## Hepatitis E

Hepatitis E is a self-limited, enterically transmitted acute viral hepatitis that occurs frequently in epidemic outbreaks and as sporadic hepatitis [6, 9].

### *Epidemiology*

The hepatitis E virus (HEV) is a hepatotropic RNA virus (see Section 63.1) [6]. Similar to HAV, HEV occurs predominantly in countries with low socioeconomic standards. It is endemic in India (Kashmir), Nepal, South-East Asia (China, Pakistan, Burma, Indonesia), Africa (Algeria, Ivory Coast, Ghana, Chad, Ethiopia, Sudan, Somalia, Kenia), and in South and Central America (Mexico). HEV epidemics mainly occur during floods or with problems of sewage disposal that can occur regularly in overcrowded refugee camps. Sporadic cases are also well documented [12]. HEV infection outside of endemic areas is usually travel associated (i.e. the disease is acquired in an endemic region and imported), though HEV hepatitis also appears to be an emerging disease in industrialized countries [5, 13].

The seroprevalence of HEV-antibodies in healthy persons in the Netherlands is < 0.5%. In patients with Non-A-Non-B-Non-C-Hepatitis HEV-seroprevalence is approximately 2%. The seroprevalence of HEV-antibodies in Central Europe is approximately 1% [6, 9].

HEV is excreted in feces and is transmitted predominantly by the fecal-oral route through contaminated water and foods. Fecal excretion and viremia are short lived [1]. Vertical transmission from infected mothers to their offspring is also documented.

The *incubation period* ranges from 8–10 weeks. In the late incubation period the virus is excreted from the liver via the bile into the feces. Viral excretion begins approximately 1 week prior to the onset of illness and persists for nearly 2 weeks. Viremia can be detected during the late phase of the incubation period [2].

In contrast to HAV, HEV is very unstable and difficult to isolate from the stools. In addition, HEV concentration in stool is markedly lower than that of HAV. Thus, HEV infection is less contagious than hepatitis A, and direct spread of HEV through contact with HEV-infected persons is very rare [9].

## Pathogenesis

The molecular pathogenesis of HEV-infection has not yet been completely clarified. An immune-mediated mechanism of liver damage is assumed. In *in vitro*-experiments, however, the virus also exhibits a direct cytopathic effect.

## Clinical Presentation

The symptoms, signs, and liver function tests in acute hepatitis E are similar to those of symptomatic acute hepatitis A and do not allow for the differentiation of different forms of acute viral infection. Hepatitis E, however, generally appears to be a more severe disease than hepatitis A [3]. The illness may be particularly severe among pregnant women.

Clinical attack rates are highest among young adults. The typical patient complains of fatigue, malaise, adynamia, lack of appetite, mild right upper quadrant pain (due to hepatomegaly), darkening of urine, acholic stools, and jaundice. Asymptomatic and anicteric infections are also known to occur. Serum aminotransferase levels and bilirubin concentrations are markedly elevated, with ALT being higher than AST.

Extrahepatic manifestations of acute hepatitis E appear to be of subordinate clinical importance.

A *cholestatic variant,* such as occurs in hepatitis A with long-standing jaundice, but with only moderately elevated aminotransferases has also been described in hepatitis E. The prognosis appears to be favorable.

*Fulminant hepatitis E* is rare with mortality rates during epidemics in Asia of 1–2%. Fulminant disease with a mortality as high as 25% occurs predominantly in women during late pregnancy. The cause for this unfavorable prognosis is unknown, although increased intravascular coagulation may play a role.

*HEV superinfection* in patients with chronic liver disease can cause severe liver decompensation and death [7, 10].

## Diagnosis

Changes in liver enzymes and the clinical presentation of hepatitis E are nonspecific and similar to other forms of acute viral hepatitis. Diagnosis of HEV infection is usually made by detection of anti-HEV antibodies or HEV-RNA in serum. The appearance in serum of immunoglobulin M antibody to HEV (anti-HEV IgM) is diagnostic for acute hepatitis E (see Section 63.1), but disappears rapidly over 3–4 months in approximately 90% of the patients. Anti-HEV IgG appears a few days later, persists for at least a few years and confers immunity [4]. The duration of HEV immunity is unclear, but it is possibly shorter term than is HAV immunity.

## Differential Diagnosis

The primary differential diagnosis is HAV infection, which cannot be made on clinical grounds, but which relies on immune-serologic findings. Additionally, the same differential diagnoses as for hepatitis A should be considered (Table 63.14).

## Natural Course and Prognosis

Hepatitis E generally causes an acute self-limited illness followed by complete recovery. After approximately 2–3 weeks the symptoms abate. Complete clinical and biochemical healing may be expected after 1–2 months. The mortality rate is usually low (0.07–0.6%) [2]. Although HEV is considered to be an agent responsible for acute hepatitis that does not become chronic, isolated cases of HEV infection evolving into chronic hepatitis are being reported with increasing frequency in immunocompromised patients, especially in organ-transplant recipients [8]. In the vast majority of patients, however, a chronic HEV-induced liver disease or a HEV carrier state does not occur.

## Prevention

The most effective mode of preventing the disease is use of clean water and proper sanitation. There is no evidence that administration of normal immune globulin can prevent HEV-infection.

Recombinant vaccines are being developed with encouraging results. One recombinant protein vaccine (rHEV) has proven to be effective in the prevention of

overt hepatitis E in a phase 2 study in a high-risk population [11]. Vaccines will be particularly useful for travelers to disease-endemic areas and for pregnant women.

### *Therapy*

No specific therapy is available and treatment is symptomatic. For therapy of fulminant disease with acute liver failure see Chapter 78.

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# Viral Infections by Nonhepatotropic Viruses 64

Henryk Dancygier

## Chapter Outline



Nonhepatotropic viruses may infect many organs, including the liver. The liver, however, is not the primary target organ, but becomes involved within the context of a generalized viral infection. Clinically and histologically, liver involvement may remain in the background or assume paramount prognostic importance when extended parenchymal necrosis leads to fulminant hepatic failure [24]. Nonhepatotropic viruses that may affect the liver are listed in Table 64.1.

The multifaceted clinical manifestations of various diseases caused by nonhepatotropic viruses is beyond the scope of this chapter. Rather, the hepatic involvement of various viral diseases will be highlighted in the following paragraphs.

## Herpes Viruses

### *Herpes Simplex Virus 1 and 2*

Primary herpes simplex virus (HSV) infection is characterized by vesicular lesions on skin and mucous membranes. Reactivation of latent infection can cause disease in many organs, including the liver [8]. Neonates, immunocompromised patients, patients with cancer or myelodysplastic syndromes, and pregnant women are at particular risk of developing HSV hepatitis, which may be caused by HSV type 1 and 2 [15, 30]. HSV hepatitis in immunocompetent persons, however, has also been reported [9, 12].

*Macroscopically* the liver is enlarged, appears mottled with multiple, partly confluent yellowish necrotic foci that are surrounded by a red rim.

*Microscopically* randomly distributed patchy areas of coagulative necrosis surrounded by dilated and blood filled sinusoids, hemorrhages, and hepatocytes

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**Table 64.1** Nonhepatotropic viruses that may infect and injure the liver

| |
|---|
| *Herpesviruses* |
| • Herpes simplex virus 1 and 2 |
| • Human herpesvirus 6 and 7 |
| • Human herpesvirus 8 |
| • Varicella-zoster virus |
| • Cytomegalovirus |
| • Epstein-Barr Virus |
| *Adenoviruses* |
| *Enteroviruses* |
| • Coxsackie B virus |
| • Echovirus |
| *Paramyxoviruses* |
| • Measles virus |
| *Togaviruses* |
| • Rubella virus |
| *Flaviviruses*[a] |
| • Yellow fever virus |
| • Dengue virus |
| • Kayasanur forest fever virus |
| • Omsk hemorrhagic fever |
| *Bunyaviruses*[a] |
| • Phlebovirus (Rift valley fever) |
| • Nairovirus (Krim Kongo fever) |
| • Hantavirus |
| *Arenaviruses*[a] |
| • Lassavirus |
| • Junin- and Machupovirus (South America) |
| *Filoviruses*[a] |
| • Marburg virus |
| • Ebola virus |
| *Parvoviruses* |
| • Parvovirus B19 |
| *Coronaviruses* |
| • SARS Corona virus (SCoV) |

*SARS* Severe acute respiratory syndrome
[a]Cause hemorrhagic fevers

containing intranuclear viral inclusions (Cowdry A: inclusion surrounded by a clear halo; Cowdry B: homogeneous, ground glass like inclusion) are seen [12]. Typically there is only a minimal inflammatory response. In neonatal HSV hepatitis multinucleated giant cells (giant cell hepatitis) occur. The viruses may be demonstrated by electron microscopy, immunocytochemistry, and by DNA-in situ hybridisation. However, the light microscopic appearance is so characteristic that these sophisticated techniques are not required to diagnose HSV hepatitis.

Hepatitis is a rare complication of HSV infection, but when it occurs it usually presents as a fulminant disease with a high mortality rate of up to approximately 80% [15, 21].

HSV hepatitis in *pregnant women* (usually caused by HSV type 2) occurs in the late second and in the third trimester. The disease is heralded by nonspecific influenza-like symptoms, right upper quadrant pain, and eventually signs of hepatic encephalopathy. Indeed, the first case of HSV infection associated fulminant liver necrosis in adults was described in a pregnant woman.

Extended parenchymal necrosis in HSV hepatitis leads to marked elevation of aminotransferase levels up to several thousand U/L (AST > ALT) and to a coagulopathy demonstrated by a prolongation of prothrombin time. In contrast to aminotransferases, serum bilirubin concentration usually is only slightly (≤5 mg/dL) elevated. Pregnancy specific liver diseases, such as acute fatty liver, HELLP-syndrome and cholestasis of pregnancy should be considered in the differential diagnosis (see Section XXI). The fatality rate of HSV hepatitis in pregnancy is high, approximately 40–50% for mother and child. Therefore, *in pregnant women, HSV infection must be excluded in every case of acute hepatitis*. Liver biopsy is the definitive diagnostic test (often a transvenous approach is necessary). Additionally, vaginal, cervical and pharyngeal smears should be obtained [18].

Opportunistic HSV hepatitis in *patients after solid organ transplantation* is not as frequent as CMV and EBV infection, but usually occurs earlier after the transplant than CMV and EBV hepatitis [21]. HSV infection in these patients is mostly due to reactivation of latent virus rather than to a *de novo* infection.

HSV hepatitis requires *immediate treatment*. Acyclovir (30 mg/kg body weight i.v. daily) is life saving in many patients.

## Human Herpesvirus 6 and 7

Infections with the human herpesviruses 6 and 7 are ubiquitous in childhood. Rarely, especially in children, both viruses may cause a fulminant hepatitis during a primary infection. Viral reactivation in immunosuppressed patients after organ transplantation may also be responsible for hepatitis [4]. The laboratory findings are nonspecific, and are characterized by elevation of aminotransferases, cholestatic enzymes, leuko- and thrombocytpenia. The viruses may be isolated from peripheral blood lymphocytes, and may be identified by negative contrast and thin-section electron microscopy, DNA-hybridization, and immunofluorescence [38].

## Human Herpesvirus 8

Human herpesvirus 8 causes Kaposis's sarcoma (KS), and is linked with two other neoplasms, a B cell non-Hodgkin's lymphoma (body cavity based lymphoma) and multicentric Castleman's disease (MCD). The liver is frequently involved in visceral KS, predominantly in HIV infected persons with advanced immunodeficiency, and more rarely after organ transplantation.

Peliosis hepatis, perisinusoidal fibrosis and nodular regenerative hyperplasia have been described in few cases of MCD.

## Varicella-Zoster Virus

A disseminated varicella-zoster virus (VZV) infection is rare. It may occur in children within the context of chickenpox, while in adults immunosuppression with reactivation of VZV is the usual cause. Hepatic involvement with varicella (varicella hepatitis) is uncommon and predominantly affects immunosuppressed hosts, such as transplant recipients, cancer and AIDS patients, but also normal hosts.

*Histologically,* focal liver cell necrosis as well as massive widespread hepatic necrosis with intranuclear hepatocellular inclusions and multinucleated giant cells at the periphery of necrotic parenchyma is seen. The lesions resemble those of HSV hepatitis.

*Clinically* varicella hepatitis may manifest as a symptomatic or subclinical aminotransferase elevation coincident with the onset of varicella, or, especially in the immunocompromised host, as fulminant hepatic failure leading to death [20, 31]. The varicella skin rash may precede, appear coincident with, or follow the onset of hepatitis, but varicella hepatitis with fulminant failure with widespread visceral dissemination in the absence of a rash has also been documented in bone marrow transplant recipients [32].

In fulminant hepatic failure aminotransferase levels reach several thousand U/L, with levels of AST being generally higher than ALT.

Reye's syndrome has been reported to be preceded by a VZV infection in approximately 10% of patients. A diffuse microvesicular steatosis, vomiting and signs of a hepatic encephalopathy are characteristic of Reye's syndrome [23].

*Therapy* of varicella hepatitis is early high dose acyclovir (30 mg/kg body weight i.v. daily) or liver transplantation in fulminant cases with organ failure [27, 37].

## Cytomegalovirus

*Congenital cytomegalovirus (CMV) infection* may be due to intrauterine or peripartal contagion. Histologically steatosis, focal liver cell necrosis, mononuclear inflammatory infiltrates, and occasionally multinucleated giant cells (neonatal hepatitis) are seen. The typical intranuclear inclusions surrounded by a clear halo impart the cells an "owl's eye" appearance. They are found in hepatocytes, bile duct epithelia and in endothelial cells. The affected cells are enlarged. Intrauterine CMV infection may result in biliary atresia.

*CMV hepatitis in the immunocompetent host* clinically resembles hepatitis of infectious mononucleosis [14]. The liver and spleen are enlarged and the clinical manifestations are mild, with mild increases of aminotransferase and bilirubin levels. Often hepatitis is anicteric. Occasionally alkaline phosphatase and γGT may be markedly elevated (up to >1,000 U/L), which, however, does not portend a serious prognosis. The course of CMV hepatitis is self-limited, and chronic hepatitis does not ensue. Isolated cases of Budd-Chiari syndrome and portal vein thrombosis associated with CMV hepatitis have been reported [34, 35]. Compared to viral hepatitis A, B and C, CMV hepatitis is characterized by prolonged fever, splenomegaly, atypical lymphocytosis, milder elevations of aminotransferases and milder histopathological alterations.

*Histologically,* focal liver and bile duct injury, lymphocytic sinusoidal infiltrates, and occasionally noncaseating histiocytic granulomas are seen (Fig. 64.1). Thus, CMV hepatitis should be included in the differential diagnosis of granulomatous hepatitis [3]. Viral inclusions or immunocytochemically detectable viral antigens usually cannot be demonstrated.

In *CMV hepatitis in the immunocompromised host* viral inclusions may be found in the absence of an inflammatory reaction. If such inclusions are accompanied by hepatocellular injury and by lymphocytic infiltration, hepatitis may be attributed to CMV infection.

*CMV hepatitis after liver transplantation* usually manifests 1–4 months after the operation, either as a *de*

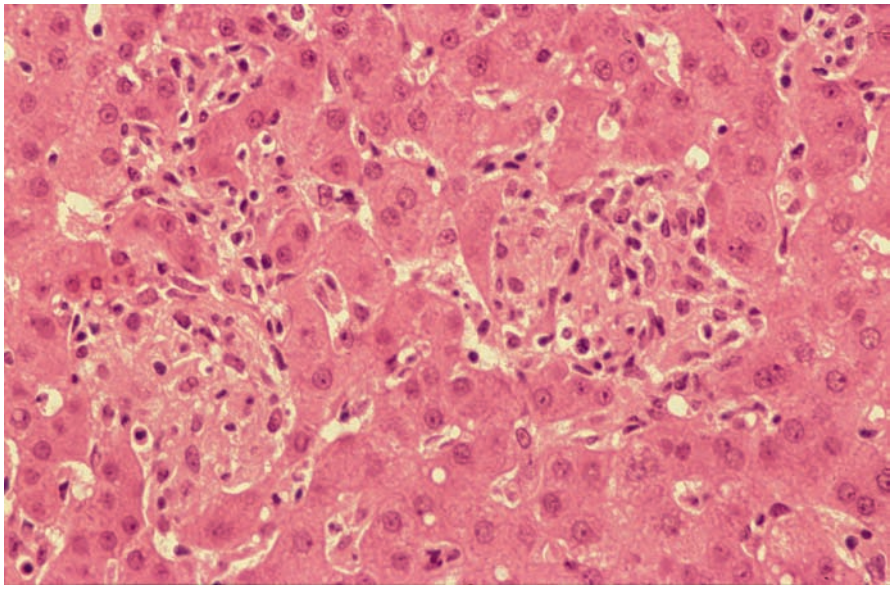

**Fig. 64.1** Histiocytic granulomas in acute CMV hepatitis. Hematoxylin & Eosin (×400)

*novo* infection of the donor liver through blood transfusion or as reactivation of a latent CMV infection in the recipient due to postoperative immunosuppressive therapy. Clinically the disease may be asymptomatic or resemble infectious mononucleosis, with mild elevation of aminotransferases, leuko- and thrombocytopenia. It must be differentiated from a rejection reaction.

The *histological appearance* of CMV hepatitis in a transplanted liver is characteristic. Focal accumulations of neutrophils form so-called microabscesses or a necrotic hepatocyte is surrounded by a mixed inflammatory cell infiltrate ("microgranuloma"). Furthermore, the nuclear inclusions described above are present. Viral antigens may be demonstrated by immunocytochemistry. The portal inflammatory infiltrate varies in density. In contrast to cellular rejection, in CMV hepatitis neither an endothelitis nor a cholangtis are seen. Patients with CMV hepatitis after liver transplantation have an increased risk of developing a vanishing bile duct syndrome.

*Therapy* of CMV hepatitis with ganciclovir (5 mg/kg i.v. bid) is usually successful.

## Epstein-Barr Virus

Epstein-Barr virus (EBV) causes infectious mononucleosis. It infects and transforms B lymphocytes, and is associated with the development of hairy leukoplakia, certain lymphomas and nasopharyngeal carcinoma. Approximately 5% of patients with infectious mononucleosis develop jaundice and 15% have elevated serum aminotransferases.

EBV hepatitis generally is a mild hepatitis accompanying a generalized EBV infection [16]. Its clinical manifestations are overshadowed by systemic signs and symptoms of infectious mononucleosis. Jaundice in a patient with EBV infection mostly is due to autoimmune hemolytic anemia and not to hepatitis. In the vast majority of cases EBV hepatitis is self-limited. Fulminant courses with liver failure are extremely rare [17, 33]. They occur predominantly in X chromosomal inherited lymphoproliferative syndrome (Duncan's syndrome) and in lymphoproliferative diseases after organ transplantation.

On *histologic examination* the portal tracts are heavily infiltrated by atypical lymphocytes and plasma cells. The inflammatory infiltrate spills over through the limiting plate to the lobular parenchyma. EBV antigens may be demonstrated in lymphocytes by immunocytochemistry and by in situ hybridization. The sinusoids are infiltrated either diffusely or in the form of small aggregates by mononuclear cells. Intrasinusoidal lymphocytosis often has a characteristic "Indian-file" appearance (Fig. 64.2). Liver cells are usually only mildly affected with scattered apoptotic bodies or foci of parenchymal necrosis filled with lymphocytes. Hepatocellular injury is clearly less pronounced than in acute viral hepatitis A

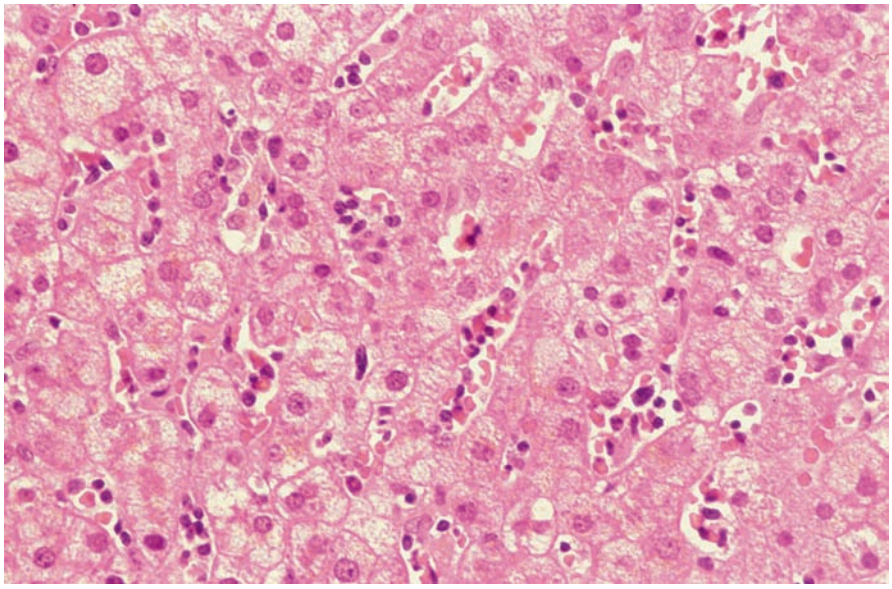

**Fig. 64.2** EBV hepatitis. "Indian-file"-like intrasinusoidal lymphocytosis. Hematoxylin & Eosin (×400)

or B. Regenerative changes and mitoses may be prominent. A steatosis or non-caseating, fibrin-ring granulomas rarely occur. Cholestasis is not part of the typical microscopical picture of EBV hepatitis, and if present should prompt one to search for granulomas [6]. The main histological differential diagnosis of EBV hepatitis is from leukemia or lymphoma.

## Adenoviruses

Adenovirus infection may cause severe hepatitis with liver failure in children and in immunosuppressed adults [1, 19]. Pathology and clinical manifestations resemble that of HSV hepatitis.

## Enteroviruses

Liver involvement is seen in systemic infections with *Coxsackie B* and *echoviruses* [36]. Coxsackie virus, and more rarely echoviruses may cause a severe, hemorrhagic-necrotizing hepatitis in newborns. The clinical manifestations in adults are milder, generally reflecting an acute cholestatic hepatitis. *Histologically* centrilobular cholestasis and ballooned hepatocytes are seen. The portal and sinusoidal inflammatory infiltrates are composed of mononuclear cells and neutrophil leukocytes.

## Paramyxoviruses

Liver involvement in *measles virus infection* is mild and self-limited [25]. The histological alterations are nonspecific, showing "hepatocellular unrest," nuclear vacuolization and a mild intrasinusoidal lymphocytosis. Isolated cases with clinically severe hepatitis and multinucleated giant cells have been documented, however, their paramyxovirus etiology has not been proven unequivocally [29].

## Togaviruses

*Rubella virus* may cause hepatitis in the newborn within the context of the congenital rubella syndrome. Focal hepatocellular necrosis, signs of cholestasis and a mild chronic inflammatory portal infiltrate are seen. In isolated cases extensive parenchymal necrosis has been described.

Intrauterine infection with the rubella virus may cause biliary atresia.

Rubella infection in adults may be accompanied by a mild anicteric, sublinical or asymptomatic hepatitis with slightly elevated aminotransferases [28].

## Arboviruses

Arthropode transmitted *Flavi-* and *Bunyaviruses* cause diseases that are characterized by disseminated intravascular coagulation with extended hemorrhages, and are therefore denominated *hemorrhagic fevers*. Liver injury in all arbovirus infections shows common features and is characterized by variably large areas of parenchymal necrosis and microvesicular steatosis, with a relatively mild inflammatory reaction. If the patient survives, scavenger and regenerative processes dominate the histological picture.

### Yellow Fever Virus

The yellow fever virus belongs to the genus of flaviviruses. The disease is endemic in Africa and in South America. It manifests acutely with fever, myalgias and headaches that are followed by jaundice after a few days. Death is due to liver and renal failure. The *histopathological appearance* depends on the stage of the disease. Confluent, centrilobular parenchymal necrosis, scattered apoptotic bodies (classic *Councilman bodies*), and eosinophilic intranuclear inclusions that are arranged concentrically around the nucleolus (*Torres bodies*) characterize the acute stage. In contrast to the marked parenchymal injury the inflammatory response is scant. Surviving hepatocytes show microvesicular steatosis and ballooning. Regeneration is evidenced by hepatocellular hyperplasia and multinucleated hepatocytes [10].

### Dengue Virus

International travel to endemic areas is a major risk factor for both primary and secondary dengue infection. The primary infection manifests as an exanthematic, influenza-like illness. Hemorrhagic fever is caused by reinfection with different serotypes of dengue virus (DEN 1–4). Dengue remains a diagnostic challenge, given its protean nature, ranging from a mild febrile illness to profound shock. Dengue shock syndrome has an estimated mortality rate close to 50%.

Liver involvement appears to occur more frequently when infections involve DEN-3 and DEN-4 serotypes. The liver is interspersed with extended, partly confluent areas of hemorrhagic parenchymal necrosis. The inflammatory reaction is mild. The surviving hepatocytes show a microvesicular steatosis. Fulminant liver failure is extremely rare in adults, but has been reported in single cases [11]. If the patient survives, diffuse parenchymal calcifications may be the only sign of previous liver involvement [7].

### Hantavirus

Certain types of hantavirus cause hemorrhagic fever with a renal syndrome (HFRS), while others are responsible for the hantavirus pulmonary syndrome (HPS). Primary involvement of the liver does not occur in either syndrome. In Chinese patients with acute hepatitis of unknown etiology hantavirus infection has been discussed as a possible cause [26].

## Arenaviruses

Arenaviruses cause Lassa fever and hemorrhagic fevers in Argentina and Bolivia.

### Lassavirus

Lassavirus infection is endemic in Central and West Africa. Fever, pharyngitis, diarrhea and a hemorrhagic diathesis characterize the clinical picture. Pain in the right upper quadrant may supervene. There is a marked rise of serum aminotransferases, while jaundice is rare. The mortality rate is approximately 30%.

The liver has a mottled appearance caused by apoptotic hepatocytes (Councilman-like bodies) and hemorrhagic necrosis of groups of liver cells which may coalesce forming bridging necrosis. Liver injury is accompanied by a marked hyperplasia of Kupffer cells and by lipofuscin deposits in hepatocytes. Cholestasis and steatosis are lacking. The viral particles can easily be demonstrated by electron microscopy [5].

### *Junin- and Machupovirus*

These viruses cause hemorrhagic fevers in South America. The clinical picture and pathological liver findings correspond to those of Lassa fever.

## Filoviruses

### *Marburg Virus*

Marburg virus disease is a highly contagious, febrile infection. It is associated with disseminated intravascular coagulation, hemorrhages, and shock. The mortality rate is 20–25%. The pathology of the liver corresponds to that of Lassa fever.

### *Ebola Virus*

Ebola fever resembles clinically and pathologically Marburg virus infection. Mortality rate is close to 50%.

## Parvoviruses

*Parvovirus B19* is the only human pathogenic parvovirus, causing *erythema infectiosum* (fifth disease) in children. Epidemiologic data suggest that parvovirus B 19 may also cause an acute hepatitis in children [39]. Adults with parvovirus B 19 infection, especially patients with underlying hemoglobinopathies (e.g. sickle cell disease) or other erythrocytic disorders (e.g. hereditary spherocytosis) may develop transient aplastic crisis. Combined with aplastic anemia massive hepatic necrosis and acute liver failure may occur [22]. Parvovirus B 19 may be demonstrated by PCR in liver tissue.

## Coronaviruses

Human coronaviruses have long been known to cause the common cold. In 2002 the *Severe Acute Respiratory Syndrome* (SARS) was described for the first time. It is caused by SARS Coronavirus (SCoV) that is genetically dissimilar from known human or animal coronaviruses. The disease presents with fever, influenza-like symptoms, dry cough, atypical pneumonia, and diarrhea.

The liver is involved in up to 60% of cases and infection of the liver by SCoV was verified for the first time in 2004 by demonstrating SCoV-RNA in liver tissue. In approximately 25% of patients aminotransferases are elevated (ALT: 200–900 IU/L). Histological examination shows signs of hepatocyte injury, such as ballooning and apoptosis accompanied by mild to moderate lobular lymphocytic infiltrates. Numerous mitoses probably denote regenerative activity [2, 13].

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# Bacterial Liver Abscess and Other Bacterial Infections

# 65

Henryk Dancygier

## Chapter Outline



Bacteria may cause liver disease by directly invading the liver parenchyma or the bile ducts, or indirectly by eliciting a hepatic response to extrahepatic, predominantly pulmonary and urogenital infections. Systemic effects of toxins and other mediators are held responsible for hepatic inflammatory and cholestatic reactions (see Chapters 19 and 52). Bacterial liver diseases encompass a broad clinical spectrum that includes asymptomatic patients, cases with slightly elevated liver enzymes, patients with fulminant hepatitis and acute liver failure, and chronic liver disease with formation of abscesses. There is no characteristic constellation of liver enzymes in bacterial infections. Mostly AST is elevated accompanied by varying elevation of alkaline phosphatase levels. Jaundice is often present in septic patients, in pneumococcal pneumonia, toxic shock syndrome, leptospirosis and in relapsing fever, but its absence does not allow to exclude these conditions. A high index of suspicion, derived from the clinical context, must be kept to diagnose involvement of the liver in bacterial diseases. The diagnosis is confirmed by serologic, microbiologic, noninvasive (ultrasound, CT, MRI) and invasive (liver biopsy) imaging techniques.

The following paragraphs focus on hepatic alterations in bacterial infections. It is not intended to present a detailed account of the various infectious conditions. The interested reader is referred to textbooks of Internal Medicine and Infectious Diseases.

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# Bacterial Liver Abscess

## Definition

Bacterial liver abscess, previously called pyogenic liver abscess, is a localized suppurative destruction of liver tissue due to bacterial invasion via the bloodstream (portal vein, hepatic artery), the biliary system or (rarely) by direct penetration (posttraumatic) of bacteria.

## Epidemiology

Liver abscess is the most common visceral abscess [3]. Its prevalence has been estimated to be 8–16 cases/100,000 hospital admissions. Males and females, predominantly in their fifth and sixth decades of life, are equally affected. Patients receiving immunosuppressive therapy, and those with malignant diseases, diabetes mellitus and chronic alcohol abuse are particularly at risk to develop a liver abscess. Two thirds of liver abscesses are solitary, localized in the right liver lobe in 60% of cases. Involvement of the liver in sepsis with the formation of multiple small suppurative hepatic lesions occurs in fewer than 1% of cases [11, 46, 50].

## Etiology and Pathogenesis

The most important causative agents are listed in Table 65.1. Usually a polymicrobial infection with gram-negative aerobic and anaerobic organisms, mostly of intestinal origin, is present. *Escherichia coli* and *Klebsiella pneumoniae* predominate with 60–70%, with anaerobic bacteria being associated in up to 50% of cases. If the primary infectious focus is bacterial endocarditis or a dental infection, staphylococci, hemolytic streptococci and streptococcus milleri are most commonly found. Gas forming organisms, such as *K. pneumoniae* and *Bacteroides fragilis*, occur more often in diabetic patients [74]. Rarely an infection with Listeria monocytogenes may lead to confluent hepatic microabscesses. In immunosuppressed patients (AIDS, intensive antineoplastic chemotherapy, after organ transplantation) one should bear in mind fungi and other opportunistic organisms.

**Table 65.1** Agents that may cause a liver abscess

- *Escherichia coli*
- *Klebsiella pneumoniae*
- Enterococci (*S. faecalis, S. faecium*)
- Microaerophilic streptococci (*Streptococcus milleri*)
- *Proteus vulgaris*
- *Pseudomonas aeruginosa*
- *Salmonella*
- Bacteroides species[a]
- Fusobacteria
- *Yersinia enterocolitica* and *Y. pseudotuberculosis*
- *Staphylococcus aureus*
- *Listeria monocytogenes*
- *Brucella suis*[b]
- *Clostridium perfringens*
- *Francisella tularensis*
- Actinomycetaceae (*Acitonomyces israelii*, *Nocardia species*)
- *Bartonella henselae*
- *Burkholderia pseudomallei*
- *Campylobacter jejuni*
- *Fusobacterium nucleatum*
- *Legionella pneumophila*

[a]*B. fragilis* is an anaerobic gram-negative organism uncommonly present in the normal fecal flora. Due to its capsular polysaccharide complexes, however, it plays an important role in anaerobic bacteremia and in intraabdominal abscesses
[b]May cause a liver abscess after years of clinical latency

Most commonly the organisms gain access to the liver via the portal vein (suppurative pylephlebitis; *pylephlebitic abscess*; see Chapter 61) or via the biliary tract as an ascending suppurative cholangitis. Portal venous bacteremia is mostly due to inflammatory intestinal and pelvic inflammatory diseases, such as diverticulitis, stercoral rectal ulcers, pericolic and perineal abscess, chronic inflammatory bowel disease, appendicitis or (extremely rarely) phlegmonous gastritis.

Hepatic infection via the hepatic artery is seen in 10–20% of cases in children and adolescents, but only rarely in adults. In these cases generally multiple small (<1 cm) predominantly subcapsular abscesses are found. Rarely gallbladder empyema, pleural empyema or a subphrenic abscess may lead per continuity to the formation of a liver abscess.

A previous history of biliary interventions such as cholecystectomy, choledochoduodenostomy, and endoscopic sphincterotomy has been reported in 23% of patients with a liver abscess, suggesting a biliary etiology [72].

Occasionally superinfection of malignant liver tumors, for example after antineoplastic chemotherapy

**Table 65.2** Causes of liver abscess

| |
|---|
| *Portal vein* |
| • Appendicitis |
| • Diverticulitis |
| • Chronic inflammatory bowel disease |
| • Rectal ulcer(s) |
| • Perianal abscess |
| • Pelvic inflammatory disease |
| *Bile ducts* |
| • Stones |
| • Cholangiocellular carcinoma |
| • Strictures |
| *Hepatic artery* |
| • Dental infections |
| • Bacterial endocarditis |
| *Per continuity* |
| • Gallbladder empyema |
| • Perforated peptic ulcer |
| • Perforation of the stomach by foreign bodies |
| • Subphrenic abscess |
| *Posttraumatic* |
| *Iatrogenic* |
| • Liver biopsy |
| • Occluded biliary stent |
| • Arterial embolization |
| • Endoscopic or surgical interventions on bile ducts |
| *Cyst infection* |
| *Infected intrahepatic malignancy* |
| *Cryptogenic* |

or transarterial chemoembolization, results in the formation of a liver abscess [8]. Very rarely foreign bodies, such as a fish bone, may perforate the stomach or duodenum, penetrate the liver and cause a suppurative reaction [12, 14].

In approximately 15% of patients (according to some reports in up to 60%) no cause can be found (*cryptogenic liver abscess*). The most important causes of a liver abscess are summarized in Table 65.2.

## *Diagnosis*

### Clinical Manifestations

A solitary liver abscess is asymptomatic during its initial phase of development. In this stage symptoms of the underlying disease predominate. Multiple abscesses often have an acute clinical onset with dull right upper quadrant pain that may radiate to the right shoulder and be associated with right sided pleuritic pain. During the further course anorexia, fatigue and weight loss supervene. Approximately 20% of patients are icteric. However, the cardinal symptoms, present in 80–99% of patients with a bacterial liver abscess, are fever and chills. There is no characteristic fever pattern. A liver abscess may be the cause of fever of unknown origin (especially in older patients).

The liver is enlarged and painful on palpation. If the abscess is subcapsular local guarding may develop. Often a reflectory elevation of the diaphragm with impaired diaphragmatic mobility is present.

### Technical Investigations

*Laboratory findings* are nonspecific with elevated erythrocyte sedimentation rate, C-reactive protein, and a leukocytosis with a left shift in the differential blood count. In advanced cases anemia and hypoalbuminemia are present. Aminotransferases (usually <100 U/L), alkaline phosphatase, γGT and bilirubin levels are only mildly elevated, except for biliary abscesses in which the cholestatic parameters are more pronounced.

*Imaging techniques* (ultrasonography and computed tomography) are diagnostic. On ultrasound a liver abscess appears as an echopoor, often somewhat ill defined space occupying lesion (Figs. 65.1 and 65.2). Depending on the consistency of the pus (e.g. gas forming organisms) and the age of the abscess its echopoor

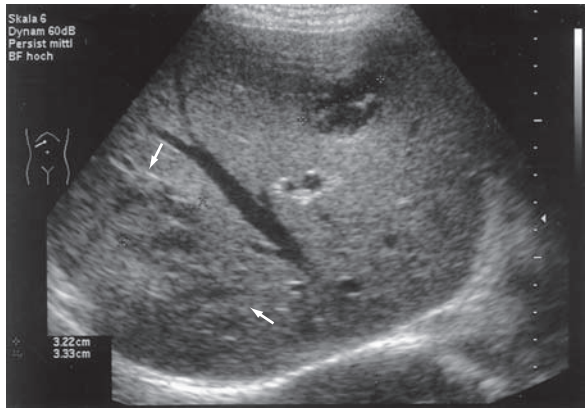


**Fig. 65.1** Two abscesses in the right liver lobe. Note the different sonomorphologic aspect. While the ventrally located abscess is predominantly echopoor, the subphrenic abscess (arrows) has a mixed, predominantly hyperechoic aspect with an irregular echo pattern. Sonographic guided aspiration yielded *Klebsiella pneumoniae* in both lesions

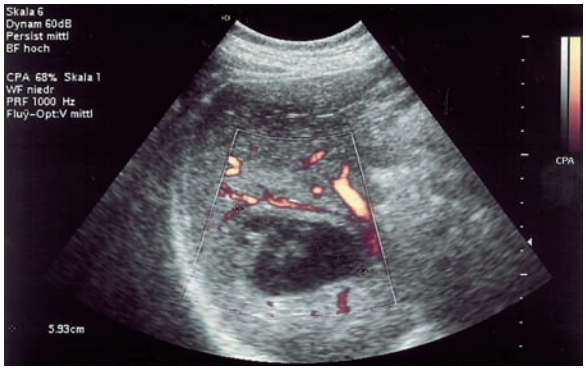


**Fig. 65.2** Liver abscess. Vascular structures surround the echo-poor avascular lesion

interior may contain variously bright reflexes. On CT a liver abscess appears as a hypodense mass lesion, whose density does not increase after administration of intravenous contrast medium. The diagnosis is confirmed by ultrasound or CT guided aspiration with culture and microbiologic differentiation of the material. Blood cultures during chills yield positive results in only somewhat more than half of the patients.

Elevation of the right hemidiaphragm and pulmonary abnormalities such as a right sided pleural effusion, pulmonary atelectasis and infiltrates may be noted on chest X-ray in a subcapsular liver abscess located close to the diaphragm.

A biliary cause of an abscess may be assumed if a communication between the abscess and the bile ducts is shown on ERCP or MRCP. Bile obtained during ERCP should be examined microbiologically [40].

## Differential Diagnosis

After the diagnosis of a liver abscess has been confirmed, underlying inflammatory and malignant gastrointestinal diseases have to be excluded. Abdominal trauma is easily discovered by the history. *In patients without an underlying disease or an obvious etiology, the two most probable causes of liver abscess are pyogenic bacteria and amebae*. A solitary liver abscess in patients residing in or visiting areas in which amebae are endemic should arouse the suspicion of an amebic etiology, even in the absence of past or present amebic dysentery (see Chapter 66). Approximately 80% of amebic liver abscesses are solitary, most of them located in the right liver lobe, whereas approximately 50% of bacterial abscesses are multiple in both lobes [26, 42]. Table 66.1 summarizes the major differences between amebic and bacterial liver abscess.

Uncommon organisms such as fungi (Candida albicans, Aspergillus fumigatus, Cryptococcus neoformans, Coccidioides immitis, Histoplasma capsulatum) should be considered in immunosuppressed patients. Personnel working in military research laboratories may develop liver abscess within the context of glanders (*Burkholderia mallei*) [65].

Burkholderia pseudomallei should be considered in patients with sepsis syndrome and multiple abscesses in several organs, especially if the patient traveled to the Far East. Sepsis with Fusobacterium necrophorum has also been reported to be associated with multiple abscesses [54].

## Course and Prognosis

Prognosis depends on the rapidity with which a liver abscess is diagnosed, on the onset of effective antibiotic therapy, the underlying disease (worse in biliary than in pylephlebitic abscess), the number of abscesses (multiple worse than single) and on the age and general condition of the patient (very old and very young patients have a worse prognosis). The early clinical application of imaging modalities and of effective antibiotic therapy in past years has led to a decline in the mortality rate from greater than 80% to currently less than 10% [62]. Factors associated with a higher mortality are septic shock, respiratory failure, immunosuppression, marked hypoalbuminemia, diabetes mellitus and an underlying malignant disease.

Depending on the size and location of the abscess, rupture into the peritoneal cavity, pleural space or into the pericardial sack can be a serious complication. Perforation into hepatic veins results in sepsis.

## Therapy

Adequate drainage, antibiotics, and effective therapy of the primary infectious focus are the cornerstones of treatment. CT or US guided percutaneous aspiration may be sufficient in a solitary abscess, and occasionally

also in several abscesses that are smaller than 6 cm in diameter. Larger abscesses require percutaneous catheter drainage [58, 67]. If percutaneous drainage is not successful or if the abscesses are very large and multiple a surgical approach will be necessary. Rarely, primary non-biliary abscesses may gain access to a major bile duct and drain spontaneously via the common bile duct.

Therapeutic ERCP (stone extraction, placement of stents) is performed in patients with ascending cholangitis with intraductal stones or bile duct strictures.

Antibiotic treatment is started as soon as possible as an empirical initial therapy. Aminopenicillins, third generation cephalosporins, aminoglycosides and metronidazole are used. The treatment is modified on demand according to the results of the antibiogram. The duration of antibiotic use depends on the number and size of the abscesses, and on the clinical response. It should be continued until the abscess has resolved completely on ultrasound or CT, which may take weeks and occasionally even months.

The following discussion of bacterial diseases involving the liver is arranged in alphabetical order rather than according to microbiological or clinical criteria.

## Actinomycetaceae

*Actinomycosis* is a chronic bacterial infection that occurs sporadically throughout the world. Actinomycetes are part of the normal mucosal flora. Abdominal actinomycosis originates from breaks in the intestinal mucosa or from genital lesions in the female [57]. The hallmark of *Actinomyces israelii* infection is the formation of suppurative inflammatory masses containing one or more spherical yellowish granules (drusen) measuring 1–2 mm, that are bordered by a wall of leukocytes. Each granule is composed of branched, gram-positive and often beaded filaments (actinomycetes) embedded in an amorphous matrix.

The liver is involved in approximately 15% of cases of abdominal actinomycosis. Usually a solitary liver abscess develops insiduously over a period of several years. The patients are initially asymptomatic. In advanced stages hepatomegaly develops associated with abdominal pain, anorexia and fever. The diagnosis is confirmed by demonstrating the organisms in the hepatic aspirate [47].

*Nocardiosis* is an acute or chronic suppurative or granulomatous infection caused by several Nocardia species, such as *N. asteroides*. Immunosuppressed individuals are at particular risk of developing opportunistic nocardia infection. The liver is affected in approximately 3% of cases of disseminated nocardiosis. The organisms reach the liver within the context of hematogeneous dissemination and lead to the formation of solitary or multiple liver abscesses. In liver biopsy acid fast filaments are seen, and the diagnosis is made by culture.

## Bartonellae

*Bartonella henselae* (less commonly *Bartonella quintana*) causes *cat scratch disease*, *bacillary peliosis* and *bacillary angiomatosis* (see Chapter 58). The liver may be involved in disseminated cat scratch disease, and the organisms may be visualized by silver staining techniques, such as Warthin-Starry stain. Histological examination reveals granulomas with stellate microabscesses at their centers. The bile ducts may display a dense periductal fibrosis and also contain the typical granulomas. Clinically the disease may present with fever and tender hepatosplenomegaly [54].

## Borreliae

*Borrelia burgdorferi* causes Lyme disease. Tick borne relapsing fever occurs worldwide and is caused by various Borrelia species (*B. duttoni, B. crocidurae, B. turicatae, B. packeri, B. hermsii*), louse born relapsing fever is caused by *Borrelia recurrentis*.

The liver may be involved in acute and chronic *Lyme disease*. A hepatomegaly is present in approximately 5% of cases, and a subclinical hepatitis or mild symptoms suggestive of hepatitis are found in 10–27% of patients. Elevated aminotransferase levels indicate mild hepatocellular injury [30, 35, 66]. *B. burgdorferi* is invasive and penetrates the liver through vascular endothelial cells. The spirochetes may be identified within hepatic sinusoids and hepatocytes, particularly in early, disseminated infection.

Histological examination reveals sinusoidal infiltration by a mixed inflammatory infiltrate [23]. Kupffer

cell hyperplasia, microvesicular steatosis, hepatocyte ballooning and granulomas are less commonly encountered [7, 16]. With appropriate antimicrobial treatment the prognosis for patients with Lyme disease associated hepatitis is excellent (a chronic hepatitis does not occur).

In severe *relapsing fever* hepatosplenomegaly and jaundice may occur in approximately 7–10% of cases [17, 33]. Laboratory findings indicate a cholestatic pattern of injury and bilirubin values may be elevated >20 mg%. Hepatic failure is a common cause of death in severe cases [64]. Histologically focal parenchymal necrosis, surrounded by a hemorrhagic rim is seen. The sinusoids contain a lymphocytic and neutrophilic infiltrate, and hypertrophied Kupffer cells may engulf erythrocytes. The organisms may be demonstrated within the sinusoids by silver staining. The current diagnostic standard for tick borne relapsing fever is detection of spirochetes in peripheral blood smears.

## Brucellae

Brucella infections occur primarily in persons having contact with cattle (*Brucella abortus*), goats (*Brucella melitensis*) and pigs (*Brucella suis*). *Brucella melitensis* is the most prevalent species worldwide and the most virulent for humans [75]. Due to its rich endowment with elements of the reticuloendothelial system the liver is involved in acute and chronic Brucella infection [2, 71].

*Histological findings* are nonspecific with reactive inflammatory changes, and diffusely scattered noncaseating, histiocytic microgranulomas and somewhat larger epithelioid cell granulomas, which may show fibrinoid necrosis at their center. The latter have to be distinguished from tuberculosis and histoplasmosis. Typically granulomas occur in the lobular parenchyma rather than in portal regions, and multinucleated giant cells are often present [31]. During the further course granulomas undergo scarring and may calcify. Although Brucellae may be cultured from the infected tissue, they are not often visible in histological preparations [22]. Focal parenchymal necrosis in *Brucella melitensis* and *suis* infection, even after years of clinical latency may lead to the formation of liver abscesses.

*Clinically* hepatomegaly is present, aminotransferase levels are only slightly increased, and jaundice is rare.

## Burkholderia pseudomallei

*Burkholderia* (formerly Pseudomonas) *pseudomallei* is the causative agent of melioidosis. The disease is endemic in the Far East [65]. Patients with diabetes mellitus and renal failure are at particular risk of acquiring the infection [41]. In *acute melioidosis* multiple small (2–3 mm), partly confluent abscesses are scattered throughout the liver [27]. The bacteria can be demonstrated by Gram stain in the aspirated abscess fluid. *Chronic melioidosis* is characterized by centrally necrotizing, epithelioid cell granulomas with giant cells. The organisms only rarely can be demonstrated within these lesions [55]. In disseminated melioidosis there is hepatomegaly, and approximately every other patient is jaundiced with serum biliribin levels greater than 5 mg%. Approximately every third patient has an elevated AST.

## Campylobacter Species

Mild elevation of AST and alkaline phosphatase levels are observed in approximately 10–20% of campylobacter infections. Histologically mild nonspecific inflammatory changes are found.

## Chlamydiae

*Chlamydia trachomatis* may cause *Fitz-Hugh-Curtis syndrome* (see below). One case of a patient with fever of unknown origin in whom C. trachomatis was isolated from the liver has been reported in the literature [10].

The hepatic alterations in infection with *C. psittaci* and *C. pneumoniae* are nonspecific, with focal parenchymal necrosis, mild inflammatory cell infiltrates and granulomas. Aminotransferases are mildly elevated (up to 100 U/L) in up to 40% of patients. In clinically severe psittacosis jaundice may supervene.

## Clostridium perfringens

The liver may be involved in gas gangrene by developing parenchymal necrosis, and forming gas containing abscesses. The causative organisms can be cultured

from affected liver tissue. Clinically, jaundice may be present that usually also has a hemolytic component.

## Ehrlichiae

Ehrlichiae are rickettsial-like bacteria. *Ehrlichia chaffeensis* causes human monocytic ehrlichiosis. *Anaplasma phagocytophila* (formerly *Ehrlichia phagocytophila*) and *Ehrlichia ewingii* cause human granulocyte ehrlichiosis.

After transmission by a tick bite the organism spreads via the lymphatics and blood stream and attacks lymphocytes and cells of the reticuloendothelial system, including those in the liver. Elevated aminotransferase levels are observed in up to 80% of patients. Jaundice occurs significantly less frequently, and liver failure is exceptional. On histological examination nonspecific lymphocytic infiltrates, focal liver cell necrosis and occasional granulomas are found [15, 49].

## Francisella tularensis

*Francisella tularensis* causes tularemia. Hepatic involvement occurs in up to 75% of patients with mild to moderate elevations in aminotransferase levels. Hepatosplenomegaly is uncommon in the acute stage but becomes more frequent as the disease progresses [20]. Jaundice and marked cholestatic hepatitis are seen in more severe cases [51, 69]. Cholangitis and a liver abscess occur infrequently [24]. Histological examination shows parenchymal necrosis, sinusoidal dilatation and a mixed inflammatory infiltrate. Occasionally granulomas are seen. Organism can rarely be demonstrated by a Gram stain.

## Gonococci

Hepatic infection by *Neisseria gonorrhoeae* may occur as a complication of gonococcal septicemia or by dissemination from the genital tract.

Histologically focal necrosis with widespread infiltration of the parenchyma by neutrophils, abscess formation and perihepatitis may be present. In perihepatitis the liver capsule is fibrosed and thickened, showing multiple thread-like adhesions with the parietal peritoneum. The liver parenchyma is normal. The organisms may be isolated from the liver capsule.

*Fitz-Hugh-Curtis syndrome* (perihepatitis) occurs by dissemination of *Neisseria gonorrhoeae* (or *Chlamydia trachomatis*) from the genital tract through the peritoneal cavity to the liver. It occurs nearly exclusively in females, with only isolated cases having been reported in males [38]. Most patients have fever. Marked right upper quadrant pain radiating to the right shoulder with local guarding may mimic acute cholecystitis. Liver enzymes are not elevated.

*Gonococcal sepsis* may be associated with marked jaundice.

## Legionella pneumophila

This gram-negative pathogen causes *Legionnaire's disease* (pneumonic infection) and *Pontiac fever* (nonpneumonic infection). The liver may be involved in both diseases, and patients with impaired cellular immunity are at increased risk of acquiring the infection. Liver alterations are nonspecific and vary from case to case. Mild inflammatory portal infiltrates, intrasinusoidal neutrophils, focal liver cell necroses, cholestasis, Kupffer cell hyperplasia, granulomas, and abscesses have all been described. Occasionally the organisms may be isolated from the liver [39].

## Leptospira

Leptospira interrogans serotype *L. icterohaemorrhagiae* causes icteric leptospirosis (*Weil's disease*) [70]. After penetrating the skin the organisms reach the liver via the bloodstream.

The histological picture of the liver is variable with relatively mild signs of hepatic injury (apoptotic bodies), but marked regenerative activity (binucleated hepatocytes, variation in nuclear and cell size, numerous mitoses). In addition, there is cholestasis, Kupffer cell hypertrophy and hyperplasia, and erythrophagocytosis by Kupffer cells. Portal and sinusoidal lymphocytosis is mild. Leptospiral antigens can be demonstrated in liver tissue by immunocytochemistry [21].

The clinical course of icteric leptospirosis is severe with signs of renal and cardiac injury, neurologic disturbances and mucosal bleeding, which is not due to coagulopathy of liver disease, but to vasculitis. Icterus appears on the 2nd or 3rd day of illness, reaches its maximum in the second week, and may persist for several days to weeks. Serum bilirubin may reach levels greater than 40 mg%! The aminotransferases rise to levels five to ten times the upper limit of normal, γGT to ten times, and alkaline phosphatase to approximately three times the upper limit of normal. An important differential diagnostic laboratory finding with regard to acute viral hepatitis is the marked increase in serum levels of creatine phosphokinase, which occurs in approximately 50% of patients with Weil's disease, but not in viral hepatitis. The diagnosis is made by demonstrating leptospira in blood (more commonly by serological tests than by culture), cerebrospinal fluid and during the further course of the disease in urine.

## Listeria monocytogenes

Miliary microabscesses containing numerous gram-positive rods are found in the liver [44]. Some patients have a granulomatous hepatitis.

A Listeria hepatitis is more prevalent in newborns. Disseminated listeriosis in adults may mimic an acute viral hepatitis with elevations of AST levels up to 150–300 U/L [76].

## Mycobacteria

The involvement of the liver in *Mycobacterium tuberculosis* infection is characterized by a wide clinical spectrum ranging from an asymptomatic affection to severe icteric liver disease with acute liver failure [4, 5, 43]. The hallmark hepatic lesions are epithelioid cell, caseating granulomas with giant cells that most commonly occur within the context of miliary tuberculosis (rarely hepatic granulomas may also develop after BCG-vaccination). However, caseating necrosis is not always present and in this case the tuberculoid granuloma may be indistinguishable from a sarcoid granuloma. Acid fast rods are demonstrable on Ziehl-Neelsen staining in fewer than 15% of tuberculoid granulomas. It is not uncommon in patients with pulmonary tuberculosis with completely normal liver function tests to find granulomas in liver biopsy. Granulomas can also be present in the intra- and extrahepatic bile ducts and in the hilar lymph nodes. In addition to granulomas nonspecific hepatic changes, such as steatosis and focal necrosis may be present [19]. The laparoscopic aspect in miliary tuberculosis with disseminated whitish nodules on the surface of the parietal and visceral peritoneum is characteristic. The major differential diagnosis is peritoneal carcinomatosis.

If the patient is massively debilitated and quasi immunologically anergic, a *sepsis tuberculosa acutissima Landouzy* may supervene, in which the liver is peppered with multiple areactive parenchymal necrotic areas. The human organism is no longer able to mount a granulomatous reaction.

Clinically the patient with pulmonary tuberculosis is generally asymptomatic with regard to the liver. Mild hepatomegaly and slight increases of alkaline phosphatase and γGT levels usually are incidental findings. The aminotransferase levels are elevated in a minority of patients. Hepatic calcifications on the plain film of the abdomen are found in approximately every other patient with pulmonary tuberculosis, denoting calcified granulomas. Jaundice may point at an involvement of bile ducts (tuberculous cholangitis) or at a bile duct obstruction by enlarged lymph nodes. Ascites is generally due to peritoneal dissemination.

The differential diagnosis of pathological liver function tests in patients with extrahepatic tuberculosis should always consider drug-induced liver damage by antituberculous medication.

In AIDS patients, infection with atypical mycobacteria such as *M. avium intracellulare* (MAI) may occur in addition to tuberculosis. In MAI infection focal aggregates of foamy, PAS-positive macrophages, packed with acid fast bacilli are found in the liver parenchyma (Fig. 65.3).

A significant hepatic involvement in leprosy (*Mycobacterium leprae*) is uncommon, and usually due to drug-induced liver injury or to amyloidosis. Twenty to 60% of patients have mild increases of liver enzymes in serum. Histologically, tuberculoid granulomas or small clusters of foamy macrophages containing acid fast bacilli (Virchow's cells) may be seen [34].

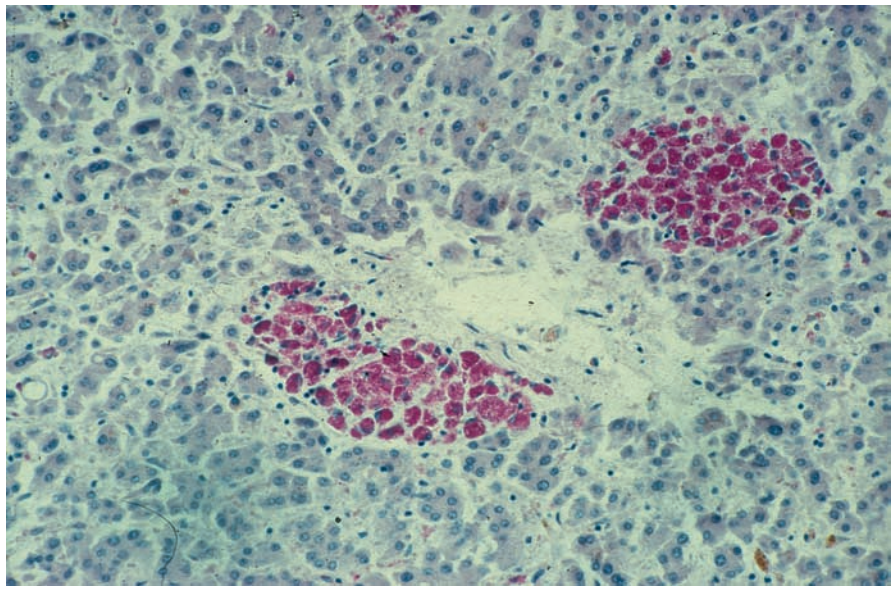

**Fig. 65.3** Atypical mycobacteriosis in the liver in a patient with AIDS. PAS stain (×400)

## Mycoplasma pneumoniae

*Mycoplasma pneumoniae* has been associated with acute cholestatic hepatitis, especially, but not exclusively in children [6, 9, 28]. In adults the liver usually is not involved in *M. pneumoniae* infection, but in a few patients mild increases of serum aminotransferase levels occur. Mild acute hepatitis associated with *M. pneumoniae* infection without lung involvement has also been reported [48, 61].

## Pneumococci

Approximately 25% of patients with classic lobar pneumonia develop jaundice, with a mild elevation of aminotransferase levels and more rarely of serum alkaline phosphatase. Histologically focal liver cell necrosis and a nonspecific inflammatory cell infiltrate may be seen.

## Rickettsiae

In *Q fever* (*Coxiella burnetii*) liver enzymes are usually mildly elevated. Two thirds of the patients have hepatomegaly, but fewer than 5% are jaundiced. Histologically, focal hepatocellular necrosis and characteristic, but nonspecific (may also be seen in Hodgkin's lymphoma and infectious mononucleosis) "doughnut granulomas", consisting of a ringlike fibrinoid material surrounding a clear lipid center ("fibrin ring granuloma") are seen [53, 68].

Hepatic granulomas, focal liver cell necrosis and nonspecific lymphocytic infiltrates also occur in *boutonneuse fever* (Mediteranean spotted fever) (*R. conorii*).

Hepatic involvement in *Rocky Mountain spotted fever* (*R. rickettsii*) manifests usually as a nonspecific reactive hepatitis with mild elevations of aminotransferase and bilirubin levels. Histologically inflammatory portal tract infiltrates with mononuclear cells and neutrophils, vasculitis of portal vessels and focal hepatocellular necrosis may occur. In single cases the organisms have been demonstrated in portal tracts.

A vasculitis of gallbladder vessels may manifest clinically as acute acalculous cholecystitis [1, 60].

## Salmonellae

Salmonella enteritis and typhoid fever still represent health problems in the less developed countries. The liver is involved in 25–30% of cases of salmonella typhi infection [56, 59].

*Pathogenetically,* local and systemic endotoxin effects and nonspecific inflammatory responses to intestinal ulcerations are discussed.

*Histologically,* nonspecific portal and sinusoidal lymphocytic infiltrates, hepatocyte ballooning, focal parenchymal necrosis and small lesions formed of hypertrophic and hyperplastic Kupffer cells ("typhoid nodules") are found. These macrophage nodules may increase in size and become necrotic at their center. True granulomas are rare. Often hepatocytes show a variable degree of diffuse, mixed macrovesicular and microvesicular steatosis.

*Clinically,* the involvement of the liver ranges from an asymptomatic nonspecific reactive hepatitis with slightly (<100 U/L) elevated aminotransferase levels to overt icteric hepatitis with hepatomegaly, AST elevations up to 800 U/L, elevation of alkaline phosphatase and coagulopathy. Jaundice is present in 1–8% of patients with typhoid fever, while hepatic encephalopathy is exceptionally rare [63]. Salmonella hepatitis may be clinically indistinguishable from an acute viral hepatitis. High fever, relative bradycardia, relatively mild elevation of AST in the presence of a marked increase in LDH and a left shift in the leukocyte differential count favor the diagnosis of typhoid hepatitis [13, 18, 37].

Chronic asymptomatic salmonella carriers may develop cholecystitis, suppurative cholangitis and rarely a liver abscess. Cholecystitis in a patient with typhoid fever is very rare.

Prognosis generally is good, since the underlying condition either resolves spontaneously or responds well to antibiotic therapy, for example with trimethoprim-sulfamethoxazole, ciprofloxacin or a third generation cephalosporin. However, in undernourished and anemic patients prognosis may be severe with mortality rates up to 20%, especially if treatment starts too late or when complications of salmonella infection, such as renal failure, supervene [36].

## Shigellae

The liver usually is not involved in shigella dysenteric infection. Rarely it may be accompanied by cholestasis and a nonspecific mild infiltration of portal tracts with neutrophils and lymphocytes. In severe infection steatosis, focal hepatocellular necrosis and a dense mixed inflammatory cell infiltrate that extends from the portal tracts into the parenchyma may be present [29].

## Staphylococcus aureus

Toxin (TSST-1) producing strains of staphylococcus aureus cause the *toxic shock syndrome* (TSS). Liver damage in TSS may result from impaired perfusion of the liver due to circulatory shock and from the action of circulating exo- and endotoxins. Additionally, TSST-1 as a superantigen may activate a large number of T cells, whose excessive cytokine production contributes to liver injury. Damage of intestinal mucosa during TSS enhances portal venous endotoxinemia which may aggravate the liver damage.

Histologically a dense mural, intraluminal and periductal neutrophil infiltration of intrahepatic bile ducts (suppurative cholangitis and cholangiolitis) and microvesicular steatosis are present [32]. Approximately half of the patients show centrilobular cholestasis [25].

Elevation of aminotransferase (<100 U/L) and alkaline phosphatase (1.5–3 times the upper limit of normal) serum levels is mild. Bilirubin levels rarely rise to greater than 5 mg%, and usually return to normal within 1 week.

## Treponema pallidum

Extended parenchymal necrosis and inflammatory lesions may occur in *congenital syphilis*. With silver staining techniques the spirochetes may be demonstrated in the liver.

*Primary syphilis* does not affect the liver. In *secondary syphilis* focal liver cell necrosis, portal inflammatory infiltrates, vasculitis of portal vessels and occasional granulomas may be encountered. Spirochetes are only rarely found in the liver. Clinically the infection may manifest as a cholestatic hepatitis. The typical lesion of *tertiary syphilis* is the gumma.

## Tropheryma whipplei

Whipple's disease is a very rare cause of hepatic granulomas.

## Yersiniae

Isolated cases of granulomatous hepatitis have been reported in infections with *Yersinia enterocolitica* and *pseudotuberculosis*. A rare but important complication of Yersinia infection is a liver abscess, with alcoholic cirrhosis, hemochromatosis and polycythemia vera being important predisposing factors [45, 52, 73].

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# Amebic Liver Abscess and Other Protozoal Diseases

# 66

Henryk Dancygier

## Chapter Outline



## Amebiasis

Amebiasis is caused by *Entamoeba histolytica* which exists as an amotile, infectious cystic and a motile, invasive trophozoite form. *Entamoeba dispar* is a harmless colonizer of the colon. Most amebic infections are asymptomatic. Symptomatic disease primarily manifests as amebic diarrhea or dysentery, or as a liver abscess. Amebic involvement of heart, lungs and brain is rare. In this chapter only amebic liver abscess is discussed.

### *Definition*

Amebic liver abscess is a liquefaction of liver parenchyma by invasive *E. histolytica*.

### *Epidemiology*

Worldwide approximately 600 million people are infected with amebae. The yearly incidence of new infections approaches 40–50 million people, predominantly in economically underdeveloped countries. In tropical and subtropical countries 20–30% of the population is infected with amebae. Yearly 500,000–700,000 deaths are due to invasive amebiasis. In Central Europe up to 3% of the population is infected. More than 95% of infections are noninvasive, i.e. the parasites remain confined to the intestinal lumen.

Most cases in Central Europe are imported from tropical and subtropical countries; however, indigenous infections do also occur. Amebic liver abscess is the most frequent extraintestinal manifestation of amebiasis. It may occur a few days to weeks, but also

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years after a sojourn in the tropics. Contrary to bacterial liver abscesses which affect males and females equally, amebic abscess is 7–10 times more prevalent in men, despite the fact that intestinal amebiasis affects both sexes with equal frequency. HIV-infected and immunosuppressed patients carry a higher risk of infection.

## Etiology, Pathogenesis and Pathology

The invasive trophozoite form of E. histolytica causes liver abscesses. The disease is transmitted by cysts in the drinking water or in contaminated food. The mature cyst contains four nuclei and is very resistant against gastric acid. After passage through the stomach and small intestine the cyst transforms into vegetative, motile trophozoites (magna form) capable of invasion and proliferation. The so-called minuta forms of trophozoites probably represent apathogenic amebae that are confined to the gut. With the help of proteolytic enzymes and pore-forming proteins, the trophozoites disintegrate the intestinal wall (*E. histolytica* = histolysis), penetrate the gut wall, and proliferate locally, creating the typical undermined ulcers. After gaining access to intestinal veins the organisms reach the liver via the portal venous circulation. *Colonization of the liver occurs only in up to 20% of cases of invasive amebiasis*. The trophozoites lyse the liver parenchyma, lead to small liquefied necrotic foci that increase in size to finally form double fist sized abscesses with an orange-brownish, anchovy-colored content. *Amebic pus is sterile*; amebae or their remnants may often be demonstrated only in the marginal areas of the abscess, i.e. in the transition zone between the liquid content and the intact hepatic parenchyma (PAS- or Giemsa stain). Unlike bacterial abscesses amebic abscesses do not have a capsule. They are surrounded by compressed liver tissue. The perifocal inflammatory reaction is only moderate.

## Diagnosis

### Clinical Manifestations

During its initial stages the abscess is asymptomatic. Symptoms develop insiduously and depend on the size and location of the abscess. Most patients present with fever (38.5–39.5°C) of 1–2 weeks duration, associated with right upper quadrant pain. Occasionally subfebrile or febrile temperatures and night sweats exist for months before the abscess becomes visible. Most patients complain of anorexia and weight loss, some of vomiting. A diarrhea is present in less than one third of cases, and may date back weeks or years, so that the patient might not recall it at all. *Only approximately 10% of patients with an amebic liver abscess have concomitant amebic colitis*. Jaundice is observed in fewer than 10% of patients with an amebic liver abscess.

Physical examination often reveals hepatomegaly that is tender on palpation. Subcapsular abscesses, located near the diaphragm may cause diaphragmatic elevation, pleuritic pain, pleural effusion and atelectasis of the adjacent lung.

### Technical Investigations

The *laboratory findings* reveal a nonspecific inflammatory response with an elevated ESR, leukocytosis without eosinophilia, anemia, elevation of $\alpha_1$- and $\alpha_2$-globulins and hypoalbuminemia. Aminotransferase levels are elevated in approximately 50%, serum alkaline phosphatase in 80% of patients.

Serological findings may be negative in the first weeks of the disease but eventually anti-amebic antibodies appear in 92–97% of patients. Serum antibodies against amebae are present in up to 25% of residents in endemic areas, without current active infection.

Microscopic demonstration of amebae in stool succeeds in approximately 10% of patients. Stool cultures yield positive results in up to 75% of cases.

*Imaging tools* (ultrasound, computed tomography) are the mainstay of diagnosis. Seventy percent of amebic abscesses are solitary, 80% are located in the right liver lobe, mostly in a subphrenic position. On ultrasound the amebic abscess appears as an echopoor focal lesion often containing scattered reflexes (detritus). On CT abscesses appear as hypodense lesions.

Aspiration of abscess contents does not yield amebae, and *aspiration is not necessary for diagnosis*. Trophozoites may be demonstrated microscopically in fewer than 20% of patients provided that material from the edge of the abscess has been aspirated.

## Differential Diagnosis

The main differential diagnosis is against bacterial liver abscess (Table 66.1) [4, 5]. Patients with a bacterial liver abscess generally have a more sudden onset of illness and leukocytosis is more marked (>20,000 /µl). Aspiration of a bacterial abscess usually yields several bacteria on Gram stain, while amebic pus is sterile. Further differential diagnoses include echinococcal and dysontogenetic congenital cysts. Necrotizing malignant tumors, especially hepatocellular carcinoma or metastatic pancreatic and ovarian cancer should also to be considered.

## Course and Prognosis

Complications are rare. Rupture into the peritoneal cavity with consequent peritonitis occurs in 2–7% of cases. Rupture into the pleural space, the lungs or the pericardial sack is less common. Hematogeneous dissemination may lead to abscesses in the spleen, lungs, brain and other organs.

Under adequate drug treatment the fever subsides within a few days. Even large amebic abscesses resolve completely (without scar formation) without the need for percutaneous drainage in the vast majority of cases. Complete resolution, however, may take months and in individual cases even years. Resolution should be monitored by ultrasound. Ultrasound follow-up should be performed initially in intervals of 2–3 weeks, thereafter every 2–3 months, in order to recognize relapse timely.

Early diagnosis and adequate treatment have reduced the mortality rate of an uncomplicated amebic abscess to <1%. In complicated cases mortality may increase up to 20%. Large and multiple abscesses, elevation of serum bilirubin levels >3.5 mg%, hypoalbuminemia <2 g/dL and signs of hepatic encephalopathy portend a grave prognosis [6, 13].

**Table 66.1** Characteristics of amebic and bacterial liver abscess

| | Amebic abscess | Bacterial abscess |
|---|---|---|
| **Age** | Usually <50 years | Usually >50 years |
| **Gender** | Males > > females | Males = females |
| **Diarrhea** | More often than in bacterial liver abscess, but not obligatory | Rare |
| **Concomitant diseases** | Rare | Frequent (bile duct diseases, diverticulitis, tumors) |
| **Jaundice** | Rare | Frequent |
| **Number** | Usually solitary | Often multiple |
| **Serologic tests** | Positive in 95% of cases | Negative |
| **Blood cultures** | Negative | Often positive |
| **Abscess contents** | Yellowish-brown to anchovy colored, odorless | Yellowish, malodorous |

## Therapy

Contrary to bacterial liver abscesses the vast majority of amebic abscesses do not require percutaneous drainage but may be managed successfully with pharmacotherapy. Nitroimidazoles are amebicidal.

*Metronidazole* 750 mg p.o. tid, or 500 mg p.o. or i.v. qid for 7–10 days

(potentially longer) leads to cure rates of greater than 90%. Resistance of E. histolytica trophozoites to metronidazole has not been documented yet. Alternatively,

*Tinidazole* 2 g p.o. qd for 5 days

or

*Ornidazole* 1 g p.o. bid for 3 to 5 days

may be given. Cure rates of nearly 100% after only 2–3 days of treatment have been reported with both substances [2, 11].

Treatment of invasive amebiasis needs to be followed by luminal agents to eradicate residual cysts in the intestinal lumen, even if stools are negative for amebae.

*Paromomycin* 10 mg/kg p.o. tid for 10 days

or

*Diiodohydroxyquin (iodoquinol)* 650 mg p.o. tid for 10–20 days

or

*Diloxanide furoate* 500 mg p.o. tid for 10 days

are administered. (Note: diloxanide furoate is no longer available in the Unites States).

The response to treatment is monitored clinically (resolution of fever and abdominal pain) and by ultrasonography. At the end of drug treatment most abscesses are still visible on ultrasound. This finding, however, should not lead to prolongation of treatment, since nearly all abscesses will be completely resorbed within a few weeks after cessation of drug treatment.

Aspiration of an amebic abscess should be avoided, because of the risk of amebic peritonitis. However, large subcapsular abscesses (>6 cm in diameter) at risk of rupture may be aspirated under ultrasound or CT guidance *after* antibiotic therapy has been instituted.

Surgical treatment is only indicated in case of rupture, in nonresponders to medical therapy or with ineffective percutaneous drainage.

## Babesiosis

*Babesia microti* and *Babesia divergens* are the most common causative agents of babesiosis. Infection may be asymptomatic or produce a malaria-like illness. The elderly, immunosuppressed, and splenectomized patients are at particular risk of acquiring infection. Mild elevations of liver enzymes may be observed, although they may be normal in subclinical infection [10]. In asplenic patients infection may be fulminant associated with multiorgan and hepatic failure. The histologic changes of the liver in mild infection resemble those of malaria, without, however, the deposition of hemozoin (see below).

## Cryptosporidiosis

*Cryptosporidium parvum* is an intestinal parasite that causes a self-limiting diarrheal illness in immunocompetent persons. In immunosuppressed, predominantly AIDS patients it causes a chronic intestinal infection. The organisms may be demonstrated histologically on the surface of the intestinal mucosa (on electron microscopy Cryptosporidia reside beneath the brush border inside the cell). In 10–25% of AIDS patients Cryptosporidium causes a sclerosing cholangitis ("AIDS-cholangiopathy") or a cholecystitis. Diagnosis is made by MRCP, ERCP or by biopsy.

There is no effective therapy for cryptosporidiosis. Immunocompetent persons usually will recover spontaneously. If therapy is required a trial of

*Nitazoxanide* 500 mg p.o. bid for 3 days

is warranted [12]. Restoration of the immune status with highly active antiretroviral therapy is the best means to eliminate the parasites in AIDS patients.

## Giardiasis

Most patients harboring *Giardia lamblia* in their intestine are asymptomatic. The most common complaints are crampy abdominal pain and diarrhea. The organisms may also invade the pancreatic and bile ducts and cause a pancreatitis, cholangitis or cholecystitis. Granulomatous hepatitis may be encountered occasionally.

*Metronidazole* 500 mg p.o. qid or 750 mg p.o. tid for 7–10 days

is the drug of choice.

## Leishmaniasis

Visceral leishmaniasis (Kala-Azar) is caused by *Leishmania donovani, infantum* and *chagasi*. The infection involves the mononuclear phagocyte system of various organs, including the liver. The survival of parasites within macrophages, among other factors depends on T-cell cytokines. Interferon-γ activates macrophages to kill the Leishmaniae via a nitric oxide (NO)-dependent mechanism. Activated $TH_2$-cells predominantly secrete interleukin-4 and interleukin-10 which inhibit NO activity thereby promoting survival and persistence of the organisms [7]. Untreated visceral Leishmaniasis is fatal.

In *acute Leishmaniasis* Kupffer cells are hypertrophied and hyperplastic, the portal tracts are infiltrated by inflammatory cells and the sinusoids contain plasma cells. Macrophages harboring parasites are scattered throughout the lobule and may form small clusters. Epithelioid cell granulomas, and occasionally so-called fibrin ring granulomas may also occur. Steatosis of hepatocytes is common.

In *chronic Leishmaniasis* panacinar fibrosis develops. The histological findings are nonspecific. Demonstration of parasites in macrophages, which succeeds in approximately 75% of cases is diagnostic.

On physical examination most patients show a hepatosplenomegaly. Laboratory examination reveals a hypoalbuminemia and a hypergammaglobulinemia. Bilirubin, AST, ALT and alkaline phosphatase levels are mildly elevated in 20–40% of cases [8].

Treatment of visceral Leishmaniasis is difficult and should be performed by a specialist in infectious diseases. The reader is referred to the respective textbooks.

## Malaria

Malaria is the most prevalent tropical parasitosis. There are between 300 and 500 million people infected worldwide. The most important causative organism is *Plasmodium falciparum*. Plasmodia transmitted by the mosquito Anopheles enter into and pass through Kupffer cells before invading hepatocytes [9]. Within the hepatocyte schizonts develop and produce merozoites which are released into the bloodstream when the hepatocytes rupture. *P. vivax* and *P. ovale* may persist as dormant forms (hypnozoites) in the liver for up to 3 years and cause relapses.

The hepatologic alterations in malaria result from activation of Kupffer cells, hepatocyte drop out and from disturbances of the microcirculation. The latter are due to clumping of erythrocytes containing Plasmodia and to disseminated intravascular coagulation. Additionally, endotoxin-mediated liver damage has been incriminated.

### *Pathology*

In severe malaria the liver is enlarged and dark brown in color. Histologically black malarial pigment (hemozoin) is deposited as fine granules within red blood cells and as somewhat coarser pigment granules within Kupffer cells and portal macrophages (see Fig. 24.14). Hemozoin is an iron-porphyrin-protein complex that is derived from the degradation of parasitized red blood cells. Possibly, hemozoin impairs the immunologic functions of macrophages [15]. The sinusoids may harbor groups of red blood cells containing the parasites. The portal tracts are mildly infiltrated with plasma cells and lymphocytes, however, *a "malarial hepatitis" proper does not exist*. Severe malaria with circulatory shock may lead to centrilobular necrosis and cholestasis.

### *Clinical Manifestations*

The clinical involvement of the liver in malaria is variable. Usually hepatic involvement is asymptomatic. However, a painful hepatospenomegaly, conjugated hyperbilirubinemia, hypoalbuminemia, hypoglycemia and lactic acidosis with prolongation of prothrombin time may occur. Hypoglycemia, often combined with lactic acidosis, is an important complication of hepatic involvement in malaria, and is associated with a severe prognosis. Impaired hepatic gluconeogenesis (with hepatic glycogen stores depleted) and increased glucose utilization by the host and the parasites are pathogenetically important. Quinine and quinidine stimulated pancreatic insulin secretion may contribute to hypoglycemia. Mild jaundice in malaria is primarily due to hemolysis, whereas a marked icterus in tropical malaria results from the combination of hemolysis, liver injury, and cholestasis. In endemic areas, jaundice is seen in approximately 2.5% of patients with falciparum malaria [1]. Elevated liver enzymes in malaria may also be drug induced. Quinine may cause granulomas, while amodiaquine may evoke a hepatitic reaction.

*Tropical splenomegaly syndrome* (hyperreactive malarial splenomegaly; "big spleen syndrome") denotes a disease that is characterized by a huge splenomegaly (the organ weighs 2–4 kg) and a relatively mild hepatomegaly. It is thought to result from an abnormal immune reaction to recurrent infections with P. falciparum with excessive formation of macromolecular IgM aggregates. Nonspecific cellular hyperplasia is found in the spleen, while a sinusoidal T-cell lymphocytosis is present in the liver. Kupffer cells are hypertrophied and contain immune complexes, but no hemozoin [3].

## Toxoplasmosis

Neonatal toxoplasmosis of the liver may present as giant cell hepatitis. The organisms may be demonstrated in sinusoidal cells.

Toxoplasmosis in adults generally is a latent, lifelong infection, which usually does not affect the liver. Immunosuppressed individuals, however, may develop a toxoplasma hepatitis with focal necroses and cholestasis [14]. The parasites may be demonstrated within the hepatocytes, either in intracellular cystic spaces or lying freely in the cytoplasm. Acute epithelioid cell granulomatous hepatitis has also been described in acquired toxoplasmosis [16].

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# Helminthic Infections

# 67

Henryk Dancygier

## Chapter Outline



Liver involvement in helminthic diseases is discussed according to the following outline:

- Trematodes (Flukes)

Schistosomiasis, Fascioliasis, Dicroceliasis, Clonorchiasis, Opisthorchiasis

- Cestodes (Tapeworms)

Echinococcosis (Hydatid disease)

- Nematodes (Roundworms)

Ascariasis, Toxocariasis, Capillariasis, Enterobiasis, Strongyloidiasis

## Hepatic Schistosomiasis (Bilharziasis)

### *Definition*

Infectious liver disease caused primarily by *Schistosoma mansoni* and *S. japonicum. S. mekongi* and *S. intercalatum* cause hepatic infections in Southeast Asia and in Western Africa, respectively. *S. haematobium* causes bilharziasis of the urinary bladder.

### *Epidemiology*

Worldwide 200 million people are infected with schistosomes and approximately 200,000 deaths per year are attributable to schistosomiasis, which is the second most common human parasitic disease after malaria [7]. Schistosomiasis is one of the most common causes of portal hypertension worldwide. In endemic areas the maximal incidence of the disease is between the 10th and 14th year of life.

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## Pathogenesis

The parasites (cercariae) penetrate through the intact skin and transform to schistosomulae. During this stage the parasites lose surface antigens and acquire blood group and MHC antigens of the host [17, 28, 30]. Due to this antigenic change the parasite surface immunologically resembles host tissue, which circumvents an immune reaction and facilitates the spread of the organisms. The schistosomulae migrate through the subcutaneous tissue with the help of proteolytic enzymes. They pass through the venous capillaries and lymphatics into the general circulation and reach the intestinal wall and the liver by hematogenous dissemination. The adult male and female worms live and copulate within the mesenteric veins, where the females deposit their eggs. Some eggs penetrate the intestinal wall and are passed in the stool, while others reach the liver via the portal circulation, where they become stuck within the small intrahepatic venules, whose diameter is smaller than that of the eggs (50 µm). Here they develop into adult worms and may also migrate in a retrograde fashion back into the mesenteric veins.

Schistosomal eggs elicit an immunological reaction in the portal tracts which leads to granuloma formation. The development of granulomas is mediated by MHC class II-restricted CD4 T helper cells [18]. The early phase of granuloma formation is characterized by a TH1-type immune response, i.e. synthesis of interferon-γ and interleukin (IL)-2 prevails. During further progress there is a switch to a TH2-type immune response with the generation of predominantly antiinflammatory cytokines (IL-4, IL-5, IL-10, IL-13), the production of antibodies by B cells, and the recruitment of eosinophils, which themselves are an important source of IL-4. The TH2-type immune response has a protective effect trying to limit the extension of the inflammation. Also hepatic granulomas are protective. They impair the invasion of eggs and the permeation of their secretory products into the liver parenchyma without eliciting a significant inflammatory reaction [32]. Since schistosomal eggs reach the liver at different points in time, formation of granulomas is asynchronous. New and old granulomas lie side by side and newly forming granulomas in a liver that already harbors granulomas are smaller than the older ones. IL-10 is believed to play an important immunomodulatory role in restricting granuloma formation.

The course of hepatolienal schistosomiasis depends on many factors. The intensity of exposure, differences in parasite species, the nutritional state of the patient, coinfections with Plasmodia, Brucellae or hepatitis viruses are among the most important ones. The risk of severe hepatic fibrosis is also determined by genetic host factors, such as the polymorphism of the interferon-γ receptor gene [11].

The humoral immune response to a schistosomal infection is complex, and is characterized by the formation of protective antibodies and antibodies enhancing the infection. IgM and $IgG_2$ antibodies may block the eosinophil-dependent killing of schistosomulae. IgE and IgA antibodies protect against reinfection.

## Pathology

The histological picture of chronic hepatic schistosomiasis is characterized by noncaseating portal granulomas and by an increasing fibrosis. The eggs are initially surrounded by a fibrinoid material and demarcated by an eosinophilic inflammatory reaction (Hoeppli-Splendore reaction). As the disease process progresses epithelioid cell granulomas develop that contain lymphocytes, macrophages, eosinophils and neutrophils, as well as Langhans giant cells, embedded in a collagenous matrix. Like in malaria, hemozoin pigment is deposited. Within approximately 3 weeks the eggs die and may be completely digested or calcify. Granulomas increasingly become deprived of cells, leaving behind fibrous scars. Portal fibrosis affects predominantly the portal vein branches ("pipe stem fibrosis" according to Symmers) and results in their partial or complete obliteration, while the arterial and ductal structures remain relatively undamaged [3]. A presinusoidal portal hypertension develops in advanced stages. Despite the marked fibrotic changes the acinar architecture remains widely intact.

## Diagnosis

### Clinical Manifestations

The disease may manifest initially as a cercarial dermatitis with an itchy, urticarial rash. Passage of the parasites through the lung is associated with fever, cough, basal pulmonary opacities and a pronounced peripheral blood eosinophilia.

*Katayama fever* denotes an acute schistosomal infection, generally in previously noninfected patients,

that occurs approximately 4–6 weeks after skin penetration by cercariae. The disease is regarded as a serum sickness-like reaction with circulating immune complexes. Fever, muscle pain, arthralgias, dry cough, abdominal pain and diarrhea characterize the clinical picture. Physical examination reveals a painful hepatosplenomegaly, lymphadenopathy and a skin rash. The symptoms abate spontaneously after several weeks in most patients.

An asymptomatic period leading to chronic disease follows. The chronic stage is characterized by intestinal and/or hepatosplenic involvement. Abdominal cramps, mucous-bloody diarrhea and tenesmus characterize the clinical picture. In addition, a portal hypertension of variable severity may develop with splenomegaly, ascites and esophageal variceal bleeding. Liver function remains intact for long periods of time due to a compensatory increase of hepatic perfusion via the hepatic artery, so that signs of liver failure or of hepatic encephalopathy (in contrast to cirrhosis) rarely occur in schistosoma-induced liver fibrosis, unless viral co- or superinfection occurs. Jaundice, spider nevi, palmar erythema, testicular atrophy and gynecomastia also are not part of the clinical picture of "pure" hepatic schistosomiasis.

### Technical Investigations

There is peripheral blood eosinophilia and a mild elevation of serum ALT levels in the acute phase of the illness [9]. Aminotransferases are normal and alkaline phosphatase is mildly increased in the chronic stage. Elevated aminotransferase levels point towards coinfection with HBV or HCV, or to other cofactors such as alcohol abuse. Leuko- and thrombocytopenia as well as a mild hemolytic anemia may be the expression of hypersplenism. In the very early stages of disease a decrease in the levels of several coagulatory proteins may occur that, however, is not relevant clinically. During the further course a hypoalbuminemia appears.

The diagnostic gold standard is the demonstration of schistosomal eggs in stool. If negative, examination of stool should be repeated several times. If there is continuing clinical suspicion of schistosomiasis despite repeatedly negative stool examinations, a rectal biopsy should be performed.

Measurement of anti-schistosomal antibodies in serum is useful in demonstrating an infection in travelers to endemic areas. Since this immunologic test, however, cannot discriminate between acute and past infection, due to the high prevalence of schistosomiasis, it is not useful in residents of endemic areas.

Ultrasound can be used to visualize major architectural changes such as fibrous thickening of the portal vein wall and its branches as well as the sequelae of portal hypertension. Enlargement of the left liver lobe is considered typical for hepatic schistosomiasis.

Liver biopsy is only diagnostic if granulomas, parasitic eggs or its remnants are present in the tissue specimen. Otherwise histological changes are nonspecific.

## Differential Diagnosis

Differential diagnosis is primarily against liver cirrhosis. Mixed pictures of hepatic schistosomiasis and chronic hepatitis B or C are not uncommon.

## Course and Prognosis

Because of preserved liver function patients with schistosomiasis tolerate bleeding from esophageal varices better than patients with liver cirrhosis. Coinfections with HBV and HCV are frequent, often are responsible for hepatic decompensation, and affect prognosis.

## Therapy

*Praziquantel* 20 mg/kg p.o. bid (for *S. haematobium*, *S. mansoni*, and *S. intercalatum*) and 20 mg/kg p.o. tid (for *S. japonicum* and *S. mekongi*)

is effective against all schistosomal species. Cure rates are 70–95%. Patients should be examined for retention of living eggs 3 and 6 months after treatment, and retreated if egg excretion has not decreased markedly.

Drug and endoscopic treatment of portal hypertension and its sequelae is performed according to the principles outlined in Chapter 80. A porto-caval shunt should not be performed in hepatosplenic schistosomiasis since it results in hepatic encephalopathy in 40–50% of patients and may exacerbate hemolysis induced by hypersplenism.

## Fascioliasis

*Fasciola hepatica* (large liver fluke) and *Fasciola gigantica* (up to 7.5 cm long) are distributed worldwide, and infect sheep, goat and cattle. Man is infected by eating water plants (e.g. watercress) to which the metacercariae of the parasites are attached [10, 25].

After oral intake the juvenile liver flukes penetrate the intestinal wall, reach the peritoneal cavity and penetrate through the liver capsule into the hepatic parenchyma, and find their way into the large bile ducts and gallbladder, where they finally reside. Here the worms can stay for several years and cause inflammatory epithelial lesions.

*Macroscopically*, yellow nodules, up to 2 cm in diameter, may be seen on the liver surface. *Histologically*, necrotic areas and a pronounced tissue eosinophilia are present, in addition to the organisms themselves. Large necrotic granulomas may form around Fasciola ova and develop into subcapsular scars. If the worm infection causes a cholangitis, secondary hepatic parenchymal changes due to cholestasis and cholangitis ensue.

The invasion phase usually lasts 2–3 months and is characterized *clinically* by right upper quadrant pain, vomiting and diarrhea, bloating, fever and hepatomegaly. It may be followed by a long lasting asymptomatic period and then by a chronic phase with signs of recurrent cholangitis, occasionally with cholestatic jaundice and hematobilia.

*Laboratory parameters* reveal a leukocytosis with a marked eosinophilia (up to 30%) and a mild anemia. During the invasive phase aminotransferase levels are slightly elevated. If cholangitis is present levels of alkaline phosphatase and γ-GT rise.

*Diagnosis* is made by demonstrating Fasciola ova in duodenal juice or in stool. Rarely the organisms may be isolated from bile during ERCP. Anti-Fasciola antibodies in serum appear already during the invasive phase.

*Treatment* is with

*Bithionol* 30–50 mg/kg p.o. divided in 3 doses every other day for 10 to 15 doses.

Cure rates range between 50% and 90%. A new imidazole derivative,

*Triclabendazole* 10–12 mg/kg p.o. qd on 1 day or 2 consecutive days,

where it is available, has become the drug of choice. Absorption of the drug is improved when it is taken after meals. Cure rates of nearly 100% have been reported. If results are unsatisfactory treatment may be repeated after several weeks.

Patients with Fasciola demonstrated in the bile duct and resistant to oral pharmacotherapy may be treated by flushing the biliary system during ERCP with a fasciolicidal povidone iodine solution [12].

## Dicroceliasis

The small liver fluke, *Dicrocoelium dendriticum* or *lanceolatum* is a parasite that affects bile and pancreatic ducts. Humans become infected by contact with sheep or by inadvertently ingesting ants that contain infectious metacercariae.

Most infections are asymptomatic or present with mild noncharacteristic abdominal complaints. Chronic infection leads to fever, hepatomegaly, recurrent cholangitis and rarely to bile duct occlusion. Diagnosis is made by demonstrating parasitic ova in stool. Treatment is with

*Praziquantel* 25 mg/kg p.o. tid.

## Clonorchiasis and Opisthorchiasis

*Opisthorchis felineus* occurs in Eurasia, *Opisthorchis viverrini* in Thailand and Laos, and *Clonorchis sinensis* in the Far East. The worms are endemic in river and lake areas. Seventeen million people are estimated to be infected.

Man becomes infected by ingesting raw fish that contains infectious metacercariae. The worms reach the bile ducts via the duodenum through Vater's papilla. Adult C. sinensis worms live in the bile ducts.

*Histological examination* reveals a chronic cholangitis with proliferation and folding of bile duct epithelium ("adenomatous hyperplasia"), ductal and periductal fibrosis with dilatation of small bile ducts that contain worms within their lumen [20]. Ascending suppurative cholangitis generally is due to bacterial superinfection (e.g. *E. coli*) and may rarely lead to the development of a bacterial liver abscess.

One third of chronically infected patients are asymptomatic. The *clinical picture* in the remaining patients is variable and ranges from nonspecific abdominal complaints to a severe cholangitic illness with fever, chills, jaundice and hepatomegaly.

The *laboratory data* typically show a marked eosinophilia accompanied by general signs of inflammation. Depending on the intensity of involvement of bile ducts and of liver parenchyma aminotransferase levels and cholestatic parameters are elevated.

The *diagnosis* is made by demonstrating Clonorchis ova in stool or in duodenal juice. Occasionally on cholangiography filling defects may be noted.

*Complications* of Clonorchis infection are cholelithiasis and cholangiocarcinoma, whose incidence in endemic areas is increased 20–40 times. Clonorchiasis associated cholangiocarcinoma is generally intrahepatic, multicentric and mucin producing.

*Therapy* with

*Praziquantel* 25 mg/kg p.o. tid for 1 day

achieves cure rates of nearly 100%. In severe Clonorchis infections treatment may be prolonged to 2 days. The worms disappear from stool within one week, the symptoms, however, abate gradually over weeks to several months. Infected, but asymptomatic patients should also be treated, because of the risk of potential complications.

If praziquantel is not tolerated

*Albendazole* 10 mg/kg p.o. qd for 7 days

may be given alternatively with cure rates of 90–100%. An ascending bacterial cholangitis is treated with antibacterial chemotherapy.

## Echinococcosis (Hydatid Disease)

### Definition

Parasitic infection with the canine tapeworm *Echinococcus granulosus (cysticus)* or the fox tapeworm *Echinococcus multilocularis (alveolaris)*.

*E. granulosus* causes cystic echinococcosis (usually unilocular cysts), *E. multilocularis* causes alveolar echinococcosis (generally multiloculated cysts and infiltrative growth). *Echinococcus vogelii* and *Echinococcus oligarthros* cause polycystic hydatid disease. Infection with *Echinococcus oligarthros* (South America, Africa) is extremely rare and of subordinate importance for man.

### Epidemiology

Echinococci occur worldwide. *E. granulosus* is common in cattle-breeding areas of the Mediterranean, Middle East, Australia, New Zealand, South Africa, and South America. Canines are the definitive hosts. Herbivores (e.g. sheep, horses, deer) or humans are the intermediate hosts. E. multilocularis is endemic in certain areas in southern Germany (incidence 0.5 new infections per $10^5$ inhabitants per year), Switzerland, France, Italy, Canada, USA, China and Russia (incidence 80–200 new infections per $10^5$ inhabitants per year). The definitive host is the fox, rarely the dog. The intermediate host is the field mouse. Man can also be infected as an intermediate host. Contagion from man to man does not occur. *Echinococci are the most common cause worldwide of non-congenital liver cysts*. Seventy percent of cysts of E. cysticus, and 98% of cysts of E. alveolaris are located within the liver.

### Pathogenesis

If dogs ingest organs (or feces) that contain cysts of infected animals, ingested eggs hatch in the gut. In the canine gut adult worms develop from scolices. Human beings get infected by accidental ingestion of tapeworm eggs via terminal host feces or through oral contact with saliva of infected dogs (*E. granulosus*) or for example by eating contaminated berries or vegetables (*E. multilocularis*). The egg shell is digested by gastric juice and bile acids and the oncospheres released penetrate through the intestinal wall and reach the liver through the portal venous circulation. In the liver the larvae encapsulate and cysts form.

### Pathology

Approximately 75% of persons infected with *E. granulosus* develop one or several liquid filled cysts (hydatids)

in the liver. The right liver lobe is more often affected and the cysts may attain a diameter of ≥ 30 cm. The typical hydatid cyst is unilocular with several daughter cysts. Its wall is made up of 3 layers: (1) a host derived external, fibrous envelope, (2) a middle, acellular hyaline (cuticular) layer (approximately 1 mm thick), and (3) an inner germinative membrane which develops brood capsules that embrace new protoscolices. Each capsule might contain 20–120 protoscolices. The detached brood capsules and free protoscolices accumulate in the clear cyst fluid forming the "hydatid sand". Daughter cysts might form from the germinative membrane or by direct transformation of the protoscolices or of brood capsules [13]. The inflammatory reaction of the host to the cyst is only weak. Next to the outer fibrous layer some granulation tissue may be present.

*E. multilocularis* is characterized by the formation of many small cystic spaces that contain a jelly-like material. The irregularly shaped cysts are surrounded by a hyaline layer, a germinative membrane and protoscolices are absent. The spongy cystic structures are locally invasive and infiltrate into the adjacent liver parenchyma [26]. Calcifications at the cyst margins may be observed in both E. species.

## Diagnosis

### Clinical Manifestations

The cysts of *E. granulosus* grow slowly, approximately at a rate of 1 cm per year, and only after years lead to symptoms by pressure on neighbouring organs. 5–15 years after the ingestion of echinococcal eggs nonspecific complaints occur, such as a feeling of pressure in the right upper quadrant, occasionally accompanied by fever. Colicky pain, jaundice and cholangitic signs are rare, but may occur whenever the cysts compress or penetrate the bile ducts.

In *E. multilocularis* infection hepatomegaly is in the foreground. Jaundice usually does not occur until the cysts infiltrate the bile ducts or rupture into them.

### Technical Investigations

Twenty to 40% of patients have a blood eosinophilia. A polyclonal hypergammaglobulinemia is common. IgE concentrations are elevated in 50% of patients. Serologic tests (indirect hemagglutination, complement binding reaction, immunofluorescent assay, enzyme-linked immunoassay, western blot) with purified echinococcal antigens attain a sensitivity of approximately 85%. However, especially with *E. granulosus* false-negative results are possible. Serologic tests remain positive for years after surgical cyst removal. Thus, relapses cannot be diagnosed serologically. The skin test with hydatid fluid (Casoni's test) is only of medical historical interest [22].

The diagnosis of cystic echinococcosis is usually made by ultrasound or computed tomography. Initially, on ultrasound the cyst resembles a simple liver cyst and contains pure anechoic (fluid) material. During further development the membrane may become detached, multiple septa and/or daughter cysts may appear, the cyst might increasingly exhibit high internal echoes, with bright shadowing echoes in the cyst wall indicating calcifications [15]. Microcalcifications are better seen on computed tomography than on ultrasound. *A definite differentiation between cystic and alveolar echinococcosis by imaging tools is not possible*. Positron-emission tomography (PET) using [18] F-fluoro-deoxyglucose has been reported to have the potential to discriminate active from inactive lesions in alveolar echinococcosis, having a sensitivity of 91% for the detection of active lesions [27].

Compression of bile ducts or cyst communication with the bile duct lumen will usually cause jaundice and may be demonstrated by MRCP or ERCP.

Echinococcal cysts should not be punctured, since spillage of hydatid fluid into the peritoneal cavity may cause anaphylactic reactions and dissemination of scolices. Diagnostic fine needle aspiration, however, appears to be a safe procedure [19]. The presence of hydatid sand in aspirated cyst fluid is diagnostic. Needle puncture may be hampered by the solid cyst envelope that may cause the needle to slide into the adjacent liver tissue. Direct histopathological demonstration of the organisms in the punctured tissue is rare. Molecular biological techniques in the punctured material may improve the diagnostic sensitivity.

## Differential Diagnosis

First and foremost congenital liver cysts, cystadenomas and cystadenocarcinomas, and more rarely liver abscesses should be excluded.

## Complications

Cholestatic jaundice, compression of portal vein with consequent portal hypertension (with or without portal vein thrombosis), fistulas into the pericardium or lungs, metastatic spread into the lungs, kidneys, spleen, central nervous and the skeletal system and spontaneous rupture into the peritoneal cavity or into the pleural space are rare but feared complications.

## Course and Prognosis

*E. granulosus* cysts may remain constant in size, and occasionally even regress spontaneously. The organisms in a small cyst may die and the cyst may calcify acquiring the aspect of a golf ball. Most patients remain asymptomatic for more than 10 years [14]. Calcified cysts, free of organisms may be left in the liver, and need no treatment. Relapses occur in approximately 20% of patients after operative resection of "active" *E. granulosus* cysts.

*E. multilocularis* behaves clinically as a locally infiltrating tumor, and is fatal without treatment. Only 10–30% of nonoperated patients survive 10 years.

## Therapy

Surgical resection can be curative and is the treatment of choice [8, 33]. Prior to cystectomy cyst content should be aspirated as completely as possible, and a scolicidal 0.5% Na-hypochlorite or 0.5% silver nitrate solution should be injected into the cyst in order to devitalize scolices. If there is a communication between the cysts and the biliary system these solutions must not be instilled, since they damage the biliary epithelium. Therapeutic aspiration of cyst contents may be performed in inoperable patients. The combination of percutaneous drainage and drug treatment may represent an effective alternative to surgical resection alone [4, 24, 31].

*Albendazole* 400 mg p.o. bid for 3 to 6 months, or up to 24 months in a cyclic monthly form

is the drug of choice. It should be started approximately 4 weeks before surgery and continued for up to 24 months after the operation. Pre- and postoperative pharmacotherapy is effective in the prevention of recurrences and/or secondary hydatidosis. Long-term cure rates for *E. granulosus* infection are 30–40%. It can be used to suppress growth of worms in inoperable patients [16]. Alternatively,

*Mebendazole* 200–400 mg p.o. tid for 3–6 months

may be given (Note: less than 10% of mebendazole is absorbed after oral administration).

Prognosis for E. multilocularis infection is poor and only partial hepatectomy (in localized lesions) or liver transplantation have been lifesaving in a few patients [23]. Relapses may occur up to 25 years after the operation. Pharmacotherapy is with the same drugs as in *E. granulosus* infection, albeit with higher doses. Neither duration or type of treatment, nor size of the lesion, the presence of calcifications, or regressive changes reliably indicate parasite death. Lacking definitive criteria in deciding whether a lesion is still active, i.e. if the organisms are still viable or not, treatment probably should be continued throughout life. PET might be a reliable tool for assessing metabolic activity of echinococcal lesions, thus helping in determining the duration of long-term treatment and for timely detection of relapses [27]. Immunosurveillance by determining the profile of specific antibodies against echinococcal antigens may also be helpful in monitoring alveolar echinococcosis after surgery and/or chemotherapy [2].

In recurrent and residual alveolar echinococcosis of the liver after surgery

*Albendazole* 15 mg/kg p.o. daily in two divided doses

or

*Mebendazole* 50 mg/kg p.o daily in three divided doses

are administered [21]. The maximal daily dose of mebendazole that can be given is 6 g. Both drugs are well tolerated, but with high dose long-term treatment side effects may occur: elevations of aminotransferase levels (27%), mild proteinuria (21%), transient partial alopecia (18%), nonspecific gastrointestinal complaints (16%), neurologic symptoms, such as dizziness or insomnia (11%) as well as leukopenia (6%).

## Ascariasis

Infection with *Ascaris lumbricoides* is the most common helminthic disease with approximately 1.4 billion people being infected worldwide. Prevalence in so-called third world countries ranges between 63% and 92%. The incidence in Europe and in the USA is very low. Autochthonous infections in Europe are very rare.

The adult worm lives in the small intestine and may reach a length of up to 40 cm. Man becomes infected by ingesting eggs containing larvae. Gastric juice dissolves the egg-shell, the released larva penetrates the wall of the small intestine, enters the mesenteric veins and reaches the liver via the portal venous system. The larvae pass through the sinusoids, enter the venous circulation and reach the lungs. From here they can penetrate the trachea and reach the systemic circulation. Thus, Ascaris may infect many organs, including lungs, kidneys, heart and brain.

If the eggs are deposited within the intrahepatic bile ducts, the adult worms may return to the duodenum through the common bile duct.

*Histologically* the liver passage of the organisms is associated by only a mild tissue reaction. The death of larvae in the liver leads to a marked eosinophilic inflammatory reaction with possible formation of granulomas. Within the bile ducts Ascaris may elicit cholangitic changes that may be the source of a secondary bacterial liver abscess.

*Clinically* the intestinal involvement manifests with nonspecific abdominal complaints, such as epigastric pain, nausea and vomiting. Migration of worms into the biliary system may be associated with right upper quadrant pain, biliary colic (56%), signs of ascending cholangitis (24%), acalculous cholecystitis (13%), obstructive jaundice or rarely with bile duct perforation and consequent peritonitis. Retained worm fragments in the bile ducts form a nidus for recurrent ascending bacterial cholangitis and pyogenic liver abscesses (<1%). Obstruction of the pancreatic duct by Ascaris may elicit an acute pancreatitis.

The *diagnosis* is made by demonstrating worm eggs or worm fragments in stool. Occasionally worms may be seen during endoscopic examination or as filling defects on cholangiography. A characteristic, albeit rare sonographic sign of ascaris is linear, parallel, nonshadowing reflexes delimiting a central echo poor lumen, which corresponds to the gut of the worm.

Pharmacotherapy with

*Albendazole* 400 mg p.o. once

or

*Mebendazole* 100 mg p.o. bid for 3 days or 500 mg p.o. once

or

*Ivermectin* 150 µg/kg p.o. once

is effective with cure rates approaching 100%. However, reinfestations are frequent and are treated the same way. Recent data suggest that

*Nitazoxanide* 500 mg p.o. bid for 3 days

is also effective. Because of possible teratogenicity mebendazole and albendazole should not be used in pregnancy, and should be substituted with

*Pyrantel pamoate* 11 mg/kg p.o. once (maximal dose 1 g).

Obstructive complications may require surgical or endoscopic extraction of adult worms.

## Toxocariasis (Visceral Larva Migrans)

*Toxocara canis* and *catis* predominantly infect dogs and cats. The disease is rare in Europe and in the USA. It occurs mostly in children with a history of geophagia playing on grounds contaminated with worm eggs. Infection occurs by ingesting Toxocara eggs which in humans cause visceral larva migrans (VLM). It is self-limiting in 6–18 months if ingestion of eggs ceases.

After ingestion larvae, released from the eggs, penetrate the gut wall, reach the liver via the portal circulation and from here the systemic circulation [5]. The larvae may stick in small intrahepatic vessels and elicit a tissue reaction. *Macroscopically* many yellowish nodules, up to 1 cm in diameter, are seen. *Microscopically* migrating larvae are surrounded by an eosinophilic, epithelioid cell granulomatous reaction with multinucleated giant cells

that with time transforms into a scar. Larvae are difficult to find in tissue sections.

*Clinically* the infection may be mild and asymptomatic being suspected only because of a marked eosinophilia. Severe disease is characterized by fever, anorexia, hepatosplenomegaly, and an itching rash. Pulmonary, ocular, heart and brain involvement is not discussed here.

In addition to eosinophilia *laboratory findings* show a marked leukocytosis and a hypergammaglobulinemia.

Rarely the liver nodules appear on ultrasound as echopoor space occupying lesions surrounded by an echogenic rim, in which many eosinophils may be demonstrated by biopsy.

*Diagnosis* may be made by demonstration of larvae in liver biopsy specimens. Generally, however, biopsies are low yield and only show a nonspecific eosinophilic granulomatous reaction. Thus, diagnosis is based on clinical, epidemiologic, and serologic findings (highly specific enzyme-linked immunosorbent assay for antibodies to *T. canis*). Stool examinations are worthless [29].

VLM is a self-limiting disease that abates slowly and usually requires no therapy. Patients with continuing reinfection or severe disease can be treated with

*Albendazole* 400 mg p.o. bid for 5 days

or

*Mebendazole* 100–200 mg p.o. bid for 5 days.

Despite the efficacy of these drugs, their success in VLM has not been proven and they are considered investigational by the United States Food and Drug Administration [6].

Milder symptoms may require only antihistamines.

## Capillariasis

Infection with *Capillaria hepatica* is very rare. Man is infected by accidentally ingesting feces containing eggs of infected animals (rats, squirrels, beaver, pigs, cats). The larvae hatch in the small intestine, penetrate the gut wall and reach the liver via the portal venous system. The larvae mature, mate, and produce eggs in the liver, which elicit an eosinophilic granulomatous reaction.

*Symptoms* include fever, hepatomegaly and blood eosinophilia. The clinical picture may be identical to that of VLM. Mild infection may subside spontaneously, whereas severe disease may be fatal.

*Diagnosis* is made by liver biopsy.

There is no proven therapy.

*Thiabendazole* 25 mg/kg p.o. bid for 7–10 days

or

*Albendazole* 200 mg p.o. bid for 10 days

have been reported to be successful.

## Enterobiasis

Occasionally *Enterobius vermicularis* may reach the abdominal cavity via the genital tract. If it penetrates into the liver white-greenish nodules, measuring approximately 1 cm in diameter ("enterobiomas") may form. Histologically a necrotic center, occasionally still containing worm remnants, is surrounded by a granulomatous and connective tissue rim.

Treatment is with

*Mebendazole* 100 mg p.o. once

or

*Albendazole* 400 mg p.o. once.

After 1–2 weeks treatment is repeated in order to avoid relapse. Alternatively

*Pyrantel pamoate* 11 mg/kg p.o. once (maximal dose 1 g)

may be given. Cure rates are approximately 100%.

## Strongyloidiasis

*Strongyloides stercoralis* primarily infects immunosuppressed persons with malnutrition, long-term corticosteroid therapy, antineoplastic chemotherapy or HTLV-1 or

HIV infection. Within the liver the worms may lodge in the small portal vessels and the sinusoids without eliciting an appreciable inflammatory tissue reaction. However, a chronic inflammatory infiltrate with giant cells and some eosinophils may also occasionally be present.

Treatment is with

*Thiabendazole* 25 mg/kg p.o. bid for 3–5 days

or

*Ivermectin* 200 µg/kg p.o. qd for 1–2 days.

Both drugs achieve cure rates of nearly 100%, but ivermectin is better tolerated than thiabendazole.

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# Fungal Infections

# 68

Henryk Dancygier

## Chapter Outline



Fungal infections of the liver predominantly occur in severely immunocompromised patients. With the exception of Candida and Mucor species all potentially hepatotoxic fungi are inhaled and affect the liver within the context of hematogeneous dissemination. Special stains, such as Grocott's hexamine silver or PAS, are helpful in demonstrating fungi within the liver tissue. Cultures of blood and liver tissue, and to a lesser degree serological and immunocytochemical investigations, complete the diagnosis.

## Candidiasis

*Candida albicans* is the most common pathogen. Infections of the liver are seen primarily in the newborn and in neutropenic patients. Liver disease is due to direct fungal invasion with the formation of granulomas and, most importantly, liver abscesses.

The diagnosis of *hepatic candidiasis* is often difficult to make because of a nonspecific clinical presentation. Fever of unknown origin unresponsive to antibiotics associated with elevated levels of serum alkaline phosphatase in an immunocompromised patient should raise the possibility of an invasive fungal infection of the liver.

*Hepatosplenic candidiasis* (chronic disseminated candidiasis) is a characteristic syndrome that in more than 90% of cases affects patients with acute leukemia and aplastic anemia. In approximately 25% of patients the liver is the only organ involved, in 15% solely the spleen, and in the remaining cases both organs are affected concomitantly. *Histologically,* multiple granulomas with abscess formation are found. They heal by scar formation. The *clinical picture* of the severely ill patients is determined by the underlying disease,

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and not by the involvement of the liver. Hepatosplenic candidiasis should be considered if, despite normalization of leukocyte numbers in previously neutropenic patients high fever persists, patients complain of right upper quadrant pain, and focal lesions are seen on computed tomography (hypodense areas) or ultrasound (target-like focal lesions) [7]. Serum levels of alkaline phosphatase are elevated in all patients. *Diagnosis* is confirmed by demonstrating fungi in liver biopsy. Mortality reaches 40–50%. Not uncommonly the changes in the liver are incidental findings at autopsy.

*Biliary tract candidiasis* may lead to bile duct obstruction. Mycelia in the bile duct system may be observed endoscopically. Aspiration of bile during ERCP and subsequent microbiological analysis in combination with cholangiographic findings help establish the diagnosis [2]. Treatment includes endoscopic therapy, such as bile duct drainage, lavage, or debridement combined with systemic

*Fluconazole* 200–400 mg i.v. qd.

Long-term prognosis, however, depends primarily on the successful treatment of the underlying disease.

## Aspergillosis

The liver is involved in approximately 20% of cases of disseminated infections with *Aspergillus fumigatus* and *A. flavus*. Thrombotic occlusion of vessels by fungi causes hemorrhagic-necrotic foci, similar to those seen in mucormycosis.

## Mucormycosis (Phycomycosis; Zygomycosis)

Hyphae of various *Mucor* species may occlude vessels, and lead to ischemic necrosis of affected organs. In the liver multiple necrotic nodules may develop. *Histologically*, hyphae are found within the necrotic foci.

## Cryptococcosis

Hepatobiliary infection with *Cryptococcus neoformans* is primarily seen in AIDS patients with advanced immunodeficiency. Occasionally focal parenchymal necrosis or epithelioid cell granulomas with giant cells containing fungi are observed in the liver. AIDS cholangiopathy caused by cryptococci is a sclerosing cholangitis [1].

## Histoplasmosis

*Histoplasma capsulatum* occurs worldwide, *Histoplasma duboisii* is present only in Africa. Initial infection is in the lungs and may spread hematogeneously. Histoplasma, analogous to tuberculosis bacteria, may remain latent in the body for decades after the primary infection, and become reactivated once the host's immune defenses are diminished. Two thirds of the patients with disseminated histoplasmosis have a hepatomegaly, often combined with a splenomegaly [3, 6]. Numerous tuberculoid granulomas are found in the liver. Occasionally they may coalesce to form large "histoplasmomas" and calcify. Infiltrating and resident macrophages contain histoplasms, whose microscopic demonstration may be difficult, because of their small size. *Clinically*, involvement of the liver may be asymptomatic, may manifest as granulomatous hepatitis with mild increases in serum levels of aminotransferases, or present a differential diagnostic challenge as cholestatic hepatitis of unknown origin [4].

## Coccidioidomycosis, Blastomycosis, and Paracoccidiomycosis

*Coccidioides immitis*, *Blastomyces dermatidis* (North American Blastomycosis) and *Paracoccidioides brasiliensis* (South American Blastomycosis) infect primarily the lungs. The liver becomes occasionally involved within the context of hematogeneous dissemination. Microscopically the inflammatory response may be granulomatous, pyogenic or mixed as suppurative

granulomas. Purulent lesions dominate in patients with diminished resistance.

Involvement of the liver may be clinically asymptomatic or lead to hepatomegaly with initially slightly elevated aminotransferases followed in time by an increase in alkaline phosphatase and bilirubin levels in serum. Diagnosis is made by demonstrating the organisms in liver biopsy.

## Penicilliosis

*Penicillium marneffei* is endemic in South East Asia and is a common cause of opportunistic infections in immunocompromised patients. As a result of international travel, penicillium marneffei infection may be occasionally imported to Europe or the United States. Clinically penicilliosis resembles miliary tuberculosis with infectious foci present in the bone marrow, spleen, lungs and liver. The infection may also primarily involve the liver, and the patients present with fever of short duration, hepatomegaly, and markedly elevated serum alkaline phosphatase levels. Microscopically, hepatic granulomas are seen and organisms are present within macrophages. The diagnosis is confirmed by demonstrating the causative agent in the liver or in the blood [5].

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# Infections

# 69

Henryk Dancygier

## Chapter Outline



## HIV Infection

HIV itself may be taken up by liver macrophages and sinusoidal endothelial cells which express CD4 surface receptors. To date, no specific histopathological findings in the liver has been associated with HIV infection. The histopathological spectrum includes nonspecific lobular inflammation, Kupffer cell hyperplasia (probably due to HIV itself) with secondary hemosiderosis involving Kupffer and sinusoidal endothelial cells, occasionally scattered non-necrotizing granulomas, and a variable degree of steatosis [42, 44]. These findings are usually modified by co- or superinfection with other viruses, opportunistic pathogens, drug induced changes and neoplastic disease.

Phagocytosis of hemopoietic cells by Kupffer cells occurs in many livers of patients with AIDS. A *hemophagocytic syndrome* (not specific for AIDS patients) should be suspected in immunodeficient patients with fever, jaundice, and hepatosplenomegaly. Hepatic lesions are characterized by nonspecific sinusoidal dilatation with hemophagocytic histiocytosis [18, 68].

Recently *hepatoportal sclerosis* has been linked to HIV infection, adding HIV to the many etiologies of noncirrhotic portal hypertension (see Chapter 53). Thus, hepatoportal sclerosis should be considered in the differential diagnosis of HIV patients presenting with variceal bleeding [53].

## Viral Hepatitis

End stage liver disease (predominantly due to chronic hepatitis B and C) is becoming a leading cause of death among people infected with HIV worldwide. The risk of death related to liver disease is inversely related to

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the CD4 cell count [30, 51]. Therefore, persons with HIV infection should be vaccinated against both hepatitis A and B virus, although antibody titers after vaccination are lower and less durable than in HIV negative persons [30].

## Hepatitis A

The clinical course of viral hepatitis A in early HIV-infection, when CD4 cell counts are still normal, is comparable to that in HIV-negative persons, and complete resolution is the rule. However, with increasing immunodeficiency hepatitis A virus (HAV) infection poses a potentially serious health risk in HIV-infected persons. HIV patients with advanced immunocompromise have a prolonged course of hepatitis A with longer duration of HAV viremia, and persistent HAV viremia even after clinical symptoms have disappeared and ALT levels have returned to normal [17, 28]. HAV infection may be associated with a high case-fatality rate in severely immunocompromised patients with high HIV viremia and coinfections with other viruses (e.g. HCV), such as is commonly the case, for example, in intravenous drug users [59].

While the inactivated HAV vaccine affords protection to immunocompetent persons >95% of the time, HIV-positive persons develop protective anti-HAV antibodies at a considerably lower rate (40–50%) [38]. CD4 T cell count and HIV viremia are the major determinants of the response to HAV vaccine [38, 70].

## Hepatitis B

Viral hepatitis B is more prevalent in men having sex with men, intravenous drug users and recipients of blood products. Occult HBV infection appears to be more common in HIV-infected patients than in the general population, and approximately 90% of HIV-infected persons have serologic markers of past HBV infection, and might therefore represent a reservoir of HBV infection [9].

In HIV-infected patients who are still for the most part immunocompetent, the course of acute HBV infection is comparable to that in HIV negative individuals. With advancing immunodeficiency, however, HIV-infected patients become less and less able to mount an adequate immune response to HBV and to eliminate HBV [26]. This fact explains why prior to the introduction of highly active antiretroviral therapy (HAART) HBV/HIV-coinfections were often ignored. Despite ongoing active HBV replication hepatitis was not apparent in these patients due to the absence of immunologically mediated liver injury. Upon restoration of immune reactivity with HAART, HBV infection became clinically apparent. Chronic hepatitis B in HIV-positive men having sex with men is associated with higher levels of HBV replication and a higher risk of progression of liver disease to cirrhosis than infection with HBV only and may have important ramifications on choice of antiviral medications [16].

The optimal treatment of chronic hepatitis B in HIV-infected persons has not been established, although general consensus statements have been published [1, 64]. The nucleos(t)ide analogs lamivudine, adefovir, entecavir, tenofovir, and emtricitabine maintain their viral suppressive activity on HBV-DNA also in HBV/HIC coinfected patients. When treating HBV/HIV coinfected patients combination therapies containing antiviral agents active against both viruses should be used or a nucleos(t)ide analog active against HBV should be added to effective antiretroviral combination therapy. Tenofovir, emtricitabine and adefovir are active against both HIV and HBV. These drugs result in clinically important suppression of serum HBV-DNA and should be used as a part of anti-retroviral therapy in HBV/HIV-coinfected patients (see also Chapter 63.3) [6, 41]. *Therapy of HBV with only one anitiviral agent in HBV/HIV coinfected patients should not be performed because of the emergence of drug resistant HIV*.

Patients with chronic hepatitis B and HIV infection with CD4 T cell counts ≥ 400/μL are treated like HIV-negative patients (for drugs and dosage see Section 63.3). All nucleos(t)ides mentioned above lead to a marked decline in HBV-DNA levels in 90–100% of coinfected patients. Withdrawal of therapy or the development of resistance (for example lamivudine resistance due to YMDD mutation) may lead to exacerbations of HBV infection in 17% and to acute liver failure in 5% of patients [3]. The incidence of YMDD mutation in HBV/HIV coinfected patients is approximately 20% per year [5].

The efficacy of interferon is limited in HBV/HIV coinfected patients. However, if treatment is begun early, i.e. before overt immunodeficiency is manifest, interferon

may be given. Coinfected patients with advanced immunodeficiency have lower response rates to interferon, higher rates of HBV reactivation after treatment, increased progression to cirrhosis and increased cirrhosis related mortality than HBV monoinfected patients [19]. There are no published data regarding the efficacy of pegylated interferon in HBV/HIV coinfected subjects.

Standard vaccination protocols are less effective among HIV-infected persons than in the general population, particularly in patients with low CD4 T cell counts and high HIV-RNA levels [39]. However, HIV-infected patients with CD4 T cell counts ≥ 400/μL may be vaccinated successfully against hepatitis B. With lower CD4 T cell counts, doubling the dose of vaccine antigen may yield satisfactory results.

## *Hepatitis D*

Data on hepatitis D virus (HDV)/HIV coinfection are scant. HDV infection does not seem to affect clinical, virological, or immunologic responses to HAART in patients with HBV/HDV/HIV coinfection. However, HDV infection increases the risk of hepatitis flares, liver cirrhosis, hepatic decompensation, and death due to liver disease in patients with HBV/HIV coinfection [54].

Long-term efficacy and toxicity of interferon treatment do not seem to be different in HIV-infected and HIV-uninfected patients with delta hepatitis; given the overall poor rate of long-term response, interferon treatment should be considered only in immunocompetent HIV/HDV-coinfected patients [46]. Recently, lack of anti-HIV activity of entecavir in an HIV patient coinfected with hepatitis B and delta viruses was reported [58].

## *Hepatitis C*

Nearly 40% of HIV-infected persons in the United States are coinfected with hepatitis C virus (HCV). In comparison only approximately 2% of the general HIV-negative population show serological evidence of HCV infection [10, 56, 65]. HCV/HIV-coinfected pregnant women transmit HCV to the newborn at a rate two times that seen in HIV negative mothers. Coinfection also increases sexual transmission rates of HCV to 3%.

### Effects of HIV Infection on Chronic Hepatitis C

Infection with HIV appears to adversely affect the outcome of hepatitis C, leading to increased viral persistence after acute infection, higher levels of viremia, accelerated progression of HCV-related liver disease, and higher mortality rates than HCV monoinfection [35, 63]. End-stage liver disease is the primary cause of death in HIV/HCV-coinfected patients under HAART [43]. The interaction between HIV and HCV in the liver is not completely understood. Alteration of intrahepatic HCV-specific IL-10 cytokine response and reduction in HCV specific CD4 T cells might provide a cellular mechanism for the loss of control of HCV in coinfected individuals [24, 27].

Steatosis is more common and more severe in HIV/HCV coinfected patients than in those with HCV monoinfection [23]. Steatosis and steatohepatitis were present in 23% and 30%, respectively, of patients with HIV/HCV coinfection, and both were associated with an increased risk of having advanced fibrosis. Genotype 3, increased body mass index, and diabetes mellitus were identified as independent risk factors [60]. Steatosis does not seem to affect efficacy of treatment in any HCV genotype/HIV coinfected patient, but successful anti-HCV therapy with viral eradication was reported to reduce steatosis in genotype 3 patients [48]. Progression of steatosis and fibrosis to cirrhosis in HCV/HIV-coinfected patients appears to be particularly accelerated if cofactors, such as a significant consumption of alcohol supervenes [4, 7]. On the other hand successful HAART combined with effective treatment of HCV infection with pegylated interferon/ribavirin may slow the rate of fibrosis progression [11, 49].

Hepatic fibrosis in HIV/HCV coinfected patients may be assessed by noninvasive tests based on simple serum parameters, such as albumin, aspartate aminotransferase, platelet count, INR and hyaluronic acid [12, 29, 34, 61]. Although these tests have been reported to accurately distinguish mild from advanced fibrosis, liver biopsy still remains the gold standard in determining various stages of hepatic fibrosis.

*Chronic HCV infection* in HCV/HIV coinfected persons is treated with pegylated interferon and ribavirin the same way as HCV monoinfection (for details see Section 63.3), despite the fact that medical management of hepatitis C in HIV-infected persons is complicated by immune suppression, potential drug interactions and toxicities, and other forms of liver disease [14, 40, 52,

63, 73]. The combination of pegylated interferon and ribavirin is more effective than interferon and ribavirin. Amantadine addition and interferon intensification do not improve the response rates [47].

Treatment ideally should begin with CD4 T cell count ≥ 400/μL and low HIV-viremia, i.e. in an early stage of HIV infection or after immune restoration under HAART. At the onset of HAART (usually during the first 3 months of therapy) a paradoxical increase in HCV viremia may occur, accompanied by a rise in serum levels of aminotransferases. This effect is most pronounced among patients with low CD4 T cell counts. The mechanism for this rise is unclear, but it must not be misinterpreted as a sign of drug toxicity which then erroneously might lead to withdrawal of effective antiretroviral therapy [50, 55]. Aminotransferase levels usually normalize within a few weeks.

Although coinfection status does not affect key HCV kinetic parameters under treatment and, as in HCV monoinfection, efficiency is associated significantly with early viral clearance, the sustained viral response rates (SVR) in coinfected patients are lower than in HCV monoinfection. The overall SVR range between 27% and 44%, those for HCV genotype 1 are 14–38%, and for non-genotype 1 44–73% [13, 15, 32, 36, 57, 67]. Thus, overall, therapy provides cure to approximately one third of patients, a rate significantly lower than that seen in HCV-monoinfected individuals and relapses are common [36]. When treating HCV/HIV coinfected patients one should also be aware of new side-effects of HCV treatment, such as pancreatitis and severe weight loss, that may result from the interaction of ribavirin with antiretroviral drugs [31].

As stated above, HIV coinfection reduces considerably the survival of patients with HCV-related end stage liver disease. Therefore, adequate timing of liver transplantation in HIV-coinfected subjects must be assured [43].

Early treatment of *acute hepatitis C* in HIV-positive individuals with pegylated interferon monotherapy leads to high rates (61%) of SVR, albeit lower than in HIV negative patients [69].

#### Effects of Chronic Hepatitis C on HIV Infection

Reports regarding the impact of HCV infection on the progression of HIV disease are conflicting. Some data suggest that HCV seropositivity in HCV/HIV-infected patients is associated with an accelerated progression to AIDS compared to those infected with HIV only [25]. On the other hand, a study among patients in an urban US cohort showed no evidence that HCV infection substantially alters the risk of dying, developing AIDS, or responding immunologically to HAART [62]. In a cohort-study of hospitalized veterans, coinfection with hepatitis C was even associated with a significant decrease in the mortality of HIV-infected patients, although this effect was less pronounced during the HAART era [21].

#### Diseases of other Systems in HCV/HIV Coinfected Patients

The prevalence of *cardiovascular disease* and of *depressive symptoms* (especially in those with a history of alcohol problems) seems to be elevated in HCV/HIV coinfected persons compared to those without HCV. The mechanisms for this association are unclear but it has been suggested that hepatitis C may be independently associated with increased risk of cardiovascular disease among those coinfected with HIV [22, 33].

### *Hepatitis E*

Data on HEV/HIV coinfection are very scant. A study of anti-HEV antibodies in 145 HIV-1 infected subjects found that 14.4% also had anti-HEV antibodies [37]. In Russia, 13 out of 117 HIV-infected patients (11.1%) were found to be anti-HEV seropositive (compared to a frequency of anti-HEV of 1.7% in the normal population). The rate of anti-HEV seropositivity increased with the progression of HIV infection, reaching 43.3% in AIDS patients [2]. Data on the interaction of HEV and HIV are not available.

### *Hepatitis G*

Hepatitis G virus (GB virus C; GBV-C) is related to HCV, however, it does not appear to cause liver disease. 40–75% of HIV-infected persons show serologic evidence of GBV-C infection. In patients with concurrent GBV-C/HIV coinfection progression of HIV-disease

appears to be slower and is associated with prolonged survival as compared to HIV monoinfection [66, 71, 72]. The mechanisms of the interaction between GBV-C and HIV are unclear. Possibly GBV-C inhibits HIV replication or it is a surrogate marker for other, poorly understood factors that have a favorable impact on HIV infection.

GBV-C has no effect on the course of liver disease in HCV monoinfection. Interestingly in HCV/HIV-coinfected patients, GBV-C RNA was associated with a significant reduction in the severity of HCV-related liver disease [8].

## Opportunistic Infections

Life threatening opportunistic infections in HIV-infected patients, including those affecting the hepatobiliary tract, usually develop with advanced immunodeficiency and CD4 T cell counts <50–100/μL [45]. They were regularly seen before effective antiretroviral therapies were available, and have become rare in the HAART era.

*Mycobacterium avium intracellulare* infection occurs in approximately 50% of patients in the late stages of AIDS. Ill defined granulomas of foamy histiocytes containing acid fast rods may be present in the liver (see Fig. 65.3).

*Extrapulmonary tuberculosis* occurs in 25–75% of HIV-infected persons. The liver usually is involved within the context of miliary dissemination. Multiple small granulomas, and, if immunocompromise is far advanced, multiple areactive parenchymal necroses are scattered throughout the liver. Furthermore, tuberculous abscesses and bile duct tuberculomas may form.

*Extrapulmonary pneumocystis carinii (jiroveci)* infection may be seen in patients treated with inhalational pentamidine. A pneumocystis jiroveci hepatitis is extremely rare.

*Bacillary peliosis hepatis* is caused by *Bartonella henselae* and *B. quintana* (see Chapter 65). Peliosis may simulate hepatic mass lesions on imaging. Long-term treatment with erythromycin or with cephalosporins is usually effective.

Five to 25% of HIV patients with elevated liver enzymes have evidence of *cytomegalovirus infection*. On histological examination sinusoidal mononuclear cell infiltrates are present. Well defined granulomas are rare. Occasionally typical viral inclusion bodies are seen in Kupffer cells, hepatocytes or endothelial cells. CMV itself is difficult to demonstrate in liver biopsy, but CMV antigens may be visualized by immunocytochemistry or by in situ-hybridization.

*Hepatic histoplasmosis* may occur in patients with pulmonary histoplasmosis. The fungi are present within liver macrophages, which are arranged in granuloma-like clusters.

Rarely, *Cryptococcus neoformans* may be encountered in the sinusoids.

In high incidence areas the liver may be involved within the context of general *coccidioidomycosis*. The organisms appear as small spherules in fibrosed granulomas.

Compared to mucosal candidiasis, *hepatobiliary candidiasis* is rare. In HIV-infected neutropenic patients with malignant lymphomas micro- and macroabscesses caused by candida species may occur after chemotherapy.

Biliary tract candidiasis may mimic sclerosing cholangitis (see Chapter 68) [20].

*Microsporidia, cryptosporidia* and CMV may infect the bile ducts and cause a chronic sclerosing cholangitis (*AIDS-cholangiopathy*) that on ERCP or MRCP is indistiguishable from primary sclerosing cholangitis.

CMV, microsporidia, cryptosporidia and *Isospora belli* may be rare causes of *acalculous cholecystitis*.

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# Neoplastic Diseases

# 70

Henryk Dancygier

## Chapter Outline



## Hepatocellular Carcinoma

HIV infected patients are often coinfected with HBV and HCV. The course of chronic viral hepatitis is accelerated in coinfected patients with an increased rate of progression to cirrhosis, decompensated liver disease, hepatocellular carcinoma (HCC), and death [6, 8, 9]. HIV-positive HCC patients are younger and more frequently symptomatic than HIV-negative patients. Survival rates are similar, but high HIV viremia is associated with a worse prognosis [1].

The multiple virus–virus and virus–host interactions that underlie viral hepatocarcinogenesis are poorly understood and the exact risk of HCC in HIV and HBV and/or HCV coinfected patients remains to be fully assessed. It is controversial whether the prevalence of HCC in coinfected patients is different in the pre-HAART and the current HAART era [4, 7]. In a large cohort study HCV coinfection dramatically promoted the development of cirrhosis (10- to 20-fold) and of HCC (fivefold) [4]. To reduce the incidence of HCC in coinfected patients more effective treatment of chronic HBV and HCV infections is needed [4, 5]. Coinfected patients with cirrhosis should be screened at 6-month intervals using ultrasonography and measurement of alpha-fetoprotein levels.

Treatment options for HCC are the same as in HIV negative patients (see Chapters 102 and103) [2].

## Kaposi's Sarcoma

The causative agent of Kaposi's sarcoma (KS) is human herpesvirus 8. In the pre-HAART era KS occurred in 10–15% of AIDS patients [3]. The tumor may involve the entire gastrointestinal tract and the liver, where it

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forms dark red, violaceous nodules lying within an otherwise unremarkable hepatic parenchyma. The gallbladder and bile ducts may also be affected.

Microscopically a granulation tissue-like aspect is observed with proliferating endothelial and spindle-shaped fibroblast-like cells and with slit-like vascular clefts within the stroma.

Predominant involvement of the liver is clinically rare. Aminotransferase levels may be elevated and jaundice may be present.

## Malignant Lymphomas

Malignant lymphomas in AIDS patients usually derive from B cells. Hepatic involvement within the context of a primary extranodal Non-Hogkin's lymphoma is more common than primary hepatic lymphoma (see Chapter 102).

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# Drug-Induced Liver Injury

# 71

Henryk Dancygier

## Chapter Outline



Drug-induced liver injury should be considered in every HIV-infected patient presenting with elevated liver enzymes. It is axiomatic that all potentially hepatotoxic drugs in HIV-negative patients may also cause liver injury in HIV-infected persons (see Chapter 93). Due to their frequent use in HIV-infected patients antibiotics, antituberculous and antifungal drugs have to be especially considered. Among the antibacterial agents, sulfonamides rank first. On liver biopsy, noncaseating granulomas containing eosinophils are found. Over the counter substances and herbal drugs are commonly consumed by HIV-infected patients, often without the physician's knowledge. Occasionally these xenobiotics may also be hepatotoxic.

The development of highly active antiretroviral therapy (HAART) has led to a pattern of liver injury (antiretroviral-associated liver disease) quite characteristic for HIV-patients [7, 10]. The relative frequency of severe hepatotoxic events during HAART is reported to be approximately 6% for the nucleosidic reverse transcriptase inhibitors (NRTIs), 8–15% for the non-nucleosidic reverse transcriptase inhibitors (NNRTIs), and 3.5–18% for the protease inhibitors (PIs). Withdrawal of HAART because of hepatotoxic side effects is required in approximately 9% of cases, while death due to drug-induced injury is reported to occur in up to 2.5% of cases [5, 6].

HAART and HCV infection appear to act synergistically in HIV-infected patients to increase the risk of fulminant hepatic failure, which has been reported to be several fold higher during the HAART era than prior to the introduction of HAART [3]. Furthermore, non-alcoholic fatty liver disease, as can be induced by HAART, may predispose to acute on chronic liver failure when these patients are subjected to acute insults such as hepatitis viruses and other drugs [2].

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## Nucleosidic Reverse Transcriptase Inhibitors

Potentially hepatotoxic drugs belonging to this group include zidovudine, lamivudine, stavudine, abacavir, zalcitabine and didanosine. Most NRTIs are directly hepatotoxic by interfering with hepatic energy metabolism. In addition, abacavir is believed to cause liver injury by an idiosyncratic mechanism.

NRTIs do not interact exclusively with viral DNA-polymerase (reverse transcriptase), but also inhibit and deplete mitochondrial DNA-polymerase γ, thereby impairing oxidative phosphorylation, cellular ATP-synthesis and fatty acid β-oxidation. Pronounced decreases in hepatic mitochondrial DNA and impairment of energy metabolism lead to hyperlactatemia and steatosis [8, 11]. The hepatotoxic spectrum encompasses mild increases in serum aminotransferase levels, pronounced parenchymal injury with steatosis, steatohepatitis, cholestasis, ranging clinically from mild hepatic dysfunction to fulminant liver failure.

NRTI induced liver injury may occur within the context of a *lipodystrophy syndrome*, characterized by weight loss, peripheral lipoatrophy, central adiposity, "buffalo hump", hyperlipidemia, lactic acidemia, insulin resistance, and hepatic dysfunction [1]. Possibly, pregnant women have a higher risk of developing lactic acidosis during NRTI therapy.

The interaction of ribavirin and NRTIs (competing for phosphorylation) in HCV/HIV-coinfected patients might, at least theoretically lead to a diminished antiviral efficacy of NRTIs. Mitochondrial damage and lactic acidosis may occur in isolated cases of ribavirin treated HIV-patients [4].

## Non-nucleosidic Reverse Transcriptase Inhibitors

NNRTIs may lead to dose-independent, idiosyncratic liver damage. Possibly, the function of mitochondrial polymerase γ is also impaired by NNRTIs. In addition to lactic acidosis, steatosis and steatohepatitis occur. Among the NNRTIs associated with the highest hepatotoxic potential is nevirapine. It may lead to severe liver injury and it is the antiretroviral drug most commonly associated with treatment withdrawal, due to liver damage. Typically, 4–6 weeks after the onset of therapy serum levels of aminotransferases begin to rise, the patient complains of nausea, fatigue and abdominal pain, occasionally accompanied by an allergic skin rash, fever and arthralgias.

## Protease Inhibitors

PIs are the most common antiretroviral drugs associated with hepatotoxicity. However, withdrawal rates and death rates are lower than with NRTIs. Ritonavir, indinavir, saquinavir, and nelfinavir are all potentially hepatotoxic. Although hepatotoxicity may be more common in persons with chronic viral hepatitis, evidence does not support withholding protease inhibitor therapy from persons coinfected with hepatitis B or C virus [9]. PIs induce hepatotoxicity by an indirect mechanism, i.e. by the accumulation of potentially toxic metabolites. Competitive inhibition of UDP-glucuronyl transferase with consequent elevation of indirect bilirubin in serum occurs. Up to 40% of all patients treated with indinavir develop mild to marked hyperbilirubinemia. In addition, lipodystrophy syndrome (see above) may complicate therapy with antiretroviral regimens containing PIs.

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# Autoimmune Hepatitis

# 72

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## Chapter Outline



In 1950 Waldenström was the first to describe a chronic form of hepatitis in young women that was associated with jaundice, hypergammaglobulinemia, and amenorrhea, and led to cirrhosis [77]. The association with Lupus erythematosus cells in the blood and with other autoimmune syndromes led to the term "lupoid hepatitis". In 1965 Mackay et al. coined the term "autoimmune hepatitis" [50].

## Definition

Autoimmune hepatitis (AIH) is a chronic (> 6 months) progressive inflammation of unknown origin, that most commonly is characterized histologically by an interface hepatitis, biochemically by a marked hypergammaglobulinemia and high titers of autoantibodies in serum. The definitive diagnosis requires the exclusion of metabolic, genetic, viral, drug-induce and purely cholestatic liver disease.

## Epidemiology

AIH is distributed worldwide and makes up for approximately 15–20% of all cases of chronic hepatitis in Western Europe and in North America. The incidence in Central Europe is 0.1–1 per $10^5$ persons per year, and 1.9 per $10^5$ persons per year in North America. The prevalence in Western Europe and in North America ranges between 3 and 17 per $10^5$ persons, with the lowest figures found in southern regions of both continents. Females are affected 4–5 times (in children up to 9 times) more often than males. AIH occurs at all ages

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with a bimodal age distribution peaking at the ages of 10–20 and 45–70 years. Thus, initial presentation in older patients is not rare [2, 52, 54]. 2.6% of all liver transplants in Europe and 5.9% in the United States are performed because of AIH.

## Etiology and Pathogenesis

Etiology and pathogenesis of AIH are unknown. Immunologic reactions based on a genetic predisposition are among the etiopathogenetic factors most intensely discussed [14, 15, 55].

### *Immunologic Factors*

The pathophysiological hypotheses encompass viral induced immunologic reactions and a dysregulation of the cellular and humoral immune system with loss of self-tolerance and impaired recognition of foreign antigens.

Anecdotal reports postulate a relationship between AIH and viral infections (hepatitis A, B and C, Epstein-Barr virus, herpes simplex virus) assuming molecular mimicry and immunologic cross reactions [10, 53, 56]. Even if the exact triggering mechanisms are unknown it is clear that *liver injury in AIH is due to an immunologically-mediated inflammatory process*. Different pathophysiologic mechanisms may be operating at different times in this chronic disease. Livers from patients with AIH contain antigen sensitive, disease specific cytotoxic T lymphocytes that may damage and kill hepatocytes with the help of cytokines (IL-2, IL-12, TNF-α). The molecular target antigens of cytotoxic T lymphocytes are not known [48]. Furthermore, regulatory T cells decreased in number and with diminished ability to expand may favor the emergence of liver-targeted autoimmunity [49]. In addition to T cell mediated cytotoxicity, antibody dependent cellular cytotoxicity (ADCC) against antigen-antibody complexes on the surface of hepatocytes was demonstrated to occur in patients with AIH. The immune complexes bind to Fc-receptors of natural killer cells that subsequently initiate cytolysis. IL-4 and IL-10 are the most important mediators of this immune reaction (see Chapter 18). Recently it has been demonstrated that hepatocyte membrane autoantigens (liver arginase, cytokeratins 8 and 18, heat shock proteins 70 and 90, valosin-containing protein) may represent candidate targets for autoantibodies in AIH type I [71].

The diagnostically defining serum antibodies against nuclei and smooth muscle (ANA and SMA) are not disease specific and not pathogenic. The antibodies that characterize AIH type II (directed against cytochrome IID6; anti-LKM1) might possibly represent an exception in this regard. Anti-LKM1 antibodies recognize CYP2D6 expressed on the membrane of isolated hepatocytes and have been shown to inhibit enzyme activity [51, 59].

### *Genetic Factors*

AIH type I is a polygenic disorder with a strong genetic predisposition [19]. The exact genetic risk factors that affect occurrence, clinical phenotype, severity, and outcome are still being investigated. Susceptibility is associated in northern Europeans and in white North Americans with the *DRB1* gene. *DRB1*0301* is the most important susceptibility allele, *DRB1*0401* is a secondary but independent risk factor in AIH type I. It is assumed that the allelic configuration affects antigenic presentation by class II molecules of the major histocompatibility complex on CD4 helper T cells thereby modifying intensity of the immune response [15, 27, 28, 57]. Eighty-five percent of patients with AIH type I have *DRB1*0301, DRB1*0401* or both alleles. The alleles bear also prognostic relevance. Patients with *DRB1*0301* have a worse prognosis than those with *DRB1*0401*, their response rates to corticosteroids being lower and relapse rates after withdrawal of therapy being higher. Disease severity is associated with the number of alleles encoding lysine at DRβ71 (gene dose) and the number of polymorphisms, including those of the tumor necrosis factor-α gene, cytotoxic T lymphocyte antigen-4 gene, and tumor necrosis factor–receptor superfamily gene [15]. In contrast *DRB1*1501* seems to confer protection from the development of AIH type I in whites [21]. Polymorphisms of the gene encoding Fas might also contribute to the development of AIH [36].

The distribution of susceptibility alleles in AIH type I exhibits geographic and ethnic variation, and not all patients with the same susceptibility alleles show an identical clinical phenotype. Already the substitution

of one amino acid by another alters the susceptibility, thus leading, for example, to an earlier onset of disease, to changes of immune response and to a reduced response to therapy. The first genome-wide scan of Japanese AIH patients revealed at least 26 candidate AIH susceptibility or resistance regions other than HLA class II loci, suggesting that the products of several genes interact to determine heritable susceptibility to AIH [82].

Gentic factors in AIH type II are not so well established as in AIH type I. *DRB1*07* seems to be a risk factor for the development of AIH type II in German patients [54].

## Pathology

The histologic aspect of AIH presents some characteristic features, although the microscopic changes are not pathognomonic. *The spectrum of histologic lesions in AIH ranges from acute to chronic hepatitis with varying degrees of activity to cirrhosis* [25]. The classical histological finding in AIH is lymphocytic infiltration of the portal triads and periportal zone with periportal hepatocyte necrosis (see Figs. 25.3 and 72.1). Usually there are only a few plasma cells intermingled. Thus, the term "plasma cell hepatitis" that was occasionally applied to AIH is misleading and should not be used anymore. The necroinflammatory process involves primarily the interface between the portal tracts and the lobular parenchyma (interface hepatitis) leading to the death of zone 1 hepatocytes. Groups of necrotic hepatocytes occasionally may coalesce to form more extensive areas of parenchymal collapse. Liver cell rosettes are quite characteristic (but not pathognomonic) for AIH and represent hepatocyte regeneration (see Fig. 25.4; see Chapters 25 and 26).

Depending on the severity of the disease, inflammatory cell infiltrates consisting primarily of lymphocytes accompanied by activated Kupffer cells are not confined to the portal tracts and the periportal areas but may also be found within the lobules. Hepatocyte lesions range from ballooning degeneration to lytic cell necrosis. Lytic group necroses of liver cells result in confluent collapse of the reticulin fiber network.

AIH with only minor portal changes but with marked centrilobular liver cell necrosis as well as anecdotal cases with numerous eosinophils within the inflammatory cell infiltrate have also been documented [62, 68, 72]. Bile duct lesions, signs of cholestatis, deposits of copper and iron and granulomas are not a feature of classic AIH, and according to some authors they even represent histologic exclusion criteria. These changes rather suggest the presence of an autoimmune bile duct disease or of an overlap syndrome, and should be further differentiated by biochemical tests [8, 25, 26].

## Diagnosis

There are no single pathognomonic features that allow for the diagnosis of AIH. The definititive diagnosis of AIH is a diagnosis of exclusion. Hereditary (Wilson's disease, hereditary hemochromatosis, $\alpha_1$-antitrypsin deficiency), viral (hepatitis A, B and C), drug-induced (minocyclin, isoniazide, α-methlydopa, nitrofurantoin) and cholestatic liver disease must be excluded [4].

Since symptoms and signs are nonspecific the diagnosis of AIH may be objectified by applying a numerical *scoring system* that includes typical features of the disease as well as less characteristic criteria. The response to therapy with corticosteroids is also included (Table 72.1) [5, 40]. However, the application of this scoring system in daily clinical practice is quite cumbersome. Therefore, *simplified diagnostic criteria*, based on the following four major criteria

- Hypergammaglobulinemia with selective or predominant increase in IgG
- Presence of autoantibodies (ANA, SMA, anti-LKM, anti-SLA/LP)
- Compatible histological features
- Exclusion of viral hepatitis, drug-induced, genetic and metabolic liver disease

may be used as a first approach to a patient with suspected AIH. If all four criteria are present in a female patient the diagnosis of AIH may be regarded as definite, and if only three criteria are met then diagnosis is probable (see also Table 29B) [7, 34a].

The determination of HLA class genes plays no role in the clinical diagnosis of AIH.

Traditionally three subtypes of AIH have been distinguished based on the clinical presentation and the autoantibody profile (Table 72.2) [40]. Type I and II differ in epidemiology, while type III clinically

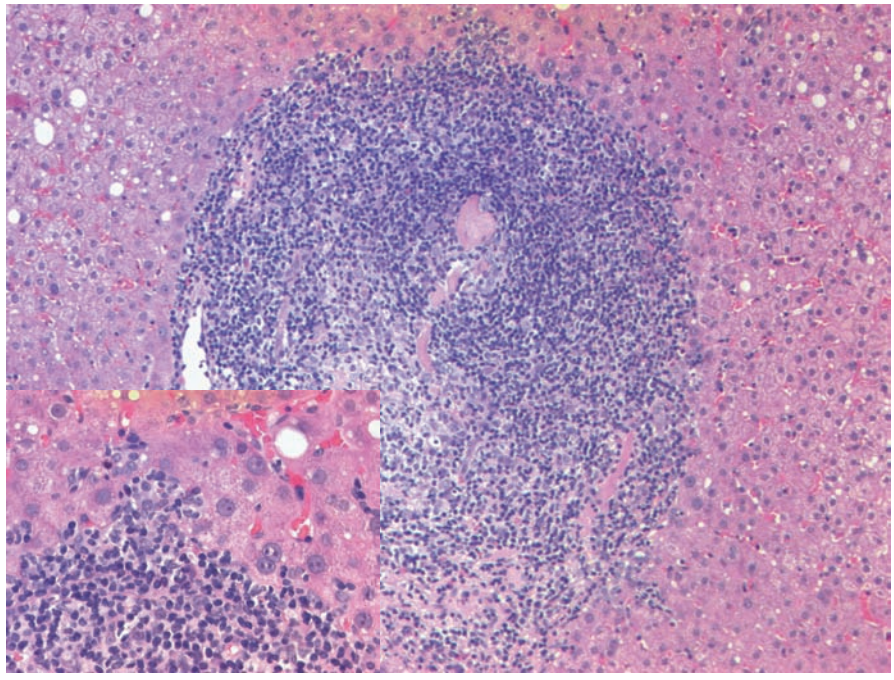


**Fig. 72.1** Autoimmune hepatitis. The portal tract is expanded by a dense, predominantly lymphoplasmacytic infiltrate. Hematoxylin & Eosin stain. **Inset**: Mild to moderate interface hepatitis with inflammatory cells breaching the limiting plate and injuring/killing zone 1 hepatocytes

resembles type I, but may be differentiated from type I AIH by antibody profiling [22]. It is controversial, however, whether these subtypes in fact represent distinct clinical entities with different clinical presentations, courses and responses to therapy. It might be more reasonable to diagnose AIH and add the actual autoantibody profile, for example ANA positive AIH, anti-LKM1 positive AIH, etc.

The International Autoimmune Hepatitis Group recognizes AIH types I and II, while AIH type III is regarded as a subtype of AIH type I.

## *Autoimmune Hepatitis Type I*

AIH type I is the most frequent form of AIH worldwide, accounting for approximately 80% of all cases. It is most common in Northern Europe and in North America. Seventy percent of patients are young women between 20 and 40 years old, but presentation in older age groups is not uncommon. More than 30% of affected patients have concurrent immunological diseases, such as autoimmune thyroiditis, synovitis or ulcerative colitis.

The clinical course is insidious in most cases and manifestations are protean with noncharacteristic symptoms, such as fatigue and malaise (see below) leading to a delay in diagnosis. Thus, it is not surprising that up to 25% of patients already have a cirrhotic liver on initial diagnosis of AIH. In approximately every fourth patient, however, AIH may have an acute clinical onset, including rare presentations as fulminant hepatitis with liver failure.

AIH type I is characterized by the occurrence of antinuclear antibodies (ANA) and/or anti-smooth muscle antibodies (SMA). ANA are directed against functional and structural nuclear components, the nuclear membrane or against DNA. SMA are directed against cytoskeletal components, such as actin, troponin and tropomyosin (see Chapter 36) [18]. The exact target antigens in AIH type I are unknown. Asialoglycoprotein receptor is one candidate. In addition to disease defining antibodies p-ANCA are found in 50–90% of patients wit AIH type I [83]. They mostly belong to the isotype $IgG_1$, which differentiates them from p-ANCA present in primary sclerosing cholangitis.

## *Autoimmune Hepatitis Type II*

AIH type II is primarily a disease of childhood, worldwide in distribution, but rare in North America. Approximately 20% of adult cases of AIH in Europe

**Table 72.1** Diagnostic scoring system for autoimmune hepatitis in adults (see also Table 29.2B) [5, 40]

| Category | Score |
|---|---|
| **Female gender** | + 2 |
| **Male gender** | 0 |
| **Alk Phos: AST (or ALT) ratio** | |
| > 3 | – 2 |
| 1.5– 3 | 0 |
| < 1.5 | + 2 |
| **γ-globulins oder IgG in serum** | |
| > 2 × ULN | + 3 |
| 1.5–2 × ULN | + 2 |
| 1–1.4 × ULN | + 1 |
| < 1 × ULN | 0 |
| **Autoantibodies** | |
| ANA, SMA or anti-LKM-1 titers | |
| > 1:80 | +3 |
| 1:80 | + 2 |
| 1:40 | + 1 |
| < 1:40 | 0 |
| AMA positive | – 4 |
| **Presence of other autoantibodies in ANA, SMA and anti-LKM1 negative patients** (e.g. anti-SLA/LP, ASGPR, actin, p-ANCA) | + 2 |
| **Viral markers of active infection** | |
| Positive | – 3 |
| Negative | + 3 |
| **Hepatotoxic drugs** | |
| Yes | – 4 |
| No | + 1 |
| **Alcohol** | |
| < 25 g/day | + 2 |
| > 60 g/day | – 2 |
| **HLA-DR 3 or HLA-DR 4** | + 1 |
| **Concurrent immune disease** (any nonhepatic disease of an immune nature) | + 2 |
| **Histologic features** | |
| Interface hepatitis | + 3 |
| Plasmacellular infiltrate | + 1 |
| Rosettes | + 1 |
| None of above | – 5 |
| Biliary changes (includes destructive and nondestructive cholangitis, ductopenia) | – 3 |
| Atypical features (steatosis, iron or copper overload, alcohol-induced hepatitis, granulomas, ground-glass hepatocytes, viral inclusions) | – 3 |
| **Treatment response** | |
| Complete remission | + 2 |
| Remission with relapse | + 3 |
| **Interpretation** | **Score** |
| **Pretreatment** | |
| Definite diagnosis | > 15 |
| Probable diagnosis | 10–15 |
| **Posttreatment** | |
| Definite diagnosis | > 17 |
| Probable diagnosis | 12–17 |

*ULN* upper limit of normal

and 4% in the USA fall upon AIH type II. Compared to AIH type I AIH type II more often clinically manifests as an acute hepatitis. Extrahepatic immune diseases, especially type 1 diabetes mellitus, vitiligo and autoimmune thyroiditis are also more common with AIH type II.

AIH type II is characterized by the presence of antibodies against microsomes from liver and kidney (anti-LKM1). These antibodies react with the epithelia of proximal tubules and liver cell cytoplasm of rodents and may be demonstrated by immunocytochemistry. They must be distinguished on immunofluorescence microscopy from antimitochondrial antibodies (AMA). The target antigen of anti-LKM1 is cytochrome P450 IID6 (CYP2D6) and less often uridine 5′-diphosphate glucuronyl transferase [7, 7, 51, 81]. Anti-liver cytosol autoantibody (anti-LC1) may be associated, and occurs predominantly in children. Anti-LC1 may be the only autoimmune marker of AIH type II in children [11].

Ten to 15% of patients with *autoimmune polyglandular syndrome type 1* (APS1) have also AIH type II. Patients with APS1 have autoantibodies against CYP1A2 and CYP2A6 [13]. However, while anti-CYP1A2 are associated with AIH, antibodies against CYP2A6 also occur in patients with APS1 without liver disease. Antibodies against CYP1A2 are specific for AIH associated with APS1, and are not encountered in APS1-patients without hepatitis, nor do they occur in any other autoimmune liver disease. Approximately 50% of patients with APS1 and 90% of those with APS1 and AIH type II have antibodies against aromatic L-amino decarboxylase.

The knowledge of AIH type II as part of the APS1 is important since in these patients the course of AIH is more aggressive with acute and fulminant presentations and the response to corticosteroids is worse than in AIH without APS1.

APS1 is an autosomal recessive disease caused by mutations in a single gene. The gene is located on the long arm of chromosome 21 and is called autoimmune regulator (AIRE) [75]. It is assumed that AIRE is involved in establishing and maintaining immune tolerance. Patients with APS1 have a defective immune response to Candida albicans and present with recurrent mucocutaneous candidiasis, and with many organ specific autoimmune diseases, leading especially to hypothyroidism, hypoparathyroidism and ovarian insufficiency. In addition, ectodermal dystrophies such as keratopathy, enamel hypoplasia and nail dystrophy are observed.

**Table 72.2** Different types of autoimmune hepatitis [4, 38]

| Feature | AIH type I | AIH type II | AIH type III[a] |
|---|---|---|---|
| **Characteristic autoantibodies** | ANA, SMA | anti-LKM1 | Anti-SLA/LP |
| **Associated autoantibodies** | p-ANCA, anti-actin, anti-ASGPR | anti-LC1, anti-ASGPR | ANA, SMA, anti-ASGPR |
| **↑ Gamma-globulin** | +++ | ++ | |
| **Age at onset of disease** | All age groups; bimodal (10–20 and 45–70 years) | 2–14 years | As AIH type I |
| **Female** | 80% | 90% | As AIH type I |
| **Other immune diseases** | Autoimmune thyroiditis, ulcerative colitis (rare), synovitis | Vitiligo, Diabetes mellitus type 1, autoimmune thyroiditis, autoimmune polyendocrine syndrome 1 | As AIH type I |
| **HLA association** | B8, DR3, DR4 *DRB1*0301* and *DRB1*0401* (Northern Europeans), *DRB1*1501* (protective), *DRB1*0404* (Middle American), *DRB1*0405* (Japanese), *DRB1*1301* (South American) | B 14, DR3, *C4A-QO, DRB1*07* | Not precisely defined |
| **Target antigens** | ANA: Centromere, ribonucleoproteins (not precisely defined) | LKM1: P-450 IID6 (CYP2D6), P-450 IA2 (APS1), P-450 IA6 (APS1) LC1: Formiminotransferase cyclodeaminase | SLA/LP: UGA-suppressor tRNA-associated protein |

*ASGPR*: Asialoglycoprotein receptor, *LC1*: Liver cytosol type 1, *SLA/LP*: Soluble liver antigen/liver pancreas, *APS 1*: Autoimmune polyendocrine syndrome 1, *p-ANCA*: perinuclear antibodies against neutrophil cytoplasm

[a]The distinct existence of AIH type III is controversial. Probably AIH type III may be allocated to AIH type I

## Autoimmune Hepatitis Type III

AIH type III is the most uncommon and immunologically less well characterized AIH. Ninety percent of affected patients are women between the ages of 20 and 40 years.

AIH type III is characterized by antibodies against soluble antigen from liver and pancreas (anti-SLA/LP) [43, 76]. Contrary to previous views, the target antigens are neither cytokeratins nor glutathion-S-transferases, but the cytosolic UGA-suppressor transfer RNA-associated protein [78, 80]. Anti-SLA/LP cannot be visualized by immunofluorescence; instead, they are demonstrated by standardized immunoassays based on recombinant antigen [6].

Patients with AIH type III do not differ from those with classic AIH type I with respect to age, gender, presence of different autoantibodies (except for anti-SLA/LP) and the response to corticosteroids. Therefore, antibodies against SLA/LP do not define a clinically discrete form of AIH, but they may be regarded as a specific diagnostic marker for AIH, occurring in approximately 20% of patients with AIH [35]. Thus, the search for anti-SLA/LP attains special importance in patients with chronic hepatitis of unknown cause. Approximately 25% of these patients present with anti-SLA/LP in serum, allowing for a change in diagnosis from previously cryptogenic hepatitis to AIH in which conventional autoantibodies are absent [42].

## Clinical Presentation

The presentation of AIH is heterogeneous with non-specific symptoms common to all autoimmune liver diseases. Patients complain of fatigue, lethargy, malaise, anorexia, nausea, weight loss, myalgias, arthralgias involving small joints, fever, skin rashes, itching

and noncharacteristic upper abdominal discomfort. The spectrum of presentations ranges from no symptoms (with the diagnosis made incidentally by finding elevated serum aminotransferase levels and hypergammaglobulinemia) to an acute onset even with fulminant hepatic failure [44]. The majority of patients have an insiduous course, but in 25–30% of cases presentation is acute. Acute onset AIH may be difficult to diagnose by serological data alone and liver biopsy with histologic evidence of chronic disease might be helpful in this situation [1]. In acute onset AIH the diagnosis of AIH may be made on the initial presentation without awaiting the period of 6 months required for the diagnosis of a chronic hepatitis.

Physical examination may be unremarkable, but it may also reveal hepatomegaly, splenomegaly, jaundice, and signs and symptoms of chronic liver disease (see Chapters 79 and 80).

**Table 72.3** Extrahepatic diseases in autoimmune hepatitis [47, 52]

- Hemolytic anemias
- Immune thrombocytopenic purpura
- Glomerulonephritis
- Lupus erythematosus
- Immune thyroid disease
- Immune vasculitis
- Celiac disease (more common with primary biliary cirrhosis)
- Ulcerative colitis (rare)
- Iridocyclitis
- Fibrosing alveolitis
- Raynaud's disease
- Localized scleroderma
- Sjögren's syndrome
- CREST-Syndrome
- Synovitis
- Rheumatoid arthritis
- Primary adrenal insufficiency
- Diabetes mellitus type 1
- Autoimmune polyendocrine syndrome 1
- Polymyositis
- Febrile panniculitis
- Vitiligo
- Lichen planus
- Alopecia
- Nail dystrophy

Extrahepatic immune mediated diseases are associated with AIH in 10–50% of patients and may be one clue to diagnosing AIH (Table 72.3) [45].

## *Laboratory Findings*

Aminotransferase levels in serum are markedly elevated (AST > ALT) while parameters of cholestasis (alkaline phosphatase and γGT) are normal or only slightly increased. *The level of aminotransferase elevation does not always correlate with the histological activity of the disease* (possibly GLDH levels reflect intralobular inflammatory activity better than aminotransferases). A polyclonal hypergammaglobulinemia with a marked increase in IgG levels is characteristic for AIH. IgA levels might be decreased [20]. Once cirrhosis develops, the synthetic parameters of liver function decrease.

Laboratory diagnosis of AIH is based on the demonstration of autoantibodies in serum, which is done increasingly by ELISA using recombinant, well defined antigens, rather than by immunofluorescence microscopy (see above and Chapter 36). While the measurement of routine antibodies such as ANA and AMA is standardized, assays for many other antibodies, for example anti-actin and anti-ASGPR (asialo glycoprotein receptor) are available in specialized laboratories only. They are not always standardized and, because of different measurement systems, results from different laboratories are not always comparable. One must therefore be very critical in drawing conclusions regarding the course and prognosis of AIH based on these determinations alone. The dictum that *the level of antibody titers does not correlate with the activity of hepatitis* still holds true. It is possible, however, that antibodies against ASGPR are an exception to this rule. They may be regarded as general markers of all AIH and their presence might be associated with a more severe histological and clinical activity. The prevalence of autoantibodies in AIH is summarized in Table 72.4. Note that *in up to 10% of patients with AIH no autoantibodies in serum are found*. These patients, however, are hypergammaglobulinemic with predominant elevation of IgG. Very rarely patients with AIH, in the absence of the typical antibody profile, and no histological or clinical evidence for primary biliary cirrhosis, are AMA positive. An

**Table 72.4** Prevalence of autoantibodies in autoimmune hepatitis [7]

| Autoantibody | Prevalence in AIH |
|---|---|
| ANA[a] | 40–60% |
| SMA | 40–50% |
| LKM1 | 0–5% |
| SLA/LP | 15–30% |
| No standard autoantibody | Up to 10% |

[a]The combined prevalence of ANA and SMA in AIH type I is about 80%

algorithm for the rational use of serum autoantibodies in autoimmune liver disease is presented in Fig. 72.2.

### *Imaging Tools*

Imaging techniques (ultrasound, CT scanning, MRI) are of subordinate importance in the diagnosis of AIH. Only liver biopsy is diagnostically useful. Although the microscopic findings are not disease specific, only histology allows one to reliably assess the degree of inflammatory activity and the stage of fibrosis (see Chapters 28 and 29). *Liver biopsy is mandatory in the initial diagnosis of AIH*. Liver biopsy should also be performed *before* scheduled withdrawal of immunosuppressive therapy. In this clinical situation the histological findings are crucial in deciding whether treatment may be terminated or should proceed (see below).

On laparoscopy the liver is swollen in the early stage of AIH, displaying a fine fibrotic rim at its lower margin. During the further course the consistency of the organ increases. The subcapsular lobular architecture becomes increasingly blurred, the number of vessels increases, and fibrotic scars and dilated lymph vessels become visible. The stage of AIH may be diagnosed reliably by an experienced laparoscopic examiner. However, despite the diagnostic value of laparoscopy, the procedure is nowadays rarely performed and not necessary in the diagnosis of AIH. Signs of cirrhosis and portal hypertension may be seen on ultrasound examination. CT scanning and MRI do not provide significant additional information. They are useful, however, in addition to ultrasound in characterizing focal lesions that develop in a cirrhotic liver.

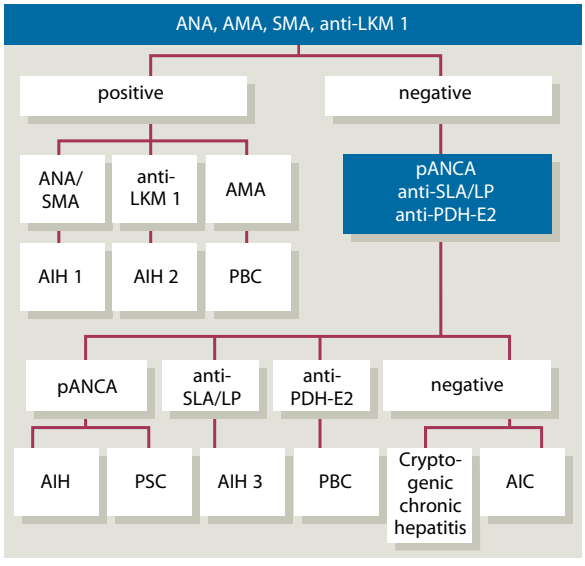


**Fig. 72.2** Suggested algorithm for the use of autoantibodies in the diagnosis of autoimmune liver disease. *AIH* autoimmune hepatitis, *PSC* primary sclerosing cholangitis, *PBC* primary biliary cirrhosis, *AIC* autoimmune cholangiopathy, *PDH-E2* pyruvate dehydrogenase E2

## Differential Diagnosis

The differential diagnosis of AIH includes all chronic hepatitides, and cryptogenic liver cirrhosis. In addition, in every acute hepatitis (especially in the elderly) AIH has to be excluded, since a significant proportion of patients with AIH have an acute onset. The definitive diagnosis of AIH is established after the exclusion of metabolic, hereditary, viral, drug-induced and cholestatic liver diseases.

The measurement of anti-SLA/LP in patients with cryptogenic chronic hepatitis or cirrhosis of unknown origin, negative for ANA, AMA and anti-LKM1 yields positive results in up to 25% of cases, allowing for the reassignment of the disease to the group of autoimmune disorders.

The delineation of AIH from variant (overlap) syndromes is discussed in Chapter 77 [8].

The differentiation of AIH from virus-associated autoimmunity is relevant clinically, since it has a direct impact on treatment. Anti-LKM1 antibodies are present in 2–12% of cases of chronic viral hepatitis C and D. The titers of anti-LKM1 are lower, and the antibodies in virus associated autoimmunity are directed against different LKM-epitopes on recombinant CYP2D6 than in AIH type II. The basis for autoantibody production in chronic hepatitis C and D is a genetic predisposition of

the host and homologies between CYP2D6 and the viral genome (molecular mimicry) [32]. Differentiating between autoimmune liver disease and virus-induced autoimmunity is therapeutically important, since patients with autoimmune liver disorders receive immunosuppressive treatment, while those with chronic hepatitis C (with or without autoantibodies) are treated with pegylated interferon (contraindicated in autoimmune disease) and ribavirin. The simultaneous occurrence of a chronic viral hepatitis and AIH has been documented anecdotally, but is extremely rare.

Up to 25% of patients with nonalcoholic fatty liver disease present with low titer (≤ 1: 320) ANA, which might lead to misdiagnosing NAFLD for AIH with consequent corticosteroid treatment of steatosis. The astute clinician will avoid this error, and in dubious cases liver biopsy should be performed.

## Course and Prognosis

AIH often is an aggressive and progressive disease that, without adequate immunosuppressive treatment leads to death in many patients. If a marked inflammatory activity with bridging necrosis and fibrosis is present already on initial diagnosis, without treatment 50% and 90% of patients will die within 3 and 10 years, respectively. In contrast, patients with mild disease have 5 year survival rates comparable to the normal population and the risk of having cirrhosis 5 years after diagnosis is 17%.With adequate treatment the 5 and 20 years survival rates are > 90% and 80%, respectively [20, 63, 69]. Patients with AIH who are asymptomatic at presentation have a good prognosis and may not require immunosuppressive therapy [31]. Thus, course and prognosis of AIH crucially depend on the activity of the inflammatory process, the degree of liver cell death and on timely adequate therapy in patients with active disease.

Certain autoantibodies may have relevant prognostic value. Antibodies against actin identify a subgroup of SMA-positive patients with AIH type I in whom the disease starts at an earlier age and who respond less well to corticosteroids than anti-actin negative patients. Antibodies against ASGPR seem to correlate with the histological and clinical activity, and the presence of anti-ASGPR possibly identifies patients who will relapse after withdrawal of therapy.

There are only few data on the impact of gender and ethnicity on the natural history of AIH. Men with AIH appear to have a higher relapse rate and younger age of disease onset which may relate to increased prevalence of HLA A1-B8-DR3. Despite this, men have been reported to have a significantly better long-term survival and outcomes than women [3]. Blacks, especially black men with AIH, have more aggressive disease at the initial presentation, are less likely to respond to conventional immunosuppression, and have a worse outcome than non-blacks [74]. A recent report describes Somalian men with AIH presenting with cholestatic features and responding poorly to standard immunosuppressive regimens [29].

AIH may influence pregnancy outcome, and pregnancy may affect the activity of AIH. A rate of 26% of adverse pregnancy outcome in AIH has been reported, and unexplained adverse pregnancy outcomes were highly associated with the presence of anti-SLA/LP [67]. The course of AIH in pregnancy is variable, but liver tests often improve, assumed to be due to a state of immune tolerance during pregnancy. Post-partum flare-ups and AIH first presenting in the early post partum period are well documented [12, 64].

## Therapy

All types of AIH are treated by immunosuppression for several years and some patients must receive lifelong therapy. AIH patients with no autoantibodies in serum (up to 10%) respond to immunosuppressive therapy like patients with classical AIH. Patients with AIH and bile duct injury but lacking features of primary biliary cirrhosis respond as well to corticosteroid therapy as patients with classical disease. Background bile duct changes should not alter the treatment of AIH [23]. The absolute and relative indications for therapy are listed in Table 72.5. Histologically inactive AIH without fibrosis only rarely proceeds to cirrhosis. Therefore, immunosuppressive treatment in these patients must be pondered critically, since the side effects of corticosteroids may offset their slight therapeutic benefit in this clinical situation. Examination of liver tissue remains the best method of evaluating both treatment response and need for treatment in patients who have little biochemical activity [34].

Immunosuppressive therapy also should not be administered to patients with inactive cirrhosis and only

**Table 72.5** Indications for treatment of autoimmune hepatitis [4, 20, 39]

| Absolute | Relative |
|---|---|
| Serum AST ≥ 10 × ULN | Symptoms (fatigue, arthralgia, jaundice) |
| Serum AST ≥ 5 × ULN and γ-globulin level ≥ 2 × ULN | Serum AST and/or γ-globulin increased, but less than absolute criteria |
| Marked inflammatory activity with bridging necrosis or multiacinar necrosis on histologic examination | Interface hepatitis |

*AST*: aspartate aminotransferase level, *ULN*: upper limit of normal

mildly elevated aminotransferase levels [53]. However, liver cirrhosis is not a contraindication for immunosuppressive treatment.

The goals of therapy are

- Induction of remission
- Maintaining complete remission
- Preventing relapse, and if necessary
- Management of relapse after drug withdrawal

Two treatment regimens are comparable with each other in the management of severe AIH in adults: (1) *monotherapy with prednisone* or (2) the *combined administration of prednisone and azathioprine* irrespective of the serological subtype of AIH [9, 20, 52]. Prednisone is transformed in the liver to its active moiety prednisolone. Since this metabolic step is not impaired even in advanced chronic liver disease, there is no advantage to administer prednisolone instead of prednisone [66]. Various treatment strategies are outlined in Table 72.6. *Prednisone in combination with azathioprine is the preferred initial treatment* because of its lower frequency of side effects [20, 39, 70]. Phenotyping and genotyping of thiopurine methyltransferase activity (key enzyme in azathioprine transformation to its active metabolites) before institution of azathioprine therapy does not predict response of AIH to the drug [46].

The clinical picture in addition to aminotransferase and γ-globulin levels are the initial parameters of determining treatment response. Ninety percent of adults have improvements in the serum aminotransferase, bilirubin, and γ-globulin levels within 2 weeks [24]. Normalization of increased IgG levels in > 90% of cases correlates with histologic improvement.

Although there is no prescribed minimum or maximum duration of treatment, experience has shown that

**Table 72.6** Standard treatment of autoimmune hepatitis [53]

| Initial therapy | | |
|---|---|---|
| | **Daily oral dose** | **Duration** |
| 1. Predniso(lo)ne Monotherapy | 60 mg | 1 week |
| | 40 mg | 1 week |
| | 30 mg | 2 weeks |
| | 15–20 mg or less | Maintenance |
| or | | |
| 2. Predniso(lo)ne | 30 mg | 1 week |
| | 20 mg | 1 week |
| | 15 mg | 2 weeks |
| | 10 mg or less | Maintenance |
| plus | | |
| Azathioprine[a] | 50 mg | |
| **Therapy of relapse** | | |
| 1. Repeat initial therapy | | |
| or | | |
| 2. Predniso(lo)ne | 30 mg | 4 weeks |
| plus | | |
| Azathioprine | 150 mg | |
| or | | |
| 3. Predniso(lo)ne | 60 mg | |

[a]Treatment to maintain remission may also be performed with azathioprine monotherapy (2 mg/kg per day) sparing corticosteroids. Induction therapy with azathioprine monotherapy is not promising

usually 2–4 years of therapy are required to maintain complete remission and to avoid relapses after cessation of treatment. *The most common error made in treating patients with AIH is too early withdrawal of therapy*. The administration of prednisone every other day during the induction phase aiming at reducing corticosteroid side effects is not recommended, since this practice reduces the rate of histological remissions. On the other hand, enforcing a rapid and early remission by increasing drug doses also has no advantage with respect to the overall success rate of treatment.

After reaching complete remission, the prednisone dose is slowly and continuously tapered to a daily maintenance dose of 2.5–10 mg. The dose should be adjusted to the minimal dose required to maintain remission. After complete remission is stable for 6–12 months prednisone usually can be withdrawn completely and therapy may be continued with azathioprine alone at a daily dose of 50 mg for approximately 2 more years [41].

Treatment withdrawal should not be based on biochemical parameters only. Before complete treatment withdrawal a liver biopsy should be performed. Histological improvement lags approximately 3–6

months after clinical and biochemical improvement. In addition to inflammatory changes, fibrosis also commonly improves, and if it is not too far progressed may even regress to a certain degree during corticosteroid therapy of AIH [16]. Fibrosis progresses in only a minority of patients during immunosuppressive therapy, and progression is associated with HLADR3/DR4 [17]. If histological and biochemical findings are normal, treatment may be terminated. Despite normal histology, relapses after drug withdrawal occur in approximately 20% of cases. Inflammatory portal infiltrates are associated with a relapse rate of 50% and virtually every patient with a continuing interface hepatitis and/or progression to cirrhosis during drug treatment will experience a relapse after drug withdrawal. Thus, *the histological findings after approximately 6–12 months of clinical and biochemical remission are essential in deciding whether therapy may be terminated or not*.

Conventional treatment regimens should be continued in adults until remission, treatment failure, incomplete response, or drug toxicity. Once disease remission (criteria: disappearance of symptoms, normal serum bilirubin and γ-globulin levels, serum aminotransferase level normal or less than twice normal, normal hepatic tissue or minimal inflammation and no interface hepatitis within 1 year of treatment and for at least 6 months) has been achieved, drug withdrawal should be attempted. Overall, complete remission rates of 60–70% are reached. Despite treatment, 30–40% of patients proceed to cirrhosis within 10 years.

*Treatment failure* (criteria: worsening clinical, laboratory, and histologic features despite compliance with therapy; increase of serum aminotransferases by 67%; development of jaundice, ascites, or hepatic encephalopathy) is observed in approximately 10% of cases. Onset at an early age, acute presentation, hyperbilirubinemia, and presence of HLA DRB1*03 characterize patients who fail corticosteroid treatment [58]. In these patients prednisone dose should be increased to 60 mg daily or to 30 mg daily combined with azathioprine, 150 mg daily, for at least one month, then slowly tapering the prednisone dose over months to 2–2.5 mg daily and azathioprine to 25–50 mg daily. Despite dose escalation, only 20% of patients with treatment failure achieve histological remission, and maintenance dose probably has to be given lifelong.

*Relapses* occur most commonly within the first 6 months after treatment withdrawal and are characterized by an increase of serum AST to ≥ 3 times the upper limit of normal, reappearance of symptoms, and inflammatory lesions on liver histology. However, liver biopsy is not required to document a relapse. Prolonged time to complete clinical and biochemical remission and increased numbers of portal plasma cells prior to treatment withdrawal are associated with > 90% probability of relapse [73]. Treatment of a relapse corresponds to the standard initial regimen as outlined in Table 72.6. Long-term maintenance therapy with azathioprine helps to spare corticosteroids. However, with each relapse the probability of side effects of drugs increases and that of a new remission decreases. Lifelong combination therapy with prednisone (e.g. 7.5–10 mg daily) and azathioprine (50 mg daily) may be necessary.

In patients receiving long-term corticosteroid therapy supporting measures, such as the administration of vitamins D and K, and bisphosphonates should not be forgotten.

In patients with AIH not responsive or intolerant to standard immunosuppressive therapy, prednisone monotherapy at a dose of 60 mg daily or another immunosuppressive drug may be tried. Encouraging results have been reported for cyclosporine (5–6 mg daily), tacrolimus (3 mg bid), mycophenolate mofetil (1 g bid), 6-mercaptourine (1.5 mg/kg daily), and budesonide (3 mg tid). These drugs, however (possibly with the exception of budesonide), should be regarded as investigational in AIH and should therefore be used only in controlled trials [34, 37, 53, 61, 79].

Ursodeoxycholic acid (UDCA) should not be used in patients with classical AIH. Possibly, UDCA has some value in cholestatic variants of AIH.

With multiple relapses, in treatment failure not responding to drug therapy, and in decompensating cirrhosis liver transplantation should be considered [65]. AIH presenting as acute liver failure and a failure of elevated serum bilirubin to fall in a patient with multiacinar necrosis on histologic examination within 2 weeks of corticosteroid therapy is considered an absolute indication for liver transplantation.

The 5-year survival rates after transplantation for AIH are 80–90% and 75% after 10 years. Recurrence of AIH in the transplanted liver occurs in approximately 17–41% of patients followed for more than 10 years after the procedure, mainly in those with HLA-DR3 and/or HLA-DR4. Recurrence is usually relatively mild and can be mastered by adjusting immunosuppressive therapy. Histological recurrence precedes clinical and biochemical recurrence, thus, regular liver biopsy is

warranted after orthotopic liver transplantation for AIH [30, 60].

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# Primary Biliary Cirrhosis

# 73

Henryk Dancygier

## Chapter Outline



"Chronic idiopathic obstructive jaundice" occurring chiefly in middle-aged women has been already noted by Hanot in 1876. Denominated later "primary biliary cirrhosis," this entity continued to puzzle clinicians throughout the following century, as it was extremely difficult to differentiate from jaundice due to obstruction of the main bile ducts [2, 115]. In a landmark study published in 1965 a close association between anti-mitochondrial antibodies and primary biliary cirrhosis was demonstrated [132].

## Definition

Primary biliary cirrhosis (PBC) is an autoimmune cholestatic liver disease characterized by progressive, non-suppurative, inflammatory destruction of interlobular bile ducts resulting in ductopenia, and leading to cirrhosis.

Synonym: chronic non-suppurative destructive cholangitis.

## Epidemiology

There is a striking female predominance with 80–90% of affected patients being women between 20 and 60 years of life. Anecdotal cases of pediatric-onset PBC have also been reported [17].

PBC is more common than assumed previously, and incidence and prevalence show geographical variations [40]. The incidence in the USA and in Germany is approximately 2.7–3.5/$10^5$ persons per year. The incidence for women older than 40 years is 10/$10^5$ persons per year. The prevalence rates are 25–40/$10^5$

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persons and for women older than 40 years 65–100/$10^5$ persons [55]. The highest prevalence rates are reported from Northern Europe, while low prevalence rates are found in Australia.

## Etiology and Pathogenesis

Etiology and pathogenesis are not exactly known. Immune mechanisms based on a genetic susceptibility and triggered by environmental factors appear to be operative.

### *Genetic Factors*

The concordance rate of PBC in monozygotic twins is 63%. First-degree relations of index PBC patients have a PBC prevalence of 4–6% [124]. The incidence of AMA in first-degree relatives of patients with PBC is increased to 13% [59]. An enhanced X monosomy has been reported in women with PBC [78]. This preferential involvement of X chromosome gene products might be one explanation for the female predisposition to PBC. There is supporting evidence for the genetic association of PBC with major histocompatibility complex-encoded genes. PBC is associated with the DRB1*08 family of alleles. HLADR8 positive persons have a 2–7 times higher risk of developing PBC. However, the presence of HLA DR8 is neither a necessary nor a sufficient condition for PBC to develop [48]. A protective association has been described with DRB1*11 and DRB1*13. Significant variation is seen, however, between different ethnic groups both in susceptibility and in protective associations. To date, none of the genetic associations identified in PBC have proved sufficiently strong to be useful clinically in the prediction of disease risk [38, 44].

### *Environmental Factors*

Geographical clustering ("hot spots") of PBC cases in urban, former industrial and/or coal mining areas and in the environs of highly toxic federal waste disposal sites may reflect environmental factors in the etiology of PBC [3, 103]. Chemicals contained in cigarette smoke, may induce PBC in genetically susceptible individuals [26].

Exposure to infectious organisms may represent another environmental factor involved in triggering the autoimmune process in PBC. Retroviral antibodies have been demonstrated in the serum of patients with PBC [73]. Recently a human betaretrovirus was identified both in liver tissue and draining lymph nodes from PBC patients [136, 137]. Bacteria may contain amino acid sequence homologies with PDC-E2 (see below) which by molecular mimicry may cause immunologic crossreactivity. There is epidemiological evidence of infection with Escherichia coli, Mycobacterium gordonae, Novosphingobium aromaticovorans (an ubiquitous xenobiotic-metabolizing bacterium), Paracoccus denitrificans, and Chlamydia pneumoniae in PBC patients [1, 10, 27, 67, 109, 111]. Although these epidemiological data are attractive *there is no objective evidence confirming a role of bacteria or viruses in the pathogenesis of PBC*.

### *Immunologic Factors*

It is widely accepted that liver damage in PBC ultimately results from an "immunologic attack" on portal bile ducts, despite the triggering factors being still largely unknown. Autoreactive T cells (against PDC-E2) occur in high concentrations in the liver and in the hepatic hilar lymph nodes in patients with PBC, and aberrant expression of mitochondrial autoantigens on biliary epithelia has been described. Autoreactive T cell-mediated cholangiocyte injury leads to the destruction of interlobular bile ducts. The diagnostically defining anti-mitochondrial antibodies (AMA) are neither cytotoxic nor do they damage the cholangiocytes [87].

Innate immune mechanisms may also play a role in the induction and persistence of abnormal humoral immune responses in PBC. The increase in serum IgM levels that is present in patients with PBC is the result of chronic B-cell activation induced via the Toll-like receptor signaling pathway. Bacterial molecules have been shown to induce hyper-IgM production in CD27+ memory B cells in PBC [53].

Additional factors may contribute to bile duct damage in PBC, such as (1) the secretion by eosinophils of

eosinophilic cationic protein, (2) apoptosis of biliary epithelial cells, secondary to the invasion of inflammatory cells or induced by dimeric IgA, and (3) ischemia due to loss of peribiliary vessels commonly seen in the early stages of PBC and probably secondary to the inflammatory process [75, 128, 133].

## Pathology

The denomination "chronic non-suppurative destructive cholangitis" and PBC already indicates that the disease starts as a cholangitis and terminates as cirrhosis. The development of PBC is characterized by different histological stages that are distributed irregularly throughout the liver. They cannot always be sharply separated from each other and overlap is common (Table 73.1) [72, 110].

On histological examination a dense lympho-plasmacytic infiltrate of portal tracts is seen, occasionally arranged in a lymph follicle like pattern. In the early stages it is often accompanied by epithelioid granulomas (Fig. 73.1). The portal tract inflammatory infiltrate is mixed in phenotype, T cells ([CD4 and CD8], with the latter predominating in the periductal areas), B cells, natural killer cells, macrophages, eosinophils and mast cells [22, 24, 44, 126]. The portal inflammation spills over to the neighbouring lobular parenchyma (interface hepatitis) resulting in damage and destruction of periportal hepatocytes ("biliary piece meal necrosis") [96]. Occasionally, Mallory-Denk bodies may be found predominantly in periportal and periseptal liver cells.

**Table 73.1** Histopathologic stages of primary biliary cirrhosis

| | According to Scheuer (1967) | According to Ludwig (1978) |
|---|---|---|
| **Stage 1** | **Florid Duct Lesion** | **Portal Stage** |
| | Bile duct lesion | Portal hepatitis |
| | Portal hepatitis | |
| **Stage 2** | **Ductular Reaction** | **Periportal Stage** |
| | Periportal (interface) hepatitis | Periportal (interface) hepatitis |
| | Ductular proliferation | |
| **Stage 3** | **Scarring** | **Septal Stage** |
| | Bridging necrosis | Bridging necrosis |
| | Septal fibrosis | Septal fibrosis |
| | Ductopenia | |
| **Stage 4** | **Cirrhosis** | **Cirrhosis** |

Continuing inflammatory activity is associated with increasing portal and periportal fibrosis. The typical stage I lesion is the inflammatory injury of interlobular bile ducts (*florid bile duct lesion*) characterized by a breach in continuity of ductal basal membranes, ectasia and rupture of bile ducts, intraepithelial lymphocytic infiltrates and cholangiocyte injury (Fig. 73.2). At the edges of the portal tracts ductular proliferation is present. Ultimately the continuing inflammatory process leads to the loss of bile ducts (ductopenia). Focal scars within the portal tracts represent remnants of previously destroyed interlobular bile ducts.

With continuing disease the inflammatory infiltrate and the number of interlobular bile ducts diminish. Progression of periportal hepatocellular damage leads to septum formation. Incomplete septa from the enlarged portal tracts and portal to portal bridges appear first, while portal to central septa form later [85]. Finally the process results in cirrhosis.

## Diagnosis

PBC must be excluded in every patient with long-standing increased serum levels of enzymes indicating cholestasis and normal appearing bile ducts on ultrasound or CT scan. The most sensitive diagnostic parameter is serum AMA. The diagnosis of PBC is made when two of three following criteria are fulfilled.

- Presence of serum AMA
- Increased levels of enzymes (e.g. alkaline phosphatase) indicating cholestasis for longer than 6 months, and
- Compatible or diagnostic liver histology

### *Clinical Presentation*

The disease begins insidiously with the patient being asymptomatic despite ongoing hepatic inflammation. The appearance of symptoms lags months (to years?) behind the development of histological lesions, and the increase in levels of cholestatic liver enzymes usually lags behind the appearance of symptoms. Thus, once symptoms develop the inflammatory process probably

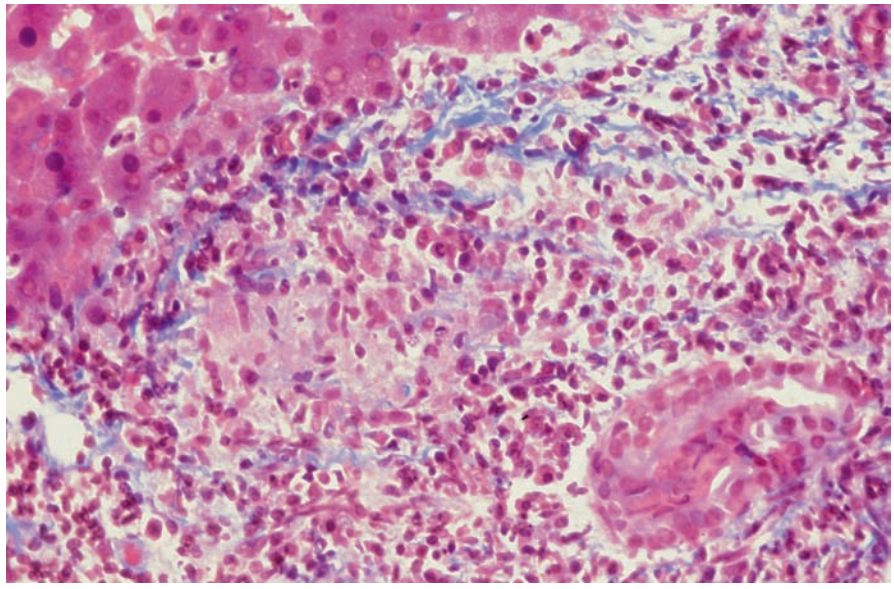

**Fig. 73.1** Primary biliary cirrhosis (stage 1). Portal inflammatory infiltrate containing an epithelioid granuloma. The interlobular bile duct is damaged. Masson trichrome (× 400)

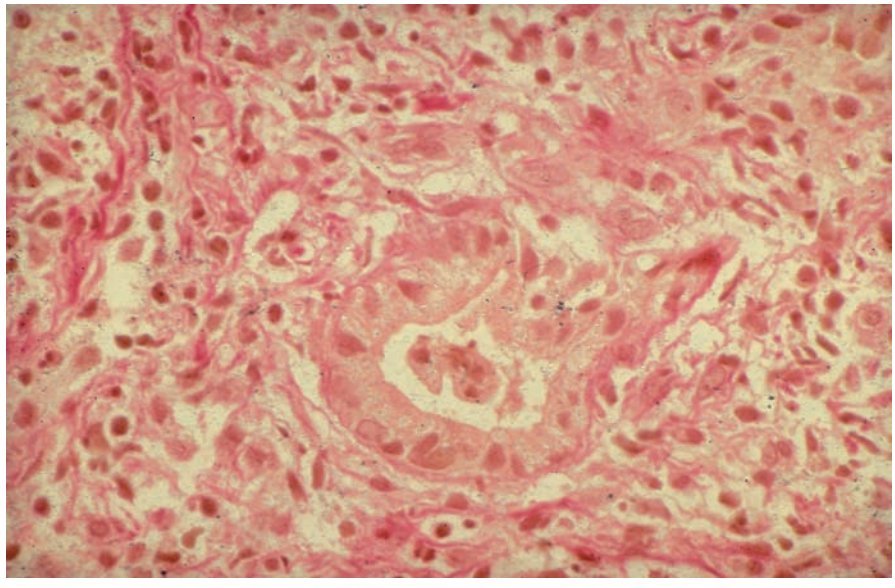

**Fig. 73.2** Primary biliary cirrhosis (stage 1). A portal lymphocytic infiltrate damages an interlobular bile duct. Domagk stain (× 400)

has been active already for a long time. Symptoms are nonspecific. Initially the patient barely notices a mild fatigue which with time, however, may become so severe as to compromise markedly his quality of life, long before the development of cirrhosis and hepatic encephalopathy [23, 101, 119]. The overall quality of life deteriorates mainly due to the decrease in energy and emotional reactions, and increased vulnerability to emotional stress, all associated with fatigue [8]. Noncharacteristic upper abdominal discomfort and bloating may supervene. The prevalence of a depressive disorder in patients with PBC is not higher than in the general population, and fatigue cannot be explained by depression [130]. Fatigue, which is seemingly unrelated to the severity of the underlying liver disease usually is accompanied by a generalized pruritus that may become excruciating. Both, fatigue and pruritus may precede the elevation of serum AP and γ-GT and the diagnosis of PBC by months or even years. Thus, the first symptoms in PBC are not equivalent to symptoms of early disease. *In a patient with fatigue and pruritus of unknown origin, even with normal serum levels of*

*enzymes indicating cholestasis, AMA in serum should be determined*. Jaundice is a symptom of advanced disease. However, occasionally it may occur in non-cirrhotic but ductopenic patients [131].

As a consequence of chronic cholestasis xanthomas and xanthelasms, vitamin deficiencies (A, D, E, K), steatorrhea and bone loss may develop (see Chapter 80) [36]. Additional genetic factors probably contribute to the development of PBC associated osteopathy.

### Extrahepatic Diseases

PBC may be associated with extrahepatic immune-mediated diseases. Conjunctivitis, myalgias, arthralgias, scleroderma, sicca syndrome, Sjögren's syndrome and Hashimoto's thyroiditis have been reported. Liver disease seems to have a slower progression in patients with PBC and systemic sclerosis compared with matched patients with PBC alone [108]. The cause for this interaction is unclear. Seven to 9% of patients with PBC have CREST (calcinosis cutis, Raynaud phenomenon, esophageal dysfunction, sclerodactyly, telangiectasia)-syndrome.

Celiac disease, often asymptomatic, is encountered in 6% of patients with PBC, and patients with celiac disease have an increased risk of developing PBC [56].

PBC and autoimmune pancreatitis (AIP) may coexist, but AIP is more often associated with primary sclerosing cholangitis than with PBC.

Skin and nail lesions, particularly plantar mycoses, onychomycoses, and interdigital mycoses are reported to be significantly more common in patients with PBC than in the general population [57].

Autonomic dysfunction (reduced heart rate variability and baroreflex sensitivity, orthostatic dysregulation of blood pressure) is found at all stages of PBC and correlates with fatigue severity [88–90]. Despite increased serum cholesterol levels PBC does not confer an increased risk for cardiovascular disease [71].

## *Laboratory Findings*

Serum levels of alkaline phosphatase and γ-glutamyl transpeptidase rise continuously over the years, while aminotransferase concentrations remain only slightly elevated. A polyclonal hypergammaglobulinemia is primarily due to increased IgM levels (also in AMA-negative patients). As a nonspecific sign of cholestasis, serum cholesterol level is increased. The nonspecific laboratory alterations of liver cirrhosis of all etiologies are found in the cirrhotic stage of PBC (see Chapter 79). A platelet count of less than 140,000/mm$^3$ and/or a Mayo-Risk-Score of 4.5 or greater (see below) appears to identify PBC patients with esophageal varices [65].

*The serologic diagnosis of PBC is made by demonstrating high titer antibodies against mitochondria (AMA)* [121]. Serum AMA at titers higher than 1:40 are considered the most specific marker for the diagnosis of PBC, being present in the sera of > 90% of patients who have PBC when tested using routine indirect immunofluorescence. Serum AMA include all three major immunoglobulin isotypes, IgG, IgM, and IgA, but the AMA activity resides predominantly in the $IgG_1$ and $IgG_3$ subclasses. Elevated AMA titers in serum (> 1:40) occur already prior to the clinical manifestations, histological changes and to a rise of serum levels of alkaline phosphatase. Thus, the finding of an AMA titer greater than or equal to 1:40 is strongly suggestive of PBC even in the absence of symptoms and in the presence of a normal alkaline phosphatase concentration [79]. *The M2 subtype of AMA is the most sensitive and specific diagnostic marker*. It is present in > 95% of cases and regarded as pathognomonic for PBC [87, 121].

The mitochondrial antigens for AMA are heterogeneous. AMA are directed primarily against components of the 2-oxoacid dehydrogenase multienzyme complex on the inner mitochondrial membrane, including the E2 subunit of the pyruvate dehydrogenase complex (PDC-E2), the branched chain 2-oxoacid dehydrogenase complex (BCOADC-E2), and the oxoglutarate dehydrogenase complex (OGDC-E2), as well as the dihydrolipoamide dehydrogenase (E3)-binding protein (E3BP) and the E1α subunit of pyruvate dehydrogenase complex (PDC-E1α) [25]. Autoantibodies against individual members of this complex (especially anti PDC-E2) represent the anti-M2 subtype of AMA.

Approximately 5% (according to some authors up to 15%) of PBC patients are consistently AMA-negative on immunofluorescence microscopy, yet they share the same clinical, biochemical and histological features of AMA-positive PBC, raising the question of the existence of a separate AMA-negative PBC [82, 112]. However, most patients who are initially

AMA-negative, become AMA-positive when investigated with more sensitive immunoblot techniques based on recombinant antigens [91]. Thus, the number of truly AMA-negative patients diminishes with increasing sensitivity of the detection assay used. The very few "true" AMA-negative cases might represent patients with autoimmune cholangitis (especially when ANA are positive) (see Chapter 74).

AMA are not the only disease specific autoantibodies in PBC. In addition, highly specific (99%) *antinuclear antibodies* (ANA) directed against the lamin B receptor (nuclear envelope), centromeres and the nuclear pore complex (gp210, Sp100, p62) have been described in up to 25% of PBC patients (on immunofluorescence a "nuclear rim" or a "multiple nuclear dots" pattern is usually seen). These autoantibodies are highly indicative of PBC and are associated with more serious liver disease with a significantly higher rate of progression to end-stage liver disease with hepatic failure. Thus, in addition to their high specificity these antibodies seem to have prognostic significance in patients with PBC [31, 37, 81, 83, 84, 139]. Recently, *small ubiquitin-related modifiers* (SUMO), a novel class of nuclear autoantigens has been described in PBC [41]. The significance of autoantibodies against SUMO is unclear. Measurement of PBC specific antibodies against nuclear components is still restricted to specialized laboratories and has not gained entrance into general clinical practice.

### *Imaging Tools*

A *liver biopsy* is not mandatory in diagnosing PBC. Nevertheless, biopsy is recommended to grade and stage the disease with regard to inflammatory activity and fibrosis. In order to minimize sampling error several large biopsy specimen are better suited than a wedge biopsy. These may easily and safely be obtained during laparoscopy, which in addition is a highly sensitive technique in visualizing cirrhotic alterations of the liver surface. A liver biopsy is also helpful in disclosing histological changes of PBC in AMA-positive patients with (still) normal serum levels of alkaline phosphatase. Nearly all these patients will develop elevated cholestasis parameters during a follow-up of 10–15 years.

*Ultrasound* and *CT-scanning* only yield nonspecific findings. Since biliary cirrhosis is micronodular, both methods are not sensitive in diagnosing cirrhotic transformation in PBC. Sequelae of portal hypertension, such as splenomegaly and ascites, however, are well documented by both methods.

*Endoscopic* and *magnetic retrograde cholangiography* yield normal findings in the early stages of the disease. In end-stage PBC nonspecific secondary alterations of intrahepatic bile ducts due to cirrhosis are seen.

## Differential Diagnosis

The differential diagnosis of PBC includes all autoimmune hepatobiliary diseases. In addition, metabolic, drug-induced, viral and cholestatic liver diseases must be excluded. The demonstration of the PBC specific antibody profile will make diagnosis straightforward in the vast majority of patients. Diagnostic difficulties often arise in patients with AMA-negative PBC, with overlap syndromes or with a switch in diagnosis during long-term follow-up (see Chapter 77).

Involvement of the interlobular bile ducts in chronic viral hepatitis C, to the less experienced pathologist, may sometimes cause confusion with a primary biliary disorder. However, in chronic HCV infection, unlike in PBC, interlobular bile ducts show only reactive but no destructive changes, and chronic hepatitis C does not lead to ductopenia.

The cholangiographic bile duct changes seen in end-stage PBC may mimic those of primary sclerosing cholangitis (PSC). However, AMA are not a feature of PSC.

Bile duct damage associated with epithelioid granulomas may be observed in conditions such as sarcoidosis and infection with Fasciola hepatica.

## Natural Course and Prognosis

PBC is a chronic, slowly progressive disease, often unnoticed for years by the patient and the physician. Smoking may accelerate the progression of PBC [140]. Nowadays, diagnosis is increasingly made in asymptomatic AMA-positive patients with still normal serum levels of alkaline phosphatase. Patients with AMA but no other signs or symptoms of PBC

eventually develop symptoms over an 11- to 24-year follow-up period [112]. AMA profiles do not predict prognosis, while anti-nuclear core complex antibodies identify patients likely to experience an unfavorable clinical course [47].

The mean time of progression from stage 1 or 2 to cirrhosis among patients receiving no medical treatment is reported to be four to six years [15, 69]. The mean life expectancy of symptomatic PBC patients (without treatment) may be estimated to be approximately 10–15 years from diagnosis. In a community-based study (which might reflect the overall situation better than most studies reported from tertiary referral centers) which included treated and untreated PBC patients the median time until death or referral for liver transplantation was only 9.3 years from diagnosis. Patient age, the presence of fatigue, alkaline phosphatase, albumin, and bilirubin levels at diagnosis independently predicted survival [43, 104]. As in cirrhosis of other etiologies, the risk of developing hepatocellular carcinoma (HCC) in patients with PBC is also increased to approximately 6%, with men having a relatively higher risk [42]. HCC develops mostly in old male patients with advanced-stage PBC, and often does not affect the patient's survival [117, 122].

It is now appreciated that there is a subgroup of patients within the PBC population who have a low risk of disease progression and who are unlikely to develop end-stage disease during a normal lifetime [45, 134]. The most reliable prognostic parameters of PBC are serum bilirubin and the Mayo-Risk-Score (www.mayo.edu/int-med/gi/model/mayomodl.htm), a model based on patient age, total serum bilirubin and serum albumin concentrations, prothrombin time and severity of edema [20, 58, 114]. Factors that decrease survival are jaundice, irreversible loss of bile ducts, cirrhosis, and the presence of other autoimmune diseases. *Neither the presence nor the titer of AMA affect disease progression, patient survival, or response to treatment* [129].

Since the early 1980s, significant changes in mortality from PBC have occurred in the USA. The most noticeable change was an increase in the age of death, which indicates prolongation of survival. These changes may be attributable to earlier diagnosis and treatment with liver transplantation or ursodeoxycholic acid (UDCA) [51, 76, 77, 95, 118].

One should be aware that in some patients with PBC autoimmune hepatitis (AIH) may develop and result in rapid progression toward cirrhosis and liver failure. Superimposed AIH necessitates a change in treatment adding immunosuppressive therapy to UDCA (see Chapter 72) [102].

## Therapy

Many immunosuppressive, antiinflammatory and antifibrotic drugs have been used alone or in combination in PBC. Healing of the disease, however, is still not in sight. Current evidence indicates that treatment of early stage PBC with UDCA probably prolongs life [4, 33, 64, 68, 97]. Drugs and drug combinations that have been used in PBC are listed in Table 73.2.

The results of treatment with corticosteroids, azathioprine, methotrexate, cyclosporine, D-penicillamine and colchicine have been disappointing [6, 7, 11, 12, 29, 35, 49–52, 66, 70, 80]. Monotherapy with these drugs is no longer warranted, and at present, there are insufficient data to support the use of immunosuppressive therapy for PBC [33].

The *current drug of choice in PBC is UDCA*, 13–15 (–20) mg/kg p.o. daily in two divided doses. Treatment should begin at an early stage, and is lifelong [16, 64, 98, 99].

UDCA is a physiologic tertiary bile acid that is nontoxic due to its hydrophilia and polarity, and has virtually no serious side effects. An interesting "side effect" might be the observation (awaiting confirmation in controlled trials) that prolonged administration of UDCA significantly decreases the probability of colorectal adenoma recurrence following removal [113]. Its mechanism of action in limiting bile duct injury is unclear, and hypotheses include membrane stabilizing effects, immune modulatory functions, and inhibition of eosinophil degranulation [14, 138].

Although some metaanalyses failed to show a benefit of UDCA in the treatment of PBC, the majority of studies confirmed the long-term beneficial effect of UDCA, especially if treatment is begun in the early stages of the disease [13, 16, 28, 30, 39, 46, 92, 93, 116, 125]. In approximately 30% of patients with PBC who are treated with UDCA a complete response is observed, characterized by normalization of liver enzymes, reduction of increased IgM levels up to 30–40% from baseline values, and stabilized or improved histologic findings [63]. In the remaining 70%, especially in

**Table 73.2** Drugs that have been used in primary biliary cirrhosis

| Drug(s) | Comments | Indication in PBC |
|---|---|---|
| **Glucocorticoid monotherapy** | May improve some laboratory parameters and have a positive effect on liver histology. Aggravates osteoporosis. | Not indicated. |
| **Azathioprine monotherapy** | No survival benefit. | Not indicated. |
| **Methotrexate monotherapy** | Mortality and transplantation rate 2.9 times higher than under placebo! | Not indicated. |
| **Chlorambucil monotherapy** | Lowers serum bilirubin levels. Long-term treatment leads to bone marrow suppression. | Not indicated. |
| **D-Penicillamine monotherapy** | No effect on PBC. | Not indicated. |
| **Colchicine monotherapy** | Improvement of some laboratory parameters, such as albumin and bilirubin. No effect on liver histology. High rates of therapy withdrawal. | Not indicated. |
| **Cyclosporine A monotherapy** | No survival benefit. Many side effects. | Not indicated. |
| **Tacrolimus monotherapy** | Too few data. | Not indicated. |
| **Ursodeoxycholic acid (UDCA)** | See text. | Indicated. |
| **UDCA plus colchicine** | Too few data. | Not indicated. |
| **UDCA plus methotrexate** | Only few data. No clinical or biochemical benefit. | Not indicated. |
| **UDCA plus prednisolone** | Few data. Possibly better effect on liver histology than UDCA monotherapy. | Possibly indicated. Further studies required. |
| **UDCA plus budesonide** | See text. | Possibly indicated. Further studies required. |
| **UDCA plus prednisolone plus azathioprine** | Fee data. | Possibly indicated as second line therapy in UDCA non-responders.Further studies required. |
| **Antiretroviral therapy (lamivudine plus zidovudine)** | Preliminary results in pilot studies suggest some histological and biochemical improvement, but too few data. | Not indicated. |
| **Statins** | Atorvastatin effectively reduces serum cholesterol levels, but no effect on cholestatic parameters in PBC patients. | Not indicated. |
| **Tamoxifen** | Only anecdotal data [107] | Not indicated. |

patients with high initial AP and γ-GT concentrations, response to UDCA is incomplete, i.e. AP and γ-GT levels fall, but do not normalize. UDCA has no impact on AMA-titers. UDCA therapy influences the process leading to bile duct destruction. A 2-year UDCA treatment reduced periportal necroinflammation and improved ductular proliferation, and when initiated at the earlier stages I-II of the disease also delayed the progression of histologic stage [19, 100]. UDCA treatment was associated with a fivefold lower progression rate from early stage disease to extensive fibrosis or cirrhosis (7% per year under UDCA vs. 34% per year under placebo) [15]. Overall, UDCA seems to improve the 10-year prognosis for most UDCA-treated patients with PBC as manifested by increased survival rates and prolonged survival free of liver transplantation. Ten year survival of UDCA-treated PBC patients with normal bilirubin and albumin serum concentration is comparable to that of a matched general population [93, 125].

There is some evidence, but no proof that combining UDCA with antiinflammatory and immunosuppressive agents might improve treatment results compared to UDCA monotherapy. In one study, after 1 year therapy with UDCA and prednisolone, improvement of liver histology was more pronounced in the combination group than in patients treated with UDCA only [61]. Corticosteroid related side effects, especially osteoporosis limit long-term treatment.

Budesonide is characterized by a high hepatic first-pass effect, with 90% of the drug being metabolized in the liver and only 10% reaching the systemic circulation. In cirrhotic patients with PBC the pharmacokinetics and pharmacodynamics of budesonide are altered, and administration of budesonide leads to markedly elevated plasma levels with serious adverse

drug reactions [34]. This effect is not present in earlier stages of PBC nor do multidrug resistance 1 gene polymorphisms affect disposition of budesonide in early PBC [21, 105]. The effects of combination therapy with UDCA and budesonide are controversial. In one US study oral budesonide appeared to add minimal, if any, additional benefit to UDCA in patients with prior suboptimal response to UDCA, and was associated with a significant worsening of osteoporosis [5]. In another European trial the combination of UDCA (10–15 mg/kg p.o. daily) and budesonide (3 mg p.o. tid) improved liver histology and laboratory findings compared to UDCA monotherapy [62]. However, patients with a suboptimal initial response to UDCA benefited only marginally from the later addition of budesonide. Thus, combined treatment with budesonide and UDCA may be considered in early-stage PBC but not in cirrhotic patients [106]. It is recommended that bone mass density is monitored during budesonide therapy.

Whether or not adding prednisone and azathioprine to UDCA has an additional beneficial effect on symptoms and biochemical, fibrogenetic and histological parameters is controversial. It remains to be determined whether or not this triple therapy represents a second line option in UDCA nonresponders [135]. Azathioprine may be substituted for mycophenolate mofetil since azathioprine is a potential risk factor for the development of nodular regenerative hyperplasia. One must remember, however, that the evidence supporting this combination treatment is very weak.

Two studies suggested histological and biochemical improvements with antiretroviral therapy combining lamivudine 150 mg p.o. bid and zidovudine 300 mg p.o. bid (Combivir®) [73, 74]. However, this approach is experimental and should not be used in clinical practice.

Statin therapy may target both hypercholesterolemia and cholestasis in PBC. However, despite effectively reducing serum cholesterol levels atorvastatin does not improve cholestasis in PBC patients with an incomplete biochemical response to UDCA [120].

Pruritus in PBC patients may be severe and agonizing, and therapeutic options are very limited. Recently improvements of pruritus with sertraline and extracorporeal albumin dialysis were described in two small trials [9, 94].

In a placebo controlled trial alendronate (70 mg p.o. per week over 1 year) significantly improved PBC-related bone loss and was more effective than etidronate for increasing bone mass in osteopenic patients with PBC [32, 141]. (For treatment of pruritus, osteoporosis and vitamin substitution in chronic cholestatic liver disease see also Chapter 52.)

Fatigue remains a major clinical problem in patients with PBC and no specific therapies are available. In recent trials only modafinil (started at a dose of 100 mg/day and titrated according to tolerability and response) showed some improvement in excessive daytime somnolence and associated fatigue in PBC [45]. Ondansetron and fluoxetine did not improve fatigue in PBC [123, 127].

Even with optimal drug treatment PBC is a progressive disease and in most younger patients *liver transplantation* must be considered, which is the treatment of choice in end-stage PBC. A Mayo-Risk-Score of 7.8 seems to correlate best with the optimal timing for transplantation [54]. Intractable pruritus and severe osteoporosis also are indications for transplantation. Recurrence rates of PBC in the transplanted liver increase with time after transplantation, from 8–18% at 5 years to 22–30% at 10 years after transplantation [18, 86]. Progression of PBC in the transplanted liver, however, is very slow. Thus, the concern of disease recurrence in the liver graft should not deprive the patient from this valuable treatment option.

Interestingly, the need for liver transplantation in PBC decreased in the last 10–15 years, despite an increase in the total number of liver transplants and no change in transplant rates for primary sclerosing cholangitis [60, 86]. The reasons for the fall in numbers transplanted for PBC are unclear, but it is tempting to speculate that this trend might be due to the efficacy of UDCA treatment.

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# Autoimmune Cholangitis

# 74

Henryk Dancygier

In 1987 Brunner and Klinge have described three women with the clinical, biochemical and histological features of primary biliary cirrhosis (PBC) without, however, the presence of serum antimitochondrial antibodies (AMA) [2]. Instead, these patients had antinuclear antibodies (ANA) in serum. The authors have called this condition "immune cholangitis".

Currently, autoimmune cholangitis (AIC) denotes a disease displaying the clinical and pathological features of PBC with absent AMA, but with positive tests for ANA [7, 13, 19]. More recently, a broader definition has been suggested that includes: (1) serum ANA and/or smooth muscle (SMA) positivity and/or hypergammaglobulinemia, (2) serum AMA negativity by immunofluorescence, (3) biochemical and/or histologic features of cholestatic and hepatocellular injury, and (4) exclusion of chronic viral, metabolic, or toxic liver disease [5, 17].

However, these definitions and the term autoimmune cholangitis is problematic, since they imply the existence of a distinct cholangiopathy (autoimmune cholangitis), while PBC, small-duct primary sclerosing cholangitis and idiopathic adulthood ductopenia may also be considered as (auto)immune cholangiopathies [10].

There is evidence to suggest that AIC is not a separate disease entity, but rather an AMA-negative PBC [11, 17, 18]. The epidemiological, clinical and histopathological features, as well as the response to treatment are similar in AIC and PBC [9, 12, 15]. AIC, like PBC may also be associated with extrahepatic immune disorders, such as systemic lupus erythematosus, antiphospholipid antibody syndrome and celiac disease [8, 14, 16]. The AST and bilirubin levels in serum seem to be higher, while the serum IgM concentrations lower in AIC than in PBC. However, the most important distinguishing feature is the autoantibody profile (see discussion of AMA-negative PBC in Chapter 73). Most PBC patients, initially negative for AMA on immunofluorescence turn out to be AMA positive as soon as more sensitive techniques for their demonstration are applied. Thus, the question – is it AIC or AMA-negative PBC? – may be focused on the sensitivity of the technique used in detecting AMA. Moreover, approximately 40% of patients with PBC have also PBC-specific ANA (see Chapter 73).

As long as an etiologic classification of immune cholangiopathies is not available, AIC should not be regarded as a distinct entity but rather a variant of PBC or a disease of the spectrum of overlap syndromes (see Chapter 77) [1, 3, 4, 6]. Thus, for the time being patients lacking detectable AMA, especially when indirect immunofluorescence is used, but otherwise presenting signs and symptoms of PBC should be regarded as affected by "AMA-negative PBC" or having an overlap between PBC and autoimmune hepatitis [9, 17].

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# Primary Sclerosing Cholangitis

# 75

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## Chapter Outline



## Definition

Primary sclerosing cholangitis (PSC) is a chronic fibro-obliterative inflammation of intra-and/or extrahepatic bile ducts of unknown origin. *Small duct primary sclerosing cholangitis* (formerly called *pericholangitis*) is an intrahepatic variant of PSC in patients with chronic cholestasis and hepatic histology compatible with PSC, but normal findings on endoscopic retrograde or magnetic resonance cholangiography.

## Epidemiology

Since it was first described by Delbet in 1924, fewer than 80 cases of PSC had been reported in the literature up to the mid-1970s. Not until the introduction and increasing use of endoscopic retrograde cholangiography (ERC) did the numbers of patients diagnosed with PSC began to rise continuously.

The epidemiological characteristics of PSC vary somewhat between different countries. A recent study in white Americans reported an incidence of PSC in men of 1.25 per $10^5$ person years compared with 0.54 per $10^5$ person years in women. The prevalence of PSC was 20.9 per $10^5$ men and 6.3 per $10^5$ women. Both incidence and prevalence of PSC were approximately one third of those previously described for primary biliary cirrhosis in the same population [4]. In a population-based study from Scandinavia the incidence of PSC was 1.3 per $10^5$ person years and the prevalence 8.5 per $10^5$ persons [12]. A recent epidemiological study from the UK reported an incidence of 0.4 per $10^5$ person years and a prevalence of 3.8 per $10^5$ [15]. All epidemiological investigations agree that approximately two thirds of

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the affected patients are men, most commonly in their third and fourth decade at presentation. PSC, however, is also recognized in children and adolescents [20]. First-degree relatives of patients with PSC have a disease prevalence of 0.7%, equivalent to a nearly 100-fold increased risk of developing PSC compared with the general population [8].

Approximately 70–80% of PSC cases are associated with inflammatory bowel disease (IBD), particularly (up to 90%) with ulcerative colitis [4]. On the other hand 5–6% of patients with ulcerative colitis have concurrent PSC. Generally IBD precedes PSC. There seems to be no close association between the inflammatory activity of IBD and the severity of PSC.

## Etiology and Pathogenesis

The etiology and pathogenesis of PSC are unknown. Toxic, infectious, genetic and (auto)immune factors have been discussed widely but their role remains speculative [1, 41, 52].

Hypotheses incriminating chronic portal bacteremia and/or toxic bile acids in damaging the biliary system have not been confirmed. The markedly increased copper concentration in liver tissue found in patients with PSC does not seem to trigger the disease process but rather is a secondary storage phenomenon that may be observed regularly in chronic cholestatic liver disease.

Animal experiments and serological findings in neonates with biliary atresia suggest that viral infections (reovirus type 3, cytomegalovirus) may elicit a fibro-obliterative cholangiopathy. AIDS-cholangiopathy is another example of infectious agents (cryptosporidium parvum, microsporidia, cytomegalovirus) causing a PSC-like cholangiopathy. However, a direct causal relationship between an infectious agent and PSC has not yet been established.

Various immunological findings may be recorded in PSC patients. A wide variety of nonspecific autoantibodies may be detected in serum of patients with PSC [2]. Increased levels in blood and bile of circulating immune complexes and secretory immunogobulins, a prolonged clearance of immune complexes from the circulation, decreased numbers of circulating T lymphocytes particularly due to diminished CD8 cells, and a reduced CD4/CD8 ratio within the liver tissue have also been documented in PSC patients. Inhibition of leukocyte migration by bile duct epithelial constituents and an increased expression of HLA-DR antigens on bile duct epithelia in the early stages of PSC also corroborate the idea of PSC as an immune-mediated disease. All these findings, however, are nonspecific and represent immunologic epiphenomena in chronic inflammation rather than pathophysiologically significant reactions.

The importance of genetic factors is underscored by the increased prevalence of PSC among first-degree relatives (see above) [8]. MHC-haplotypes with an increased risk of PSC include several risk alleles such as MHC class I chain-like α-molecules (MICA)*008, DRB1*0301, DRB1*1301 or DRB1*1501, with the strongest association being found for the MICA*008-homozygosity [52]. Combinations of killer immunoglobulin-like receptors and HLA class I ligands that reduce natural killer (NK) cell inhibition have been shown to increase the risk for autoimmune diseases. Particular variants of ligands for NK cell receptors encoded at three neighbouring genes in the HLA complex may contribute to the HLA associated risk of PSC [23].

## Pathology

PSC may affect the entire biliary system from interlobular bile ducts to the ampulla of Vater. Macroscopically the bile ducts appear thickened with their lumen being irregularly narrowed. Bile duct ectasias of various degree occur even without the presence of nearby duct strictures. The chronic inflammatory process may also involve the gallbladder, with dysplasia and carcinoma of the gallbladder being more prevalent in the PSC population [43].

PSC probably initiates as an inflammation of the interlobular bile ducts. This small duct variant of PSC (formerly called pericholangitis) was first recognized in 1985 [51]. It is characterized by a marked periductal edema and a dense portal inflammatory infiltrate consisting of plasma cells, lymphocytes and polymorphonuclear leukocytes. Not uncommonly the portal inflammatory process leads to interface hepatitis with the formation of so-called piece meal necroses which may be difficult to differentiate from chronic hepatitis of autoimmune or viral origin [29, 31]. The inflammatory changes in PSC are irregularly distributed, portal and periportal inflammation being focal with varying severity in different locations. Like in the early stages of primary biliary

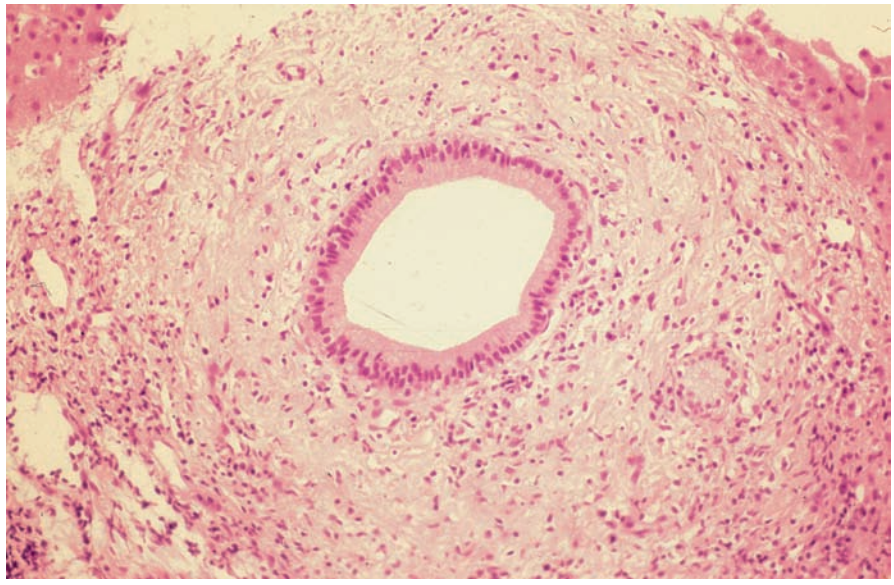

**Fig. 75.1** Concentric peribiliary fibrosis in primary sclerosing cholangitis. The histological finding is nonspecific and does not allow for differentiating from secondary sclerosing cholangitis. Hematoxylin and Eosin

cirrhosis (PBC) the portal tracts in PSC may also contain lymphoid follicles. Portal granulomas occur much more rarely in PSC than in PBC. Despite PSC being primarily a mural inflammatory process, the cholangiocytes as well may display alterations ranging from degenerative and atrophic changes to cell death [18]. Loss of peribiliary vascular plexus has been described in the early stages of PSC and is probably secondary to the inflammatory process [50]. It is unknown whether or not ischemia contributes to the formation of bile duct strictures in PSC.

During the further course of the disease, portal cellular infiltrates diminish with a concentric, onion-skin like, peribiliary fibrosis becoming increasingly prominent (Fig. 75.1). Finally, the chronic fibro-obliterative process transforms the bile ducts into fibrous cords which are either solid or contain remnants of bile duct epithelium. Complete focal loss of bile ducts may be observed in advanced stages of PSC [30]. The final stage of PSC is represented by secondary biliary cirrhosis. A histological staging system for PSC is reported in Table 75.1 [29].

**Table 75.1** Staging of primary sclerosing cholangitis

| | |
|---|---|
| Stage 1 | Portal cholangitis |
| Stage 2 | Periportal hepatitis and/or fibrosis |
| Stage 3 | Septal fibrosis and /or bridging necrosis |
| Stage 4 | Cirrhosis |

Source: According to Ludwig et al. [29]

## Diagnosis

The diagnosis of PSC is based on the typical cholangiographic appearance of the bile ducts in a patient with a cholestatic laboratory profile, and on the exclusion of secondary causes of chronic cholangitis. If small duct PSC is suspected liver biopsy is required.

### *Clinical Manifestations*

PSC usually develops insidiously as a chronic smouldering disease initially unnoticed by the patient and may be in an advanced stage by the time it becomes clinically apparent. The main signs and symptoms are reported in Table 75.2. Increasing fatigue, malaise and

**Table 75.2** Symptoms and signs in primary sclerosing cholangitis

| | |
|---|---|
| Jaundice | 61% |
| Pruritus | 49% |
| RUQ-pain | 42% |
| Weight loss | 38% |
| Fever | 36% |
| Hepatomegaly | 48% |
| Esophageal variceal bleeding | 9% |
| Ascites | 6% |
| Asymptomatic | 19% |

*RUQ* Right upper quadrant

pruritus are usually followed by the appearance of jaundice. On average at the time of diagnosis the symptoms date back 2 years in 80% of patients. With the increasing use of cholangiography (ERC and MRC) in patients with a chronic cholestatic laboratory profile, the proportion of PSC patients who are asymptomatic at diagnosis has increased from 7–10% to 25–44% [37, 53]. While PSC is a chronic disease with protean symptoms in the vast majority of patients, in approximately 1% of all patients it can present with acute liver failure [9]. In long-standing PSC signs and symptoms of malabsorption of fat soluble vitamins and of chronic hepatic failure may supervene.

### Extrahepatic Diseases

Like other autoimmune diseases PSC also may be associated with extrahepatic diseases. Immune thyroiditis, rheumatic disorders, sicca syndrome, and retroperitoneal fibrosis have been reported [1, 37, 53]. Occasionally, irregularly narrowed lesions of the pancreatic duct may also be observed. In these cases an autoimmune pancreatitis should be considered (see below). The association of PSC with IBD, particularly ulcerative colitis has been mentioned above. In addition to an increased prevalence of cholangiocarcinoma, patients with longstanding PSC also have an increased risk for colorectal, pancreatic, gallbladder and hepatocellular carcinoma compared with that of the general population (see below) [7].

## Laboratory Findings

The main laboratory findings in patients with PSC are reported in Table 75.3. A marked and long-standing increase in alkaline phosphatase and γ-glutamyl transpeptidase serum levels with only minimally elevated serum aminotransferase concentrations is a typical but nonspecific finding. In very few patients with typical bile duct changes on ERC, serum alkaline phosphatase levels remain normal while aminotransferase concentrations are mildly elevated. The erythrocyte sedimentation rate, although a very nonspecific indicator of systemic inflammation, together with anemia of chronic disease, can provide an indication of PSC activity. Four percent of PSC patients have a peripheral blood eosinophilia.

**Table 75.3** Serum laboratory findings in primary sclerosing cholangitis

| | |
|---|---|
| ↑ Alkaline phosphatase | 98% |
| ↑ Aminotransferases | 90% |
| ↑ Bilirubin | 57% |
| ↑ Immunoglobulin M | 39% |
| ↓ Albumin | 28% |
| pANCA positive | 20–80% |
| ANA positive | 25% |
| AMA positive | 15% |

Patients with PSC have increased antibody titers to a wide range of autoantigens (Table 75.3). Antinuclear antibodies have been described in 7–77%, anticardiolipin antibodies in 4–66%, anti-smooth muscle antibodies in 13–20%, anti-thyroid peroxidase antibodies in 16% and rheumatoid factor in 15% of patients with PSC [2]. pANCA occurs in 20–80% of patients and has been thought of as more specific for PSC than other autoantibodies. However, all the serum autoantibodies are nonspecific and are not required for diagnosing PSC. Moreover, there is no correlation between the titers of autoantibody (including pANCA) and clinical parameters, so they are not helpful in clinical management.

## Imaging Tools

ERC is the diagnostic technique of choice and despite the rapid advancement of magnetic resonance technology it is still regarded in many centers worldwide as the gold standard in the diagnosis of PSC. One should be aware, however, that *even mild cholangiographic bile duct changes are not equivalent to early disease but rather represent long-standing cholangitis*. The typical cholangiographic pattern presents as irregularly placed multiple bile duct strictures and ectasias of varying length and severity (Figs. 75.2 and 75.3). The hepatic bifurcation is the region that is most often and most severely affected. An increasingly diffuse rarefaction of bile ducts may be observed in advanced stages. So-called high grade dominant stenoses may be superimposed on diffuse bile duct sclerosis in approximately 10% of cases. If cholangiography is technically adequate, lesions of intrahepatic bile ducts may be visualized in virtually all patients, while the extrahepatic bile ducts are spared in up to 20% of PSC patients. Cholangiographic classification systems have not attained relevance in the management of patients with PSC.

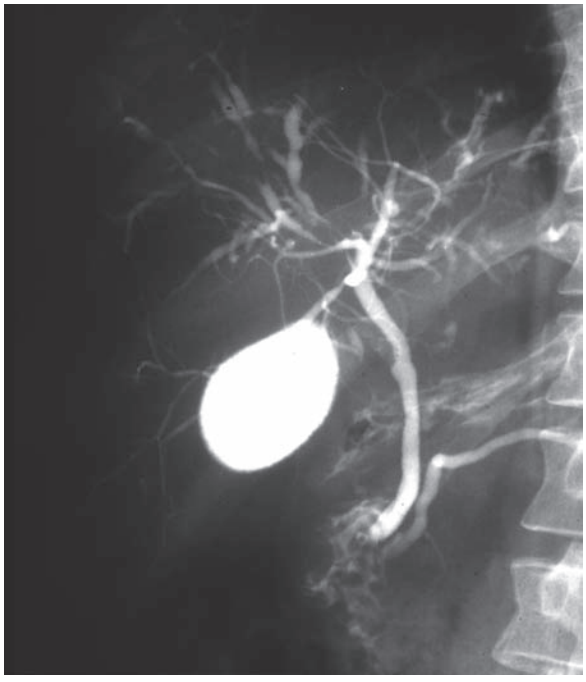

**Fig. 75.2** Endoscopic retrograde cholangiography in primary sclerosing cholangitis. Multiple irregularities of intra- and extrahepatic bile ducts are seen

Transcutaneous and endoscopic ultrasound, intraductal ultrasound, CT scanning, MRI of the liver, and nuclear functional imaging with 99m-technetium imidoacetate show no characteristic changes and yield nonspecific findings. These techniques contribute little, if at all, to the diagnosis of pre-cirrhotic PSC, but ultrasound, CT and MRI are helpful in ruling out space occupying lesions as a cause for elevated alkaline phosphatase levels.

These methods also are not helpful in the early detection of cholangiocellular carcinoma that may develop as a complication of PSC. Recently, FDG-PET has been reported superior to conventional imaging techniques for both the detection and exclusion of cholangiocellular carcinoma in patients with advanced PSC, but further studies must be conducted before this costly imaging modality can be recommended in the routine diagnostic work-up of patients with PSC [39].

There is no specific histological picture of PSC and, as mentioned above, the inflammatory changes are irregularly distributed. Therefore, with the exception of small duct PSC, the diagnostic importance of liver biopsy in PSC generally is limited [29]. However, laparoscopy and (laparoscopic or percutaneous) liver biopsy are helpful in staging PSC.

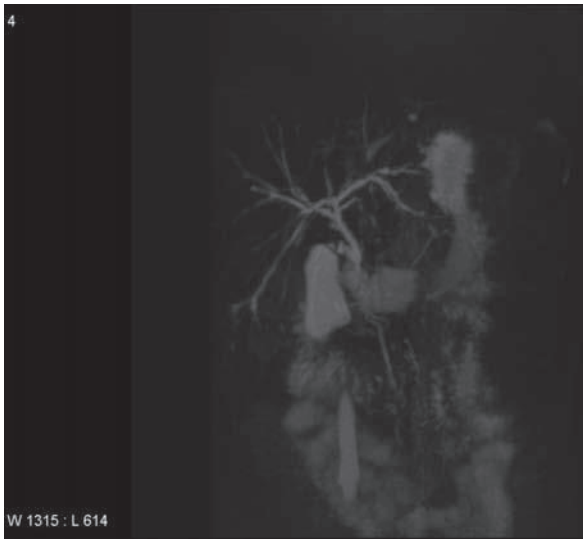


**Fig. 75.3** Magnetic resonance cholangiography. Mild primary sclerosing cholangitis

## Differential Diagnosis

The differential diagnosis of PSC includes all chronic cholestatic liver disorders, particularly those leading to secondary sclerosing cholangitis (see Chapter 52, Table 52.1). Due to their relatively high prevalence, however, first and foremost intraductal stones, *postoperative bile duct strictures, cholangiocarcinoma* (Klatskin tumor), and PBC have to be excluded. *Ischemic cholangiopathy* in critically ill patients following circulatory shock (e.g., in polytraumatized and septic patients) should be considered increasingly more often in the differential diagnosis [21].

Isolated *small duct PSC* is diagnosed in patients with inflammatory bowel disease showing a cholestatic laboratory profile, biopsy features compatible with PSC, but a normal cholangiogram [31]. If ductopenia (absence of interlobular bile ducts in ≥50% of portal tracts) is found histologically in the absence of PSC, inflammatory bowel disease, and other specific cholestatic syndromes such as drug reaction or sarcoidosis, the most likely diagnosis is *idiopathic adulthood ductopenia* (see Chapter 76) [26].

*Drug-induced liver injury* usually does not present with a PSC-like pattern on cholangiography. Bile duct lesions due to local intraarterial (hepatic artery) infusional chemotherapy with 5-fluorouracil and floxuridine are exceptions and may lead to the formation of ischemic bile duct strictures.

The bile duct lesions on ERC in *PBC stage IV* may resemble PSC-induced changes. PBC primarily affects women, while two thirds of PSC patients are men. Moreover, the characteristic antibody profile of PBC is absent in PSC.

*Biliary infections* in AIDS patients, for example with cryptosporidia, microsporidia and cytomegalovirus may lead to cholangiographic bile duct changes identical to those of PSC. The clinical constellation of a cholestatic laboratory profile with bile duct irregularities on ERC in a patient with a known HIV-infection is characteristic of *AIDS-cholangiopathy*.

*Immunglobulin $G_4$ associated cholangitis* (IAC) appears to be a newly emerging clinical entity that often is associated with autoimmune pancreatitis (AIP), and patients with unexplained biliary strictures associated with elevated serum $IgG_4$ concentrations should also be examined for the presence of AIP [22, 33, 49]. Patients with IAC more often have a rather abrupt clinical presentation with obstructive jaundice in comparison with patients with classic PSC [10]. Portal inflammation with or without interface hepatitis, portal sclerosis, and large bile-duct obstructive features may be present. Corticosteroid therapy reduces $IgG_4$-bearing plasma cell infiltration in the liver, and in the majority of patients requiring biliary stents these can be removed after successful corticosteroid treatment.

Biliary strictures occurring *after orthotopic liver transplantation* may resemble ischemic type biliary lesions. They are, however, not caused by ischemia but probably result from bile duct damage induced by biliary debris and biliary casts.

*Rare differential diagnoses* include, for example, Langerhans cell histiocytosis, mast cell cholangiopathy, angioimmunoblastic lymphadenopathy, local irradiation and congenital immune deficiency syndromes that may lead to a PSC-like bile duct pattern in children (see Table 52.1) [5, 6, 40].

Differentiating a *central cholangiocarcinoma* (Klatskin tumor) spreading along the bile ducts from PSC may be extremely difficult.

## Course and Prognosis

PSC is a chronic, progressive disease that leads to secondary biliary cirrhosis and chronic hepatic failure without adequate treatment. It carries an increased risk of recurrent bacterial and fungal (candida species) biliary infections, of forming intraductal stones and, most notably, of developing malignancies [1, 4, 7, 14, 15, 27, 37, 48].

Survival among children, adolescents and adults with PSC is significantly less than expected for the general population of similar age and gender [4, 20]. In a recent (2008) epidemiological study from the United Kingdom, mortality rate was increased three-fold in people with PSC compared to the general population [15]. The estimated median survival from the time of diagnosis to death or liver transplantation of patients who are symptomatic at the time of diagnosis is approximately 10–12 years, while 93% of patients who are asymptomatic at the time of diagnosis still live longer than 10 years. Age, low albumin, persistent bilirubin elevation for longer than 3 months (>4 times the upper limit of normal), hepatomegaly, splenomegaly, dominant bile duct stenosis, and intra- and extrahepatic ductal changes at the time of diagnosis are independent risk factors correlating with poor prognosis [48]. A Mayo model for predicting survival in PSC is available at http://www.mayoclinic.org/gi-rst/mayomodel3.html.

The most important complication of PSC is cholangiocarcinoma (CCA). It occurs in 6–20% of patients after a mean follow-up of approximately 11–15 years, with a yearly incidence of approximately 1%. The prevalence of CCA in autopsy studies is as high as 30–40% [1, 4, 14, 37, 48]. Approximately one third of biliary malignancies are diagnosed within 1 year after the diagnosis of PSC [7].

The early detection of a CCA associated with PSC is a diagnostic challenge and even with a high index of suspicion is extremely difficult, as the numerous inflammatory bile duct strictures are indistinguishable from CCA on cholangiography. Rapidly developing dominant bile duct strictures and a worsening of the general state of the patient may be clues to the development of a biliary malignancy. Screening patients with PSC with serum tumor markers (e.g., carcinoembryonic antigen, carbohydrate antigen 19-9) is not helpful. Brush cytology from dominant strictures and

bile cytology yield unsatisfactory results with low sensitivities and predictive values. There is also no additional diagnostic benefit from p53 immunocytochemistry and K-ras mutation analysis [38]. High resolution (12.5–20 MHz) intraductal ultrasound with miniprobes introduced into the bile ducts also is not able to differentiate between benign and malignant strictures.

In addition to CCA, the risk of hepatocellular carcinoma is increased 40 times, that of pancreatic carcinoma 14 times, and that of colorectal carcinoma 10 times in patients with PSC compared to the general population [7, 15].

The natural history of small duct PSC (SD-PSC) differs from that of large duct PSC. SD-PSC is a disease of progressive potential but associated with a better long-term prognosis. The survival of patients with isolated SD-PSC is similar to that of the general population, and the risk of CCA in these patients is not increased. In a small proportion of patients SD-PSC may progress to large duct PSC. SD-PSC may also recur after liver transplantation [3, 11].

## Therapy

Biliary tract infections in patients with PSC are treated with antibiotics or antifungals. Deficiency states due to malabsorption are corrected appropriately (see Chapters 52 and 80).

An effective *drug treatment* for PSC is not available. Corticosteroids, colchicine, methotrexate, azathioprine, mycophenolate mofetil, tacrolimus, penicillamine, and metronidazole have no effect on the natural course of the disease [19, 46, 47].

Only ursodeoxycholoic acid (UDCA) has shown some promise. UDCA 13–15 mg/kg p.o. daily reduces the serum levels of cholestatic liver enzymes in some patients. However, a beneficial effect of UDCA on the natural course of PSC is not proven [28, 35].

The administration of high-dose UDCA (20–30 mg/kg p.o. daily) is well tolerated, safe and leads to biliary enrichment of UDCA [42]. Although results are not uniform, high-dose UDCA may be of clinical benefit in PSC and may be associated with an improvement in survival. However, trials with a large number of participants and of long duration are currently lacking and are required to establish whether the effect of high-dose UDCA on liver biochemistry, histology, and cholangiography in patients with PSC is translated into improved long-term survival [17, 34, 36]. UDCA also improves liver biochemistries in SD-PSC. As in large duct PSC it is not clear whether UDCA delays disease progression in SD-PSC [16].

Results of *endoscopic* or *percutaneous dilatation* of dominant stenoses are promising and repeated dilatation with opening of dominant stenoses appears to slow the progression of PSC [44, 45]. However, a beneficial effect on survival has yet to be demonstrated.

The only life saving procedure for patients with PSC associated cirrhosis is *orthotopic liver transplantation*. The 5-year survival rates are approximately 75% and even PSC patients with CCA have a 35% 5-year survival following transplantation [13]. Xanthogranulomatous cholangiopathy (XGC) at the hilum of the native liver occurs in 20–30% of patients with PSC and negatively impacts graft and patient survival. Unfortunately there are no clinical features or laboratory tests to identify the presence of XGC prior to transplantation [24, 25].

Compared to patients with PBC, PSC patients have higher retransplantation and lower survival rates. Preemptive liver transplantation in PSC, for example, aiming to prevent the development of a CCA, is not recommended [32].

For treatment of CCA see Chapter 116.

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# Biliary Ductopenia (Vanishing Bile Duct Syndrome)

# 76

Henryk Dancygier

## Chapter Outline



## Definition

Vanishing bile duct syndrome (VBDS) encompasses a group of different cholestatic liver diseases characterized by the destruction and permanent loss of predominantly interlobular bile ducts. If more than 50% of portal tracts in an adequately large biopsy specimen do not contain a bile duct, then VBDS is present. VBDS may be idiopathic or secondary to other causes.

## Epidemiology

Due to the rarity of idiopathic VBDS reliable data on the incidence and prevalence of idiopathic adulthood ductopenia (IAD) is not available. An analysis of approximately 2,000 patients with small bile duct disease gives an idea of the frequency of ductopenia. Sixty-five percent of cases fell upon primary biliary cirrhosis, 28% on primary sclerosing cholangitis, 1.2% on idiopathic VBDS and 0.5% on drug-induced biliary ductopenia [8]. The median age at diagnosis is in the third and fourth decade of life and IAD occurs with a distinct male preponderance [6–8, 14]. Manifestations of IAD late in life, however, have also been reported [10].

## Etiology and Pathogenesis

The causes of VBDS are reported in Table 76.1. The common pathophysiological denominator of all conditions is the progressive loss of interlobular bile ducts. The pathophysiological pathways leading to bile duct loss, however, differ according to the underlying condition.

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**Table 76.1** Causes of biliary ductopenia (vanishing bile duct syndrome)

| Cause/disease | Comment |
|---|---|
| **Congenital, developmental and genetic** | |
| Biliary atresia | See Chapter 56 |
| Alagille's syndrome | See Chapter 85 |
| $\alpha_1$-Antitrypsin deficiency | See Chapter 83 |
| Cystic fibrosis | See Chapter 86 |
| Progressive familial intrahepatic cholestasis | See Chapter 85 |
| Zellweger syndrome | Congenital absence of peroxisomes. Anomalies in bile acid metabolism |
| Trisomy [17, 18, 21] | |
| **Immunologic** | |
| Primary biliary cirrhosis | See Chapter 73 |
| Primary sclerosing cholangitis | See Chapter 75 |
| Cholestatic overlap syndromes | See Chapter 77 |
| Sarcoidosis | Granulomas occur preferentially in the portal and periportal areas. In a few cases granulomatous destruction of interlobular bile ducts occurs. The clinical manifestations in these patients resemble those of primary biliary cirrhosis |
| Acute and chronic hepatic cellular rejection | The bile ducts are the targets of immune defense. The bile duct lesions usually are reversible with adequate immunosuppressive therapy. However, in a small percentage of patients destruction of bile ducts continues despite immunosuppression. Ischemic factors, in addition to immune reactions possibly are also implicated in bile duct injury |
| Chronic graft versus host disease | After bone marrow transplantation a few patients develop progressive bile duct loss |
| Stevens–Johnson syndrome | Usually drug-related |
| Macrophage activating syndrome [1] | Histologically, liver involvement is characterized by acute lobular hepatitis, marked hepatocyte apoptosis and small bile duct injury (see Chapters 52 and 97) |
| **Vascular (ischemic/hypoxic)** | |
| Thrombosis of hepatic artery | |
| Traumatic injury | |
| Intraarterial infusion | Anti-tumor chemotherapy (5-FU, FUDR), transarterial chemoembolization (TACE) |
| Vasculitis | 1. Collagen vascular disease, for example, polyarteritis nodosa<br>2. Irradiation damages the endothelium of peribiliary arterial plexuses leading to secondary bile duct injury. A direct damage of small bile ducts by irradiation seems also possible |
| Ischemic cholangiopathy [3] | Rarely sclerosing cholangitis like lesions following septic shock may result in loss of bile ducts and rapidly progress to cirrhosis |
| **Infectious** | |
| Cytomegalovirus<br>Reovirus 3<br>Rubella virus | Neonatal exposure has been implicated in biliary ductopenia |
| Cryptosporidium parvum | AIDS-cholangiopathy |
| Echinococcus granulosus | Rupture of hydatid cysts may lead to a circumscribed loss of bile ducts, not to a true VBDS |
| Hepatitis B virus | Fibrosing cholestatic hepatitis in patients after liver transplantation |
| Hepatitis C virus | Questionable. Only anecdotal reports in renal transplant recipients with HCV infection |
| Epstein-Barr virus | Very rare |
| **Drug-induced** | See Table 76.2 |
| **Neoplastic** | |
| Langerhans' cell histiocytosis | see Chapter 97 |
| Hodgkin's disease | Hepatic involvement in Hodgkin's disease is seen in up to 50% of autopsy cases. Cholestasis with bile duct loss is a very rare presentation of Hodgkin's disease |
| **Idiopathic** | |
| Idiopathic adulthood ductopenia | |

**Table 76.2** Drugs reported to cause chronic cholestasis and ductopenia [2, 11]

- Ajmaline
- Amineptine
- Amitryptyline
- Amoxicillin/clavulanic acid[a]
- Ampicillin
- Androgenic anabolic steroids
- Arsenic derivatives
- Azathioprine
- Barbiturates
- Carbamazepine
- Carbutamide
- Chlorothiazide
- Chlorpromazine
- Cimetidine
- Clindamycin
- Co-trimoxazole
- Cromolyn sodium
- Cyamemazine
- Cyclohexyl proprionate
- Cyproheptadine
- Diazepam
- Erythromycin
- Estradiol
- Flucloxacillin
- Glibenclamide
- Glycyrrhizin
- Haloperidol
- Ibuprofen
- Imipramine
- Methyltestosterone
- Norandrostenolone
- Phenylbutazone
- Phenytoin
- Prochlorperazine
- Tetracyclines
- Thiabendazole
- Tiopronin
- Tolbutamide
- Trioleandomycin
- Xenalamine

[a]Cholestasis may appear *after* the drug has been withdrawn

In newborns and infants, developmental and genetic factors as well as malformations of the ductal plate predominate, possibly due to viral infections or metabolic factors [13].

Immune mechanisms, hypoxic/ischemic factors and drugs predominate in adults. While (auto)immune cholangiopathies, such as primary biliary cirrhosis and primary sclerosing cholangitis are well recognized causes of VBDS, permanent loss of bile ducts in chronic ascending bacterial cholangitis is rather the exception.

In contrast to hepatocytes, which receive a dual blood supply via the hepatic artery and the portal vein, the bile ducts are supplied solely by the branches of the hepatic artery. Cholangiocytes are therefore more susceptible to ischemic injury than hepatocytes [13].

Individual drugs only rarely lead to VBDS. Due to their frequent worldwide use, however, drugs always must be taken into consideration as a potential cause of VBDS (Table 76.2) [2, 11].

It is possible that viral infections and immune reactions in genetically susceptible individuals are important in IAD [12, 14]. White probands with the *DRB1*1502* allele seem to have an increased susceptibility to VBDS and multiple HLA-DRB1 alleles (**04, *11, *15*) occur more often in VBDS patients [4].

## Pathology

A portal inflammatory and fibrotic process leading to the destruction and loss of interlobular bile ducts is the histological hallmark of ductopenia (see paragraphs on Pathology in Chapters 73 and 75) [5]. By definition, VBDS is present if interlobular bile ducts are absent in at least 50% of portal tracts (normally each hepatic arterial branch is accompanied by at least one portal bile duct profile) [7]. The irregular distribution of lesions may impair the diagnostic accuracy of a liver biopsy and an adequately large tissue sample is mandatory for the diagnosis of VBDS. In order to avoid a sampling error, at least 11–20 portal tracts are required for accurate semiquantitative assessment, paying special attention to bile duct(s) accompanying the hepatic artery branches. An adequate biopsy size is 3 cm in length and 16 gauge in caliber. Diagnostic accuracy declines with specimen size, especially when smaller than 2 cm in length [11]. Alternatively, several biopsies may be easily obtained during diagnostic laparoscopy (see Chapter 45). The diagnosis can be established on routine hematoxylin and eosin sections. However, on PAS stained sections after pretreatment with diastase, the basement membranes of bile ducts (which are PAS positive and diastase resistant) are well visualized and even small bile ducts lying within an inflammatory infiltrate may be discerned easily. Immunocytochemical demonstration of cytokeratins 7 and 19 also allows for

an excellent identification of interlobular bile ducts. Ductular proliferation may coexist with interlobular duct loss, and ductules must be distinguished from bile ducts [11].

## Diagnosis

### *Clinical Manifestations*

A literature review of 39 cases of IAD reported a median age at diagnosis of 27 years (range 15–67 years), and most patients presented with a progressive cholestatic clinical picture with fatigue, episodic jaundice, pruritus and occasional fever [6]. A mild apparently nonprogressive clinical course with no symptoms of liver disease has also been reported [9].

### *Laboratory Findings*

Nonspecific elevations of alkaline phosphatase, γ-glutamyl transpeptidase and alanine aminotransferase levels are observed. There is no specific autoantibody profile of VBDS.

### *Imaging Techniques*

The diagnosis of VBDS is made histologically on large liver biopsy specimen (see above) or in the explanted liver. Ultrasound, CT scanning and MRI do not yield significant diagnostic findings in the precirrhotic stages.

## Differential Diagnosis

The differential diagnosis has to consider all the conditions listed in Table 76.1.

## Course and Prognosis

Apparently two different clinical courses of IAD exist. While one half of the patients progresses relatively rapidly to cirrhosis and liver failure, the other half has a long lasting benign course with only mildly abnormal cholestatic parameters in serum and a cholangitic histologic picture, but with subjective well-being.

## Therapy

If the cause of VBDS is known, then the underlying illness is treated. Potentially incriminating drugs should be withdrawn. Some cases respond to lifelong, high dose (10–25 mg/kg) UDCA. Patients with rapidly progressive VBDS must undergo liver transplantation.

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# Autoimmune Overlap Syndromes

# 77

Henryk Dancygier

## Chapter Outline



The advancement of immunological, molecular biological, and morphological techniques as well as the introduction of diagnostic scoring systems allows for precisely allocating the majority of patients to one of the three major hepatobiliary (auto)immune diseases, i.e. autoimmune hepatitis (AIH), primary biliary cirrhosis (PBC) or primary sclerosing cholangitis (PSC). Up to 20% of these patients, however, share overlapping clinical, histological, and immunological features that do not allow for establishing a precise diagnosis [12]. These patients are said to suffer from a variant or overlap syndrome [10, 23]. The clinician should be aware, though, that this term does not define an exact diagnosis, but rather results from our still unsatisfactory understanding of the precise etiology and pathogenesis of autoimmune liver diseases.

The degree of overlap may be minor with signs and symptoms of one disease clearly prevailing, or major with the clinical, serological and histological features of two diseases fully overlapping. In addition to coexistence, two diseases may appear sequentially during follow up. The evolution of one autoimmune liver disease to another does not represent a true overlap, but a sequential syndrome.

The knowledge of variant (overlap) syndromes of autoimmune liver disease is not only interesting diagnostically, but also has therapeutic implications. Predominantly cholestatic overlap syndromes are generally more responsive to ursodeoxycholic acid (UDCA) than to immunosuppressive therapy, while hepatitic overlap syndromes respond to immunosuppressive treatment with corticosteroids and azathioprine. If both components, cholestatic and hepatitic, are present in equal measure combination therapy of UDCA and immunosuppressive drugs is warranted.

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## Overlap Between AIH and PBC

PBC–AIH overlap syndrome is a clinical entity characterized by the occurrence of both conditions at the same time in the same patient. It occurs in approximately 5–13% of patients with AIH, and in approximately 19% of patients diagnosed with PBC [7, 9, 12, 17]. In addition to the serological features of AIH (elevation of alanine aminotransferase > 5 × the upper limit of normal (ULN), IgG > 2 × ULN, typical autoantibody profile), these patients have elevated alkaline phosphatase and IgM levels in serum and bile duct lesions on histologic examination. In two thirds of cases ANA and SMA occur in addition to AMA. Moreover, the presence of anti-SLA/LP in patients with PBC is highly specific for AIH-PBC overlap [15]. Patients with true AIH–PBC overlap syndrome usually respond well to immunosuppressive therapy (prednisone plus azathioprine) combined with UDCA [8].

In addition to PBC–AIH overlap syndrome, a rare *sequential syndrome of AIH superimposed on PBC* can occur. In a Mayo Clinic series of 1,476 patients with PBC who had no features of AIH, only eight patients developed AIH over a 9-year period [14]. The development of superimposed AIH cannot be predicted from baseline characteristics and initial response of PBC to UDCA therapy. If not detected and treated early, superimposed AIH can result in rapid progression toward cirrhosis and liver failure in PBC patients. Most patients respond well to treatment with prednisone plus azathioprine [19, 21].

*Autoimmune cholangitis* may be regarded as an overlap between PBC and AIH, a subtype of AIH type I with cholestatic features, or a distinct variant of PBC (AMA-negative PBC) (see Chapter 74) [3, 18].

## Overlap Between AIH and PSC

AIH–PSC overlap syndrome is rarer than overlap between AIH and PBC. The diagnosis is established in a patient with AIH and concomitant features of PSC, such as typical bile duct changes on ERCP or MRCP [1, 13]. The presence of an inflammatory bowel disease hints at the diagnosis of PSC.

If the scoring system for the diagnosis of AIH is applied to patients with PSC confirmed by cholangiography, a diagnosis of definite or probable AIH is made in approximately 1.4%–8% of cases, respectively [5, 6, 16, 22]. Patients with AIH-PSC overlap syndrome benefit from immunosuppression (prednisone and azathioprine) combined with UDCA [11].

AIH and PSC may also be sequential in their appearance, whereby patients have features of AIH and then after an average duration of 4–5 years develop clear features of PSC on ERCP. There are no specific features at presentation that can predict this sequence of events [2].

## Overlap Between PBC and PSC

PBC–PSC overlap syndrome is very rare, with only anecdotal reports being found in the literature [20]. In the early stages, the distinction between these two disorders is easier to discern than in advanced stages, where it may be difficult to distinguish both diseases even with the help of histological and immunological changes. Secondary alterations of bile ducts in PBC stage IV may be indistinguishable from PSC on ERCP/MRCP.

Some differentiating features between AIH, PBC and PSC are summarized in Table 77.1, and a diagnostic and therapeutic algorithm in AIH-overlap syndromes is depicted in Fig. 77.1.

**Table 77.1** Some differentiating features between autoimmune hepatitis (AIH), primary biliary cirrhosis (PBC), and primary sclerosing cholangitis (PSC)

| | PBC | PSC | AIH |
|---|---|---|---|
| **Occurrence in childhood** | No (only anecdotal cases) | Yes | Yes |
| **Percentage females** | 90% | 40% | 60% |
| **HLA B8, DR3** | (+) | + | +++ |
| **IBD** | (+) | +++ | (+) |
| **Predominant immune globulin in serum** | IgM | (IgG) | IgG |
| **Autoantibodies** | AMA | (pANCA) | ANA |
| **Alteration of major bile ducts** | – | +++ | – |
| **Response to corticosteroids** | – | – | +++ |

*IBD* Chronic inflammatory bowel disease

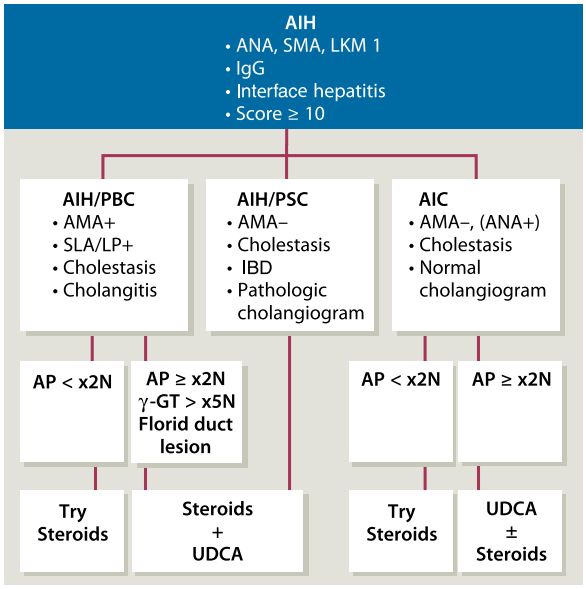


**Fig. 77.1** Diagnostic and therapeutic algorithm in autoimmune hepatitis overlap syndromes [4]. *AIH* Autoimmune hepatitis, *PBC* primary biliary cirrhosis, *AIC* autoimmune cholangitis, *IBD* chronic inflammatory bowel disease, *N* upper limit of normal, *UDCA* ursodeoxycholic acid

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# Acute Liver Failure

# 78

Alexander Koch and Christian Trautwein

## Chapter Outline



## Definition

Acute liver failure (ALF) is defined by loss of liver function in patients with no pre-existing chronic liver disease. This definition differentiates ALF from end-stage liver disease, which can also result in terminal loss of liver function. Acute liver failure is characterized by a related group of typical findings, which include severe liver insufficiency clinically presenting with coagulation disorders (usually an INR ≥ 1.5) and any degree of mental alteration caused by hepatic encephalopathy. The poor prognosis of ALF is due to the combination of liver insufficiency and hepatic encephalopathy.

Depending on the length of illness, ALF has been historically differentiated into fulminant or hyperacute (< 7 days), acute (7–21 days) and subacute (> 21 days and < 26 days) liver failure [74]. Duration of the clinical course has, unlike the cause of the illness, no prognostic significance.

## Epidemiology

The worldwide incidence of ALF is uncertain. Approximately 2,000 cases of ALF occur yearly in the United States, representing 0.1% of all deaths and around 6% of liver-related deaths [27].

## Etiology

The major causes of ALF are medication toxicity, viral hepatitis and indeterminate causes. Data collected by the Acute Liver Failure Study Group revealed that

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during the period of 1998 to 2001 acetaminophen overdose was responsible for 39% of ALF cases, indeterminate causes for 17%, idiosyncratic drug reactions for 13% and acute viral hepatitis A or B for 12% [57]. Amoxicillin-clavulanate has been identified as the most common agent in drug induced liver injury, accounting for almost 13% of cases [1].

ALF is more rarely caused by amanita intoxication, acute Wilson's disease, and Budd-Chiari syndrome (Table 78.1).

**Table 78.1** Causes of acute liver failure

| |
|---|
| **Infectious (viral)** |
| Hepatitis A |
| Hepatitis B |
| Hepatitis D |
| Hepatitis E |
| Hepatitis NANBNC |
| *Rare viral causes* |
| Herpes simplex virus |
| Herpes virus Type 6 |
| Varicella virus |
| Cytomegalovirus |
| Ebstein-Barr virus |
| Toga virus |
| Paramyxovirus |
| Parainfluenza virus |
| **Drugs/Toxins/Chemicals** |
| Acetaminophen |
| Amanita phalloides |
| Halothane |
| Isoniazid |
| Valproate |
| NSAIDE |
| *Other drugs see Table 78.5* |
| **Cardiovascular** |
| Budd-Chiari syndrome |
| Hypotension (circulatory shock) |
| Heart failure (e.g. right ventricular) |
| Hyperthermia |
| Veno-occlusive disease |
| Portal vein thrombosis |
| Sepsis |
| **Metabolic and infiltrative** |
| Wilson's disease |
| Reye's syndrome |
| Acute fatty liver of pregnancy |
| HELLP syndrome (**h**emolysis, **e**levated **l**iver enzymes, **l**ow **p**latelet count) |
| Galactosemia |
| Hereditary fructose intolerance |
| Hereditary tyrosinemia |
| Malignant tumors |
| Anorexia nervosa (with extremely poor nutritional status) |

A distinct cause for ALF can be established in approximately 60–80% of cases; identification of the underlying disease directly influences treatment options and determines prognosis [46].

## Prognosis

The probability of spontaneous hepatic recovery cannot be predicted by any single factor alone. The most important variables for predicting the outcome in ALF are the grade of hepatic encephalopathy, the patient's age, the underlying etiology and the progression in clinical course. Spontaneous recovery of liver function is more likely with lower grades of hepatic encephalopathy: 65–70%, for grade I–II 40–50% for grade III and < 20% for grade IV [56].

The overall prognosis is determined by different pathophysiological factors of ALF: the metabolic consequences of diminished functional liver cell mass, the release of toxic metabolites from destroyed liver cells and the ability of regeneration of the remaining liver tissue.

The potential for liver regeneration is higher in fulminant liver failure than in subacute liver failure due to its sudden occurrence. In patients with fulminant liver failure, intensive care medicine can more often bridge to spontaneous recovery of liver function; acetaminophen toxicity should be considered in most cases of fulminant ALF. In contrast, continuous damage in subacute liver failure reduces the possibility of liver regeneration and leads to a worse prognosis.

With regard to its cause, the prognosis of ALF due to Wilson's disease, Budd-Chiari syndrome and Non A-, B- or C-hepatitis is more severe whereas the clinical course of ALF caused by hepatitis A is more favourable. The overall prognosis of ALF for children younger than 10 years and adults older than 40 years in all underlying diseases is poor.

Based on developments in intensive care medicine over the past 20 years the prognosis of ALF has improved; nevertheless, the mortality of ALF with conservative treatment is approximately 40–70% depending on patients' characteristics and underlying disease. In other words, 50% of patients with ALF will need a liver transplantation.

**Table 78.2** King's college criteria

| |
|---|
| **Acetaminophen-induced ALF** |
| Arterial pH < 7.3 (following adequate volume resuscitation), irrespective of coma grade |
| ***OR*** |
| PT > 100 s (INR ≥ 6.5) + serum creatinine > 3.4 mg/dL in patients in grade III/IV coma |
| **Non-acetaminophen-induced ALF** |
| PT > 100 s irrespective of grade of encephalopathy |
| ***OR*** |
| Any three of the following, irrespective of coma grade: |
| Drug toxicity, indeterminate cause of ALF |
| Age < 10 years or > 40 years |
| Jaundice or coma interval > 7 days |
| PT > 50 s (INR ≥ 3.5) |
| Serum bilirubin > 17.5 mg/dL |

Survivors of ALF usually recover completely; residual cerebral, renal or hepatic damage is rare. To assess prognosis of ALF, several scoring systems have been developed. Most frequently the King's College Score is used (Table 78.2). The accuracy of the King's College Criteria has been evaluated in separate cohorts, proving that fulfillment is associated with an inferior outcome; however, sensitivity of this scoring system is poor. Relatively low negative predictive values indicate that King's College Criteria are better able to predict prognosis in patients with a poor than those with a good prognosis. The Model for Endstage Liver Disease (MELD) score is suited to predict mortality in patients with chronic liver disease; currently, its use cannot be recommended in patients with ALF [80]. In patients with acute acetaminophen intoxication APACHE-II-Score > 15 and serum lactate concentration > 3 mmol/l discriminates patients with an adverse prognosis.

Liver histology can be an important tool for establishing a diagnosis but its accuracy is not proven for predicting outcome and its routine use is therefore not recommended [26].

## General Considerations

In all patients with clinical evidence of moderate to severe hepatitis, measurement of prothrombin time and careful evaluation of the mental status should be performed. If the prothrombin time is prolonged, with an INR ≥ 1.5, and alterations in mentation are present, the diagnosis of ALF is established.

All patients with the diagnosis of ALF should be treated in an intensive care unit (ICU), preferably in a transplant center, since the condition may progress rapidly.

When possible, a *detailed history* should be obtained from the patient, patient's family or friends, and/or paramedics, with particular attention to possible exposures to viral infection, medications, illicit drugs and toxins.

On admission, *physical examination* is important to assess and document the patient's initial mental status so that any subtle progression can be more accurately detected. Jaundice is a common clinical sign but not always seen at presentation.

One should look carefully for signs of cirrhosis such as spider nevi, gynecomastia, atrophic tongue and glabella, so as to exclude underlying chronic liver disease. Due to massive hepatocyte loss the physical examination can reveal a decreased size of the liver. Otherwise an enlarged liver might be observed in cases of early viral hepatitis, malignant infiltration, congestive heart failure or Budd-Chiari syndrome.

In order to evaluate the etiology and severity of ALF, initial laboratory examination must be immediate and extensive.

*Laboratory examination* should include coagulation parameters, routine chemistries (glucose, electrolytes, AST, ALT, AP, γGT, bilirubin, albumin, creatinine, urea nitrogen), arterial blood gas measurement, complete blood count, blood typing, toxicology screening and acetaminophen level. Arterial ammonia at presentation is predictive of outcome in patients with acute liver failure and may be an independent risk factor for the development of both hepatic encephalopathy and intracranial hypertension.

ALF due to hepatitis A and B, Wilson's disease and autoimmune hepatitis should be excluded. To identify the underlying etiology of ALF, liver biopsy, preferably via transjugular route because of coagulopathy, might be useful, especially in cases of suspected autoimmune hepatitis, malignancy or lymphoma (see Chapter 44).

All women of child-bearing age with ALF should undergo pregnancy tests.

According to ALF etiology specific therapeutic procedures should be initiated and the indication for transplantation should be evaluated as early as possible and considered continuously.

## Special Entities

### Acetaminophen Intoxication

Acetaminophen toxicity is a typical example of a dose-related toxicity. Severe hepatotoxicity and ALF has to be expected after ingestion of > 10 g per day. However, severe liver injury can occur rarely when doses as low as 3–4 g per day are taken, especially in patients consuming regularly ethanol (see Table 93.3) [67]. Medical history can reveal excessive ingestion either in the intention of suicidal overdose or in accidental overdose as pain medication. Medical history provided by relatives (e.g. preexisting depression, conflict in partnership) and information from paramedics (blister packages or empty bottles at location) can be helpful. Very high levels of aminotransferases are a typical laboratory finding of acetaminophen intoxication.

In therapeutic doses acetaminophen is detoxified in the liver by glucoronidation or sulfation. High acetaminophen dosage and liver enzyme induction can lead to metabolism by cytochrome P450 IIE1 pathway which results in production of n-acetyl-p-benzoquinone-imine (NAPQI). As long as there are adequate hepatic glutathione reserves NAPQI is detoxified; otherwise, NAPQI binds covalently to cellular proteins. These NAPQI-protein-complexes induce hepatocellular apoptosis.

N-acetylcysteine (NAC) replenishes diminished hepatic glutathione reserves so that NAC given in high dosage can inhibit the progression of ALF due to acetaminophen toxicity.

As NAC is a very safe and easily administered drug, it should be given early on suspicion of acetaminophen intoxication, though it may still be of value 48 h or more after ingestion [36]. If NAC is administered early, the full extent of ALF can be nearly always avoided (Table 78.3). Additional activated charcoal (1 g/kg p.o.) may be useful for gastrointestinal decontamination as long as 3–4 h after ingestion [25].

Because acetaminophen is the leading cause of ALF in the United States and Western Europe and NAC is an adaptive antidote, acetaminophen levels should be measured wherever applicable to confirm the diagnosis of acetaminophen intoxication. High serum drug levels and rising aminotransferases indicate imminent liver injury or ALF.

If acetaminophen levels are below 200 µg/mL 4 h after ingestion, administration of NAC can be deferred.

**Table 78.3** N-acetyl-cysteine treatment of acetaminophen intoxication

| |
|---|
| **Oral administration** |
| Initial: 140 mg/kg b.w. by mouth or nasogastric tube |
| Following: q4h × 17 doses: 70 mg/kg by mouth or nasogastric tube |
| **Intravenous administration** |
| 150 mg/kg b.w. in 200 mL 5% glucose over 15 min, followd by 50 mg/kg b.w. in 500 mL 5% glucose over 4 h, followed by 100 mg/kg b.w. in 1000 mL 5% glucose over 16 h |
| **Primary gastrointestinal decontamination** |
| Up to 4 h after ingestion using activated charcoal 1 g/kg b.w. orally administered |

Patients at particular risk for acetaminophen toxicity (e.g. alcohol abuse, long-term fasting, enzyme-induction by tuberculostatic and anti-epileptic medications) and with laboratory-confirmed serum concentrations greater than 100 µg/mL should be treated.

A recently published study shows that the measurement of serum acetaminophen-protein adducts can reliably identify acetaminophen toxicity in cases of acute liver failure in which no clinical or historic data are given to reveal the cause of acute liver failure [12].

### Mushroom Poisoning

Amanita phalloides poisoning is an uncommon cause of acute liver failure with an especially rapid course (see Table 93.3).

Amatoxins of Amanita phalloides are hepato- and nephrotoxic. One mushroom of the species Amanita phalloides (50–100 g) contains 8–20 mg amatoxin; LD50 is about 0.2 mg/kg b.w. amatoxin. In Germany, returned settlers from Russia are at particular potential danger because Amanita phalloides looks similar to edible mushrooms in their country of origin.

As there are no typical laboratory findings and no commercially available blood tests to confirm the presence of amatoxins, the diagnosis has to be based on clinical symptoms and a detailed history.

In the typical clinical course severe diarrhea, vomiting and abdominal cramps start approximately 6–10 h after mushroom ingestion. The climax of liver deterioration takes place after 2–3 days; at that time, gastrointestinal symptoms usually have already subsided. An interval between ingestion and diarrhea of < 8 h is a very early predictor of a fatal outcome [19].

There are no controlled clinical studies for proper treatment of Amanita phalloides intoxication. Recommendations are based on case reports, experts' opinion and experimental data.

The main goal after (suspected) ingestion of Amanita phalloides is primary gastrointestinal decontamination by gastrointestinal lavage, administration of activated charcoal and laxatives and forced diuresis to prevent toxin absorption in the small intestine. Fluid resuscitation is also fundamental. Amanita intoxication frequently necessitates liver transplantation, but complete recovery has been described with supportive care and medical treatment [40, 60].

Liver transplantation should be strongly considered in patients with evolving clinical symptoms (diarrhea, vomiting, abdominal cramps) less than 8 h after mushroom ingestion. From day 4 after ingestion, INR ≥ 6 is a reliable tool for deciding emergency transplantation [19].

Specific antidotes for Amanita phalloides are silibinin and penicillin G, despite no controlled trials proving their efficacy [28, 40, 60]. Silibinin is not available as a licensed drug in the United States, although it is widely available in Europe and has been reported to be more effective than penicillin G in Amanita poisoning [19]. Silibinin is administered in a dosage of 30–50 mg/kg (intravenously or orally) for an average period of 3–4 days (Table 78.4). Penicillin G is given intravenously in doses of 300,000–1,000,000 UI/kg/day.

Charcoal plasma perfusion and MARS (see below) have been described as effective procedures in combination with conservative therapies within the first 36–48 h after amanita ingestion [10, 32]. There is no distinct scientific evidence for the effectiveness of other substances, such as α-liponic acid, cortisone, cephalosporins and cimetidine. Future clinical research has to concentrate on the efficacy of silibinin, *N*-acetylcysteine, and detoxification procedures.

**Table 78.4** Therapy of *Amanita phalloides* intoxication

| |
|---|
| **Fluid resuscitation** |
| **Primary gut decontamination:** |
| – Gastric lavage (up to 36 h after ingestion) |
| – Activated charcoal 1 g/kg b.w. p.o. |
| *In proof of Amanita phalloides ingestion* |
| **Antidot-therapy:** |
| – Silibinin i.v. 20 mg/kg b.w. in 4 doses over 2 h (diluted in 5% glucose or 0,9% NaCl) |
| – Additive i.v. NAC-therapy as in acetaminophen-intoxication |
| **Secondary gut decontamination:** |
| – Hourly application of 0.25–0.5 g/kg b.w. activated charcoal |

## Drug-Induced Liver Injury

Drug-induced liver injury (DILI) is caused by direct hepatotoxic effects of a drug, or a reactive metabolite of a drug (see Section XVIII). Parenchymal cell injury induces activation of immune cells, which produce proinflammatory and tissue hepatotoxic mediators and may lead to acute liver failure.

In clinical practice medication history should be taken carefully to reveal type, duration and quantity of all substances ingested over the past year. Attention has to be paid to the fact that ALF can result from therapeutic doses in patients with underlying liver disease (particularly with ongoing alcohol abuse) and in patients who are taking medications known to induce the cytochrome P450 system such as anti-convulsants.

In contrast to dose-related acetaminophen toxicity, a variety of drugs can cause idiosyncratic liver injury which may lead to ALF. Idiosyncratic drug toxicity usually occurs within the first 6 months of intake. Drugs used for more than 1–2 years are unlikely to cause de novo liver damage. Drug-induced ALF is a diagnosis of exclusion; all other causes for ALF have to be ruled out. In addition to prescribed medications, non-prescription over-the-counter-drugs, herbal preparations and nutritional supplements can also cause liver injury [70]. "Usual suspects" for drug-induced liver injury are antibiotics, non-steroidal anti-inflammatory agents (NSAID) and anti-convulsants, especially isoniazid, valproic acid, phenytoin and amoxicillin-clavulanate. Table 78.5 gives an overview of drugs which are known to cause drug-induced liver injury. There are no specific therapies or antidotes for idiosyncratic drug injuries; discontinuation of all but essential medication is necessary. Unless allergic drug-reactions are suspected, there is no indication for corticosteroids in drug-induced liver injury.

## Viral Hepatitis

In the United States, acute viral hepatitis is causative in approximately 12% of ALF (hepatitis B 8%, hepatitis A

**Table 78.5** Selected drugs which may cause liver injury

| | |
|---|---|
| **Isoniazid/Rifampicin**[a] | Isoflurane |
| **Trimethoprim-sulfamethoxazole** | Lisinopril |
| **Amoxicillin-clavulanate** | Nicotinic acid |
| Sulfonamides | Ofloxacin |
| Phenytoin | Dapsone |
| Statins | Imipramine |
| Propylthiouracil | Etoposid |
| Halothane | Allopurinol |
| Disulfiram | Methyldopa |
| Valproic acid | Ketoconazole |
| Amiodarone | Efavirenz |
| Diclofenac | Metformin |

[a]bold: combination agents with enhanced toxicity

4%) [57, 66]. Implementation of hepatitis B vaccination has led to a striking reduction in the occurrence of HBV-related ALF. Nevertheless, acute hepatitis B still is a significant cause of fulminant hepatitis. Acute hepatitis due to precore or pre-S mutant hepatitis B viruses may be difficult to diagnose by routine serology; thus, liver failure in this group may be misinterpreted as non A, B or C hepatitis or of cryptogenic etiology. In unclear cases hepatitis B infection can be detected by polymerase chain reaction (PCR) [83].

Acute hepatitis C alone seems not to cause ALF [21]. In countries where hepatitis E is endemic (e.g. Russia, Pakistan, India or Mexico) it is a significant cause of ALF; especially in pregnant women [37, 66]. Acute hepatitis E infection has to be considered in patients with ALF and recent travels to endemic areas.

In acute hepatitis B virus infection related ALF the nucleoside analog lamivudine in doses of 100 mg per day was used – rather based on case reports and experts' opinion than on distinct scientific data – to improve liver function and reduce the necessity of liver transplantation [44]. In a randomized controlled clinical trial the efficacy of lamivudine 100 mg daily was compared to placebo. The lamivudine group showed a greater decrease in levels of HBV-DNA but no significantly greater biochemical or clinical improvement compared to placebo could be demonstrated [41].

Therapy in viral hepatitis A- and B- and E-related ALF is based on supportive care, as no virus-specific treatment has been proven effective.

Chemotherapy or immunosuppression can lead to reactivation or acute flare of hepatitis B and by this means even to ALF. Therefore patients found to be positive for HBsAg should be treated prophylactically with a nucleoside analog before initiating immunosuppressive therapy. Prophylaxis should be continued for 6 months after cessation of immunosuppression [48].

Herpes and varizella zoster, Epstein-Barr virus and cytomegalovirus infections rarely cause ALF. While herpes virus ALF has been reported in healthy individuals, it is typically immunocompromized patients and pregnant woman who are at increased risk [34, 58]. The diagnosis is established by clinical presentation usually with high fever and direct virus detection via PCR; typical skin lesions are only present in 50%. Liver biopsy can be helpful to clarify the diagnosis. Recommended therapy for herpes infection is intravenous treatment with acyclovir, which should be initiated immediately on suspicion.

## *Wilson's Disease*

ALF due to Wilson's disease typically occurs in young patients (see Chapter 81). Patients typically present with acute-onset jaundice and hemolytic anemia, with serum bilirubin levels > 20 mg/dL (mainly indirect-reacting bilirubin). Hemolysis is induced by copper ions leaking from necrotic hepatocytes into the circulation and causing lysis of red blood cells [38].

Laboratory diagnostic tests for establishing the diagnosis of Wilson's disease should include measurement of serum ceruloplasmin which is typically low, but can be normal in up to 15% of cases, and high serum and urinary copper levels. Indirect indicators of the presence of Wilson's disease are very low serum alkaline phosphatase or uric acid levels, and a bilirubin to alkaline phosphatase ratio > 2 [62]. Slit lamp examination reveals Kayser-Fleischer rings in about 50% of cases [18]. Measurement of hepatic copper concentration is a powerful diagnostic tool as long as (transjugular) liver biopsy is feasible in the acute situation (Table 78.6).

To acutely lower serum copper concentrations albumin dialysis, continuous veno-venous hemofiltration and especially plasmapheresis and plasma exchange are recommended [62]. These procedures lead to amelioration of hemolytic anemia and provide clinical stabilization until liver transplantation can be performed; recovery without transplantation is unusual. In ALF due to Wilson's disease d-penicillamine is not indicated. Family members of patients with ALF due to Wilson's disease need genetic counseling and screening.

**Table 78.6** Diagnostic characteristics in ALF due to Wilson's disease

**Clinical presentation:** young patients, acute onset, jaundice
**Laboratory findings:**
- Hemolysis (LDH, haptoglobin)
- Hyperbilirubinemia (indirect > direct)
- Low serum ceruloplasmin
- High serum and urinary copper levels (urine Cu > 600 nmol/day)
- Very low serum alkaline phosphatase levels
- Very low serum uric acid levels
- Bilirubin (mg/dL) to alkaline phosphatase (IU/L) ratio > 2.0
- Renal impairment due to tubular copper toxicity

**Examination:**
- Slit lamp: Kayser-Fleischer ring (50% of cases)

**Liver biopsy:**
- High hepatic copper levels (> 250 µg/g dry weight) and compatible histology

### Autoimmune Hepatitis

If autoimmune hepatitis is assumed to be the underlying cause of ALF, liver biopsy should be performed to establish this diagnosis. Without histological findings, making a definite diagnosis might be difficult, especially if autoantibodies are absent.

If feasible in the clinical setting, therapy with prednisone, starting with doses of 40–60 mg daily is indicated as a therapeutic trial; nevertheless, the anticipatory clinical course frequently necessitates listing for transplantation.

### Acute Fatty Liver of Pregnancy

Acute fatty liver of pregnancy is associated with increased maternal and fetal mortality and affects a small number of women usually in the last trimester (see Chapter 99) [59]. The clinical triad of jaundice, coagulopathy and low platelet count is commonly associated with signs of pre-eclampsia such as hypertension and proteinuria. Obstetrical services should always be consulted immediately. Early recognition of the symptoms and expeditious delivery is crucial and directly affects outcome. After delivery liver function usually is completely restored. All fertile female patients with ALF should undergo pregnancy tests.

### Budd-Chiari Syndrome

Depending on the progression of the disease, Budd-Chiari syndrome may result in acute liver failure when sudden obstruction of all three main liver veins occurs (see Chapter 59). Clinically, the acute Budd-Chiari syndrome presents with ascites, abdominal pain, jaundice and hepatomegaly [20]. Budd-Chiari syndrome is frequently associated with primary myeloproliferative disorders and hypercoagulable states, such as a factor V Leiden mutation, anti-cardiolipin antibodies, or protein C and S deficiency [14]. In general, acute Budd-Chiari syndrome necessitates liver transplantation [52]. Transjugular portosystemic acute stent shunt (TIPSS) or percutaneous transjugular direct portocaval shunt, in patients with inaccessible hepatic veins, seems to be a therapeutic option to decrease portal pressure gradient, improve synthetic function, reduce elevated aminotransferase levels and control ascites [39].

### Indeterminate Etiology

As long as a clear-cut diagnosis cannot be established by routine evaluation, liver biopsy has to be considered as it can be helpful in diagnosing malignancy, autoimmune hepatitis, viral infection and Wilson's disease. Furthermore, unidentified toxins or drug ingestions have to be reconsidered.

## Therapy

### General Considerations

The clinical course and prognosis of ALF is determined by the underlying disease. Different etiologies necessitate differentiated therapeutic approaches. There is no proven conservative therapy for ALF in general, so management consists of supportive intensive care and, as far as available, specific treatments. Main challenges of intensive care medicine in ALF are fluid management and hemodynamic stabilisation, treatment of hepatic encephalopathy and coagulation disorders, maintenance of metabolic homeostasis, prevention and treatment of complications, adequate

nutrition, as well as surveillance for and therapy of infection [50].

The only therapy documented to improve patient outcome in ALF is orthotopic liver transplantation, which is associated with 1-year survival rates of greater than 80% [46]. Patients with ALF should therefore always be transferred early to and managed in an intensive care unit at a facility capable of performing liver transplantation, since transportation may be hazardous if complications develop [45].

## *Encephalopathy and Cerebral Edema*

Hepatic encephalopathy is one of the major clinical findings in ALF and directly influences outcome. Cerebral edema, resulting increased intracranial pressure and cerebral herniation, is the main cause of death in ALF [77]. The pathophysiology of hepatic encephalopathy is multifactorial and not completely understood [13, 75]. Ammonia increasingly produced from nitrogenous substances in the gut lumen is of particular importance in the development of hepatic encephalopathy [61]. Therefore treatment has been directed towards reducing the production and absorption of ammonia via administration of lactulose, oral neomycin, and a low protein diet.

Ammonia causes osmotic derangements in astrocytes, changes in cellular metabolism and alteration in cerebral blood flow [3]. These factors mainly determine clinical course and prognosis of encephalopathy and cerebral edema. Brainstem herniation as the most advanced stage of cerebral edema correlates with arterial ammonia concentration. Ammonia measurements could possibly be a tool for risk stratification for hepatic encephalopathy and intracranial hypertension, which might help to identify patients for ammonia-lowering therapies and invasive monitoring.

The level of hepatic encephalopathy has prognostic impact (Table 78.7). Prognosis is favorable, if the patient is accessible and orientated (encephalopathy grade I and II). With higher levels of encephalopathy the prognosis is generally worse and unpredictable. The risk of multi-organ failure and the danger of cerebral edema directly correlate with the grade of encephalopathy. Independent from the etiology of ALF, 65–75% of patients with ALF and encephalopathy grade IV develop cerebral edema [53].

**Table 78.7** Grades of hepatic encephalopathy

| | |
|---|---|
| **I. Prodrome** | Mild confusion, slurred speech, slowness of mentation, disordered sleep rhythm, euphoria/depression |
| **II. Impending coma** | Accentuation of grade 1: drowsy but speaking, inappropriate behavior, incontinence |
| **III. Stupor** | Sleeps most of the time but arousable to vocal stimuli, incoherent or no speech, marked confusion |
| **IV. Coma** | Comatose, unresponsive to pain |

Younger patients and patients with rapidly developing clinical symptoms are at particular risk.

Arterial ammonia at presentation is predictive for outcome in patients with acute liver failure. Patients with encephalopathy grade III and IV show significantly higher serum ammonia levels than patients with lower grade encephalopathy. It may be possible to use a serum ammonia cut-off value of ≥ 124 µmol/L to predict the presence or development of advanced cerebral dysfunction. Ammonia levels can be used for risk stratification [2].

## Diagnosis of Increased Intracranial Pressure

Cerebral hypertension causes diminished cerebral perfusion. Cerebral perfusion pressure (CPP) is defined as the difference between intracranial pressure (ICP) and mean arterial pressure (MAP). Hence, in the situation of rising cerebral pressure and falling MAP, the CPP rapidly decreases. Critical values of CPP are about 60 mmHg; conditions with CPP under 40 mmHg can rarely be survived. Maintaining CPP above 70 mmHg may improve neurological outcome. Indication for lowering cerebral pressure is given at intracranial pressure above 25 mmHg. As clinical signs of elevated ICP, such as hypertension, bradycardia and irregular respirations are not present in either case, ICP measuring devices are the only effective tools to detect rising ICP in early stages. Neurological manifestations of intracranial hypertension (ICH) include increased muscle tone, hyperreflexia and altered papillary responses; however, in the early course they may be absent or difficult to detect [45].

The risks of insertion of intracranial devices, especially infection and bleeding, have to be balanced accurately against the anticipated benefits. In the majority of cases, intracranial pressure measuring devices are inserted in patients with hepatic encephalopathy grade III and IV and concomitant indication for liver transplantation. In higher grade encephalopathy and especially following transplantation, measuring intracranial pressure can be very useful due to possible fluctuations of intracranial pressure during operation [47]. Four different types of catheters have been used to measure ICP: epidural, subdural, parenchymal and intraventricular devices (Table 78.8). The advantage of epidural catheters is that they are less invasive and therefore they cause fewer complications, but they cannot be used to drain cerebrospinal fluid to lower ICP. Before an ICP monitor is placed, any existing coagulopathy should be corrected. The number of complications in the use of modern epidural ICD measuring devices is about 4% compared to significantly higher rates of subdural (20%) and parenchymal catheters (22%) [4]. Invasive ICP monitoring with epidural catheters is safe and reliable though it is unclear whether the use of such devices improves overall survival. Besides invasive monitoring of intracranial pressure, the cranial computed tomography (CCT) is a useful tool to diagnose cerebral edema or intracranial bleeding; however, it does not reliably demonstrate evidence of edema, especially in early stages.

Repeated use of transcranial doppler ultrasound and measurement of serum S-100 protein and neuronal specific enolase have not been proven reliable and adequate tools in examination of ICP and cerebral edema [43, 72].

By relating oxygen concentration of jugular venous to arterial oxygen concentration, evaluation of cerebral oxygen consumption is possible. Also, elevated lactate levels in jugular venous blood can be a sign of inadequate cerebral oxygen supply.

**Table 78.8** Monitoring intracerebral pressure: different types of catheters

| | |
|---|---|
| **Epidural** | Placed outside the dura mater |
| **Subdural** | Placed beneath the dura mater |
| **Parenchymal** | Placed directly into the brain parenchyma |
| **Intraventricular** | Placed within a cerebral ventricle |

## General Therapeutic Measures in Cerebral Hypertension

Patients at risk for cerebral edema should be cared for in a silent environment, and direct manipulation of the patient should be minimized to avoid agitation. Although induced sedation should be used carefully, distinct agitation can be treated with short-acting benzodiazepines in small doses. The upper part of the body should be positioned at a 30–45 degree elevated angle, and the mental status should be checked frequently [16].

Unconscious or comatose patients (encephalopathy grade III and IV) should be electively intubated and mechanically ventilated to prevent aspiration and to facilitate hyperventilation to lower intracranial pressure. Propofol is often used for sedation, because it may reduce cerebral blood flow [81]. An increased cerebral blood flow due to loss of cerebrovascular autoregulation is a typical phenomenon during the various stages of hepatic encephalopathy before overt cerebral edema supervenes.

By the use of gut lavage, the additional systemic impact of intestinal toxins can be reduced. Lactulose and intravenous application of L-ornithin and L-aspartate can decrease serum ammonia levels, but there is lack of clinical data proving that lowering ammonia levels leads to prevention of cerebral edema. Lactulose therapy in ALF is associated with a small increase in survival time but has no effect on severity of hepatic encephalopathy or overall outcome [29].

## Drug Therapy of Intracranial Hypertension

Mannitol is an important drug for osmotic therapy of cerebral edema. It is especially effective in early and middle grades of cerebral edema but loses its efficacy in higher grades. Mannitol is usually administrated at a dose of 0.5–1.0 g per kg body weight, given intravenously over a period of 30 min. The administration of mannitol can be repeated as long as serum osmolarity does not exceed 320 mosmol/L. In renal failure the usage of mannitol is limited due to the insufficient elimination by extracorporal renal replacement therapies.

Maintaining serum sodium in patients with ALF at levels of 145–155 mmol/L via administration of 30%

hypertonic NaCl-solution can possibly control ICP [54]. Induced hypernatremia might have a potential role in the prophylaxis of intracranial hypertension or cerebral edema. Preliminary neurosurgical data validate that hypertonic saline is a safe and effective treatment for elevated ICP in patients after traumatic brain injury [78]. Further studies are needed to prove the clinical impact especially in ALF.

Thiopental is a widely used agent in refractory intracranial hypertension in neurology and neurosurgery. In hepatic encephalopathy grade IV, administration of thiopental can reduce cerebral oxygen consumption. Systemic hypotension is a classic adverse reaction which can necessitate the use of systemic catecholamines [22]. Phenobarbital sedation is usually induced using a bolus of 3–5 mg/kg b.w. intravenously.

Although corticosteroids are regularly used for therapy of intracranial hypertension due to cerebral tumors, they have not been proven effective in patients with intracranial hypertension in ALF and should not be used [5].

## Supportive Therapy in Intracranial Hypertension

Hyperventilation to $CO_2$ levels of 25–30 mmHg can provide a short-term amelioration of intracranial pressure by cerebral vasoconstriction and subsequently decreased cerebral perfusion [42]. In patients with ALF the loss of autoregulation of cerebral blood flow can be restored after a short time of mechanical hyperventilation [71]. Hyperventilation should be initiated if intracranial hypertension or cerebral edema is proved and mannitol infusion is ineffective; continuous prophylactic hyperventilation in ALF showed no reduced incidence of cerebral edema or intracranial hypertension [17]. Attention should be paid to the fact that long-term hyperventilation carried out in grades of progressive intracranial pressure with already reduced cerebral perfusion can furthermore impair oxygen delivery [79].

Few uncontrolled studies have shown a protective effect of mild hypothermia in acute liver failure and cerebral edema [30, 31, 63]. Hypothermia (32–35°C) can be applied safely and easily. The risk of complications (arrhythmias, myocardial ischemia, infections, coagulopathy) increases with the degree and duration of hypothermia mainly with body temperatures below 32°C. Hypothermia reduces intracranial pressure and re-establishes disturbed autoregulation of cerebral blood flow. Some animal studies suggest that hypothermia can reduce the extent of liver injury in acute liver failure [23]. In contrast, hypothermia might also lead to impaired liver regeneration. Further research and controlled clinical studies are required to clarify the significance of hypothermia in acute liver failure.

## *Infections in Acute Liver Failure*

Patients in ALF show severe disturbances in immunological defense which become apparent in deranged function of neutrophils and Kupffer cells and a lack of opsonins (complement-factors and fibronectin). In ALF bacterial infiltration via the portal vein is commonly observed, with subsequent activation of neutrophils and Kupffer cells by endotoxins, resulting in the increased synthesis of cytokines like IL-6 and TNF.

Infections are the second most common cause of death in ALF. Positive cultures are observed in 80% of patients with ALF and 32% have laboratory-confirmed mycosis. Mortality of systemic mycosis is about 50% in ALF. Most common are infections of the respiratory tract (approximately 50% of all cases) followed by infections of the urogenital tract and infections of inserted foreign materials (e.g. central-venous catheters, urinary catheters and intracranial pressure measurement devices). Typical clinical findings of infection – fever and leucocytosis – are infrequently found in ALF. Therefore a regular microbiological screening of all reachable visceral cavities and body fluids should be provided to obtain maximum information about the actual bacterial flora and resistance in order to not only detect bacterial and fungal infection as soon as possible, but also to provide the most appropriate and effective antimicrobial therapy. By administration of a prophylactic intravenous antibiotic regimen, the general rate of infections can be lowered to about 20%. Concurrently the progression of hepatic encephalopathy towards higher grades and coma can be significantly reduced; but there is no evidence of improved overall outcome, so prophylactic antibiotic therapy in ALF can not be recommended generally [64, 76]. Enteral administration of amphotericin B has shown to be able to minimize the rate of fungal infections to 4%.

Prophylactic application of fluconazole is also effective in prevention of invasive mycosis. Based on these data in all patients with ALF a prophylactic treatment with antibiotic and antimycotic drugs should be discussed. Attention has to be paid to the potential hepatotoxicity of these substances. For enteral gut decontamination, neomycin and paromomycin plus amphotericin B can be used, however systemic antibiotic therapy showed no benefit in overall survival [76].

### *Multi-Organ-Failure in Acute Liver Failure*

ALF patients typically can rapidly develop multi-organ-failure (MOF). A distinct dysfunction of immune defence occurs early in the clinical course of the disease. Amongst others this is caused by diminished production of opsonins by damaged hepatocytes. As a consequence bacterial infection and endotoxemia are common. This leads to activation of macrophages and release of cytokines like interleukin-6 (IL-6) and tumor-necrosis-factor-alpha (TNFα). The clinical situation is similar to sepsis with peripheral vasodilation and consecutive hypotension up to shock and disturbances in microcirculation. Intravascular volume deficits on admission are often due to decreased oral intake resulting from altered mental status, fluid shift in the extracellular space and possibly blood loss. Cardiac output is increased to compensate for reduced vascular filling; initial fluid resuscitation is crucial. Due to tissue hypoxemia secondary deterioration of extrahepatic organs, as gut with bacterial translocation and admittance of toxic substances, emerges. This may result in accelerating hepatic damage. The extent of deterioration in the function of kidney, lung and central nervous system (CNS) determines the patient's prognosis. Histological findings in ALF are confluent hepatic necrosis and loss of hepatocytes; the histological extent of hepatic necrosis does not significantly correlate with the anticipated prognosis [15].

## Aspects of Cardiovascular System and Hemodynamics

Similar to the situation in sepsis and septic shock, patients with ALF present with hypovolemic hyperdynamic circulation and low intravascular pressure (CVP, PAP, PCWP). Patients with hepatic encephalopathy grade III-IV and high risk for intracranial pressure should obtain extensive hemodynamic monitoring. In comparison to the classic pulmonary catheter (Swan-Ganz catheter) the PiCCO-System yields a lower rate of infections and complications [9]. Using invasive hemodynamic monitoring, patients can be hydrated in an optimal manner and the hemodynamic situation can be accurately controlled and be properly influenced by the administration of catecholamines. Agents that promote vasoconstriction should generally be avoided unless significant systemic hypotension is present.

Refractory shock despite optimal hydration is associated with poor prognosis. The following benchmarks concerning the hemodynamic situation should be obtained for a favorable outcome: cardiac index (CI) > 4.5 L/min/m$^2$, peripheral systemic resistance (PSVR) > 700 dyn/s/cm$^5$, and oxygen consumption ($VO_2$) > 170 mL/min/m$^2$.

Fluid resuscitation is performed with colloids and crystalloids. Additionally, plasma albumin and, especially in severe coagulopathy, fresh frozen plasma can be used. Systemic vasopressors like norepinephrine, epinephrine and dopamine are catecholamines of choice in severe shock due to peripheral vasodilation following adequate volume replacement; vasopressin should be avoided.

## Renal Failure

Acute renal failure (ARF) complicates the clinical course in about 30–70% of patients with ALF and significantly affects overall prognosis [51]. ARF in ALF is mainly caused by dehydration, hepatorenal syndrome or acute tubular necrosis [82]. Especially in acetaminophen intoxication – depending on the dose ingested – ARF can occur due to direct toxicity [6]. Renal failure in hepatic failure is typically characterized by extreme intrarenal vasoconstriction, with very low urine sodium concentration and fractional excretion. In early stages vasoconstriction is reversible; in prolonged courses kidney damage is permanent. Renal replacement therapy should be started early before conventional indications in order to avoid complications of hyperhydration such as pulmonary edema and cerebral edema.

Due to the often attending cardiovascular instability of patients with ALF, continuous-veno-venous-hemodialysis/hemofiltration (CVVHF/CVVHD) is superior to intermittent renal replacement therapies in terms of hemodynamical parameters [11]. To protect kidney function, nephrotoxic drugs such as aminoglycosides, non-steroidal anti-inflammatory drugs (NSAID) and intravenous contrast agents should be avoided as far as possible.

Terlipressin has been used effectively in treating hepatorenal syndrome in chronic liver disease, but clinical data indicate worsening of cerebral hyperemia and hypertension by administration of terlipressin in ALF with high-grade hepatic encephalopathy [69]. At present there is no indication for terlipressin to treat ARF in acute liver failure.

## Coagulation Disorders

Severe coagulation disorders define the clinical presentation of ALF and are the major cause for bleeding complications in ALF. The nature of coagulation disorders is complex and includes a lack of pro-coagulation factors as well as a deficit in inhibitors of coagulation and fibrinolysis. Due to consumption, platelet counts are often below 100,000 /mm$^3$. With no evident signs of bleeding correction of coagulation disorders is generally not recommended [24]. Using a threshold platelet count of < 10,000 /mm$^3$ in the absence of bleeding seems to be safe although some experts suggest more conservative levels of 15–20,000 /mm$^3$. When invasive procedures are planned or in setting of severe coagulopathy, substitution of fresh frozen plasma (FFP), single factors or platelets can be useful and should be taken into consideration. Invasive procedures can be safely performed with platelet counts of 50,000–70,000 /mm$^3$. In the setting of severe coagulopathy and hemorrhage, transfusion of platelets is recommended at levels below 50,000 /mm$^3$ and administration of FFP is indicated if prothrombin time is prolonged (INR ≥ 1.5). Excessive substitution of FFP can lead to volume overload, resulting in pulmonary and cerebral edema.

If correction of coagulopathy is necessary and cannot be achieved adequately with FFP, particularly in patients with prominent volume overload, substitution with recombinant activated factor VII (rFVIIa, 40 μg/kg b. w. i.v.) has been shown to be effective in improving prothrombin time and controlling bleeding [33, 68].

Vitamin K should be given routinely, despite existing malnutrition, in a dose of 5–10 mg per day subcutaneously or intravenously, usually over a period of 3 days.

The main origin of bleeding with relevant loss of blood is the mucosa of the upper intestine. The most important identifiable risk factors for intestinal bleeding in all critically ill patients are mechanical ventilation for more than 48 h and coagulopathy [8]. Additional risk factors are hepatic and renal failure, sepsis and shock [49]. Therefore patients with ALF should receive, balancing potential hepatotoxicity, $H_2$-receptor blockers – which are well studied – or proton-pump inhibitors (PPI) as prophylactic measure to prevent bleeding [7]. Sucralfate can be used additionally as second-line therapy.

## *Metabolic Disorders in Acute Liver Failure*

As a result of diminished gluconeogenesis and reduced release of glycogen, ALF patients often develop severe hypoglycemia. Therefore, blood glucose concentrations have to be monitored carefully and glucose should be administered early.

Disturbances of acid-base balance are common. In acute liver failure both acidosis and alkalosis might be present. Metabolic alkalosis is more frequent in early stages of ALF as impaired urea synthesis of the liver results in the accumulation of the two precursor substrates bicarbonate and ammonium. Alkalosis is associated with hypokalemia that is further aggravated by high sodium reabsorption in patients with acute liver failure. As ALF progresses patients can develop metabolic acidosis due to lactic acidosis, which portends an unfavorable prognosis.

Nearly 30% of the patients with acetaminophen intoxication display metabolic acidosis. Metabolic acidosis considerably worsens prognosis; dropping of arterial pH below 7.3 on day 2 or later after ingestion of acetaminophen is associated with 90% mortality.

## *Basic Aspects of Nutrition in Acute Liver Failure*

Nutrition in ALF strives for two main goals: first, catabolism should be minimized, since loss of body

cell mass worsens spontaneous recovery and negatively influences the outcome of potential liver transplantation; secondly, liver regeneration should be supported and homeostasis of glucose should be assured.

In the catabolic stage an energy input of 30 kcal/ kg body weight should be achieved. Glucose infusions are essential to prevent hypoglycemia and to reach a blood glucose concentration of 8–10 mmol/L. According to glucose control in severe sepsis blood glucose levels should not exceed 140 mg/dL. Careful monitoring and exact administration of insulin is crucial.

The demand of protein and amino acids is about 1–1.2 g/kg b.w. and should be calculated independent of ammonia levels. Substitution of branched-chain amino acids has not been proven superior to other preparations in ALF [55].

Lipids are an important energy source in ALF, and the use of lipids is particularly indicated during long-term (>3 days) parenteral nutrition. Serum triglyceride concentrations and cholesterol levels should be monitored daily to avoid fatty degeneration of the remaining liver parenchyma due to disturbed hepatic lipid utilization.

Minimal enteral nutrition is reasonable to avoid atrophy of intestinal mucosa and to prevent consecutive bacterial translocation from the gut into bloodstream. Besides monitoring energy demands, the surveillance and substitution of electrolytes is important. Prevention of hypophosphatemia is crucial as it is an independent risk factor in critical care medicine. Vitamins and micronutrients have to be substituted, generally in standardized forms of application.

## Liver Replacement Therapies

The liver captures multiple functions in the human organism. The role of the liver in homeostasis of acid-base balance, glucose metabolism or protein synthesis can be partially replaced by therapeutical interventions. A pending problem is to replace detoxification function of the liver. In the early 1970s initial approaches were undertaken to imitate and support detoxification function of the liver. At this time extended dialysis therapies were studied. The rationale for extracorporal systems in ALF is to provide an environment facilitating recovery or the opportunity for transplantation.

Actually besides the renal replacement therapies such as hemodialysis, hemofiltration, plasmapheresis and hemadsorption via activated charcoal, two procedures to cover partially liver function in ALF are used in studies: *molecular adsorbents recirculation systems* (MARS) and *Prometheus-System*. Both use albumin as a scavenging molecule. These liver support systems can transiently improve hepatic encephalopathy and biochemical markers but studies showing significant improvement of hepatic function or long-term benefit are pending [35]. One of the main clinical problems of both systems is the loss of platelets and worsening of coagulation parameters across the device [21].

There is lack of well-designed controlled studies with homogeneous patient populations. The practices of different specialized centers with regard to synthetic function, detoxification and homeostasis are too different; hence liver replacement therapies should only be used in prospective controlled clinical trials. Therefore, at present it is unclear if these devices will become clinically relevant to treat patients with ALF.

## Liver Transplantation and Prognosis

*Orthotopic liver transplantation* (OLT) is the only definitive therapy for patients with ALF who are unable to achieve regeneration of sufficient hepatocyte mass. Due to the fact that outcome in ALF is hard to predict, early referral to a transplant center is important to offer the best chance for a favorable outcome.

Without liver transplantation patients with ALF will either have recovery of liver function or die from the underlying disease; hence patients with ALF are given highest priority on the transplant list [57].

Contraindications to listing for transplantation are center-dependent. Severe cardiopulmonary disease which cannot be ameliorated acutely and influences surgical mortality, extrahepatic malignancy and active alcohol or drug abuse are contraindications for liver transplantation at most centers. Relative contraindications such as advanced age and HIV disease are commonly considered center-specific on a case-by-case basis. During intensive care management in ALF uncontrolled extrahepatic sepsis, MOF, irreversible brain damage or intractable cerebral edema with sustained elevation of ICP > 50 mmHg are contraindications for liver transplantation [65].

Limited organ availability, specific risk of transplant surgery in ALF and lifelong immunosuppression necessitate an accurate evaluation of indication for OLT. Actually, there are no single prognostic indicators or even scoring systems to predict outcome in ALF precisely. Therefore the decision on listing a patient for transplantation might be difficult and should be based on regard of the clinical course and a variety of clinical and biochemical parameters.

Patient age and etiology of the underlying disease have shown a correlation with patient survival. Survival rates in patients with ALF between the ages of 10 and 40 years are approximately 30–35%, whereas survival rate in patients older than 40 years or younger than 10 years is less than 10%.

ALF due to drug-induced liver injury and cryptogenic causes is associated with poor prognosis, while higher survival rates are observed in patients with hepatitis B and D virus infection. Acute hepatitis A and acetaminophen toxicity usually lead to a favorable outcome.

Clinical and biochemical parameters which are routinely used to predict outcome of ALF include level of hepatic encephalopathy, prothrombin time, factor V level, serum bilirubin, serum creatinine and arterial pH [56].

While in chronic liver disease severity of illness is assessed by using the MELD system, this score currently cannot be recommended in ALF.

Timing of OLT in ALF is critical, as a delay in the availability of the donor organ may often result in the onset of hazardous infections or sepsis and severe neurological damage, and therefore poor outcome.

Considering clinical data and individual variables, the indication for liver transplantation in ALF has to be continuously re-evaluated, particularly in patients who fail to show clinical and biochemical improvement despite appropriate intensive care management. The advance in intensive care medicine and advent of transplantation resulted in survival rates of > 60% in ALF (from 15% in the pre-transplant area). Post-transplant short-term survival rates for ALF are as high as 80–90%. The long-term survival rate for ALF 5 years after transplantation is about 60%, which is 5–10% inferior to outcomes in elective transplantation in chronic liver disease [57].

*Hepatocyte transplantation* might be a new approach for "bridging to transplantation" in ALF. In a study with 11 patients who underwent hepatocyte transplantation in ALF, 6 patients could be successfully bridged to liver transplantation. One patient even showed regeneration of sufficient hepatocyte mass with no further need for transplantation [73]. Follow-up studies are needed to prove the clinical impact of this technology.

In the future, patient survival might be improved by developing prognostic indicators for early identification of patients who benefit from liver transplantation. Enhancements in artificial liver support systems and hepatocyte transplantation in combination with evidence-based accurate intensive care management could help to optimize "bridging" to transplantation and improve outcomes in ALF.

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# Liver Cirrhosis

# 79

Henryk Dancygier

## Chapter Outline



## Definition

Cirrhosis is the end-stage manifestation of every chronic progressive liver disease. It is a diffuse process characterized by *loss of hepatic parenchyma*, formation of *fibrous septa* and *structurally abnormal nodules*, resulting in the *distortion of the normal architecture* and of gross vascular anatomy and microcirculation [2, 3].

## Epidemiology

Liver cirrhosis is a leading cause of death worldwide. It is the end result of a long-lasting process, usually clinically silent and unnoticed by the patient and the physician for years. Therefore, incidence and prevalence are not exactly known. In the past, up to 30–40% of cases have been discovered at autopsy [28]. Due to the widespread use of imaging techniques, such as ultrasound and computed tomography it may be assumed that currently most cirrhotic livers are discovered earlier. Moreover, geographical differences in causes, variations in incidence and prevalence from one country to another, and even between different regions in the same country, make an accurate epidemiological estimate very difficult.

Several reports suggest that coffee drinking is associated with a reduced risk of cirrhosis, supporting the hypothesis that there may be an ingredient in coffee that protects against cirrhosis, especially alcoholic cirrhosis [33, 49].

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## Etiology and Pathogenesis

The many causes of cirrhosis are summarized in Table 79.1. Approximately 40–60% of cases of liver cirrhosis in Europe and North America are due to alcohol abuse, while 25–30% result from chronic viral hepatitis. On a global scale, chronic hepatitis B and C, with more than 400 million people infected worldwide, represent the most important problem. Hereditary hemochromatosis is not as rare as assumed in the past. The genetic defect occurs with a frequency of 1:20 in its heterozygous form and in 1:400 in the homozygous form in the general population. Despite intensive diagnostic efforts the cause of cirrhosis remains unknown in approximately 10% of cases (cryptogenic cirrhosis) [25]. Approximately 70% of hitherto cryptogenic cases are nowadays believed to result from nonalcoholic fatty liver disease within the context of insulin resistance and metabolic syndrome, while the remainder are possibly due to as yet unknown viruses, metabolic derangements or (auto)immune mechanisms. Liver disease in metabolic syndrome is responsible for the majority of cases of cryptogenic cirrhosis among Hispanics and European Americans, and is unexpectedly rare among African Americans, supporting the evidence that ethnic factors play a role in the development of cirrhosis [8]. Several etiologic factors, such as hemochromatosis and alcohol, or alcohol and hepatitis C may also act in concert and accelerate the progression to cirrhosis.

**Table 79.1** Causes of liver cirrhosis

| |
|---|
| **Infectious** |
| Virus hepatitis B, C, D |
| Schistosomiasis |
| **Autoimmune** |
| Autoimmune hepatitis |
| Primary biliary cirrhosis |
| Autoimmune cholangitis |
| Overlap syndromes |
| **Metabolic-toxic** |
| Ethanol |
| Nonalcoholic fatty liver disease (insulin resistance; metabolic syndrome) |
| Indian childhood cirrhosis |
| **Drug-induced** |
| e.g. $CCl_4$, arsenic, methotrexate, isoniazid, amiodarone, α-methyldopa |
| **Genetic–hereditary** |
| Hereditary hemochromatosis |
| Wilson's disease |
| $\alpha_1$-antitrypsin-deficiency |
| Porphyria cutanea tarda |
| Glycogen storage diseases |
| Galactosemia |
| Tyrosinemia |
| Urea cycle disturbances |
| Abetalipoproteinemia |
| Cystic fibrosis |
| **Biliary** |
| Secondary biliary cirrhosis (gallstones, strictures) |
| Primary sclerosing cholangitis |
| $IgG_4$-associated cholangitis |
| Ischemic cholangiopathy |
| Ductopenia, bile duct atresia |
| Alagille's syndrome |
| **Vascular** |
| Chronic right heart failure ("cirrhose cardiaque") |
| Constrictive pericarditis |
| Budd-Chiari syndrome |
| Sinusoidal obstruction syndrome (venoocclusive disease) |
| Hereditary hemorrhagic telangiectasia (Osler-Rendu-Weber disease) |
| **Cryptogenic**[a] |

[a]Approximately 70% of cryptogenic cirrhoses develop in the context of insulin resistance and the metabolic syndrome

The following pathophysiological mechanisms are important in the development of liver cirrhosis

- Hepatocyte death with loss of hepatic parenchyma
- Fibrosis
- Changes in cell growth (hyperplasia, regeneration) and
- Vascular and circulatory alterations

### *Cell Death*

Chronic loss of hepatocytes is regarded as the primary stimulus and perpetuating factor in the development of liver cirrhosis. In order for cirrhosis to develop, liver cell loss must be sustained and long-lasting. Short-lived parenchymal losses, for example in acute necrotizing viral hepatitis or in acetaminophen toxicity, usually heal completely. If parenchymal loss is extensive, the tissue defects heal by scar formation with broad fibrous septa and regenerative nodules, but do not result in a progressive process leading to cirrhotic transformation of the liver. Continuous liver cell loss represents a stimulus for proliferation and growth of hepatocytes and for fibrogenesis.

Different pathogenetic mechanisms may underly hepatocyte injury and liver cell death (see Section III). Liver injury may be mediated by immune mechanisms (for example cytotoxic lymphocytes attacking virally

infected hepatocytes), inflammatory reactions (mediated by neutrophils and macrophages) or toxic factors (for example via oxidative stress and calcium-mediated cytotoxicity). Ischemic parenchymal necrosis of large regenerative nodules may occur especially after massive gastrointestinal bleeding and be in turn a stimulus for further nodule growth.

## Fibrosis and Circulatory Disturbances

Fibrosis plays a crucial role in nodular transformation of the liver. However, it represents just one facet of liver cirrhosis and must not be equated with cirrhosis [20]. Isolated fibrosis, even if extensive, does not necessarily result in cirrhosis. *Cirrhosis is more than just widespread liver fibrosis*. Further pathogenetic factors, such as liver cell loss and circulatory disturbances, must supervene in order for cirrhosis to develop.

The development of liver cirrhosis is accompanied by a marked increase in collagen content and by deposition of extracellular matrix, both produced mainly by stellate cells, which are activated and transformed into myofibroblasts (see Chapter 3). During this activation process the spectrum of proteins and enzymes secreted by stellate cells changes. More collagens, but also matrix metalloproteinases (MMPs) and their inhibitors (TIMPs), are expressed [35].

Progressive disease is characterized by increasing fibrosis with fibrous tissue surrounding islands of hepatic parenchyma, thus leading to the formation of *pseudolobuli*. These are called so, because the normal lobular architecture has been destroyed and the normal vascular relationship between portal vessels and central veins has been obliterated. Depending on the etiology and pathogenesis of cirrhosis connective tissue deposits may assume various aspects. Broad fibrous septa, chicken-wire fibrosis and perisinusoidal fibrosis may occur separately or combined. Fibrous septa may form bridges between portal tracts (portal-portal septa) and between portal tracts and central veins (portal-central septa). These remodeling processes are accompanied by *hemodynamic alterations*. Vascular channels within the fibrous septa lead to the establishment of *intrahepatic vascular shunts* between afferent (portal vein and hepatic artery) and efferent (hepatic vein) vessels of the liver, which are significant for the development of the sequelae of liver cirrhosis (see Chapter 80). The deposition of an increasingly dense extracellular matrix within the space of Disse results in the formation of pseudomembranes located underneath the sinusoidal endothelium and in the *capillarization of sinusoids*. Thus, an additional barrier is created between the sinusoidal lumen and hepatocytes, lengthening the diffusion pathway and impeding the exchange of substances between sinusoidal blood and parenchymal cells, rendering hepatocytes in a cirrhotic liver vulnerable to nutritive and ischemic injury.

Hemodynamic alterations are not only secondary to structural distortion of normal anatomy, but may be due to primary vascular and circulatory changes. Thus, an obliterative portal venopathy, for example, may occur and initiate a process leading to fibrosis and nodular transformation. Small (< 1 cm in 75% of cases) *focal nodular hyperplasia (FNH)-like nodules* have been described in 15% of 130 cirrhotic explant livers, believed to result from vascular alterations in the cirrhotic liver. Unlike in FNH, (considered to be a hyperplastic response to increased blood flow in normal liver) the ß-catenin pathway is not activated in cirrhotic FNH-like nodules [34, 47].

Generally fibrogenesis in liver cirrhosis is considered to represent a repair mechanism triggered by continuous loss of liver parenchyma. However, fibrogenesis may also be a primary process, initiated by stimulation of stellate cells without prior loss of liver tissue, which occurs for example in hypervitaminosis A.

## Disturbances in Hepatocyte Growth and Proliferation

Changes in hepatocellular growth combined with parenchymal destruction, fibrotic strangulation of liver tissue and vascular alterations contribute to the nodular transformation of the liver. How exactly cirrhotic nodules form, however, is still poorly understood. Several mechanisms probably act in concert, such as tissue growth after parenchymal destruction, partitioning of nodules by fibrotic septa, and remodeling due to vascular and circulatory alterations. The proliferation of hepatocytes in a cirrhotic liver is viewed as a reactive regenerative process after cell loss. Regeneration, however, is incomplete, since complete restoration of normal hepatic architecture does not occur and the parenchymal defects are replenished by surrogate tissue (see Chapter 13).

Hepatocyte growth in a cirrhotic liver manifests itself by thickened hepatocyte plates, containing more than one cell layer, by an increased number of bi- and multinucleated hepatocytes and by nuclear size variation (anisonucleosis). Lipofuscin in proliferating hepatocytes is absent and growing parenchymal nodules compress adjacent structures. The proliferative activity of hepatocytes can be highlighted by immunostaining of proliferation markers, such as Ki-67 (labelled with the antibody MIB-1) or proliferating cell nuclear antigen (PCNA). Generally, with advancing cirrhosis and increasing Child-Pugh stage the proliferation of hepatocytes decreases. However, there is no strict correlation between the proliferative activity of hepatocytes and the functional reserve of the liver or the prognosis of cirrhosis [15].

Nodular transformation in cirrhosis of biliary origin is not pronounced until the late stages of the disease. In alcoholic cirrhosis, hepatocyte proliferation is inhibited which possibly contributes to the micronodular aspect of alcoholic cirrhosis (see below).

## Pathology

Liver cirrhosis represents the advanced stage of nodular transformation of the organ. The normal lobular structure is absent in cirrhotic nodules which is the reason why they are called pseudolobuli [2, 3].

In the past, numerous terms were used to characterize cirrhosis, such as Laennec type, nutritive, post-necrotic, post-hepatitic, post-collapse, portal, septal, regular, and irregular, to name just a few [3, 20, 53]. A precise definition of these categories, however, was lacking and etiologic, pathogenetic and morphological criteria were widely overlapping. Thus, the multitude of terms did not testify to a differentiated etiopathogenetic perspective but was rather evidence of our conceptual confusion. *The macroscopic aspect of a cirrhotic liver does not allow for drawing firm conclusions regarding its etiology and pathogenesis*. What appears morphologically identical may be due to various causes and pathogenetic mechanisms. Thus, *etiologic, pathogenetic and morphological criteria of liver cirrhosis are separate but complementary categories*. The pathologist should use terms, such as alcoholic or post-hepatitic cirrhosis, only if the morphological aspect unequivocally permits it, which, as mentioned above, often is not the case. Anyway, in today's clinical practice it is generally the clinician who informs the pathologist about the etiology of liver disease, and not vice versa. Therefore, in addition to etiologic and pathophysiological interpretation the pathologist should primarily provide a detailed descriptive anatomical-pathological diagnosis. A simple, i.e. reproducible and comprehensible, macroscopical description of cirrhosis is its classification according to the size of nodules, specifically

- Micronodular
- Macronodular, and
- Mixed forms

These three categories do not describe different diseases, but rather may represent different developmental stages of one disease process.

### *Macroscopical Findings*

#### Micronodular Cirrhosis

A liver cirrhosis in which nearly all nodules measure less than 3 mm in diameter is called micronodular (Figs. 79.1 and 79.2). A noticeable feature is the uniformity of the nodules. Typical causes for micronodular cirrhotic transformation are chronic alcohol abuse, bile duct obstruction, chronic venous outflow tract obstruction, hereditary hemochromatosis, Indian childhood cirrhosis, and many

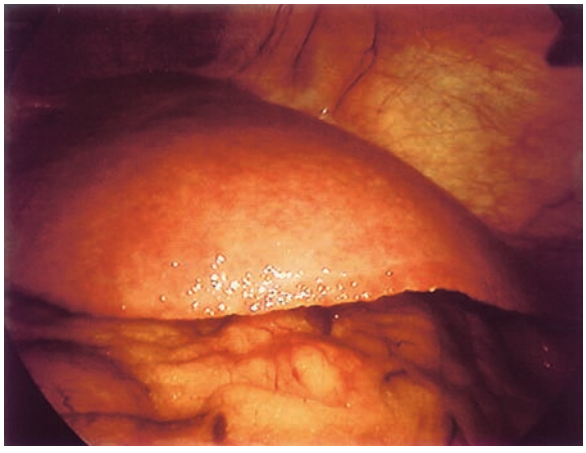

**Fig. 79.1** Laparoscopic appearance of micronodular alcoholic liver cirrhosis. At first glance the liver surface looks smooth. However, the light reflexes are split and the margin of the liver is finely undulated. As a rule such findings are underdiagnosed on ultrasonography as fatty liver without cirrhotic change

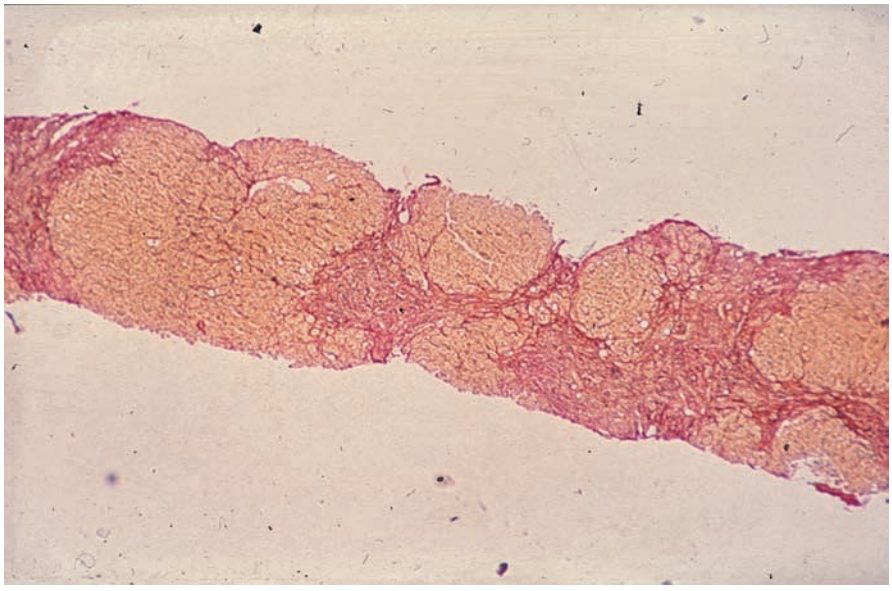

**Fig. 79.2** Biopsy specimen of the liver shown in Fig. 79.1. The pseudolobular cirrhotic transformation is easily recognizable in connective tissue staining. Domagk Staining (× 50)

hereditary metabolic diseases in pediatric hepatology. In micronodular cirrhosis the pseudolobuli usually contain portions of only one acinus ("monoacinar cirrhosis"). Portal tracts (for example in venous outflow obstruction) and efferent veins (for example in biliary obstruction) are only rarely found in micronodules. The fibrous septa contain new blood vessels that form a "fibrovascular compartment" which bypasses the sinusoids and shunts the blood through portal-venous bridges (connections between portal tracts and centrilobular veins) directly into the systemic circulation. The ratio of extracellular matrix to liver parenchyma is higher than in macronodular cirrhosis, which is why micronodular cirrhosis usually has a more firm consistency than macronodular cirrhosis. The early stages of alcoholic cirrhosis, the most frequent cirrhosis in Europe and North America, are an exception. Due to marked steatosis in the early stages alcoholic cirrhosis presents with an enlarged and relatively soft liver. Since alcohol inhibits hepatocyte proliferation, the nodules remain uniformly small at first. During the further course the nodules become irregular, and mixed forms (micro- and macronodular) are common.

The micronodular transformation is best appreciated on the cut surface of the organ, while the liver surface, as seen on ultrasound and CT-scanning may appear smooth. The cut surface of the liver in alcoholic cirrhosis has a yellowish appearance (fat in hepatocytes), in hereditary hemochromatosis a reddish-brown (iron overload), and in biliary obstruction a greenish-brown (bile retention) aspect.

### Macronodular Cirrhosis

Macronodular cirrhosis is characterized by nodules greater than 3 mm in size (Fig. 79.3). The size of the nodules may vary considerably and nodules several centimeters in diameter are not uncommon. The pseudolobuli of macronodular cirrhosis consist of several acini, which is why it is also called "multiacinar cirrhosis". The pseudolobuli contain portal tracts and centrilobular veins, however, their architectural relationship is deranged. Most diseases leading to cirrhosis ultimately result in macronodular cirrhosis. Overlap between micro- and macronodular forms, however, is

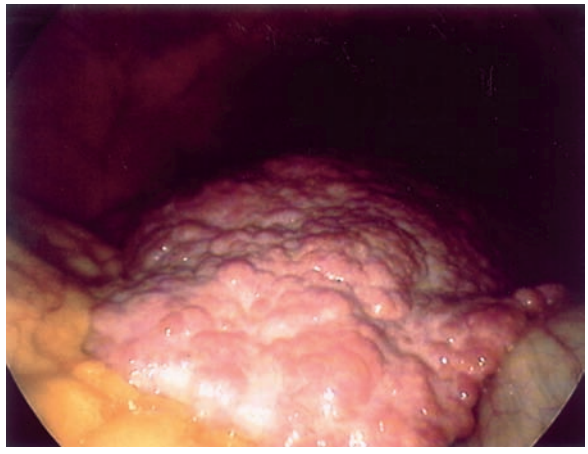

**Fig. 79.3** Macronodular liver cirrhosis

frequent. Liver cirrhosis due to chronic viral hepatitis and autoimmune hepatitis is macronodular from the outset. The growth activity within the nodules is recognizable by thickened liver cell plates consisting of several cell layers and by increased variation in hepatocyte nuclear sizes. The expansive proliferation of hepatocytes may lead to compression and pressure atrophy of adjacent parenchymal cells. Regional variation in circulation probably contributes to the non-uniform growth activity in different parts of the nodules, which results in variously shaped nodules and may lead to a "nodule-in-nodule" pattern. With progressive disease the broad fibrous septa cause increasingly deep grooves of the liver surface. Typical end-stage macronodular cirrhosis is small and hard ("shrunken liver"). Assuming that broad fibrous septa result from prior extensive parenchymal necrosis, this form of cirrhosis was designated previously as "postnecrotic" or "postcollaptic cirrhosis".

A special form of macrodular cirrhosis is "incomplete septal cirrhosis". It is seen most commonly in "burnt out" inflammation due to chronic viral hepatitis B. Its pathogenesis is unclear. Parenchymal necrosis and circulatory disturbances due to obliterative portal venopathy are assumed to play a role in its development [57]. Slender, incomplete fibrous septa extend from the portal tracts into the lobular parenchyma, where they often terminate halfway. Inflammatory cellular infiltrates within the septa are sparse or are absent completely. The hypoplastic portal tracts occasionally contain sclerotic veins. In their vicinity, an increased number of central venule profiles are present, but the normal vascular relationships are lost. The expanding parenchymal nodules have a blurred outline and compress the reticulin fiber network at their edges.

Diagnosing incomplete septal cirrhosis on liver biopsy may be extremely difficult, and its differentiation from noncirrhotic (idiopathic) portal hypertension may sometimes be impossible. The differential diagnosis should include liver changes associated with diffuse or focal nodularity, such as nodular regenerative hyperplasia and partial nodular transformation. In contrast to cirrhosis, intervening fibrous septa between the nodules are lacking in both conditions. The compression and condensation of sinusoidal collagen bundles at the edges of the nodules by the hyperplastic parenchyma may simulate fibrosis. Special reticulin fiber stains should always be employed in the diagnosis and differential diagnosis of these lesions.

### Mixed Forms

If the number of micronodules roughly equals that of macronodules, a mixed form of cirrhosis is said to be present. During the course of the disease micronodular cirrhosis may give way to the macronodular form. Viral superinfections, autoimmune processes and circulatory disturbances account for this transformation. Transformation of macrondular to micronodular cirrhosis does not occur.

## *Histological Findings*

Liver histology is the gold standard in the diagnosis of cirrhosis (see below). Histological findings allow for the assessment of inflammatory activity and may provide clues as to the etiology of cirrhosis. Cirrhosis with a marked inflammatory cell infiltrate, usually at the interface between septal connective tissue and parenchyma (interface hepatitis) is said to be active and progressive. Accordingly, fibrous septa that are infiltrated by inflammatory cells have been designated in the older literature as active, in contrast to passive septa lacking such an infiltration. Apoptotic (acidophilic bodies) and necrotic processes may occur throughout the lobular parenchyma. Portal-portal and portal-central fibrous bridging represents previous parenchymal necrosis. It significantly contributes to the structural transformation of the liver and to the progression of cirrhosis. Special stains (in addition to the routine hematoxylin and eosin stain), such as for example Masson Trichrome, Domagk, Ladewig, elastica, reticulin fiber and PAS after digestion with diastase, are helpful in visualizing the architectural transformation of liver tissue. The iron stain should be part of the routine arsenal, while staining of copper and copper-binding protein should be reserved for special diagnostic problems, such as cholestasis and Wilson's disease.

The histological lesions in different liver diseases leading to cirrhosis are discussed in the respective chapters. Following are a few selected examples hinting at histological characteristics which facilitate etiologic interpretation of cirrhosis.

In *chronic viral hepatitis B infection* "ground-glass hepatocytes" may occur that may be highlighted by immunostaining with antibodies against HBsAg (see Fig. 63.34a).

Lymphocytic aggregates within the portal stroma, often arranged in a follicle like pattern are typical of *chronic viral hepatitis C*.

A marked steatosis may be present in *alcoholic cirrhosis*. Often, however, in advanced alcoholic cirrhosis hepatocellular fat has disappeared. Damaged, ballooned and necrotic hepatocytes, Mallory-Denk bodies and neutrophilic infiltrates are signs of *alcoholic hepatitis*. Fibrosis in alcoholic liver disease usually starts in the perivenular areas and has a characteristic "chicken-wire" appearance.

Advanced *hereditary hemochromatosis* is characterized by a marked deposition of iron predominantly in periportal hepatocytes, in liver macrophages and in biliary epithelial cells of interlobular bile ducts (Fig. 79.4). Inflammation is only scant. However, even an extensive iron overload is no proof of hereditary hemochromatosis. A variously intense siderosis may be found in cirrhosis of different etiologies, with the exception of biliary cirrhosis, which usually contains only little iron [37].

*Wilson's disease* may produce various histologic patterns, such as steatosis, steatohepatitis, Mallory-Denk bodies, chronic inflammatory cell infiltrates with hepatocyte necrosis and apoptosis and increased deposition of copper in hepatocytes (see Fig. 81.4). Albeit characteristic, the latter, however, is not pathognomonic of Wilson's disease, and may also be observed in biliary cirrhosis and in longstanding obstructive cholestasis of any cause.

PAS-positive round intracytoplasmic inclusions resistant to diastase digestion hint towards *$\alpha_1$-antitrypsin deficiency* (see Fig. 83.2 and 83.3).

In *biliary cirrhosis* the lobular architecture remains intact for long periods of time. In end-stage *secondary biliary cirrhosis,* complex pseudolobuli form containing segments of several adjacent liver acini and resembling the pieces of a jigsaw puzzle. The normal vascular relationships remain essentially intact. These complex structural changes, however, usually can only be detected on the cut surface of the liver and not on liver biopsy.

The inflammatory and fibrotic lesions in *primary biliary cirrhosis* (PBC) are not distributed uniformly throughout the liver. Thus advanced stages of PBC and florid duct lesions coexist in the same liver and may even be observed in the same biopsy.

Chronic *venous outflow obstruction* causes perivenular fibrosis, dilatation of zone 3 sinusoids and ultimately "reverse lobulation". Nodular regenerative hyperplasia also may be a hint to venous outflow obstruction.

## Diagnosis

### *Clinical Manifestations*

The clinical spectrum of liver cirrhosis ranges from asymptomatic patients with normal laboratory findings, in whom the diagnosis of cirrhosis is made incidentally, to patients with decompensated disease and hepatic failure. At the time of diagnosis the majority of patients have compensated cirrhosis and physical

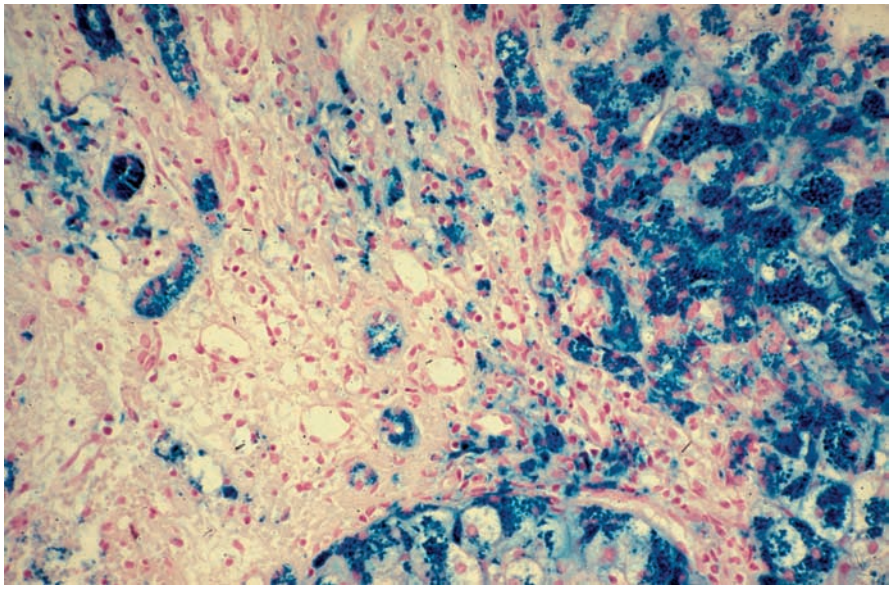

**Fig. 79.4** Hereditary hemochromatosis. Massive iron overload of periportal hepatocytes, portal macrophages and biliary epithelial cells. Prussian Blue staining (× 200)

examination discloses signs of chronic liver disease (see Section VI). A minority of patients initially present with decompensated disease manifested for example by ascites, esophageal variceal bleeding, signs of hepatic encephalopathy or the systemic manifestations of hepatocellular carcinoma.

After the diagnosis of cirrhosis has been established, its cause(s) should be clarified, since even in advanced stages the etiology is crucial in choosing the appropriate therapy. History and physical examination are important in determining the etiology of cirrhosis, but they are not definitive. Clinical and laboratory data should be interpreted conjointly (Table 79.2).

In addition to clarifying the etiology, the stage of disease must be determined. The most widely used clinical staging system of cirrhosis is that according to Child-Pugh, which also has prognostic significance (Table 79.3; see also Chapter 30). According to another classification, cirrhosis is classified into 4 stages. Stage 1 is characterized by the absence of esophageal varices and of ascites. Stage 2 patients have esophageal varices without bleeding and no ascites. Stage 3 is characterized by ascites with or without esophageal varices in a patient that has never bled, and stage 4 by gastrointestinal bleeding with or without ascites [14].

The clinical changes in patients with liver cirrhosis derive from the loss of functional parenchyma (*liver cell failure*) and the sequelae of vascular-parenchymal remodeling (*portal hypertension*). In the early stages of the disease the patients are asymptomatic. During the further course noncharacteristic complaints, such as fatigue and loss of energy supervene. The patients complain of sleep disturbances, they feel feeble and not well rested in the morning [13]. In this stage of the disease there is still no reversal of day-and-night-rhythm. Mood changes with easy irritability, occasional anxiety and depression may be observed. The history often reveals loss of libido which the patient only rarely mentions spontaneously by himself. Dyspeptic symptoms, bloating, loss of appetite and weight loss may initially divert the attention to the gastrointestinal tract, the more so as up to 10–15% of patients with liver cirrhosis have gastric and/or duodenal peptic ulcers. Disturbances in taste perception quite often occur in cirrhotics and probably are mediated centrally [38]. Increased abdominal girth described as tightness may be the first evidence of developing ascites.The full-blown clinical picture of cirrhosis is impressive and the diagnosis can hardly be missed at this stage (Fig. 79.5).

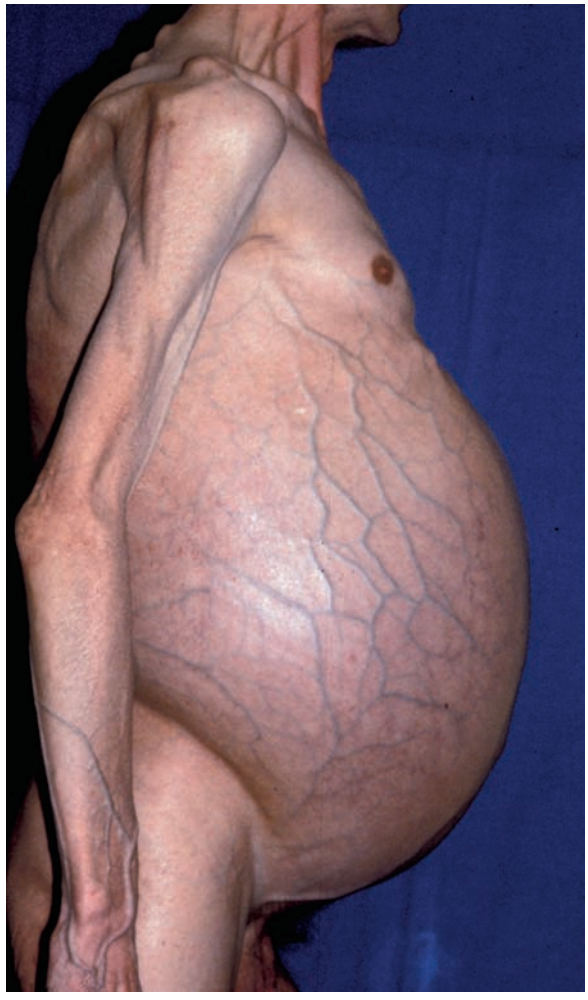

**Fig. 79.5** Patient with advanced liver cirrhosis, massive ascites and muscle wasting

The extent of physical findings depends on the stage of the disease and on the etiology of liver cirrhosis. Assigning the cause of cirrhosis based on physical finding, however, is not always possible, since findings are usually nonspecific with regard to etiology. One should also be aware that all physical findings typical of cirrhosis represent end-stage lesions common to all etiologies (Table 79.4).

Body composition is altered in patients with cirrhosis. *Malnutrition* is virtually ubiquitous in all types of cirrhosis (see Chapter 91). Liver cirrhosis is characterized by a significant reduction in body cell mass and body fat, and by a redistribution of body water. A pronounced loss of fat occurs already in the initial stages, followed by an accelerated loss of body cell mass in the advanced stages of liver cirrhosis [21]. The patient

**Table 79.2** Characteristic findings in patients with liver cirrhosis of various etiologies

| Disease | Findings |
|---|---|
| **Alcoholic liver disease** | History of alcohol intake (often negated by the patient) |
| | AST: ALT > 2 |
| | Decrease of γ-GT levels upon abstinence |
| | *Alcoholic hepatitis*: hepatomegaly, jaundice, fever, leukocytosis, AST and ALT < 500 IU/mL, AST: ALT > 2 |
| | *Alcoholic cirrhosis*: micronodular, surface on ultrasound often appears smooth, hepatomegaly |
| **Chronic hepatitis C** | Presence of anti-HCV |
| | Presence of HCV-RNA (viral load) |
| | Liver biopsy for grading and staging |
| | Vasculitic skin lesions (cryoglobulins) |
| **Chronic hepatitis B** | Persistence of HBsAg > 6 months after initial infection |
| | Liver biopsy for grading and staging |
| | "Ground glass" hepatocytes are liver cells with large amounts of HBsAg in their cytoplasm |
| **Primary biliary cirrhosis** | Male: female = 9: 1 |
| | Fatigue (early symptom), jaundice, pruritus (late symptoms) |
| | Mildly elevated aminotransferases, marked elevation of AP and γ-GT levels in serum |
| | ↑ serum cholesterol |
| | ↑ IgM, poitive AMA (subtype M2 [anti PDC-E2] is confirmatory) |
| | Histologically lesions are not distributed uniformly throughout the liver |
| | *Autoimmune cholangitis*: AMA negative, ANA positive, liver histology compatible with PBC |
| **Primary sclerosing cholangitis** | Strong association with inflammatory bowel disease, especially with ulcerative colitis. Approximately 70% of patients with PSC have ulcerative colitis |
| | Jaundice and pruritus are late symptoms |
| | Mildly elevated aminotransferases, marked elevation of AP and γ-GT levels in serum |
| | pANCA present in 75–80% of patients, but not specific for diagnosis of PSC |
| | ERCP is the diagnostic gold standard: strictures and dilatations of intra- and/or extrahepatic bile ducts |
| | *Liver biopsy* not required for diagnosis (exception: small duct PSC) |
| | *Complication*: cholangiocellular carcinoma |
| **Autoimmune hepatitis** | Female predominance |
| | Hypergammaglobulinemia |
| | *Type 1 (classic)*: ANA, SMA, anti-SLA, anti-actin, ANCA |
| | *Type 2*: anti-LKM1, ALC-1 |
| | *Overlap syndrome*: AMA |
| **Hereditary hemochromatosis** | Family history |
| | HFE gene mutation (heterozygotes usually do not develop cirrhosis) |
| | Dark skin tan in late stages |
| | Transferrin saturation elevated in approximately 90% of patients (males > 60%, females > 50%) |
| | *Hepatic Fe-index* in liver biopsy ≥ 1.9 (hepatic Fe-concentration in mmol/g: patient age) |
| **Wilson's disease** | Family history |
| | Neurologic-psychiatric symptoms in young age |
| | In each patient < 40 years with acute liver failure Wilson's disease should be included in the differential diagnosis |
| | ↓ Ceruloplasmin in serum in 95% of patients |
| | ↑ Copper excretion in urine |
| | Liver biopsy: various histologic patterns, for example steatosis, steatohepatitis, chronic hepatitis, cirrhosis |
| | ↑ copper concentration in hepatocytes (e.g. rhodanine stain) is not specific for Wilson's disease. Occurs in all chronic cholestatic liver diseases |
| **$\alpha_1$-antitrypsin deficiency** | Family history |
| | Chronic obstructive lung disease |
| | α-globulin band in serum electrophoresis may be flattened or absent |
| | $\alpha_1$-antitrypsin concentration in serum decreased |
| | *Liver biopsy*: globular, PAS-positive inclusions in hepatocytes after diastase digestion |

*AST* aspartate-aminotransferase, *ALT* alanine-aminotransferase, *AMA* antimitochondrial antibodies, *ANCA* antibodies against cytoplasm of neutrophils, *SLA* soluble liver antigen, *ANA* antinuclear antibodies, *ALC* antibodies against liver cytosol, *LKM* liver-kidney-microsomes, *PBC* primary biliary cirrhosis, *PSC* primary sclerosing cholangitis

**Table 79.3** Child-Pugh stages of liver cirrhosis

| Parameter | Points | | |
|---|---|---|---|
| | 1 | 2 | 3 |
| **Ascites** | 0 | +/+ + | + + + |
| **Encephalopathy** [a] | No | grade I/II | grade III/IV |
| **Serum albumin** (g/dL) | > 3.5 | 2.8–3.5 | < 2.8 |
| **Serum bilirubin** (μmoL/L)[b] | < 35 | 35–51 | > 51 |
| **in PBC, PSC** | < 69 | 69–170 | > 170 |
| **Prothrombin time** (%) | > 70 | 40–70 | < 40 |
| **INR** | < 1.7 | 1.8–2.3 | > 2.3 |

Scoring occurs by adding the points for the various parameters.
5–6 points: Child A (well compensated disease)
7–9 points: Child B (significant loss of hepatic function)
10–15 points: Child C (decompensated disease)
[a]Stages of hepatic coma according to Trey et al. (1966) N Engl J Med 274: 473–81 (see Section 80.4),
[b]17 μmoL/L = 1 mg/dL

**Table 79.4** Physical findings in patients with liver cirrhosis

| Finding | Comment |
|---|---|
| **Ascites** | Portal hypertension |
| **Hepatomegaly** | Facultative; small liver in posthepatitic cirrhosis |
| **Splenomegaly** | Portal hypertension |
| **Skin Changes** | |
| **Glazing lips and tongue** | Skin atrophy; papillary atrophy |
| **Oral rhagades** | Zinc deficiency |
| **Spider angiomas** | Central arteriole with radiating vessels |
| **"Banknote" skin** | Skin atrophy due to zinc deficiency |
| **Palmar erythema** | ↑ estrogen: testosterone (?) |
| **Dupuytren's disease** | Palmar fibromatosis; occurs predominantly in alcoholics |
| **Jaundice** | Advanced hepatocellular failure |
| **Purpura** | Vascular fragility; thrombocytopenia |
| **Scratch signs** | Pruritus |
| **Xanthelasms** | Chronic biliary/cholestatic diseases |
| **↑ Abdominal venous cutaneous vessels** | Portal hypertension |
| **Caput medusae** | Very rare; portal hypertension |
| **Nail Changes** | |
| **White nails** | Predominantly thumb and index finger |
| **Clubbed fingers/hour glass nails** | In hepatopulmonary syndrome |
| **Endocrine Changes** | Feminization in men |
| **Abdominal baldness** | |
| **↓ Terminal hair in men** | |
| **Testicular atrophy** | |
| **Gynecomastia** | Increased ratio of estrogen to free androgen due to decreased testosterone production, and increased peripheral conversion of testosterone to estradiol |
| **Amenorrhea** | |
| **Miscellaneous** | |
| **Foetor hepaticus** | Intestinal methylmercaptanes (?) |
| **Kayser Fleischer Ring** | Green-brown corneal ring; Wilson's disease |
| **Muscle atrophy** | Cytokines (?); malnutrition |
| **Parotid gland swelling** | Malnutrition; predominantly in alcoholics |

with advanced and decompensated *cirrhosis of viral origin* has quite a typical appearance. Due to generalized muscle wasting the extremities are lean and emaciated (just "skin and bones"), the facial fat pads have receded, and the patient is hollow-cheeked and cachectic (Fig. 79.5). The lips are dark red, the tongue is smooth and bright red due to papillary atrophy, and oral rhagades (linear scars at the angles of the mouth) are present. With massive ascites, the abdomen is markedly distended. The tense and atrophic abdominal skin appears glossy due to loss of abdominal hair (abdominal baldness). The superficial abdominal venous vessels are prominent; a caput medusae, however, is a very rare finding. Spider angiomas occur preferentially on the upper part of the body and on the face. Very rarely they also may be present in the rectal mucosa (see Fig. 80.12).

Due to hyperdynamic circulation the skin is warm and dry, and the pulse is strong. In this advanced stage of cirrhosis jaundice is usually present and if accompanied by pruritus the skin may show scratch effects. The hands display a palmar erythema and the skin on the back of the hand is thin, atrophic and puckered. Petechiae and ecchymoses preferentially occur on the extremities. Due to hormonal changes, signs of feminization in men, such as gynecomastia, testicular atrophy, decrease of secondary hair-growth, and amenorrhea in women may be observed. With advanced liver cell failure a sweetish, slightly fetid breath (foetor hepaticus) hints towards the presence of hepatic encephalopathy. Possibly it is caused by methymercaptanes generated in the gut. Disturbances of memory, concentration and coordination are further clues of hepatic encephalopathy.

In end-stage cirrhosis the liver usually is small, firm and palpable only on deep inspiration. With non-tense ascites and thin abdominal wall, its nodular surface occasionally may be palpable. The spleen is enlarged due to portal hypertension, but the degree of splenomegaly correlates poorly with the severity of portal hypertension.

In contrast to the patient with cirrhosis of viral origin, the typical patient with *alcoholic cirrhosis* appears turgid. The face is bloated due to long-standing alcohol

abuse, the gait is ataxic with a wide-based stance, lateral instability of the trunk and difficulty in maintaining balance when turning. Leg edema may assume enormous (elephantiasis-like) proportions. Dupuytren's disease (palmar fibromatosis) and bilateral parotid swelling occur more often in alcoholics. The liver in alcoholic cirrhosis is usually enlarged. In addition, the alterations described above may also be present in alcoholics.

In *biliary cirrhosis* signs of long-standing cholestasis dominate the clinical picture, such as scratch signs due to pruritus or xanthelasms and xanthomas.

## Laboratory Findings

The laboratory changes depend on the stage of the disease and the cause of cirrhosis. The most important nonspecific laboratory changes are listed in Table 79.5. The parameters specific for various etiologies, such as immune-serologic and viral findings are discussed in the chapters dealing with the respective disease.

Most cirrhotic patients have a mild normo- to macrocytic anemia. Leuko- and thrombocytopenia are due to hyperspelnism, but platelet numbers < 30,000 /μL are rare and clinically relevant thrombocytopenic bleeding is rather the exception.

Elevated serum levels of aminotransferases usually indicate the process still being active. Flares in aminotransferase levels hint at a necroinflammatory process, but there is no strict correlation between the concentration of aminotransferases and the necroinflammatory activity. A predominant increase in aminotransferase levels suggests a hepatocellular cause of cirrhosis, while a preponderance of cholestatic parameters, such as alkaline phosphatase combined with γ-glutamyl transpeptidase favors a biliary etiology. Usually, ALT levels are higher than AST concentrations in cirrhosis of viral origin (chronic hepatitis B ± D and C), while in alcoholic cirrhosis AST is higher than ALT. In addition, γ-GT levels are often elevated in the latter, and decline during abstinence from alcohol. The significance of these enzymatic patterns, however, in advanced ("burnout") stages of cirrhosis is reduced, with aminotransferase levels usually being only mildly increased (< 100 IU/L) or even normal. *Low or normal aminotransferase levels do not exclude cirrhosis, but rather support the diagnosis in a patient with an appropriate history and physical findings*.

Serum bilirubin concentration remains normal for a long time or is only slightly increased. The level of serum bilirubin, especially in biliary cirrhosis is considered to be of prognostic significance. Elevated levels of serum cholesterol and triglycerides, with lowered concentrations of cholesterol esters are also observed predominantly in cirrhosis of biliary origin.

The functional reserve capacity of the liver is high. Therefore, reductions in the synthetic capacity of the liver, such as a reduced synthesis of albumin and of hepatic clotting factors with resulting hypoalbuminemia and prolongation of prothrombin time, respectively, and a decrease in serum level of choline esterase occur only

**Table 79.5** Laboratory serum findings in patients with liver cirrhosis

| Laboratory finding | Comment |
|---|---|
| **Aminotransferases** | Viral cirrhosis: ALT > AST<br>Alcoholic cirrhosis: AST > ALT |
| **Parameters of Cholestasis** | Biliary cirrhosis: ↑ AP and γGT |
| **Bilirubin** | Serum level rises in advanced stage of cirrhosis |
| **Choline esterase** | Parameter of hepatocyte synthetic capacity. Serum level decreases in advanced stage of cirrhosis |
| **Prothrombin time** | Parameter of hepatocyte synthetic capacity. Prolonged in advanced stage of cirrhosis |
| **Albumin** | Parameter of hepatocyte synthetic capacity. Serum level decreases in advanced stage of cirrhosis |
| **γ-globulins** | Serum levels are increased with a broad based γ-band on serum electrophoresis in 80% of patients with cirrhosis. γ-globulins make up for 20–35% of all proteins<br>*Autoimmune hepatitis*: γ-globulins increased in all patients. γ-globulins > 50% of total protein<br>*Primary biliary cirrhosis*: ↑ IgM<br>*Alcoholic cirrhosis*: ↑ IgA<br>*Viral cirrhosis*: ↑ IgG |
| **Blood count** | Mild normo- to macrocytic anemia. Leukopenia, thrombocytopenia (hypersplenism) |
| **Ammonia** | Serum levels increased in advanced stage of cirrhosis. Levels do not correlate with signs and symptoms of hepatic encephalopathy |
| **Branched-chain amino acids**[a] | Serum levels decreased in advanced stage of cirrhosis |
| **Aromatic amino acids**[b] | Serum levels increased in advanced stage of cirrhosis |

*ALT* Alanine-aminotransferase, *AST* Aspartate-aminotransferase, *γGT* Gamma glutamyl-transpeptidase, *AP* Alkaline phosphatase
[a]Branched-chain amino acids: valine, leucine, isoleucine,
[b]Aromatic amino acids: phenylalanine, tyrosine, methionine

in advanced disease stages. In interpreting decreased serum levels of albumin and of choline esterase one should also take into account that these proteins are negative acute phase reactants, whose concentrations therefore also are reduced in acute infections.

Ammonia concentration in serum rises in advanced liver failure, usually accompanied by changes in the normal amino acid pattern, i.e. a decrease in branched-chain amino acids (valine, leucine, isoleucine) and a rise in aromatic amino acids (phenylalanine, tyrosine, methionine). The performance of tests of specific liver function, such as indocyanine green, MEGX and caffeine clearance (see Chapter 35) is dispensable in clinical practice.

80% of patients with cirrhosis, and all patients with an autoimmune hepatitis have a hyper-γ-globulinemia with a broad based γ-band in serum electrophoresis, which is regarded as sign of nonspecific activation of the humoral immune system. Twenty to 35% of all proteins, in autoimmune hepatitis even more than 50% fall upon γ-globulins. The distribution of immune globulin classes also to a certain degree permits to draw some conclusions as to the cause of cirrhosis. While in cirrhosis of viral origin usually IgG levels are elevated, in alcoholic cirrhosis IgA, and in primary biliary cirrhosis IgM predominates. The attentive interpretation of serum electrophoresis will not only allow for detection these frequent changes, but in isolated cases will permit one to formulate the presumptive diagnosis of $\alpha_1$-antitrypsin deficiency, in which the α-globulin band may be flattened or completely absent.

## Imaging Techniques

The diagnosis of advanced and decompensated cirrhosis can be made with sufficient accuracy by interpreting the clinical manifestations and laboratory findings. Imaging techniques play a leading role in the diagnosis of early stages of cirrhosis and in detecting focal (neoplastic) alterations in cirrhotic livers.

*Sonography*, *CT-scanning* and *magnetic resonance imaging* are the prime imaging modalities in the diagnosis of cirrhosis, and the reader is referred to Chapters 37 and 38 for a discussion of these techniques. CT and MRI are not superior to ultrasound in the diagnosis of cirrhosis, and all three techniques, when combined with contrast enhancement, are regarded as equivalent in detecting and characterizing nodular hepatocellular carcinoma in a cirrhotic liver. *Endoscopic ultrasonography* (EUS) may visualize parts of the left liver lobe. A systematic examination of the liver is not possible with EUS. Thus, except for selected findings in the left liver lobe that happen to be within the range of the EUS-transducer (and may also be biopsied through the EUS-scope), EUS has no place in the diagnosis of liver disease.

A widely underused technique in the diagnosis of cirrhosis is *laparoscopy* (see Chapter 45). *Mini-laparoscopy* is a very safe procedure, takes only a few minutes to perform, allows diagnosis of cirrhosis at a glance, and permits acquisition of several liver biopsies under direct vision, which further increases safety and diagnostic accuracy. Especially in biliary cirrhosis, which often is micronodular and not uniformly distributed throughout the liver, laparoscopy with biopsy is the best staging method currently available.

*Nuclear imaging techniques* do not play a role in the diagnosis of cirrhosis.

*Transient elastography* is an evolving technique measuring liver elasticity (stiffness) (see also Chapter 28). It has a good diagnostic accuracy for the diagnosis of advanced fibrosis, but a high variation of results is found in earlier stages of fibrosis [24]. Moreover, one should not equate fibrosis with cirrhosis. The diagnosis of cirrhosis, in addition to fibrosis, requires the demonstration of architectural distortion and loss of normal vascular relationships.

The gold standard in the diagnosis of cirrhosis is *liver biopsy* which nowadays should not be performed anymore as a "blind" biopsy, but rather under ultrasound, CT or preferentially under laparoscopic guidance (see Chapters 43, 44, and 45). Liver biopsy not only allows diagnosing pseudolobular remodeling but also may yield clues as to the etiology of cirrhosis (see above).

However, the diagnosis of cirrhosis in a biopsy specimen is afflicted with some caveats. Quite often a biopsy cylinder from a cirrhotic liver disintegrates into several fragments, which makes interpretation of architectural remodeling difficult. Percutaneous biopsy in macrododular cirrhosis is affected by sampling variability, which may lead to a false negative diagnosis in up to 30% of cases [39, 54]. Biopsy under laparoscopic guidance reduces sampling error [44].

Therefore, in examining a biopsy specimen the observance of certain changes and criteria is helpful in establishing the diagnosis of cirrhosis [52].

- The diagnosis of cirrhosis in a biopsy specimen is highly probable if at least one parenchymal nodule, completely surrounded by connective tissue is present.
- The diagnosis of cirrhosis is definite if several such pseudolobules are present, or if fibrous septa cross abnormally structured lobules.
- The presence of broad fibrous septa without portal tracts and an abnormal architectural relationship between terminal venules and portal tracts, even in the absence of fibrous septa, is highly suggestive but no prove of cirrhosis.
- If the biopsy specimen is fragmented, fibrous tissue at the edges of the fragments should be looked for, which often requires special reticulin and connective fiber stains.
- A marked variation in size of hepatocytes and hepatocyte nuclei in different areas of the biopsy specimen and changes in growth pattern, mirrored by multicellular liver cell plates or by compression effects at the edges of parenchymal nodules are compatible with cirrhosis.

Despite observing all these criteria, it still may be difficult to decide in the individual case whether complete cirrhosis is already present or if the patient has still precirrhotic fibrosis.

Characterization of liver cirrhosis nodules by analysis of gene-expression profiles in order to disclose genetic mechanisms involved in the development or progression of hepatocellular carcinoma is still experimental [41].

**Table 79.6** Different forms of ascites[a]

- Portal ascites
- Malignant ascites
- Inflammatory, bacterial ascites
- Pancreatogenic ascites
- Chylous ascites
- Hypoalbuminemic ascites

[a]See Chapter 54

**Table 79.7** Causes of non-cirrhotic portal hypertension[a]

**Prehepatic**
- Portal vein thrombosis
- Splenic vein thrombosis
- Arterio-venous splanchnic shunts
- Marked splenomegaly

**Intrahepatic**
- Alcoholic hepatitis (central sclerosis)
- Acute liver failure
- Drugs/toxins (e.g. vinyl chloride, vitamin A, 6-mercaptopurine)
- Budd-Chiari syndrome
- Sinusoidal obstruction syndrome (veno-occlusive disease)
- Peliosis hepatis
- Nodular regenerative hyperplasia
- Schistosomiasis
- Sarcoidosis
- Primary biliary cirrhosis (in precirrhotic stage)
- Hepatocellular carcinoma or hepatic metastases
- Hemangioendotheliomatosis
- Myeloproliferative diseases
- Idiopathic

**Posthepatic**
- Chronic right heart failure
- Obstruction of inferior V. cava

[a]See Chapter 53

## Differential Diagnosis

The full blown clinical picture of decompensated cirrhosis usually poses little differential diagnostic difficulties. In the first instance the various etiologic forms of cirrhosis, of ascites and the non-cirrhotic causes of portal hypertension must be differentiated (Tables 79.1, 79.6 and 79.7).

If on ultrasonography the liver surface appears undulated with nodular lesions in the parenchyma, liver metastases, multiple hemangiomas, a nodular regenerative hyperplasia and a partial nodular transformation must all be excluded (see Section XXII). Hepatic granulomas, for example in sarcoidoisis, or a chronic thrombosis of hepatic veins, rarely may cause a similar appearance.

*Indian childhood cirrhosis* is a particular form of cirrhosis in children between the ages 6 months and 5 years that was originally described in, but is not confined to India. The pathogenesis is unclear, but the very high copper concentration in the liver is believed to play an important pathogenetic role in the development of cirrhosis.

Histologically, steatosis, ballooning of hepatocytes, focal necrosis, Mallory-Denk bodies, inflammatory cell infiltrates (steatohepatitis) and a marked pericellular chicken-wire fibrosis are found, which lead to micronodular cirrhosis [55]. Clinically progressive hepatomegaly accompanied by a splenomegaly are

observed. Penicillamine is useful in the initial stages of the diseases, but in the past as a rule patients died due to liver failure. Orthotopic liver transplantation is the only therapeutic option that significantly improves outcome.

## Course and Prognosis

Advanced cirrhosis is the irreversible end-stage of chronic liver disease. Fibrosis in a cirrhotic liver is not a static condition but rather a dynamic process reflecting continuous formation, degradation and remodeling of extracellular matrix. This process results in qualitative and quantitative changes of the extracellular matrix which may be increased tenfold in a cirrhotic liver. Up to a certain degree, connective tissue may be degraded and fibrosis, including fibrosis in a cirrhotic liver, may regress [16–18, 22, 23]. Cirrhotic structural transformation, however, is permanent, and, despite occasional claims to the contrary, complete reversal of cirrhosis has yet to be demonstrated convincingly. Thus, the old dictum that *once cirrhosis is fully established it is irreversible* still holds true [16, 57].

Comorbidities affect prognosis and should be kept in mind when caring for patients with cirrhosis. Cirrhotic patients have a higher incidence of gallbladder stones and 10–15% of cases concomitantly have type 2 diabetes mellitus (insulin resistance with hyperinsulinemia) and gastro-duodenal ulcers. Sudden and unexpected deterioration of a minimal hepatic encephalopathy should prompt the search for infections, gastrointestinal bleeding, renal failure, electrolyte disturbances (diuretic therapy!) or a hepatocellular carcinoma (HCC). Patients with long-standing cirrhosis of any etiology have an increased risk of HCC. Of particular importance, because of the high prevalence of these conditions in the general populations, are type 2 diabetes mellitus and obesity. Obesity (body mass index > 30 kg/m$^2$) and type 2 diabetes mellitus represent independent risk factors for the development of both cirrhosis and HCC [11, 19, 42]. Therefore, each patient with liver cirrhosis, particularly obese and diabetic patients, should undergo *long-term routine HCC screening with ultrasound* (performed by an experienced examiner with a state of the art machine) *and by measuring serum* $\alpha_1$*-fetoprotein levels every 6 months* [12, 31, 45]. The majority (90%) of arterially enhancing nodules < 20 mm in cirrhosis, however, are benign (see Chapter 102) [10].

Mortality rates from cirrhosis show favourable trends in countries following the reduction in alcohol consumption and hepatitis B and C virus infection. In England and central and eastern European countries however, population based mortality rates from liver cirrhosis have sharply increased in the past 35 years. This upward trend in mortality rates is attributed to the persistent increase in the prevalence of alcohol consumption. Increased mortality from accidents, suicides, and mental disorders, particularly among those with alcoholic cirrhosis, indicates that prognosis is influenced by behavioral as well as by physical pathology [6, 48]. Thus, liver cirrhosis remains a disease with a very poor prognosis.

The natural history of cirrhosis is characterized by a *"compensated phase"*, defined by the absence of complications, such as ascites, variceal bleeding, encephalopathy and by preserved synthetic and excretory functions (albumin ≥ 3.5 g/dL, INR ≤ 1.5, total bilirubin ≤ 1.5 mg/dL), followed by a rapidly progressive phase marked by increasing portal pressure and declining liver function, resulting in the development of ascites, portal hypertensive gastrointestinal bleeding, encephalopathy and/or jaundice. The development of any of these complications defines the transition from a compensated to a *"decompensated phase"*. Transition from a compensated to a decompensated stage occurs at a rate of 5–7% per year. During a 10 year follow up of compensated viral cirrhosis, HCC develops in 21–32% of cases, followed by ascites (19.5–23%), jaundice (17%), upper gastrointestinal bleeding (4.5–6%), and encephalopathy (1–2%) [4, 51, 56].

Survival of patients with compensated cirrhosis is significantly longer than that of decompensated patients with median survival times of 12 years and 2 years, respectively (Fig. 79.6). The mortality risk increases as the stage and the number of complication episodes increases [14, 30].

The one-year outcome probabilities according to clinical stages (see above) are summarized in Fig. 79.7. While patients remain in *stage 1*, the mortality rate is as low as 1% per year. Patients exit this status at a cumulative rate of 11.4% per year: 7% because of the development of varices and 4.4% because of the development of ascites (with or without varices). For patients remaining in *stage 2*, the mortality rate is 3.4% per year. Patients leave this status by developing ascites (6.6% per year) or by developing variceal bleeding before or at the time of development of ascites (rate 4% per year). For patients remaining in *stage 3*, the mortality rate is

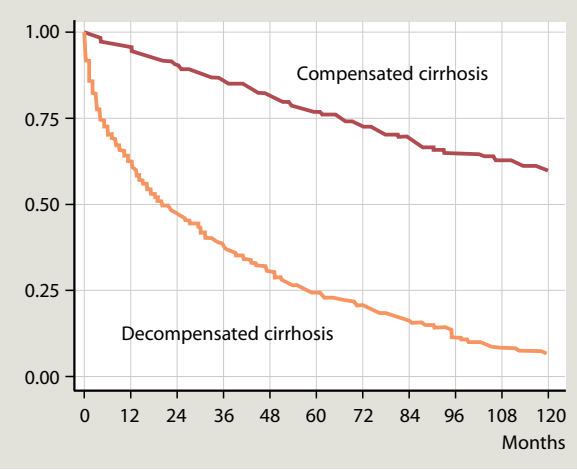


**Fig. 79.6** Survival of patients with compensated and decompensated cirrhosis. (Adapted from [14]. With permission)

20% per year. Patients exit this stage by bleeding (7.6% per year). In *stage 4* the 1-year mortality rate is 57%, with nearly half of these deaths occurring within 6 weeks from the initial episode of bleeding.

The *Child-Pugh stages* also correlate quite well (largely independent from etiology) with the 1 and 2 year survival rates:

Child A: 100 and 85%
Child B: 80 and 60%,
Child C: 45 and 35%, respectively.

The *APACHE III-score* (Acute Physiology, Age and Chronic Health Evaluation) appears to be superior to the Child Pugh-classification in estimating the short term outcome – hospital mortality – especially if information regarding ascites and prolongation of prothrombin time is integrated into the APACHE-score [9]. However, the APACHE-score is more cumbersome to determine than the Child-Pugh stage and is not widely used in clinical practice.

The *MELD score* correlates significantly with residual liver function. Increasing MELD score is associated with the onset of ascites and encephalopathy, it reliably predicts the mortality risk in patients with end-stage liver disease and is suitable for use as a disease severity index to determine organ allocation priorities [7, 29, 32, 50]. Dilutional hyponatremia (< 126 mEq/L), refractory ascites and the development of hepatorenal syndrome are associated with a poor prognosis. Addition of serum sodium to MELD can improve its prognostic accuracy [1, 5, 36, 46].

Additional parameters that have been reported to add in assessing the patient prognosis are indices of malnutrition, the presence of diabetes mellitus or a pathological oral glucose tolerance test, and low HDL cholesterol levels (< 30 mg/dL), all correlating with a poor prognosis. HDL cholesterol in noncholestatic cirrhotic patients may be regarded as a liver function test and an indicator of prognosis [26, 27, 43].

## Therapy

The first step in the treatment of cirrhosis aims at eliminating the cause(s), hoping to stop or at least to delay the progression of further architectural transformation of the liver. If the alcoholic patient abstains from ethanol, if replication of hepatitis B or C virus is arrested and the virus eliminated, the necroinflammatory process usually will abate and finally come to a standstill. Due to the high functional reserve capacity of the liver, the remaining vital parenchyma will sustain and improve organ function. However, clinical experience also teaches that despite eliminating the causative stimulus, once a critical point is reached the cirrhotic process may progress. The factors responsible for this continuing cirrhotic transformation (autoimmunity, circulatory disturbances, viral superinfections, viral persistence in liver tissue?) are poorly understood.

General measures, such as oral supplementation with branched-chain amino acids that can be administered for a long period improves event-free survival, serum albumin concentration, and quality of life in patients with decompensated cirrhosis with an adequate daily food intake [40].

Therapeutic interference with the fibrotic process, be it by inhibiting fibrogenesis or by stimulating the degradation of extracellular matrix, would be highly desirable, but currently is not possible. Thus, at *the present time therapy of cirrhosis essentially is the management of its complications and sequelae* (see Chapter 80).

A significant progress in the treatment of end stage liver cirrhosis is orthotopic liver transplantation (OLT). Since the first OLT was performed in 1963, this technique has evolved from an experimental intervention to a life saving operation. Current 1 and 5 year overall survival rates after OLT are 80–90% and 70%, respectively. In contrast, with conservative treatment the 5 year survival rate of patients with decompensated

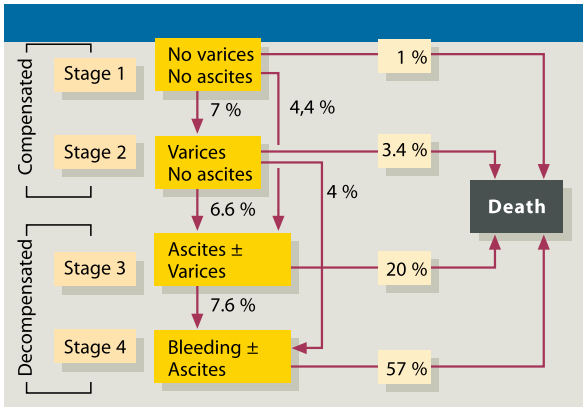


**Fig. 79.7** Clinical course of cirrhosis: 1 year outcome probabilities according to clinical stages (According to [14]. With permission)

cirrhosis is only 20%. Timely referral to a specialized liver center is mandatory in caring for patients with liver cirrhosis.

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# Complications of Liver Cirrhosis

# 80

Henryk Dancygier

## Chapter Outline



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Most complications of liver cirrhosis are either due to *portal hypertension* and/or to *progressive hepatic dysfunction*. Hepatocellular carcinoma also may be viewed as a complication of liver cirrhosis especially when it develops in the course of chronic viral infection, hereditary hemochromatosis, $\alpha_1$-antitrypsin deficiency, chronic alcoholic and nonalcoholic fatty liver disease. The malignant tumors of the liver are discussed in Chapter 102.

## 80.1 Ascites, Varices, Portal Hypertensive Gastroenteropathy

Gastrointestinal complications in patients with liver cirrhosis are primarily due to portal hypertension (see Chapter 53), with ascites, and gastrointestinal bleeding from esophagogastric varices and/or from portal hypertensive gastroenteropathy being of prime importance.

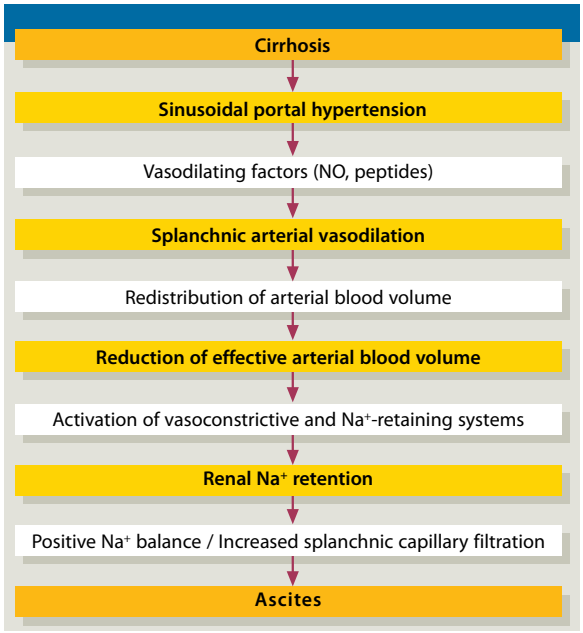


**Fig. 80.1** Simplified pathogenesis of cirrhotic ascites

### Ascites

This chapter focuses on the therapeutic approach to cirrhotic ascites [15, 25, 31]. For etiology, pathogenesis, diagnosis, differential diagnosis, prognosis and approach to the patient with ascites see Chapter 54.

Approximately 25% of patients with compensated cirrhosis develop ascites during a follow up period of 10 years. The development of ascites is a prognostically important event in the natural history of liver cirrhosis. It is associated with a mortality rate of 50% and 80% within 2–5 years respectively after the first ascites episode. A rise in serum creatinine to >1.5 mg% in a patient with cirrhotic ascites is associated with a mortality rate of up to 80% within 6–12 months [7].

A simplified scheme summarizing the pathophysiology of ascites is depicted in Fig. 80.1. Ascites is often complicated by an impairment of free water excretion by the kidneys and by renal vasoconstriction, which may lead to dilutional hyponatremia and to the development of a hepatorenal syndrome. Persistent ascites and low serum sodium (<126 mEq/L) identify patients with cirrhosis with high mortality risk even if MELD score is low [18, 28].

The *aims of ascites treatment* are the creation of a negative $Na^+$- and water-balance with a desired daily weight loss of approximately 500 mg in patients without peripheral edema and up to 1 kg in those with peripheral edema. If these goals cannot be met by dietary measures and by diuretics alone, ascites is said to be *refractory* (see below), and portal pressure has to be lowered by invasive methods creating portal-systemic shunts.

Ascites can further be classified into *simple, uncomplicated* or *problematic, complicated* (Table 80.1). An uncomplicated ascites is not infected and not associated with the development of the hepatorenal syndrome.

**Table 80.1** Types of ascites

| | Uncomplicated ascites | Complicated acites[a] |
|---|---|---|
| Ascites grade[b] | Grade 1 or 2 | Grade 3 |
| Encephalopathy | – | + |
| $Na^+$ in 24 h urine | >20 mmoL | <10 mmol |
| Serum $Na^+$ | >130 mmoL/L | <130 mmol/L |
| Serum $K^+$ | 3.6–4.9 mmol/L | <3.5 or >5 mmol/L |
| Serum creatinine | <1.5 mg% | >1.5 mg% |
| Serum albumin | >3.5 g/dL | <3.5 g/dL |

[a]Spontaneous bacterial peritonitis may be present

[b]*Grade 1*: mild ascites only detectable by ultrasound examination; *Grade 2*: moderate ascites, manifest on physical examination by moderate symmetrical distension of the abdomen; *Grade 3*: large, tense ascites or gross ascites with marked abdominal distension

**Table 80.2** Stepped care approach to cirrhotic ascites

| Step | Action | Success rate |
|---|---|---|
| 1 | Bed rest and daily $Na^+$-restriction to 2 g $Na^+$ or 5.2 g dietary salt + Fluid restriction to 1–1.5 L/day | 10–15% |
| 2 | In addition: spironolactone up to 400 mg p.o. in two divided doses | 65% |
| 3 | In addition: loop diuretic, e.g. furosemide up to 160 mg p.o. or i.v. daily | 85–90% |
| 4 (refractory ascites) | Paracentesis ≥ 5 L: albumin infusion 8 g/L ascites removed<br>Paracentesis < 5 L: albumin infusion 8 g/L ascites removed or consider infusion with synthetic colloidal plasma expander<br>If not successful: TIPS<br>If not successful or complications: consider liver transplantation | Nearly 100% |

*Predictors of an unfavourable prognosis* in patients with cirrhotic ascites with still normal blood urea nitrogen and serum creatinine are

- Impaired free water clearance (water diuresis after a water load[1])
- Dilutional hyponatremia
- Marked $Na^+$-retention (diminished $Na^+$-excretion)
- Reduction of glomerular filtration rate
- Increased plasma renin activity
- Increased plasma norepinephrine concentration
- Arterial hypotension

Cirrhotic ascites is managed by a "stepped care" approach (Table 80.2).

## General Measures

### Bed Rest

The upright posture activates the sympathetic nervous system and the renin-angiotensin–aldosterone system. Both activated systems reduce renal perfusion and the glomerular filtration rate, diminish urinary $Na^+$-excretion and decrease the response to diuretics. In single cases bed rest may improve the response to diuretics. However, there have been no clinical studies demonstrating increased efficacy of diuretics or shortened duration of hospitalization with bed rest. Bed rest is not recommended for the treatment of patients with uncomplicated ascites.

### Diet

*$Na^+$ restriction* to 90 mmol/day (corresponding to ~2 g $Na^+$/day or 5.2 g dietary salt/day) practiced as a no-added salt diet is advisable. A further reduction to ≤50 mmol/day (corresponding to ≤3 g dietary salt/day) is hardly practicable, since such an unpalatable diet will inevitably affect compliance with $Na^+$ restriction.

There is no role for water restriction in patients with uncomplicated ascites. *In the presence of dilutional hyponatremia (120–125 mmol/L) fluid should be restricted to 1–1.5 L/day* [32]. (Note: currently no clinical trials are available to assess the effect of water restriction on dilutional hyponatremia in patients with cirrhotic ascites; too rigorous water restriction may lead to central hypovolemia with consequent stimulation of ADH secretion and exacerbation of dilutional hyponatremia).

Cautious *protein restriction* should only be carried out in the presence of clinical signs of hepatic encephalopathy.

## Diuretics

In 85–95% of patients ascites can be mobilized with diet and diuretics. The simplest way to control therapeutic response is by daily weighing (Table 80.3).

The *main indications* for the use of diuretics in patients with liver cirrhosis are

- Mild to moderate ascites
- Marked ascites that cannot be mobilized by large-volume paracentesis, e.g. because of peritoneal adhesions
- Patients with edema without ascites
- Prevention of recurrence of ascites after therapeutic paracentesis

[1]Forty-five minutes infusion of glucose 5% at a dose of 20 mL/kg body weight. Fifteen minutes after the end of the infusion urine volume over 90 min is determined.
Urine volume: >8 mL/min = normal water diuresis, 3–8 mL/min = moderate impairment of water diuresis, <3 mL/min = strong impairment of water diuresis

**Table 80.3** Diuretics used in the treatment of cirrhotic ascites

| Drug | Dose (mg/day) | Onset of action after oral administration (h) | Duration of action (h) | $t_{1/2}$ (h) |
|---|---|---|---|---|
| *Loop diuretics* | | | | |
| Furosemide | 40–160 p.o. or i.v. | 0.5 | 4–6 | 1 |
| Torsemide | 5–40 p.o. | 1–2 | 6–8 | 3 |
| Bumetanide | 0.5–2 mg/dose p.o. or i.v. (maximum dose: 10 mg/day) | 0.5–1 | 4–6 | 1–1.5 |
| *$K^+$-sparing diuretics* | | | | |
| Spironolactone[a] | 50–400 p.o. | 1–2 days | 3–5 days | 1–1.5 (14–24)[b] |
| Eplerenone[c] | 25–50 p.o. | May take up to 4 weeks for full therapeutic effect | | 4–6 |
| Amiloride | 5–10 (– 30) p.o. | 2 | 24 | 6–9 |

[a]Is transformed in the liver to the active metabolites canrenone and 7α-thiomethylspironolactone
[b]Due to active metabolites
[c]Not yet approved for the use in patients with cirrhotic ascites. May be useful for patients intolerant of spironolactone

## Potassium-Sparing Diuretics

*Spironolactone* is the diuretic of choice for the initial therapy of ascites due to cirrhosis. There is a lag of 3–5 days between the initiation of spironolactone therapy and the onset of natriuresis.

Start therapy with spironolactone 100 mg p.o. daily and increase progressively by 100 mg every 3–5 days if diuretic response is insufficient. The maximal daily dose is 400 mg divided in two doses.

Spironolactone alone is superior to loop diuretics used alone and may be used as monotherapy in the treatment of cirrhotic ascites.

*Adverse effects*: Antiandrogenic activity (painful gynecomastia, decreased libido, impotence). Hyperkalemia. Metabolic acidosis with or without hyperkalemia in patients with renal insufficiency.

In patients intolerant to higher doses of spironolactone, the dose of spironolactone should be reduced and a loop diuretic added. There are some uncontrolled data that Tamoxifen at a dose of 20 mg p.o. bid may be useful in the management of painful gynecomastia.

*Triamterene* does not cause gynecomastia, it is however a relatively weak diuretic and inferior to spironolactone in mobilizing ascites.

The initial dose is 50–100 mg p.o. bid. Maximum dosage is 300 mg p.o. daily.

*Amiloride*, 15–30 mg p.o. qd, has a relatively weak diuretic action. It is not approved by the FDA for the treatment of cirrhotic ascites.

*Eplerenone*, 25–50 mg p.o. qd, is a selective aldosterone receptor antagonist with a lower incidence of endocrine related side effects than spironolactone. It might become an alternative to spironolactone in patients with painful gynecomastia. It is approved for the treatment of hypertension and heart failure (not yet for cirrhotic ascites).

The main adverse effect is hyperkalemia.

## Loop Diuretics

Loop diuretics used alone have a significantly lower efficacy than spironolactone alone. Therefore *a loop diuretic should not be used as the sole agent in patients with ascites due to cirrhosis*. Loop diuretics should be used as an adjunct to an aldosterone antagonist or to another potassium-sparing diuretic.

Furosemide is the loop diuretic of choice in patients with cirrhotic ascites.

*Furosemide* initially 20–40 mg p.o. or i.v. qd–bid. Depending on the diuretic response dosage may be increased up to maximally 160 mg p.o. or i.v. daily.

*Adverse effects* occur in up to 35% of patients: hypokalemia, hypochloremic metabolic alkalosis, hyponatremia, hypovolemia with prerenal azotemia, hepatorenal syndrome, hepatic encephalopathy, muscle cramps (often due to effective hypovolemia). In patients with severe muscle cramps albumin, quinidine, quinine, zinc sulphate or magnesium may be tried.

*Torsemide* has a longer half life than furosemide, but it is not superior to furosemide in the treatment of cirrhotic ascites. Initial dose is 5–10 mg p.o. or i.v. once daily. If needed dose may be titrated upwards up to 40 mg p.o. or i.v. daily.

*Bumetanide* is similar to furosemide. The initial dose is 1 mg p.o. or i.v. once daily, which, if necessary is titrated upwards by doubling the dose. The maximum dose of bumetanide should not exceed 10 mg/day.

## Practical Approach to Diuretic Therapy

Diuretics and dietary $Na^+$-restriction are the first line treatment of ascites.

*Do not be too aggressive in mobilizing ascites, do not overtreat*. It is better to have some ascites with normal renal function than rapid and complete ascites mobilization with potentially irreversible renal failure.

- A mild to moderate ascites may be treated initially solely with spironolactone 100 mg p.o. qd–bid. Since the onset of action of spironolactone is gradual, 3–5 days should elapse before dosage is adjusted until diuresis is achieved.
- If the diuretic response is insufficient (weight loss <1 kg in the first week and <2 kg/week in subsequent weeks) a loop diuretic, preferentially furosemide 40 mg p.o. qd is added. The dosages are adjusted according to the clinical response (daily weight loss) while monitoring serum electrolytes and renal function. If serum creatinine rises to >1.5 mg% diuretics should be temporarily discontinued.
- Alternatively, diuretic treatment can be started as a combination of spironolactone and furosemide at a ratio of 40 mg furosemide for every 100 mg of spironolactone.
- Intravenous albumin improves the response to diuretics in patients with cirrhotic ascites [8].
- Do not treat patients with cirrhotic ascites with furosemide monotherapy.
- In the absence of renal insufficiency and with urinary $Na^+$-excretion >30 mEq/L, spironolactone may be used alone, in a daily dose of 100–200 mg p.o.. Increasing the dose to >400 mg qd does not increase the diuretic efficacy.
- With a urinary $Na^+$-excretion of 10–30 mEq/L, a loop diuretic is added to spironolactone, e.g. furosemide 40–160 mg p.o. or i.v. qd or 20–80 mg p.o. bid. Thiazide diuretics, e.g. hydrochlorothiazide are rarely used.
- With a urinary $Na^+$-excretion < 10 mEq/L, an additional large-volume therapeutic paracentesis is performed.
- The optimal daily weight loss in patients with peripheral edema is 1 kg, in those without peripheral edema 0.5 kg.
- More aggressive diuresis leads to intravascular hypovolemia, which may cause renal failure, electrolyte imbalances and precipitate hepatic encephalopathy.

In hospitalized patients diuretic treatment may be monitored according to urinary $Na^+$-excretion. In patients not responding adequately to diuretics the $Na^+$-excretion in 24 h urine should be determined. *A urine $Na^+$-excretion of >80 mmol $Na^+$/24 h, in patients whose ascites is not adequately mobilized and who do not lose weight, suggests dietary noncompliance with $Na^+$-restriction*.

The presence of hepatic encephalopathy, serum sodium concentration <120 mEq/L despite adequate fluid restriction and a serum creatinine >2.0 mg/dL (180 µmol/L) should lead to at least temporary withdrawal of diuretics [32].

Errors in the therapy of cirrhotic ascites are summarized in Table 80.4.

### *Large-Volume Paracentesis*

Large volume paracentesis (LVP) is the initial treatment of choice in patients with tense (grade 3) and refractory ascites [10, 12]. Therapeutic paracentesis is the fastest method to mobilize ascites, it shortens the hospital stay and is associated with less complications than therapy with diuretics. In the absence of hepatic encephalopathy, gastrointestinal bleeding or a bacterial infection, LVP may be performed in an outpatient setting (see "Treatment of Refractory Ascites") [17].

The goal of paracentesis is the complete removal of ascites. This can be attempted in one session. Single total paracentesis is as effective as and generally safer than repeated partial paracenteses.

With paracentesis of ≥5 L removed at once, acute reduction of effective blood volume with subsequent activation of the sympathetic nervous system and renin-angiotensin-aldosterone-system may lead to *circulatory dysfunction* with a fall in arterial blood pressure and increased cardiac output and *complications*, such

**Table 80.4** Errors in the treatment of cirrhotic ascites

| Finding | Wrong action | Correct action |
|---|---|---|
| *Marked hyponatremia (≤125 mmol/L)* | Infusion of NaCl | Fluid restriction (is, however, rarely effective in complete correction of dilutional hyponatremia). Too vigorous fluid restriction may lead to renal impairment and to central hypovolemia with exacerbation of hyponatremia<br>Consider interrupting diuretic therapy or reduce diuretic dose<br>With elevated or rising serum creatinine, stop diuretics and consider volume expansion. Serum Na ≤ 120 mmol/L: stop diuretics<br>*Vasopressin 2 receptor antagonists* will probably be a pathophysiologically rational choice in the future |
| *Impaired renal function* Serum creatinine > 2 mg%; creatinine-clearance <40 mL/min in the absence of a renal disease | Increase in dose of diuretics | Cautious volume expansion with synthetic colloid solutions or albumin (4.5% albumin solution contains $Na^+$ concentrations equivalent to normal saline!)<br>Possibly vasopressin analogues, e.g. terlipressin (not yet available in the USA)<br>In a few patients reversal of type 1 hepatorenal syndrome with the administration of midodrine + octreotide was reported |
| *Marked, grade 3 ascites* | High dose diuretic therapy | Slow, stepwise increase in diuretic dosage attempting to achieve the desired daily weight loss of 0.5–1 kg |

as dilutional hyponatremia with rapid reappearance of ascites and renal dysfunction with irreversible renal failure in up to 20% of patients [41]. *The severity of post-paracentesis circulatory dysfunction correlates inversely with patient survival*. To avoid hemodynamic changes and circulatory complications, *LVP of ≥ 5 L must be accompanied by plasma volume expansion*, even in patients with peripheral edema [11, 23]. *Albumin is the plasma volume expander of choice*. Concomitant with or immediately after LVP is completed, albumin is administered i.v. at a dose of 8 g/L ascites removed. If less than 5 L of ascites is removed synthetic plasma expanding solutions may be used.

Albumin is more effective than saline or synthetic plasma expanding solutions in preventing paracentesis (≥5–6 L)-induced circulatory dysfunction [13, 39]. Thus, despite its initial high price (approximately $15/g), the effectiveness of albumin may be associated with decreased hospital costs compared with the more "cost-effective" synthetic colloids, when treatment outcomes in patients with cirrhosis and ascites are compared [26]. Despite its efficacy, a survival benefit of albumin compared to artificial plasma expanders has not yet been demonstrated.

## Practical Approach to Paracentesis

There is no evidence that spontaneous bacterial peritonitis, renal failure, hepatic encephalopathy, or severe jaundice should be considered as contraindications for paracentesis. Caution is needed when performing paracentesis on patients with severe thrombocytopenia or an INR > 2.5, but mild coagulapathy need not be corrected with blood products prior to paracentesis. Renal insufficiency might be a risk factor for iatrogenic hemoperitoneum.

- Paracentesis should preferably be performed in a single session. This can be done over a period of 1–4 h or much quicker by using vacuum-sealed containers which allow for removal of, for example, 8 L ascites in 30 min.
- Paracentesis <5 L: cautious volume expansion with synthetic colloidal solutions or albumin monitoring pulse rate and blood pressure.
- Paracentesis ≥5 L: expansion of plasma volume with infusion of albumin at a dose of 8 g/L ascites removed.
- If following paracentesis diuretic therapy is not reinstituted, ascites will recur in >90% of patients. With diuretics the recurrence rate of ascites is 18%. Therefore, *paracentesis should always be followed with maintenance diuretic therapy and dietary sodium restriction to avoid recurrence of ascites*.

## *Refractory Ascites*

An ascites refractory to therapy – either diuretic-resistant or diuretic-intractable ascites – occurs in 5–10% of patients

with cirrhotic ascites. Once refractory ascites develops, prognosis is extremely severe, with approximately 50% of patients dying within 6–12 months.

A *diuretic-resistant ascites* refers to an ascites that despite maximal diuretic treatment of at least 1 week duration (spironolactone 400 mg/day and furosemide 160 mg/day) and strict dietary $Na^+$-restriction (<90 mmol/day) cannot be mobilized (mean weight loss of <0.8 kg over 4 days and 24 h urinary $Na^+$-excretion less than oral $Na^+$-intake) or that recurs early (reaccumulation of grade 2 or 3 ascites within 4 weeks after initial mobilization). The intake of nonsteroidal anti-inflammatory drugs must be excluded because NSAIDs blunt the response to diuretics.

A *diuretic-intractable ascites* refers to an ascites that cannot be treated adequately with diuretics because diuretic associated complications preclude the application of adequate doses of diuretics. Diuretic induced complications include hepatic encephalopathy in the absence of any other precipitating factor but diuretics, renal insufficiency (increase of serum creatinine by >100% to >2 mg/dL in patients with ascites responding to treatment), hyponatremia (decrease of serum $Na^+$-concentration by >10 mEq/L to <125 mEq/L), hypokalemia (<3 mmol/L) or hyperkalemia (>6 mmol/L) [2, 24].

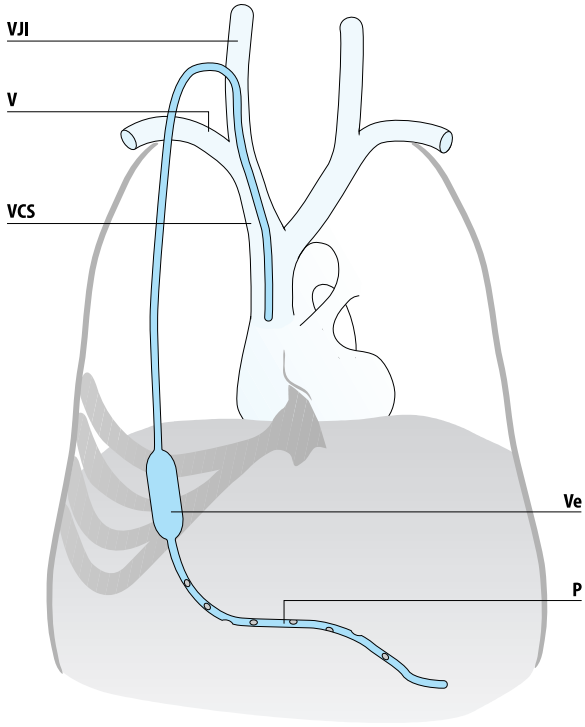


**Fig. 80.2** Peritoneovenous shunt. *VJI* Internal jugular vein, *V* subclavian vein, *VCS* superior vena cava, *Ve* valve, *P* perforated catheter

## Therapy of Refractory Ascites

**Serial Large-Volume Paracentesis**. Even in outpatients, LVP using strict aseptic techniques can be repeated safely every 10–14 days. The risk of bacterial peritonitis is low and there is no need to perform ascites cultures after each tap or to use antibiotics prophylactically [5]. However, it is advisable to determine the number of neutrophils per milliliter ascites.

**Peritoneovenous Shunt (PVS)**. PVS is a surgically implanted unidirectional connection between the peritoneal cavity and the superior vena cava, allowing ascitic fluid to flow from the abdominal cavity into the systemic circulation (Fig. 80.2). *PVS functions as a continuous paracentesis with venous reinfusion of ascitic fluid*.

Compared to LVP with albumin infusion PVS does not lead to any significant survival benefit but carries a considerable risk (up to 40%) of serious complications, such as volume overload with congestive heart failure and pulmonary edema, variceal bleeding from expansion of intravascular volume, myocardial infarction, bacterial peritonitis, septicemia, peritoneal fibrosis, disseminated intravascular coagulation, thrombosis of a central vein and/or thrombotic occlusion of the shunt. Thus, nowadays there is little role for the use of PVS in the treatment of refractory ascites and *PVS should not be performed anymore*.

**Transjugular Intrahepatic Portosystemic Shunt (TIPS)**. TIPS is a method of portal decompression based on the nonsurgical, radiologically controlled placement of an intrahepatic connection between the portal vein and the hepatic venous system (Fig. 80.3) [29]. *TIPS corresponds functionally to a side-to-side portal-caval shunt* and has largely replaced surgically placed shunts.

TIPS is a highly effective treatment for refractory ascites and decreases the need for LVP in patients with refractory cirrhotic ascites [21, 27, 30]. It leads to complete resolution of refractory ascites in up to 75% of cases [3].

TIPS should be considered as a treatment option for patients who need frequent LVP (>3/months), who are intolerant of repeated LVP or in whom a LVP cannot be performed, e.g. because of extensive peritoneal

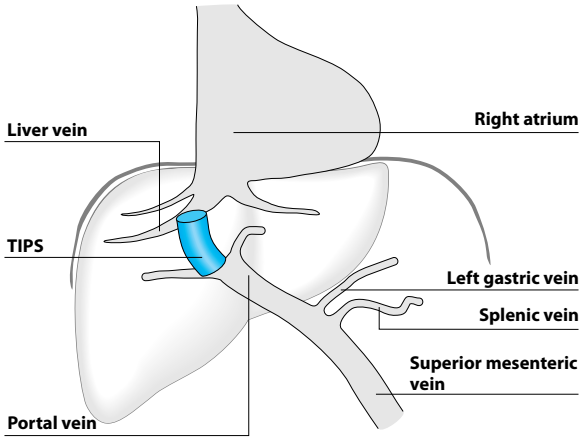


**Fig. 80.3** Transjugular intrahepatic portosystemic shunt (TIPS)

adhesions. TIPS also resolves hepatic hydrothorax in 60–70% of patients (see Section 80.5).

TIPS leads to an increase in cardiac output, a rise in right atrial and pulmonary arterial pressure with a secondary fall of systemic vascular resistance, increase of pulmonary vascular resistance and of effective arterial blood volume, improvement of renal function and to an increase in urinary $Na^+$-excretion. It is less effective in patients older than 60 years and in those with a pre-TIPS creatinine clearance of <40 mL/min.

Compared to LVP plus albumin, TIPS reduces the rate of relapse of refractory ascites in cirrhosis and the development of hepatorenal syndrome, but more often leads to hepatic encephalopathy [6, 14]. The overall survival and the quality of life, however, are not improved by TIPS compared to LVP and drug therapy of ascites [1, 35]. But TIPS significantly improves transplant-free survival of cirrhotic patients with refractory ascites and is an effective procedure to bridge the time to orthotopic liver transplantation [33, 34].

Compared to uncovered stents, the use of polytetrafluoroethylene-covered prostheses improves TIPS patency and decreases the number of clinical relapses and reinterventions [4].

Possible long-term consequences include deterioration of liver function, increased incidence of hepatic encephalopathy (approximately in 30–50% of patients; the risk is higher in patients over the age of 60 years), TIPS-stenosis or occlusion, hemolytic anemia, infections (19%), renal failure (17%), and heart failure due to a rise in preload in patients with cardiac diseases. The 3-month mortality rate after TIPS placement has been reported to be 32% [37].

TIPS should not be placed in patients with serum bilirubin >3 mg/dL, a Child-Pugh score >11, age over 70 years, or evidence of heart failure (ejection fraction <55%) [24].

**Liver Transplantation**. TIPS and LVP do not improve the long-term survival without transplantation. In any patient with cirrhosis who develops ascites, suitability for liver transplantation should be considered. In patients with refractory ascites, liver transplant evaluation should be initiated, especially when complications, such as spontaneous bacterial peritonitis or hepatorenal syndrome occur. Patients undergoing liver transplantation have a 5-year survival probability of 70–80%.

Patients with one or more of the following alterations have a poor prognosis and should be regarded as *candidates for liver transplantation*:

- Impaired free water clearance (urine volume < 8 mL/min after intravenous infusion of glucose 5% at a dose of 20 mL/kg body weight).
- Dilutional hyponatremia (serum Na <130 mEq/L without diuretic therapy).
- Arterial hypotension (mean arterial pressure < 80 mmHg without diuretic therapy).
- Diminished glomerular filtration rate (serum creatinine > 1.2 mg/dL without diuretic therapy).
- Marked $Na^+$-retention (urinary $Na^+$-excretion < 10 mmol/24 h with moderate dietary $Na^+$-restriction and without diuretic therapy).

## Outlook

Better understanding the pathophysiology of cirrhotic ascites has led to the use of new agents, primarily

- Vasopressin receptor antagonists and
- Vasoconstrictor drugs

*Vasopressin receptor antagonists* act on the distal tubule of the kidney and by counteracting arginine-vasopressin promote solute-free water diuresis. They can be combined with diuretics to improve mobilization of ascites and edema. In several randomized, placebo-controlled trials, orally active V2 vasopressin receptor antagonists (tolvaptan and satavaptan) were able to correct serum hyponatremia in patients with cirrhosis and ascites [9, 16a, 36, 42].

*Vasoconstrictor drugs* that are able to improve the hemodynamic derangements that lead to ascites

formation in cirrhosis, thereby reducing the activity of antinatriuretic factors, represent another attractive therapeutic approach to cirrhotic ascites. *Midodrine* (an $\alpha_1$-adrenergic agonist) 10 mg p.o. tid leads to a moderate increase in urine sodium excretion in patients with ascites, a mild rise in glomerular filtration rate, urine volume and to significant decreases in plasma renin activity and aldosterone concentration [19, 40]. *Terlipressin*, a vasopressin 1 receptor agonist improves renal function and induces natriuresis in patients with cirrhosis and ascites without hepatorenal syndrome. There is preliminary evidence that terlipressin may be as effective as intravenous albumin in preventing paracentesis-induced circulatory dysfunction [20, 38].

The use of these agents (vasopressin receptor antagonists and vasoconstrictor drugs) in cirrhotic ascites is limited to a small number of reports, and they are not yet approved for patients with cirrhosis and ascites. More controlled studies are needed to evaluate their efficacy and safety in treating patients with ascites.

*Clonidine*, a centrally acting $\alpha_2$-agonist and sympatholytic agent (a vasodilator), was evaluated as an adjunct treatment in patients with cirrhotic ascites. At a dose of 0.075 mg p.o. bid it led to a more rapid mobilization of ascites with fewer complications than placebo [22].

## Esophageal Varices

### Definition

Varices are dilated, often tortuous veins. They occur most often in the distal esophagus and in the gastric fundus in patients with portal hypertension. Duodenal and rectal varices or varices of gallbladder and bile ducts rarely occur and are of minor clinical importance.

### Epidemiology

Two thirds of all patients with liver cirrhosis with increasing portal hypertension develop esophageal varices and a portal hypertensive gastropathy (see below). At the time of diagnosis of cirrhosis 60% of patients with decompensated and 30% of those with compensated cirrhosis already have varices. Approximately 10–15% of patients with esophageal varices concomitantly also have gastric fundal varices.

Anorectal varices, which are fed by the superior rectal vein which drains into the portal venous system, occur in 20–60% of patients with cirrhosis. Hemorrhage from these collateral vessels is rare [54].

### Anatomy, Etiology and Pathophysiology

The veins of the esophageal wall consist of a subepithelial and a submucous plexus. Both plexus communicate through perforating veins. In the distal, precardiac esophagus the veins are mainly subepithelial. Esophageal varices are fed by the gastric coronary veins and the short gastric veins. The variceal pressure depends on the pressure gradient between the portal vein and the right atrium and varies with respiration. *The mean variceal pressure is 20–25 cm $H_2O$*. Since the veins in the distal esophagus are located beneath the epithelium and submucosal and perforating veins are scant in this location, the risk of variceal rupture is highest in the distal esophagus.

The most important pathogenetic factor in the development and increase in size of gastroesophageal varices is portal hypertension. The hepatic venous pressure gradient (HVPG), determined by the difference between wedged and free hepatic venous pressure, is a good estimate of portal pressure (see Chapter 46 and 53). Varices start developing with HVPG values ≥10–12 mmHg [14]. With increasing HVPG values both the transmural variceal pressure and the variceal wall tension rise, and the risk of bleeding increases [76].

Rarely esophagogastric varices may also develop in the absence of portal hypertension. Thus, for example, obstruction of the superior vena cava at the level of junction with the azygos vein, by increasing the outflow resistance of the azygos vein may lead to the development of isolated varices in the proximal esophagus ("downhill-varices"). Mediastinal tumors, bronchial and esophageal cancer, goiter, fibrous adhesions or inadvertent ligation of vessels during thyroid resection may cause "downhill-varices".

Isolated fundal varices are usually due to an isolated block in the splenic vein.

## Diagnosis

The diagnosis of subepithelial varices is made *endoscopically*. The dilated vessels protrude to a variable degree into the lumen, and their size and the appearance of the vessel wall may be assessed endoscopically (Figs. 80.4 and 80.5). Both have prognostic significance. The aspect of the varices forms the basis for classifying them into different grades (Table 80.5).

*Esophageal capsule endoscopy* is a new, minimally invasive technique that allows for the detection of esophageal varices. The overall agreement for detecting esophageal varices between esophagoscopy and capsule endoscopy was reported to be 86%, and in 79% of cases there was complete agreement on variceal grade [30]. Although these results are promising, it still remains to be determined whether esophageal capsule endoscopy will attain a significant role in screening and surveillance of esophageal varices in patients with portal hypertension.

**Table 80.5** Endoscopic grading of esophageal varices

| | |
|---|---|
| Grade I | Straight, pink-bluish veins within the level of the mucosa, Ø ≤ 2 mm |
| Grade II | Tortuous bluish dilated veins protruding into the esophageal lumen, Ø 2–3 mm |
| Grade III | Nodular, tortuous bluish varices, occluding approximately half of the esophageal lumen, Ø 3–4 mm |
| Grade IV | Grape-like, blue vascular convolutes protruding far into the esophageal lumen. The esophageal lumen becomes visible only after insufflation of air. Fine angiectasias are present on the surface of the vessels ("varix on varix") and pinpoint thinning of epithelium ("cherry-red-spots") |

Intra- and perimural varices are visualized very well with *endoscopic ultrasound* (Fig. 80.6). The clinical significance of these occasionally impressive endosonographic findings, however, currently is unclear. Varices around and within the gallbladder wall may be visualized by conventional abdominal ultrasound. These interesting sonomorphologic findings, however, generally have no clinical significance.

Laboratory findings, such as thrombocytopenia (<90,000/mL), splenomegaly, platelet count/spleen diameter ratio (N/mm$^3$/mm; cutoff 909), diameter of

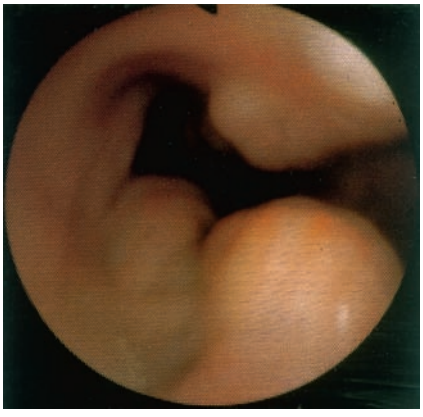

**Fig. 80.4** Third degree esophageal varices, protruding into the esophageal lumen without contacting each other

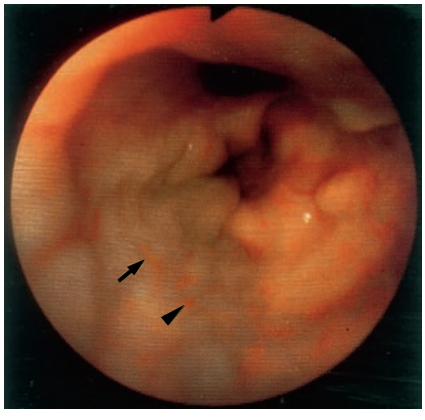

**Fig. 80.5** Esophageal varices with "cherry-red-spots" (*arrowhead*) and "red-wale-markings" (*arrow*)

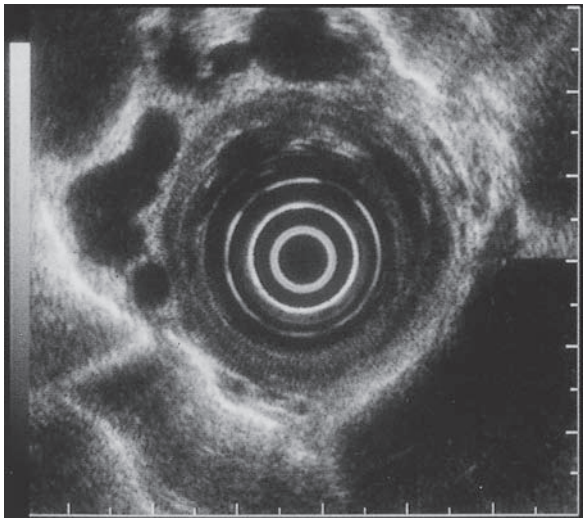

**Fig. 80.6** Endosonographic appearance of periesophageal varices

portal vein (≥13 mm), lowered serum albumin and FibroTest have been proposed as noninvasive predictors of the presence of esophageal varices [28, 40–42, 78, 93, 105]. These parameters, however, especially when esophageal varices are still small, are unreliable and do not substitute for endoscopy. In subjects with chronic hepatitis C and advanced fibrosis the risk of having varices increases with decreasing platelet counts, increasing bilirubin concentration in serum, and INR. The probability of having medium or large varices at platelet counts >150,000/mm$^3$ has been reported to be negligible [81].

## Course and Prognosis

In patients with cirrhosis, esophageal varices develop at a rate of approximately 5–12% per year. If on initial endoscopy small (<5 mm) varices are present, the rate of progression to large varices is approximately 10–15% per year [16, 28].

Varices may rupture and bleed. Approximately one third to one half of all patients with esophageal varices bleed at least once during their lifetime. Up to 25% of patients with newly diagnosed and untreated esophageal varices will bleed within the first 2 years after diagnosis. The risk of hemorrhage primarily depends on variceal size (see below). It is 7% within 2 years in patients with small varices (diameter < 5 mm) and rises to 30% in those with large varices (diameter > 5 mm) [57]. Acute variceal bleeding always is a life threatening event and the risk of dying from the first variceal hemorrhage is approximately 20% [26, 28, 58].

Without treatment, recurrent bleeding is the rule which in up to 20% of cases may occur as a fulminant hemorrhage from fundal varices. Rebleeding occurs within the first 6 weeks in 30% of cases and within 1 year after the first bleed in 70% of patients. The earlier the recurrence, the higher the mortality risk.

The MELD score (≥18) is a good predictor of short-term (6 weeks to 3 months) mortality among cirrhotic patients at first episode of bleeding from esopahgeal varices [2, 7]. Measurement of the HVPG obtained within 48 h of admission also may predict efficacy of treatment and short-term prognosis. However, it is not universally available and simple clinical variables, such as systolic blood pressure, Child-Pugh class, etiology of cirrhosis may be used instead as accurate predictors of short-term prognosis [1, 4]. Due to early and combined use of pharmacological and endoscopic therapies, and short-term antibiotic prophylaxis, in-hospital mortality of patients with cirrhosis and variceal bleeding decreased continuously over the past two decades [20].

## Predictors of Variceal Bleeding

Since variceal bleeding is associated with a high mortality risk, it is important to define predictors and to assess the risk of bleeding in order to establish effective prophylactic measures. The risk factors and the prognostic significance of endoscopic and functional criteria for variceal bleeding are summarized in Tables 80.6 and 80.7. The risk of variceal hemorrhage depends on the severity of liver disease (MELD score; Child-Pugh class) and rises with decreasing liver function. *The size of varices, variceal pressure and the appearance of the surface of the vessel wall are important predictors of variceal bleeding*. Large vessels with high wall tension are more likely to bleed than small ones [22, 72, 73]. The wall tension correlates directly with transmural pressure and the diameter of a vessel and indirectly with wall thickness. It increases with rising portal pressure, increasing vessel size and decreasing wall thickness. Thus, not surprisingly, a large vessel with a thin wall will exhibit a higher wall tension than a small vessel with a thick wall and will therefore be more likely to rupture. Direct measurement of variceal pressure is possible

**Table 80.6** Independent risk factors for esophageal variceal hemorrhage

| |
|---|
| Hepato-venous-pressure-gradient > 12 mmHg |
| Variceal characteristics |
| • Size |
| • Wall tension |
| • Intravariceal pressure |
| • Red colour signs |
| Liver function (Child-Pugh-Stage; MELD score) |
| Continuing alcohol abuse |

**Table 80.7** Prognostic significance of endoscopic and functional criteria for variceal bleeding

| Endoscopic criteria | Bleeding risk (%) |
|---|---|
| *Red wale markings* | |
| Absent | 19 |
| Mild | 33 |
| Moderate | 39 |
| Severe | 80 |
| *Variceal size* | |
| Small Ø < 3 mm | 18 |
| Medium Ø 3–5 mm | 29 |
| Large Ø > 5 mm | 49 |
| *Cherry-red-spots* | |
| Absent | 23 |
| Mild | 32 |
| Moderate | 40 |
| Severe | 55 |
| *Liver function (Child-Pugh-Stage)* | |
| Child A | 17 |
| Child B | 31 |
| Child C | 39 |

Source: According to the North Italian Endoscopic Club (1988)

either by puncturing the vessel or less invasively by a balloon technique with pressure registration by a capsule. Both methods are used in experimental studies but not in clinical practice.

The surface appearance of the vessel wall may yield important information regarding impending hemorrhage. Diffuse redness of the vessel, red color signs, such as "cherry red spots" and "red wale marks" (correspond to microtelangiectasia of the varix) and hemocystic spots looking like blood blisters (>4 mm; saccular aneurysm projections), are all thought to indicate a high risk for bleeding (Fig. 80.5). A "white nipple sign" on a varix represents a platelet-fibrin plug and indicates previous bleeding but is not predictive of rebleeding [87].

Every third patient with variceal hemorrhage, however, does not present these endoscopic signs. Thus, hemodynamic parameters are preferable in predicting the risk of variceal bleeding. The level of HVPG is a reliable and independent indicator for esophageal variceal bleeding. The normal value of HVPG is 5 mmHg. Portal hypertension starts at a HVPG > 5 mmHg, but values of >10–12 mmHg are clinically significant. With a HVPG < 10–12 mmHg, varices do not develop, and preexisting varices do not bleed. Once HVPG increases to >12–16 mmHg, the risk of bleeding is high, but the degree of portal pressure elevation over 12 mmHg does not correlate directly with bleeding.

## Prophylaxis and Therapy

The management of patients with esophageal varices aims at three goals:

- Prevention of first variceal hemorrhage (primary bleeding prophylaxis)
- Treatment of acute variceal hemorrhage, and
- Prevention of recurrent variceal hemorrhage (secondary bleeding prophylaxis)

The outcome of the patients critically depends on the success of these measures. There is an increased array of therapeutic options including pharmacological, endoscopic, mechanically compressing (balloon tamponade), radiologic-invasive (TIPS) and surgical techniques which may be applied according to the clinical situation [24, 25, 27]. Substances that lower the portal pressure are listed in Table 80.8. Most of these substances are not (yet) introduced into clinical practice. Currently β-adrenergic blocking agents, nitrates, vasoconstrictors (e.g. terlipressin) and growth hormone inhibitors, such as somatostatin and octreotide are important. Endoscopic techniques encompass sclerotherapy and band ligation; surgical procedures include the creation of various portal-systemic shunts or the staple-gun transection of the esophagus as a salvage procedure for active variceal bleeding after failure of acute endoscopic therapy [68]. The prophylactic and therapeutic options in patients with esophageal varices are summarized in Table 80.9.

### "Preprimary" Prophylaxis

"Preprimary" prophylaxis refers to the prevention of the development of esophagogastric varices in patients with liver cirrhosis. The best way to achieve this goal is to successfully treat the underlying disease that leads to cirrhotic transformation. *Beta-blockers are ineffective in preventing the development of varices in patients with cirrhosis* [50]. For cirrhotic patients without varices, screening endoscopy every 3 years, or sooner if liver function deteriorates, is recommended.

**Table 80.8** Substances that lower portal pressure

*Substances that diminish portal venous flow*
- Angiotensin-receptor agonists
- $V_{1a}$-receptor agonists
- Endothelin-receptor agonists
- $\alpha_1$- and $\alpha_2$-adrenoceptor agonists
- $\beta_1$- and $\beta_2$-adrenoceptor antagonists
- Blockers of ATP-sensitive $K^+$-channels
- Activators of $Ca^{++}$-channels
- Inhibitors of NO-synthase

*Substances that lower the intrahepatic vascular resistance*
- $\beta_2$-adrenoceptor agonists
- $ET_A$-$ET_B$-receptor antagonists
- Nitrates

*Substances whose mechanism of action is not completely understood*
- α-adrenoceptor antagonists
- $AT_1$ angiotensin-receptor antagonists
- Angiotensin converting enzyme inhibitors
- Antiglucagon
- $Ca^{++}$-antagonists
- Dimethylxanthine
- Diuretics
- 5-Hydroxytryptamine-receptor antagonists
- Somatostatin, octreotide
- Natriuretic peptides
- Parathyroid hormone
- Antagonists of platelet-activating factors

Source: According to [61]

**Table 80.9** Prophylactic and therapeutic options in patients with esophageal varices [17]. See text for use and clinical significance of individual substances and measures

| | |
|---|---|
| **Primary prophylaxis** | Nonselective β-blockers<br>Oral nitrates<br>Endoscopic variceal ligation |
| **Acute bleeding** | Octreotide/somatostatin<br>Vasopressin/nitrates<br>Terlipressin<br>Endoscopic therapy<br>Injection sclerotherapy<br>Band ligation<br>Balloon tamponade<br>Surgical therapy[a]<br>Shunt<br>Devascularization |
| **Secondary prophylaxis** | Nonselective β-blockers<br>Oral nitrates<br>Endoscopic variceal ligation<br>(TIPS)<br>Surgical therapy[a]<br>Shunt<br>Devascularization[a]<br>Esophageal transection[a] |

[a]Limited to patients with uncontrollable bleeding, unsuccessful TIPS or thrombosis of portal and splenic vein

## Primary Bleeding Prophylaxis

Because every episode of variceal hemorrhage is associated with a high mortality rate, patients with cirrhosis and varices should be treated before the first bleeding occurs. Primary prophylaxis refers to the prevention of the first variceal hemorrhage and relies on measures

- Lowering portal venous pressure, and
- Obliterating varices

The first goal can be achieved by pharmacotherapy, the latter by endoscopic techniques.

**Pharmacotherapy**. *Nonselective β-blockers* reduce portal-venous pressure by reducing cardiac output and splanchnic blood flow. In addition, splanchnic vasoconstriction is enhanced by an uninhibited activation of α-receptors [37]. *Nonselective β- adrenergic antagonists are the mainstay of pharmacologic prevention of a first esophageal variceal hemorrhage* [23, 74, 77]. The individual dose of β-blockers must be determined for each patient individually by adjusting the dose weekly with the goal of reducing heart rate by 25% from the baseline value without falling below a rate of 55/min or a systolic blood pressure of 90 mmHg (adjust to the maximal tolerated dose). The magnitude of heart rate reduction, however, is not a reliable indicator of portal pressure lowering, which ideally should be assessed by repeated measurements of HVPG during the course of treatment (see Chapter 46). However, invasive targeting of portal pressure is not routine in clinical practice [18, 49, 94]. Treatment with β-blockers is lifelong. *Only patients in whom β-blockers lead to a durable decrease of HVPG < 12 mmHg or > 20% from baseline benefit from treatment*. Falls in HVPG > 20% are associated with lower mortality [14, 25, 32]. In addition, reduction of HVPG also correlates with a reduced risk of spontaneous bacterial peritonitis or bacteremia [96 ].

The β-blocker of choice is propranolol, 80–160 mg p.o. daily in 3–4 divided doses; alternatively nadolol 20–240 mg p.o. daily can be used (Table 80.10). Nadolol is less lipophilic than propranolol and does not cross the blood–brain barrier. It is better tolerated and leads to less drug withdrawal (4%) due to side effects compared with propranolol (up to 30%). There are no data regarding pharmacologic primary bleeding prophylaxis of gastric fundal varices. Nonetheless, the use of β-blockers also seems warranted in these patients, as fundal and esophageal varices usually occur together.

**Table 80.10** Pharmacologic characteristics of propranolol and nadolol

| Substance | Half life (h) | Oral bioavailability (%) | Comments |
|---|---|---|---|
| Propranolol | 3–5 | ~25 | Extensive hepatic metabolism (first pass effect). Metabolites are excreted in urine. Marked inter-individual variation of plasma concentration after oral application |
| Nadolol | 10–20 | ~35 | Excreted largely unchanged in urine. Risk of accumulation in renal failure |

Depending on Child-Pugh class, the number of patients needed to treat in order to prevent one bleeding episode is 5–14. The primary prophylactic effect of β-blockers seems to be more pronounced in patients with large varices and a high Child-Pugh class, which means that in order to achieve the same effect, fewer patients need to be treated. Primary prophylaxis with propranolol is cost-effective, even if compared with no therapy [80, 92].

Thus, despite their effectiveness in some patients, HVPG does not fall < 12 mmHg or ≥ 20% from baseline in up to two thirds of patients treated with β-blockers despite adequate β-blockade. Possibly Doppler patterns of splanchnic hemodynamics can serve as a non-invasive clue for the a priori identification of good and poor responders to β-blockers. Cirrhotic patients who responded poorly to nadolol, in contrast to good responders, showed a pronounced arterial splanchnic vasodilatation at a baseline echo-color-Doppler study [10]. Therefore, in up to two thirds of patients treated with β-blockers and in 15–25% with contraindications to β-blocker therapy or those who cannot tolerate the required doses because of untoward side effects, the question as to an alternative pharmacologic primary prophylaxis arises.

*Long-acting oral nitrates*, isosorbide mono- or dinitrate, because of their vasolidating effect lower both the systemic, splanchnic and portal pressure. Combined with β-blockers, the drop in pressure is slightly more pronounced than with sole β-blocker therapy [47, 69]. However, the number of studies examining nitrates is relatively small compared to trials with β-blockers, and the efficacy of nitrates in primary bleeding prophylaxis is controversial [3, 12, 36, 47]. Monotherapy with nitrates may impair renal function and worsen a preexisting ascites. There are even worries that nitrates may increase the mortality rate. Therefore, nitrates should not be used as monotherapy in the prophylaxis of esophageal variceal bleeding [29].

*Carvedilol* and the long-acting somatostatin analogue *octreotide* also reduce HVPG but both substances are not used in the long-term prevention of esophageal hemorrhage [62, 89].

**Endoscopic Techniques**. *Endsocopic multiband ligation* (EVL) of esophageal varices is safe and effective (Figs. 80.7 and 80.8). It reduces the rate of first bleeding to 30–40% and the hemorrhage related mortality to 30% within 2 years. Patients with compensated Child-Pugh class A cirrhosis benefit most from ligation [58, 64, 82]. Most authors agree that in patients with

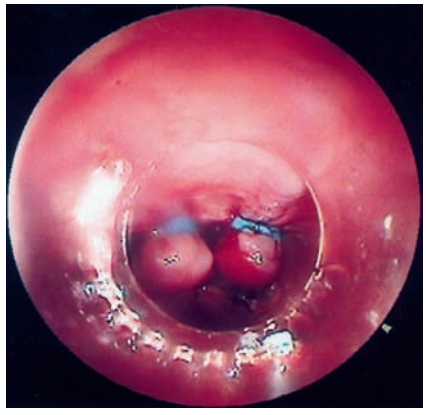

**Fig. 80.7** Variceal band ligation

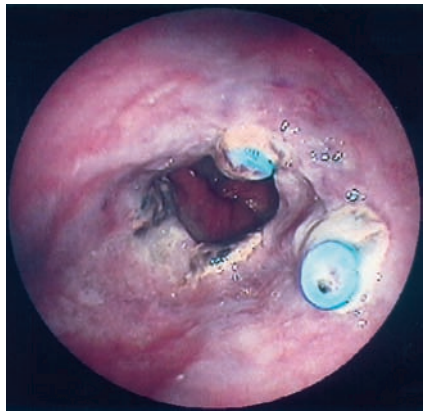

**Fig. 80.8** Several days after band ligation the ligated variceal nodules detach leaving behind ulcers

high-risk esophageal varices, EVL is more effective than propranolol for the primary prevention of variceal bleeding [56, 83, 95]. However, there are also data showing that in patients in whom propranolol lowers HVPG effectively (<12 mmHg or a decrease of >20%), its efficacy is comparable to ligation [59, 66, 85]. If quality of life is considered, then EVL is similarly cost-effective as β-blockade [55].

EVL is usually performed once every 2 weeks until varices are eradicated. Recent data show that EVL yields good results even if performed at bi-monthly intervals [104]. Postbanding ulcers occur regularly and usually are asymptomatic. Proton pump inhibitors may reduce their size [86]. Endoscopic obliteration of varices is followed by lifelong treatment with β-blockers.

*Endoscopic injection sclerotherapy* is inferior to EVL and should not be performed in primary prophylaxis of esophageal varices.

Summarizing the current evidence, the following may be stated:

- Cirrhotic patients without varices should not receive β-blockers.
- Primary prophylaxis should be performed in patients with a high risk of bleeding (high grade, large varices, "red color signs", HVPG > 12 mmHg).
- The use of β-blockers in patients with small (<5 mm) varices is not mandatory, although there are recent data suggesting that β-blocker prophylaxis of variceal bleeding in patients with compensated cirrhosis might also be beneficial when esophageal varices are still small (<5 mm) [70]. These patients should undergo endoscopic screening every 1–2 years. Primary bleeding prophylaxis should be instituted once varices increase in size.
- EVL or nonselective β-blockers are recommended for primary bleeding prophylaxis of medium or large sized esophageal varices [38].
- EVL should be followed by lifelong treatment with β-blockers.
- In clinical practice, however, β-blockers and EVL are often combined from the beginning [84].
- Nitrates alone and in combination with β-blockers are not recommended in primary prophylaxis of variceal hemorrhage [38].
- Endoscopic injection sclerotherapy and surgical portal decompression has been abandoned in primary prevention of variceal bleeding.

## Therapy of Acute Variceal Hemorrhage

Patients with acute variceal bleeding are managed in an intensive care unit. The first goal always is to secure vital functions. In somnolent patients, especially before performing the initial diagnostic endoscopy, endotracheal intubation is strongly advised. Since in one half to two thirds of patients with cirrhosis who present with bleeding, the sources are nonvariceal, early endoscopy is useful to determine the site and cause of bleeding. Erythromycin infusion (250 mg) prior to endoscopy may improve stomach cleansing and quality of endoscopic examination in these patients [21]. Prior to all hemostatic measures, stabilization of cardiocirculatory function is mandatory aiming at a systolic blood pressure of approximately 100 mmHg and a hemoglobin value not more than 10 g/dL. Higher blood pressure and Hb values lead to an increase in portal pressure with a higher risk of recurrent bleeding.

**Pharmacotherapy**. Pharmacologic therapy of acute variceal bleeding with vasoactive substances lowering portal venous and intravariceal pressure is a measure used to complement endoscopic hemostasis. In suspected acute variceal bleeding vasoactive drugs should be started as soon as the diagnosis is made, even before diagnostic endoscopy. Vasoactive drug therapy should be maintained for 2–5 days. Vasopressin, terlipressin, nitrates, somatostatin and octreotide are the drugs currently used.

*Vasopressin* lowers portal pressure by inducing contraction especially of the smooth muscle of splanchnic arterioles. However, vasopressin also causes systemic vasoconstriction which may lead to serious side effects, such as malignant cardiac arrhythmias, myocardial infarction, intestinal ischemia, cerebrovascular ischemia and local tissue necrosis. Combining vasopressin with nitrates does not lower hospital mortality. Therefore, vasopressin only assumes historical interest and has been replaced by newer agents in the treatment of acute variceal bleeding [39, 48].

*Terlipressin*, a synthetic analog of vasopresssin (N-α-triglycyl-8-lysine-vasopressin) has an intrinsic vasoconstrictory effect on splanchnic vessels and is metabolized in vivo to lysine-vasopressin. Compared to the short acting vasopressin, the action of terlipressin is prolonged to 3–4 h and, most importantly, it does not show the dreaded side effects of vasopressin. Terlipressin has been shown to be superior to placebo, and in combination with nitroglycerin to be as effective as balloon

tamponade and sclerotherapy in the treatment of acute variceal hemorrhage (at the time of writing, however, terlipressin is not yet available in the United States) [33, 35, 101]. It is administered as a bolus of 2 mg i.v. and then 1 mg i.v. every 4–6 h. Possibly the most important action of terlipressin is its beneficial effect on renal function in patients with the hepatorenal syndrome (see Section 80.3).

*Somatostatin* (250 µg i.v. bolus followed by a continuous infusion of 250–500 µg/h) has an effect comparable to terlipressin [102]. It reduces splanchnic blood flow, has only few side effects (hyperglycemia) and is well tolerated.

*Octreotide* (50 µg i.v. bolus followed by a continuous infusion of 25–50 µg/h for 5 days), a long acting analog of somatostatin, has similar efficacy. However, when analyzing critically the results of available trials both substances have no effect on hospital mortality, their use is of dubious value and therefore not mandatory in acute variceal bleeding [5, 8, 11, 19, 46, 97]. All the more than deterioration of renal function by octereotide has also been reported [51].

Patients with marked hepatic coagulopathy may benefit from *fresh frozen plasma*. Although *recombinant coagulation factor VIIa* (rFVIIa) acutely normalizes prothrombin time in patients with cirrhosis, treatment of acute variceal hemorrhage with rFVIIa fails to control bleeding or to prevent rebleeding and is therefore not indicated in this setting [13, 15].

Bacterial infections in cirrhotic patients are associated with failure to control bleeding and represent an independent risk factor for recurrent hemorrhage [45]. *Antibiotic prophylaxis* is an integral part of therapy for patients presenting with variceal bleeding and should be instituted from admission [29]. Fluoroquinolones (ofloxacin, ciprofoxacin, levofloxacin, norfloxacin) or beta-lactams (amoxicillin-clavulanate, cephalosporines) are effective and reduce the risk of bacterial infections by about 30% and mortality risk by about 9% [71, 75]. Intravenous ceftriaxone (1 g qd) seems to be more effective than oral norfloxacin (400 mg bid) in the prophylaxis of bacterial infections in patients with advanced cirrhosis and hemorrhage [34].

**Endoscopic Techniques**. Endoscopic therapy has a success rate for controlling bleeding of 90%. It is the treatment of choice in patients with acute variceal hemorrhage and should be performed immediately after initial diagnostic endoscopy. *Sclerotherapy* and urgent *band ligation* are the endoscopic techniques available to stop acute variceal hemorrhage [52, 103]. Variceal band ligation is superior to sclerotherapy. It has a significantly lower complication rate, reduces the rate of recurrent bleeding, decreases the time and number of sessions to complete obliteration of varices, and improves survival [63, 90, 100]. Moreover, a sustained rise of portal pressure after sclerotherapy, but not band ligation, in acute variceal bleeding has been reported [6]. Banding is performed regularly, initially every 7–10 days, until varices are obliterated, but the optimal time interval has yet to be determined. Banding once every 2 months may be sufficient though the current practice is every 2 weeks [104]. The occurrence of complications depends significantly on the experience of the endoscopist. Post-banding ulcers are regularly observed and occasionally may cause dysphagia and thoracic pain. The administration of proton pump inhibitors may decrease the size of these ulcers [86]. Severe complications, such as bleeding from tissue necrosis, esophageal rupture with mediastinitis and sepsis are rare, and are associated with a mortality rate of approximately 3%. Esophageal strictures may represent late sequelae.

**Balloon Tamponade**. If endoscopic emergency treatment is not readily available, if the bleeding is too rapid to permit endoscopy, or if medical and endoscopic therapy fails to control bleeding (for example, because of insufficient visualization for band ligation), balloon tamponade may achieve a satisfactory compression of the esophagogastric bleeding site in 80–90% of cases (Fig. 80.9). Rebleeding occurs in 30–50% of patients after the balloon is deflated. If bleeding continues, dislocation of the tube must be ruled out radiologically. *The application of balloon tamponade serves to gain time until definite hemostasis can be achieved*. An endoscopic diagnosis is always mandatory since patients with cirrhosis may bleed from other sites, such as duodenal and gastric ulcers. Treatment with balloon tubes may be associated with severe complications in 10–30% of cases, such as aspiration pneumonia, regurgitation of the tube with blocking of upper airways, and esophageal perforation; these complications can lead to death in 3% of patients. Therefore, treatment with balloon tubes is allowed only under continuous intensive care monitoring. Prior to inserting the balloon tube, endotracheal intubation is required in somnolent patients. Because pressure ulcers may develop rapidly, tubes must be deblocked not later than 12 h

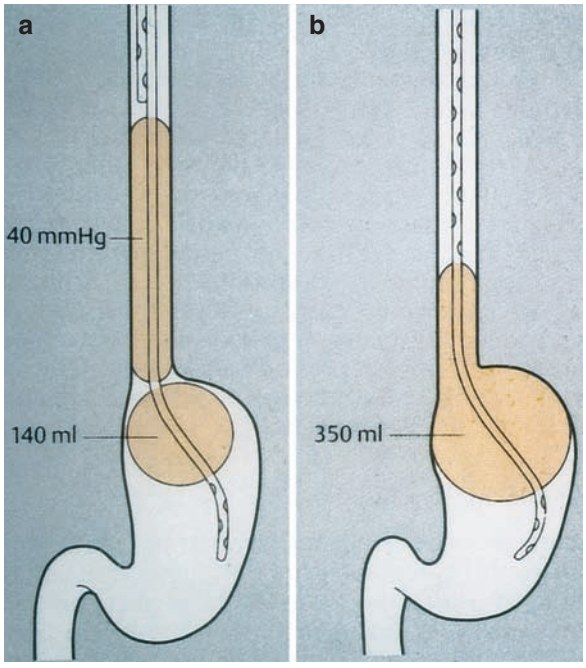


**Fig. 80.9** Balloon tubes for compression of bleeding gastroesophageal varices. (**a**) Sengstaken-Blakemore-Tube for esophageal varices. (**b**) Linton-Nachlas-Tube for fundal varices

after placement and then after each 4–6 h period for 10 min (Table 80.11).

Complete *large volume paracentesis* lowers intravariceal pressure and improves respiratory function by lowering the diaphragm. There are no data on the effect of LVP on the outcome of patients with acute variceal bleeding.

*Lactulose* 30 mL p.o. tid to qid, lactulose enemas with up to 500 mL lactulose in 500 mL physiologic saline, or poorly resorbable antibiotics, such as neomycin or paromomycin 1 g p.o. tid to qid clear the gastrointetsinal tract from old blood and are thought to prevent hepatic encephalopathy. However, there are no studies documenting the usefulness of lactulose/lactitol in this clinical setting.

**TIPS and Surgical Shunts**. If acute variceal bleeding is refractory to all of the above measures, surgical or nonsurgical shunting of portal blood to the systemic circulation is indicated as a salvage procedure. Both methods achieve acute hemostatic success rates of 90–100%. Emergency surgical shunting, however, has been reported to be associated with a mortality rate of as high as 50–80% and has become uncommon since the advent of TIPS [67]. In experienced hands, however, distal splenorenal shunt and TIPS are similarly efficacious in the control of refractory variceal bleeding [53]. Ultimately the use of each method will depend on local expertise and availability. Staple-gun transection of the esophagus nowadays should not be performed anymore.

## Prevention of Recurrent Variceal Hemorrhage

Without adequate secondary prophylaxis approximately two thirds of patients rebleed within 6 weeks after the first variceal hemorrhage.

*Prevention of recurrent variceal hemorrhage is mandatory and is performed by combining band ligation with nonselective β-adrenergic blocking agents.*

**Table 80.11** Technique of balloon tamponade

| | |
|---|---|
| Somnolent and/or agitated patients must first be intubated endotracheally | |
| Tubes must be checked for impermeability | |
| Apply tube gel generously | |
| Introduce tube through the potentially less well aerated nostril; in the wake patient, in the sitting position | |
| Push tube into the stomach | |
| *Sengstaken-Blakemore-Tube* | *Linton-Nachlas-Tube* |
| • Fill stomach balloon with 150–250 mL air | • Fill balloon with 150 mL air and pull back into the cardia |
| • Pull tube back into the cardia. Fill esophageal balloon up to 40 mmHg (control pressure) | • Apply mild tension and insufflate further 200 mL air (volume control) |
| • Fix tube at the nose without applying traction | • Apply tension via a roll with 500 mL and fix tube at the nose |
| Radiologic control of tube position | |
| Deflate after maximally 12 h | |
| All maneuvers are performed in an intensive care setting | |

Secondary prophylaxis with variceal banding begins after acute bleeding has been stopped. Banding is regularly performed (see above) until varices have disappeared completely. The result should be surveilled after 3–6 months and thereafter in yearly intervals. If varices reappear, band ligation is repeated. If initial hemostasis has been achieved by sclerotherapy, secondary prophylaxis also is performed with band ligation.

Adding β-blockers to band ligation yields superior results compared to ligation only. The additional administration of β-blockers in patients in whom esophageal varices have been obliterated by banding further reduces mortality rate from 18% to 7% [9, 31, 60]. Dosing of β-blockers corresponds to primary prophylaxis (see above). Long-term pharmacologic prophylaxis of variceal rebleeding also contributes to the prevention of community-acquired spontaneous bacterial peritonitis [44].

The results of combining β-blockers (propranolol or nadolol) with long-acting nitrates (isosorbide mononitrate) in secondary prophylaxis are controversial [65, 79, 98, 99]. At present there is no compelling evidence to add long-acting nitrates to secondary prophylaxis consisting of band ligation and β-blockers. Ideally, as in primary prophylaxis, pharmacologic treatment should be guided by hemodynamic response (HVPG) [43].

Shunt procedures should only be viewed as reserve techniques in secondary prophylaxis. Their excellent effect on portal hypertension and on lowering the rate of rebleeding is counterbalanced by the high encephalopathy rate. Surgical shunts should only be considered in recurrent bleeding in Child-Pugh class A patients. In decompensated cirrhosis, elective surgical shunts are contraindicated. TIPS may be used as a bridging procedure spanning the time to liver transplantation.

## Gastric Varices

Gastric varices are less prevalent than esophageal varices. They are present in approximately 20% of patients with portal hypertension either in isolation or in combination with esophageal varices [3, 4].

*Gastroesophageal varices* (GOV) are categorized into two types. The most common are type 1 varices (GOV1), which represent esophageal varices extending down to the cardia and along the lesser curvature. Type 2 gastric varices (GOV2) are esophageal varices which extend along the fundus. They tend to be longer and more tortuous than type 1 varices.

*Isolated gastric varices* (IGV) occur in the absence of esophageal varices and are also classified into two types. Type 1 (IGV1) are located in the fundus and tend to be tortuous and complex, and type 2 (IVG2) are located in the body, antrum, or around the pylorus [3]. The presence of isolated fundal varices (IGV1) requires excluding the presence of splenic vein thrombosis.

Variceal ligation of esophageal varices may result in gastric hemodynamic changes that increase the size of fundal varices and worsen portal hypertensive gastropathy (see below) [7]. *Secondary gastric varices* denote varices that develop in the stomach after endoscopic therapy of esophageal varices.

Bleeding gastric varices commonly are associated with large esophageal varices and the incidence of bleeding is approximately 25% in 2 years, with a higher bleeding incidence for fundal varices [4]. Patients with GOV1 have a much lower risk for bleeding. Bleeding from gastric fundal varices is usually more intensive and more difficult to control (isolated fundal varices are the most difficult to treat endoscopically) than bleeding from esophageal varices. Patients generally require more blood transfusions and mortality rates are higher. There is a high risk of rebleeding.

The best treatment of acute hemorrhage from gastric varices, as well as for primary or secondary bleeding prophylaxis, is a less researched topic and large controlled studies are lacking. The first line treatment of bleeding gastric varices is variceal obliteration by endoscopic methods using tissue adhesives and variceal band ligation [1, 2, 5]. Injection of fibrin glue, thrombin and particularly cyanoacrylate (not FDA-approved for this use in the United States) are effective [6]. Injection sclerotherapy of fundal varices must not be performed, since it causes extended ulceration and often leads to recurrent bleeding.

A TIPS or surgical procedures (devascularization, portosystemic shunts) should be considered in patients in whom hemorrhage from fundal varices cannot be controlled or in whom bleeding recurs despite combined pharmacological and endoscopic therapy [1]. Balloon tamponade may serve as a temporary measure in patients with bleeding fundal varices. Isolated fundal varices caused by splenic vein thrombosis are best treated by splenectomy, which decompresses the varices.

The effect of β-blockers or nitrates in the prevention of gastric variceal hemorrhage has not been adequately

examined. Probably they are not as effective as in the prophylaxis of esophageal variceal bleeding. This notwithstanding, long-term administration of β-bockers after successful treatment of acute bleeding from gastric varices seems reasonable.

## Portal Hypertensive Gastropathy

### Definition

A portal hypertensive gastropathy (PHG) encompasses the sum of changes of microangioarchitecture of the lamina propria and of gastric surface epithelium in portal hypertension. PHG is not inflammatory in nature and is characterized by ectasia of mucosal capillaries and submucosal veins. The sole passive hyperemia of gastric mucosa in venous congestion (congestive gastropathy) is not to be equated with PHG, but rather it represents only one aspect of PHG.

### Epidemiology

The prevalence of PHG in patients with portal hypertension ranges between 7% and 98% in published series. Ten to 50% of all upper gastrointestinal bleedings in cirrhotic patients are assumed to arise from PHG. Sixty-six to 75% of these patients experience recurrent bleeding from PHG within 1 year.

### Etiology and Pathogenesis

Per definition, portal hypertension is the condition sine qua non and plays the central role in the development of PHG. The severity of PHG is related to the level of portal pressure. However, the relation between the degree of portal hypertension and the severity of PHG is not a simple one, and the pathogenesis of PHG is only incompletely understood. Endoscopic eradication of esophageal varices may result in gastric hemodynamic changes that worsen PHG [11, 14]. Impairment of synthesis and regulatory dysfunction of nitric oxide, prostaglandins, tumor necrosis factor α and epidermal growth factor seem to play a role. It is difficult, however, to tell apart primary from reactive changes. Many experimental studies on PHG have been performed in animals and the results cannot be transferred offhand to man. Portal hypertensive rats display a defect of mitogen-activated protein kinase (ERK2) in the gastric mucosa [5]. Normally this kinase is engaged in protecting gastric mucosa from damage by free oxygen radicals. Gastric ERK2 activation induced by oxidative stress is impaired in portal hypertension leading to an increased vulnerability of gastric mucosa to exogenous substances. The gastric mucosa of patients with PHG also is increasingly sensitive to ethanol, non-steroidal antiinflammatory drugs and bile acids.

Circulatory changes contribute to PHG. While total blood flow of the gastric wall is increased in PHG, the superficial mucosal layers receive relatively less blood. These changes in blood distribution are assumed to have pathophysiologic significance.

Helicobacter pylori and gastric acid secretion do not seem to play a role in the pathogenesis of PHG [1].

### Pathology

Microscopy reveals extensive edema in more severe cases, with capillary and venous dilatation, and arteriovenous shunting in the submucosa extending into the mucosa [4, 6]. Red mucosal spots are due to extravasation of red cells through damaged endothelium or to passage of red cells through interendothelial spaces. These changes are not accompanied by an inflammatory reaction.

### Clinical Manifestations and Diagnosis

Patients with PHG may be asymptomatic or present clinically with signs and symptoms of upper gastrointestinal bleeding. Bleeding from PHG may manifest as a chronic oozing hemorrhage with iron deficiency anemia and fatigue or as an acute upper gastrointestinal bleeding with hematemesis or melena [12, 13].

Diagnosis is based upon the classic endoscopic appearance. Histologic confirmation is not necessary

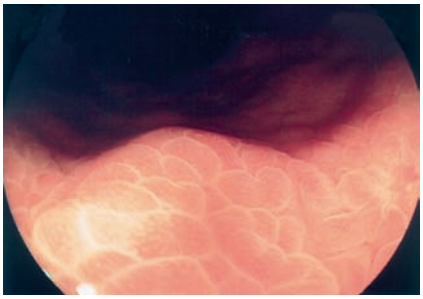

**Fig. 80.10** Portal hypertensive gastropathy. The mucosa has a reticular pattern reminiscent of a snakeskin

as biopsies are usually superficial. Endoscopic changes are usually most evident in the fundus and body and may take on several aspects

- Fine pinkish mottling of the mucosa ("scarlet fever like mucosal rash")
- Circumscribed superficial reddening, especially on the crests of gastric folds
- Fine white reticular pattern separating areas of pinkish, edematous mucosa imparting the gastric mucosa a "snakeskin" appearance [8, 13]

The last pattern is typical of PHG and is seen in more than 90% of cases (Fig. 80.10). These changes correspond to a relatively mild PHG that usually does not cause discomfort or gastrointestinal bleeding. Severe PHG is characterized by additional focal or confluent cherry red spots (similar to angiomas), diffuse subepithelial hemorrhages that lead to mucosal oozing and bleeding. Only this latter form of PHG causes clinically significant bleeding.

## Differential Diagnosis

The typical reticular pattern in the gastric body and fundus in a patient with known cirrhosis is an instant endoscopic visual diagnosis that poses no differential diagnostic difficulties. If mucosal bleeding is present one has to exclude additional damage by ethanol or nonsteroidal antiinflammatory drugs.

Gastric varices with mucosal extravasation of blood are easily visualized by endoscopic ultrasonography. The resolution of conventional endosonographic devices, however, still is too small to show dilatation of mucosal capillaries and veins typical of PHG.

Red mucosal spots may represent angiodysplasias or may result from mucosal hemorrhage in coagulation disorders.

The most important differential diagnosis of PHG is *gastric antral vascular ectasia* (GAVE) [2]. It is characterized by longitudinal rows of flat, reddish stripes radiating from the pylorus into the antrum. The endoscopic aspect resembles stripes on a watermelon ("watermelon stomach"). The red stripes represent ectatic and sacculated mucosal vessels. Histopathologically, vascular ectasia, spindle cell proliferation, and fibrohyalinosis are present. GAVE may lead to extensive loss of blood. Less than 30% of patients with GAVE have a liver cirrhosis or portal hypertension. Differentiating GAVE from PHG is clinically important, since PHG responds to therapeutic lowering of portal pressure, while GAVE must be treated with endoscopic hemostatic techniques, such as laser or argon plasma coagulation.

## Course and Prognosis

The clinical significance of PHG is its potential of severe hemorrhage. Bleeding occurs in approximately 60% of patients with severe PHG with cherry red mucosal spots. Patients with pronounced PHG have a cumulative risk of bleeding of about 75% during a follow-up of 5 years.

Few data is present relating to the natural course of PHG [3]. Prognosis is significantly influenced by the duration and severity of portal hypertension and the degree of liver dysfunction [7]. Mild forms of PHG are reversible. In a study with more than 300 cirrhotic patients PHG remained unchanged over 2 years in 29% of patients, it deteriorated in 23%, improved in 23% and findings fluctuated in 25% of cases. Acute hemorrhage occurred in 2.5% of patients, with a hemorrhage related mortality rate of 12.5%. Chronic bleeding occurred in 10.8% of PHG patients [10].

Endoscopic eradication of esophageal varices usually favors the development of PHG [13]. During a mean follow-up of 4 years the prevalence of PHG increased from 30% to 80%. In patients who develop PHG only after obliteration of esophageal varices, PHG usually is not severe and often is transient. In preexisting PHG, eradication of esophageal varices increases the risk of bleeding from PHG [11].

### *Therapy*

Nonselective β-blockers reduce the risk of bleeding from PHG and are the mainstay of therapy in acute and chronic-recurrent bleeding from PHG. The daily dose of propranolol should be adjusted on an individual basis (see above) and usually ranges between 80 and 160 mg [9]. If hemorrhage is refractory to medical treatment, TIPS or surgical portosystemic shunts should be considered.

## Portal Hypertensive Entero-, Colo- and Biliopathy

Relatively little is known about the endoscopic aspect and the histological findings of the small intestine in patients with portal hypertension. In a study of 37 patients with cirrhosis and portal hypertension, capsule endoscopy revealed mucosal abnormalities in 67.5% of cases, including telangiectasias or angiodysplastic-like changes in 24%, red spots in 62% and varices in 8% [2]. These findings attain clinical importance in the rare cases of intestinal bleeding.

In an endoscopic study of 41 patients with cirrhosis and portal hypertension, mucosal abnormalities in the ileum (*portal hypertensive ileopathy*) occurred in one-third of patients (ileal varices in 21% of cases) and were significantly associated with the presence of PHG and colopathy. Rectal varices were noted in 54% patients and 42% patients had features suggestive of colopathy [4]. Anal varices should not be mistaken for hemorroids. Both are distinct entities, and the prevalence of hemorrhoids is not increased in patients with portal hypertension. The frequency of anorectal varices, however, increases with the degree of portal hypertension and may be found in up to 40% of patients with cirrhosis (Figs. 80.11 and 80.12).

*Portal hypertensive biliopathy* may occur in patients with portal hypertension due primarily to extrahepatic portal vein obstruction. It causes alterations of the gallbladder and bile ducts with formation of strictures, dilatations and biliary varices [1, 3]. The vast majority of these patients are asymptomatic with regard to the biliary changes. A few manifest cholangitis with elevated levels of serum alkaline phosphatase, right upper quadrant pain, fever and jaundice. Intraductal stones may form as a complication of duct strictures. The bile duct changes seen on cholangiography are nonspecific.

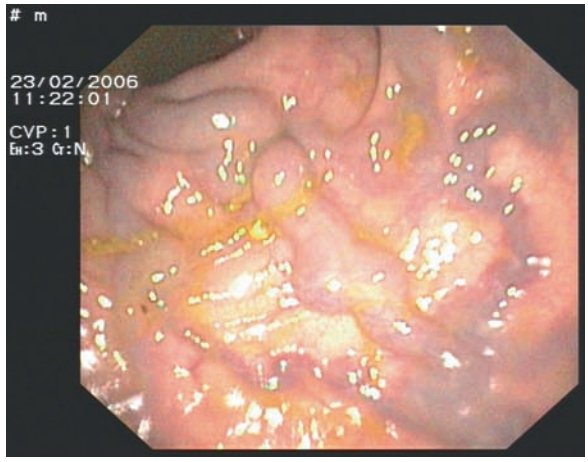


**Fig. 80.11** Rectal v\arices in a patient with cirrhosis and portal hypertension

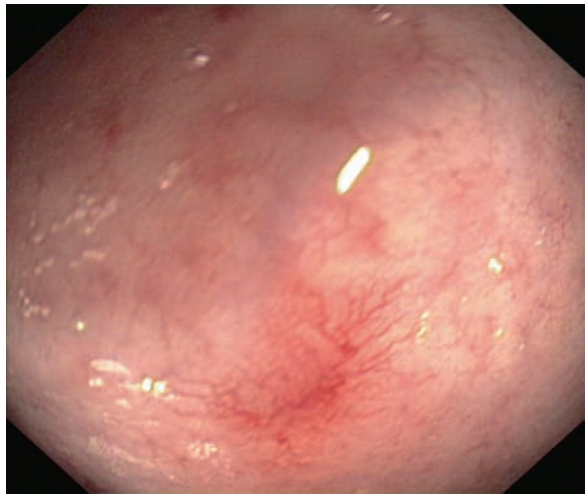

**Fig. 80.12** Spider angioma of rectal mucosa in a patient with cirrhosis

Varices in the region of the gallbladder may be visualized by sonography [5 ].

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## 80.2 Bacterial Infections

Patients with advanced liver cirrhosis are prone to bacteremia, severe infections and sepsis [33, 38, 42]. Bacterial infections occur in 30–35% of hospitalized patients with cirrhosis and in up to 50% of those admitted with gastrointestinal hemorrhage [7, 27]. The rate of nosocomial infections in hospitalized patients with cirrhosis is 15–35% compared to 5–7% in the general hospital patient population. Cirrhosis is also an independent risk factor for sepsis and moreover for a poor outcome in the setting of sepsis. Almost every third fatality of a patient with liver cirrhosis is due to an infection. Relative adrenal insufficiency is very frequent in patients with advanced cirrhosis and septic shock. Intravenous hydrocortisone (50 mg every 6 h) administration in these patients is associated with a high frequency of shock resolution and high survival rate [12, 41].

The most common bacterial infections are spontaneous bacterial peritonitis (25%), urinary tract infections (UTI) (20%), pneumonia (15%), and bacteremia (12%) [38]. The microorganisms most frequently involved in patients with cirrhosis are summarized in Table 80.12. Infections caused by Gram-positive cocci have markedly increased in the last years [9].

*Vibrio vulnificus* septicemia is the most common cause of fatality related to seafood consumption in the United States. It occurs predominantly in patients with chronic liver disease following consumption of raw oysters (especially in iron overload states which seem to increase pathogenicity of the organisms), rapidly progresses to septic shock and is associated with a high mortality. Clinicians managing patients with chronic liver disease need to educate their patients of the risk associated with the consumption of raw seafood, especially oysters. Doxycycline is the antibiotic of choice [23].

The prevalence of *Listeriosis* also is increased in iron overload states (iron overload is believed to impair monocyte function).

The pathophysiology of bacterial infections in patients with liver cirrhosis is complex and is discussed in the paragraph on spontaneous bacterial peritonitis.

**Table 80.12** Microorganisms in patients with cirrhosis and spontaneous bacteremia or spontaneous bacterial peritonitis

| Species | Spontaneous bacteremia (%) | Spontantaneous bacterial peritonitis (%) |
|---|---|---|
| Gram-negative rods | 57 | 77 |
| Staphylococci | 19 | 1 |
| Streptococcus pneumoniae | 10 | 9 |
| Other streptococci | 7 | 8 |
| Enterococcus faecalis | 0 | 2 |
| Anaerobes | 6 | 1 |
| Other bacteria | 1 | 2 |

Source: According to [2]

### Spontaneous Bacterial Peritonitis

#### *Definition*

Spontaneous bacterial peritonitis (SBP) is a life threatening infection of ascites in the absence of an intraabdominal source of infection [21, 30, 37].

#### *Epidemiology*

SBP is observed predominantly in patients with advanced cirrhosis with a prevalence of approximately 15–30% in hospitalized patients. Gastrointestinal bleeding, low protein concentration in ascites, severe liver dysfunction and a history of a previous SBP are associated with an increased risk of SBP. Compared to patients with normal protein levels in ascites the risk of SBP increases tenfold in patients with reduced ascites protein concentration (<1–1.5 g/dL).

#### *Etiology and Pathogenesis*

More than 90% of all SBP cases are monomicrobial, with approximately 80% of the organisms being Gram-negative bacteria. The most common agent is E. coli, followed by Klebsiella species, S. pneumoniae and intestinal bacteria. Gram positive cocci, mainly streptococcus species (including viridans group streptococci) are found in 20% of cases. Anaerobic bacteria occur in less than 5% of patients [4].

The pathogenesis of SBP is not well understood. It appears to be multifactorial, with immune dysfunction,

bacterial overgrowth and translocation of intestinal microorganisms thought to play a role in the development of infectious complications in cirrhosis.

*Dysfunction of the immune system* concerns both the nonspecific defense and antigen-specific reactions. The activity of the reticulo-endothelial system, chemotaxis, phagocytosis and intracellular killing of organism by neutrophil granulocytes is impaired in cirrhosis [8, 15]. Patients with cirrhosis have deficient phospholipase C activity in blood polymorphonuclear neutrophils. $O_2$-generation by neutrophils is decreased, particularly in those with more severe liver dysfunction, and suggests that this defect involves phosphatidylinositol specific phospholipase C activity [16].

*"Immune paralysis"* is defined as a reduction in monocyte human leukocyte antigen-DR (HLA-DR) expression with resulting impairment in endotoxin-stimulated proinflammatory cytokine production. More recent data point to "immune paralysis" being operative in patients with decompensated cirrhosis. Child-Pugh class C cirrhotic patients suffer from down-regulation of HLA-DR expression on monocytes. Endotoxemia, possibly mediated by IL-10, contributes to this HLA-DR down-regulation. Endotoxemia contributes to the immune paralysis in patients with cirrhosis [25].

*Bacterial translocation* refers to the passage of bacteria or bacterial products (lipopolysaccharides, endotoxins) from the lumen of the intestine through the gut wall to extraintestinal sites such as the mesenteric lymph nodes. From here they pass into the circulation and secondarily infect a previously sterile ascites [29]. Direct penetration of intestinal bacteria through the gut wall into the peritoneal cavity is controversial. Bacteria and bacterial products lead to activation of monocytes and lymphocytes with increased serum levels of proinflammatory cytokines such as tumor necrosis factor-α with the subsequent activation of nitric oxide in the splanchnic and systemic circulation, leading to vasodilation and contributing to hyperdynamic circulation (see Section 80.6). Several factors contribute to bacterial translocation including intestinal bacterial overgrowth, increased intestinal permeability, and immune dysfunction.

Levels of *antibacterial substances in ascites*, such as opsonins and complement components, which are critical in bacterial phagocytosis, are low in cirrhosis and contribute to bacterial infection of ascites.

## *Diagnosis*

### Clinical Manifestations

The clinical manifestations of SBP, in contrast to secondary peritonitis are not impressive and may misguide the physician. Patients may be asymptomatic or complain only of mild, vague abdominal discomfort. Fever, changes in mental status, abdominal tenderness, and decompensation of cirrhosis without an obvious cause should arouse the suspicion of SBP and prompt the necessary diagnostic steps.

### Laboratory Findings

The diagnosis of SBP is based on the results of diagnostic paracentesis (see Chapter 54 and Section 80.1).

A diagnostic paracentesis should be performed

- In all patients with cirrhosis and ascites on hospital admission
- In all patients with cirrhosis and ascites who deteriorate clinically (e.g. encephalopathy, azotemia, fever) without an obvious cause
- In all patients with newly formed ascites

Based on *ascitic fluid analysis* (*neutrophil count*[1] and *culture*[2]), the following entities are distinguished:

*Culture-positive, neutrocytic ascites*

Positive culture result and neutrophil count > 250/mm$^3$ ($0.25 \times 10^9$/L) [2] ascites. This combination of findings is typical of SBP.

[1]A *cutoff of 250 neutrophils/mm$^3$ ascites* has the greatest sensitivity for the diagnosis of SBP. In patients with hemorrhagic ascites with an erythrocyte count of >10,000/mm$^3$ ascites, subtraction of one neutrophil per 250 red blood cells should be made to adjust for the presence of blood in ascites. Hemorrhagic ascites is due to underlying hepatocellular carcinoma in 30% of cases or it may be the result of a traumatic paracentesis, however in 50% of cases there is no clear cause.

[2]Ascitic fluid should be inoculated into two blood culture bottles (aerobic and anaerobic) at the bedside. A specific organism can be identified in approximately 72–90% of cases.

*Culture-negative, neutrocytic ascites*

Greater than 250 neutrophils/mm$^3$ ascites with a negative culture result. With this combination of findings, consider tuberculosis and peritoneal carcinomatosis as a diagnostic possibility.

*Monomicrobial, non-neutrocytic bacterascites*

Culture positive for one pathogen with <250 neutrophils/mm$^3$ ascites.

*Polymicrobial, non-neutrocytic ascites*

Several pathogens in ascitic fluid culture with <250 neutrophils/mm$^3$ ascites. This may be the result of inadvertent intestinal puncture during diagnostic paracentesis.

Rapid diagnosis of infected ascitic fluid using leukocyte esterase dipstick testing has been advocated. The Multistix leukocyte esterase test allows for the prompt detection of an elevated ascitic fluid polymorphonuclear neutrophil count. This approach represents a promising new method for the bedside diagnosis of SBP [5, 6, 34]. A negative strip result has been reported to effectively rule out SBP [17]. The use of urine reagent strips in patients with SBP is controversial, and currently does not play a role in the diagnosis of SBP [24, 28].

## Differential Diagnosis

The most important differential diagnosis is secondary peritonitis due to intestinal perforation. Secondary bacterial peritonitis should be suspected if the neutrophil count in ascites is >5,000 neutrophils/mm$^3$ and several microorganisms are demonstrated in ascites culture. A failure of neutrophil count to fall after 48 h of antibiotic therapy also favors the existence of a secondary peritonitis. Ascites in secondary peritonitis typically is polymicrobial as compared to monomicrobial in SBP. The diagnosis of secondary peritonitis further is supported by a total protein concentration > 1–1.5 g/dL, a glucose concentration < 50 mg/L, an LDH concentration that exceeds normal serum LDH levels, ascitic levels of alkaline phosphatase > 240 U/L and CEA levels > 5 ng/mL. Ascites pH, lactate, cholesterol, fibronectin and glycosaminoglycan levels are unhelpful.

Further differential diagnoses include peritoneal tuberculosis and carcinomatosis. The sensitivity of ascites smear for mycobacteria is 0%, that of ascites culture is approximately 50%. If three still warm ascites samples are examined without delay the sensitivity of cytological examination in diagnosing peritoneal carcinomatosis amounts to 96%.

## Course and Prognosis

Early diagnosis and prompt treatment have reduced early mortality from approximately 80% to 15–20%. Still today, however, the prognosis of SBP is poor with a recurrence rate of up to 60–70% within 1 year without adequate prophylaxis and a probability of survival at 1 year of 30–50% and 25–30% at 2 years [1, 40]. Pneumococcal peritonitis has a particularly severe prognosis. SBP predisposes to renal dysfunction. Despite resolution of infection patients with SBP may develop a rapidly progressive impairment in systemic hemodynamics, leading to severe renal and hepatic failure, aggravation of portal hypertension, encephalopathy, and death [32].

## Prophylaxis and Therapy

### Primary Prophylaxis

Because of the severe prognosis of SBP the question arises whether patients with liver cirrhosis and ascites benefit from primary prophylaxis. Current evidence suggests that not every patient with cirrhotic ascites requires primary prophylaxis. Prophylaxis should be reserved for patients at an increased risk of developing SBP, i.e. those with gastrointestinal bleeding and a protein concentration in ascites < 1–1.5 g/dL.

In patients at risk for SBP

Norfloxacin 400 mg p.o. qd–bid

or

Ciprofloxacin 500 mg p.o. qd–bid

or

Ceftriaxone 2 g i.v. qd (after gastrointestinal bleeding)

should be administered for 7–10 days. They lead to a risk reduction of 9–23% and improve acute survival [3, 13, 20, 22]. Intravenous ceftriaxone is more effective than oral norfloxacin in the prophylaxis of bacterial infections in patients with advanced cirrhosis and hemorrhage [13].

Indiscriminate long-term primary prophylaxis carries the risk of the emergence of resistant gram-negative bacilli. Quinolone-resistant SBP constitutes an emergent problem in patients on long-term norfloxacin prophylaxis, with trimethoprim-sulfamethoxazole not being a valid alternative [9].

Patients with cirrhosis and low protein ascitic levels (<1.5 g/dL) with advanced liver failure (Child-Pugh score ≥ 9 points with serum bilirubin level ≥ 3 mg/dL) or impaired renal function are candidates to receive long-term prophylaxis – norfloxacin 400 mg p.o. qd or ciprofloxacin 500 mg p.o. qd – to reduce the risk of infections and improve survival [14, 31, 39].

Effective pharmacologic treatment of portal hypertension (see Section 80.1) should also be viewed as part of prophylaxis of SBP. Long-term pharmacologic prophylaxis of variceal rebleeding contributes to the prevention of community-acquired SBP [19].

## Therapy

The onset of treatment is guided by the clinical picture (fever, abdominal pain, altered mental status, diarrhea) and by the neutrophil count in ascites (result should be available 1–4 h post puncture). *In patients with >250 neutrophils/mm³ ascites, therapy with antibiotics should start without awaiting the culture result*. If needed, antibiotic therapy is modified according to the result of the culture.

Patients who have culture-negative, neutrocytic ascites and those who are symptomatic with monomicrobial, non-neutrocytic bacterascites are treated in the same way as patients with typical SBP.

In *asymptomatic* patients with monomicrobial, non-neutrocytic ascites watchful waiting is appropriate since in most of these patients ascites turns sterile without treatment. Antibiotics of choice are third generation cephalosporins (they cover 95% of the flora isolated from ascitic fluid), for example

Ceftriaxone 2 g i.v. qd

or

Cefotaxime 2 g i.v. q8h

to which 75–90% of patients respond. In asymptomatic cases

Ofloxacin 400 mg p.o. bid

or

Ciprofloxacin 500 mg p.o. bid

or

Amoxicillin/clavulanic acid 1,000/200 mg p.o. tid

may be administered [26, 37]. The routine use of an antibiotic against anaerobic organisms, e.g. metronidazole, is not warranted. It should be given only to patients with culturally proven anaerobic bacteria.

Treatment is continued until complete resolution of all clinical signs of SBP and a drop of neutrophil count in ascites to <250/mm³ (repeated paracenteses). Normally this takes less than 6 days. A reduction in ascitic fluid neutrophil count of less than 25% of the pretreatment value after 2 days of antibiotic treatment suggests failure to respond to therapy.

The recommendations for the treatment of SBP are summarized as follows:

- Upon clinical suspicion start empiric antibiotic treatment immediately after diagnostic tap with a third generation cephalosporin, for example, ceftriaxone 2 g i.v. qd or cefotaxime 2 g i.v. q8h, without awaiting the results of ascitic fluid culture.
- In milder cases (hemodynamically stable, serum creatinine < 3 mg/dL, no gastrointestinal bleeding) an oral fluoroquinolone may be given alternatively, for example ofloxacin 400 mg p.o. bid or ciprofloxacin 500 mg p.o. bid or amoxicillin/clavulanic acid 1,000/200 mg p.o. tid.
- Duration of antibiotic treatment: until resolution of clinical signs of infection and decrease of neutrophils in ascites to <250/mm³. Generally 5–6 days.
- Patients with SBP with a BUN of > 30 mg/dL or a serum creatinine >1 mg/dL should receive albumin 1.5 g/kg body weight i.v. at the time of diagnosis followed by 1 g/kg body weight i.v. after 48 h.

Renal impairment is one of the strongest predictors of mortality in patients with SBP. Albumin, but not hydroxyethyl starch, reduces the activation of the renin-angiotensin-aldosterone system, improves circulatory function, reduces the risk of renal failure in patients with SBP, and improves survival in hospital and at 3 months [10, 11, 36].

### Secondary Prophylaxis

Without secondary prophylaxis SBP recurs in up to 70% of patients within 12 months. Therefore, immediately following successful treatment patients should receive continuous secondary prophylaxis with

Norfloxacin 400 mg p.o. qd

or

Ciprofloxacin 500 mg p.o. qd [18, 35].

Alternatively,

Ciprofloxacin 750 mg p.o. qw.

May be tried

Long-term secondary prophylaxis will result in a change of the bacterial spectrum towards Gram positive cocci and carries the risk of emergence of quinolone resistant Gram negative organisms.

Given the severity of the prognosis of SBP, patients on secondary prophylaxis should always be considered as potential candidates for liver transplantation.

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## 80.3 Hepatorenal Syndrome

### Definition

Hepatorenal syndrome (HRS) is a potentially reversible functional renal insufficiency in patients with cirrhosis, advanced liver failure and portal hypertension in the absence of shock or an intrinsic parenchymal renal disease. It is characterized by changes in endogenous vasoactive systems with marked renal vasoconstriction while at the same time extrarenal arteriolar vasodilatation, decreased systemic resistance and arterial hypotension predominate [4, 6, 9].

### Epidemiology

Eighteen percent of patients with decompensated cirrhosis develop HRS within 1 year of decompensation and 40% within 5 years. The incidence of HRS in patients with liver cirrhosis who are admitted to the hospital because of ascites formation is 7–15% [7, 12].

### Etiology and Pathogenesis

HRS most often occurs in patients with advanced cirrhosis, but may also complicate acute liver failure. Older persons, especially alcoholics are more often affected. It may develop without precipitating factors or be elicited for example by alcoholic hepatitis, spontaneous bacterial peritonitis (SBP), large volume paracentesis without adequate volume replacement, nonsteroidal antiinflammatory drugs (NSAIDS), nephrotoxic medication (e.g. antibiotics) or major surgical procedures. SBP is the most common bacterial infection precipitating HRS. Approximately 30% of patients with SBP develop impairment of renal function – not necessarily a full blown HRS – that cannot be attributed to the administration of nephrotoxic antibiotics or to the presence of circulatory shock. Gastrointestinal bleeding, intensive treatment with diuretics, and diarrheal disease may elicit HRS, but more often cause prerenal insufficiency that occasionally may lead to acute tubular necrosis.

*Predictive factors* for the development of HRS in non-azotemic patients with liver cirrhosis and ascites are

- Previous episodes of ascites
- Absence of hepatomegaly
- Poor nutritional status
- Moderately reduced glomerular filtration rate (> 50 mL/min)
- Moderately increased BUN (<30 mg/dL)
- Moderately increased serum creatinine (≤1.5 mg/dL)
- Low serum $Na^+$
- High serum $K^+$
- Low urinary $Na^+$ excretion
- Low plasma osmolality
- High urine osmolality
- High plasma renin activity
- Low arterial pressure
- Reduced free water clearance following a water load
- Increased plasma norepinephrine, and
- Esophageal varices [7]

Most of these factors represent pathophysiologic parameters that express arterial underfilling with secondary activation of vasoconstricory systems. *Parameters of liver function such as bilirubin, albumin, prothrombin time or Child-Pugh stage are not able to predict the risk of HRS*.

The pathogenesis of HRS corresponds for the most part to that of ascites. Both complications of liver cirrhosis are quasi two sides of the same coin, representing changes of renal perfusion that ultimatelly lead to HRS [11]. The most important pathophysiologic change in HRS is a marked *intrarenal arterial vasoconstriction with reduced renal perfusion and reduced glomerular filtration rate*. The renal vascular resistance in non-azotemic patients with ascites is increased even before HRS ensues. Already in compensated cirrhosis a subclinical impairment of renal function occurs in some patients, characterized by a significant decrease (>50% from normal) of renal blood flow and glomerular filtration rate with retention of $Na^+$ and reduced perfusion of the outer renal cortex.

Intrarenal and systemic circulatory disturbances are closely interrelated in HRS. As in the development of ascites, *arterial splanchnic vasodilatation* is the primary event with reduced renal perfusion as its extreme sequel. Thus, the pathogenesis of hepatorenal syndrome according to the "underfilling hypothesis" may be summarized as follows:

**LIVER CIRRHOSIS**
⇓
**Portal (sinusoidal) hypertension**
⇓
Increased synthesis of NO in splanchnic vessels
⇓
Splanchnic vasodilatation
⇓
Reduction of effective arterial blood volume ("Underfilling")
⇓
Reflexive (baroceptors) stimulation of vasoconstrictory systems
⇓
Renal vasoconstriction[a]
⇓
Reduction of renal perfusion and glomerular filtration
⇓
**HEPATORENAL SYNDROME**

Arterial underfilling with reduction of effective blood volume activates vasoconstrictory systems such as the renin–angiotensin–aldosterone and the sympathetic nervous system that cause arterial vasoconstriction in the kidneys and in other vascular territories (brain, muscles, skin). Very high plasma levels of arginine–vasopressin and endothelin are also found. Primary splanchnic vasodilatation is thought to result from local overproduction of vasodilator substances, predominantly nitric oxide (NO), due to sinusoidal hypertension [5, 9].

In the early stages of HRS local vasodilating renal factors counteract reduced perfusion. Ultimately, however, with continuing arterial underfilling reactive vasoconstrictory systems prevail and lead to diminished renal perfusion. Among the local vasodilating factors in the kidneys are prostaglandins such as PGE2 and prostacyclin and NO. Other vasodilators that counteract the development of HRS belong to the family of natriuretic peptides (atrial and brain natriuretic peptide, urodilatin). NSAIDS reduce renal perfusion and decrease glomerular filtration by inhibiting the

[a]Possibly enhanced by diminished synthesis of local vasodilators

synthesis of prostaglandins. They may precipitate HRS in patients with advanced liver cirrhosis.

The functional nature of HRS is evidenced (1) by normal renal histology, (2) by the fact that transplanted kidneys from patients with HRS function normally in the recipient, and (3) that HRS is reversible after liver transplantation.

## Clinical Manifestations and Diagnosis

The clinical picture of patients with HRS mirrors renal insufficiency, cardiocirculatory dysfunction and liver failure. It is characterized by a marked retention of water and $Na^+$ with ascites, edema and dilutional hyponatremia. Circulation is hyperdynamic with high cardiac output and reduced total vascular resistance. Clinically two types of HRS are distinguished.

### *Type 1 Hepatorenal Syndrome*

*Type 1 HRS* is rapidly progressive and is characterized by doubling of the initial serum creatinine to >2.5 mg/dL or by halving of the creatinine clearance to <20 mL/min in less than 2 weeks. In addition, signs of severe hepatic insufficiency are present with marked hyperbilirubinemia, prolonged prothrombin time, hypoalbuminemia and encephalopathy.

The interpretation of serum creatinine levels in patients with liver cirrhosis is not easy. Compared to patients with other diseases that cause renal failure, serum creatinine concentration in HRS is relatively low even if glomerular filtration rate is markedly reduced. This probably is related to a decreased endogenous production of creatinine due to a reduced muscle mass in cirrhotic patients. Therefore, rather than relying solely on creatinine concentration in serum endogenous creatinine clearance should be determined in these patients.

### *Type 2 Hepatorenal Syndrome*

In *type 2 HRS* renal insufficiency (serum creatinine > 1.5 mg/dL) progresses more slowly, the decline of glomerular filtration rate is less dramatic, and renal function is more stable. Each HRS that does not meet the criteria of type 1 HRS is classified as type 2 HRS. Clinically these patients usually are characterized by a diuretic resistant ascites, which is due to a marked stimulation of antinatriuretic systems with pronounced $Na^+$-retention, and a diminished glomerular filtration rate.

Because of the lack of specific tests, the diagnosis of HRS is based on the demonstration of a reduced glomerular filtration rate in a patient with liver cirrhosis and ascites and on the exclusion of other disorders that can cause renal failure in cirrhosis. Tables 80.13 and 80.14 summarize the International Ascites Club's diagnostic criteria and the new revised criteria of HRS [4, 23]. The intrarenal hemodynamics in patients with liver cirrhosis and ascites may be assessed with Doppler-Duplex-sonography. This technique may prove useful for the diagnosis of developing HRS. Postrenal causes of renal failure can also be excluded easily with ultrasound.

The diagnosis of HRS in patients with spontaneous bacterial peritonitis should only be made after successful treatment of the infection, since improvement of initially reduced renal function after successful therapy of SBP is often observed.

**Table 80.13** International Ascites Club's diagnostic criteria of hepatorenal syndrome [4]

| |
|---|
| *Major criteria* |
| • Chronic or acute liver disease with advanced hepatic failure and portal hypertension |
| • Low glomerular filtration rate, as indicated by serum creatinine of >1.5 mg/dL or 24-h creatinine clearance < 40 mL/min |
| • Absence of shock, ongoing bacterial infection, and current or recent treatment with nephrotoxic drugs; absence of gastrointestinal fluid losses (repeated vomiting or intense diarrhea) or renal fluid losses (weight loss > 500 g/day for several days in patients with ascites without peripheral edema or 1,000 g/day in patients with peripheral edema) |
| • No sustained improvement in renal function (decrease in serum creatinine to 1.5 mg/dL or less or increase in creatinine clearance to 40 mL/min or more) following diuretic withdrawal and expansion of plasma volume with 1.5 L of isotonic saline |
| • Proteinuria < 500 mg/day and no ultrasonographic evidence of obstructive uropathy or parenchymal renal disease |
| *Additional criteria* |
| • Urine volume < 500 mL/day |
| • Urine sodium < 10 mEq/L |
| • Urine osmolality greater than plasma osmolality |
| • Urine red blood cells < 50 per high-power field |
| • Serum sodium concentration < 130 mEq/L |

**Table 80.14** New diagnostic criteria of hepatorenal syndrome in cirrhosis [23]

- Cirrhosis with ascites
- Serum creatinine > 133 µmol/L (1.5 mg/dL)
- No improvement of serum creatinine (decrease to a level of ≤133 µmol/L) after at least 2 days with diuretic withdrawal and volume expansion with albumin; the recommended dose of albumin is 1 g/kg of body weight/day up to a maximum of 100 g/day
- Absence of shock
- No current or recent treatment with nephrotoxic drugs
- Absence of parenchymal kidney disease as indicated by proteinuria > 500 mg/day, microhematuria (>50 red blood cells per high-power field), and/or abnormal renal ultrasonography

**Table 80.15** Differential diagnosis of renal insufficiency (serum creatinine > 3 mg%) in patients with cirrhosis and ascites

| Parameter | Hepatorenal syndrome | Prerenal kidney failure | Acute tubular necrosis |
|---|---|---|---|
| **$Na^+$-concentration in 24 h urine** (mEq/L) | <10 | <10 | >30 |
| **Urine-to-plasma osmolality ratio** | >1 | >1 | <1 |
| **Urine-to-serum creatinine ratio** | >30 | <30 | <20 |
| **Urinary sediment** | Normal | Normal | Cylinders, epithelia |
| **Improvement after volume expansion** | No | Yes | No |

## Differential Diagnosis

First and foremost other disorders (prerenal, acute tubular necrosis, postrenal) that can cause renal failure in cirrhosis must be excluded. Prerenal kidney failure may result from volume deficiency, due for example to gastrointestinal (vomiting, diarrhea) or renal (excessive administration of diuretics) fluid losses. Acute tubular necrosis usually occurs within the context of hypoxia due to circulatory shock in gastrointestinal bleeding, in severe bacterial infections, or after administration of nephrotoxic drugs such as aminogylcosides or NSAIDS. In prerenal failure and in HRS the absorptive capacity of tubular cells and the ability of the kidney to concentrate urine remain preserved, while in acute tubular necrosis these capacities are compromised. The criteria that help in differentiating prerenal kidney insufficiency from acute tubular necrosis are listed in Table 80.15. Monitoring renal function after withdrawal of diuretic treatment with subsequent intravenous volume expansion allows for important diagnostic conclusions. In prerenal failure, renal function rapidly improves, while in HRS this measure remains without effect.

Occasionally patients with liver cirrhosis and preexisting kidney disease (for example, pyelonephritis, urinary tract obstruction or drug induced interstitial nephritis) may also develop renal failure.

## Course and Prognosis

HRS is a life-threatening complication of decompensated liver disease. Without appropriate therapy the prognosis is extremely poor, with a median survival time from the time of diagnosis of approximately 2 weeks (!) for patients with type 1 HRS and 6 months for patients with type 2 HRS (Fig. 80.13). The presence of systemic inflammatory response syndrome (SIRS), with or without infection, is a major independent prognostic factor in patients with cirrhosis and acute functional renal failure [28].

The MELD score is a useful indicator allowing for the estimation of the outcome of patients with cirrhosis and HRS. While practically all patients with type 1 HRS have a high MELD score (≥20) and an extremely poor outcome, patients with type 2 HRS may be stratified according to a MELD score >20 with a median survival of 3 months and those with a MELD score <20 with a median survival of 11 months [1].

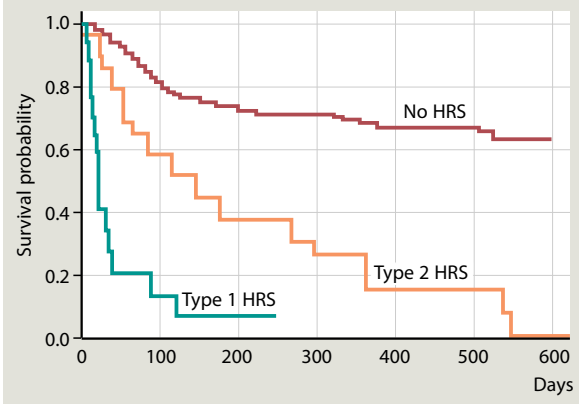


**Fig. 80.13** Survival of patients with cirrhosis after the diagnosis of type 1 or type 2 hepatorenal syndrome (From [5]. With permission)

## Therapy

Therapeutic options in HRS are very limited and treatment is often ineffective. The available evidence is based on relatively few studies with small numbers of patients.

Precipitating factors, such as SBP and gastrointestinal bleeding, should be treated. In patients with SBP, administration of albumin 1.5 g/kg body weight i.v. at the time of diagnosis and 1 g/kg body weight i.v. after 48 h may reduce the incidence of HRS and prolong survival [27].

Potentially nephrotoxic drugs, e.g. aminoglycosides, NSAID, diuretics, and contrast media, should be discontinued.

### Drug Therapy

Pharmacologic therapy aims at correcting hypovolemia as well as reversing splanchnic vasodilation and renal vasoconstriction. Drug treatment of HRS can bridge the time to liver transplantation.

Vasoconstrictor therapy combined with volume expansion is the current pharmacological approach to HRS (and to paracentesis-induced circulatory dysfunction) [10, 14, 16, 20, 21, 25, 26, 29]. Terlipressin and albumin improve renal function in patients with cirrhosis and hepatorenal syndrome [19, 24].

Patients with HRS should receive

*Terlipressin*[a] 0.5–2 mg q4h as an i.v. bolus or as a continuous i.v. infusion 2–12 mg/day
combined with
*Albumin* 1 g/kg body weight i.v. on day 1, thereafter 40 g i.v. qd.

Duration of therapy is generally between 5 and 15 days. The main endpoint of treatment is a reduction in serum creatinine to <1.5 mg/dL. Monotherapy with terlipressin is not advised.

Alternatively, a selective $\alpha_1$-receptor agonist, such as

*Midodrine*, initially 2.5–7.5 mg p.o. tid, increasing slowly to 12.5 mg p.o. tid

combined with

*Octreotide*, initially 100 µg s.c. tid, increasing slowly to 200 mg s.c. tid

and

*Albumin* 40 g i.v. qd

may be tried [3, 18, 30]. Both substances should be dosed in such a way as to increase the mean arterial pressure by 15 mmHg.

In an unblinded pilot study, *noradrenaline* was reported to be as effective and safe as terlipressin in patients with HRS. Since it is a cheap and widely available drug it was suggested that it may be used in the management of these patients instead of terlipressin [2]. However, the results with dopamine, norepinephrine, prostaglandins (misoprostol), and N-acetylcysteine are still preliminary and controversial, and more studies are needed before evidence-based recommendations can be made.

Nonselective endothelin antagonists do not improve renal function in patients with HRS [31].

### TIPS

In patients with HRS, TIPS may improve renal function for a short period of time and reduce the activity of the renin–angiotensin–aldosterone and the sympathetic nervous system [15]. Preliminary data in few patients with a Child-Pugh score < 12 showed a sustained improvement of renal function with the combined approach of oral midodrine + subcutaneous octreotide + intravenous albumin followed by TIPS [8].

### Hemodialysis

Hemodialysis has no impact on the pathophysiologic mechanisms underlying HRS and does not improve

[a]Start terlipressin at a dose of 0.5 mg q4h. If after 3 days serum creatinine does not decrease, escalate dose stepwise every 3 days to 1, 1.5 and 2 mg q4h until serum creatinine falls. Terlipressin has fewer, in approximately 5–10% of cases, ischemic side effects than vasopressin. *At the time of this writing, terlipressin is not yet FDA-approved for use in the USA.*
*Vasopressin* and its analog *ornipressin* are long-acting vasoconstrictors with significant ischemic side effects in approximately one third of patients. These agents are no longer in use in Europe. They should not be used in patients with HRS.

renal function in these patients. Hemodialysis is ineffective in patients with HRS.

### *Liver Transplantation*

Liver transplantation is the best treatment for HRS, with a 3-year survival rate of approximately 70%. The duration of renal dysfunction is a main predictor of the outcome of renal function after transplantation [13]. Therefore, the evaluation for orthotopic liver transplantation should be part of the initial management of patients presenting with the above mentioned predictive factors or suspected of having type 1 HRS, even before a diagnosis of HRS is made.

If patients are treated successfully with pharmacologic therapy, the outcome after transplantation seems to be similar to that of patients who do not have HRS [22].

The combined transplantation of liver and kidneys does not offer an advantage compared to orthotopic liver transplantation alone [17].

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## 80.4 Hepatic Encephalopathy

### Definition

Hepatic encephalopathy (HE) is a complex metabolic mental state disorder with a spectrum of reversible neuropsychiatric abnormalities seen in patients with severe acute or chronic liver dysfunction after exclusion of other brain diseases [6, 10]. Hepatic abnormalities may be associated with acute liver failure (*Type A HE*), portosystemic bypass with no hepatocellular disease (*Type B HE*) or with cirrhosis and portal hypertension or portosystemic shunts (*Type C HE*) (Table 80.16) [16].

### Epidemiology

HE is much more commonly seen in patients with chronic liver disease than in acute liver disorders. However, exact data regarding incidence and prevalence of HE are lacking and statements about its frequency in patients with liver cirrhosis vary considerably. Up to 70% of patients with liver cirrhosis, while clinically unremarkable have pathologic changes on EEG and psychometric tests. These subclinical alterations currently are termed minimal HE (see below). Prevalence of minimal HE is 53.3% in patients with extrahepatic portal vein obstruction [50].

Approximately 50% of patients with liver cirrhosis develop HE after surgical portosystemic bypass procedures. Compared to other bypass operations, the prevalence of HE is lower in patients receiving a distal splenorenal shunt. After placement of a TIPS approximately one third of patients develops HE. Patients older than 60 years are particularly at risk.

### Etiology and Pathogenesis

In the vast majority of patients, liver cirrhosis with reduced functional hepatic mass underlies the development of HE. In chronic liver disease, HE is usually triggered by *precipitating factors* (Table 80.17). More rarely, progressive liver dysfunction per se, i.e. without an accompanying precipitating event or spontaneous formation of portosystemic shunts, leads to HE. The

**Table 80.16** Nomenclature of hepatic encephalopathy

| HE type | Nomenclature | Subcategory | Subdivisions |
|---|---|---|---|
| A | HE associated with acute liver failure | | |
| B | HE associated with portosystemic bypass and no intrinsic hepatocellular disease | | |
| C | HE associated with cirrhosis and portal hypertension or portosystemic shunts | Episodic HE | Precipitated<br>Spontaneous (i.e. without recognized precipitating factors) Recurrent |
| | | Persistent HE | Mild, severe, treatment-dependent |
| | | Minimal HE | |

Source: According to Ferenci et al. [16]

**Table 80.17** Precipitating factors of hepatic encephalopathy

*Increased ammonia production*
- Nutritional protein intake
- Constipation
- Gastrointestinal bleeding
- Blood transfusion
- Infections, sepsis
- Renal insufficiency

*Hypovolemia, hepatic hypoxia*
- Overdiuresis
- Large volume paracentesis (without adequate volume replacement)
- Diarrhea, vomiting
- Circulatory shock

*Drugs*
- Tranquillizers
- Narcotic analgesics
- Diuretics

*Electrolyte and acid–base disturbances*
- Hypokalemia
- Metabolic acidosis and alkalosis

*Portal-systemic shunts*
- Spontaneous
- Surgical
- TIPS

*Development of hepatocellular carcinoma*

most frequent precipitating cause of HE is *gastrointestinal bleeding*. Patients with liver cirrhosis are at particular risk of bleeding from esophageal and/or gastric varices, from mucosal lesions of portal hypertensive gastropathy, from gastric erosions and from peptic gastroduodenal ulcers. One hundred milliliters of blood contains 15–18 g of protein which is metabolized by intestinal bacteria and generates ammonia.

An *increased dietary protein load* also leads to an enhanced nitrogen burden that may precipitate and perpetuate HE.

*Bacterial infections* may elicit HE by inducing a systemic inflammatory reaction (response to the action of proinflammatory cytokines such as tumor necrosis factor-α, interleukin-1β and interleukin-6) with catabolism, fever, dehydration, arterial hypotension, hypoxia and renal insufficiency. It has been postulated that the inflammatory response may "unlock" the blood-brain barrier to the effects of toxins [25, 46, 51]. Urease-forming organisms (E. coli, Proteus mirabilis) may lead to hyperammonemia. Helicobacter pylori also exhibits urease activity and leads to elevated intragastric $NH_3$ levels. These levels, however, are too low to increase serum $NH_3$ concentration significantly in cirrhotic patients [61]. Reduced $NH_3$ levels in serum have been reported after successful eradication of Helicobacter pylori, but this may also be due to the effect on intestinal bacteria of antibiotics used in H. pylori eradication. Most investigations could not prove a relation between H. pylori infection, $NH_3$ levels in serum and clinical signs of HE in patients with liver cirrhosis. HE may also be the first manifestation of a hitherto asymptomatic spontaneous bacterial peritonitis.

*Malnutrition* and *diabetes mellitus* are related to Type C HE, and diabetic patients have been reported to have more severe HE at earlier stages of biochemical decompensation and portal hypertension compared with nondiabetic patients [27, 52].

*Surgical procedures* in patients whose hepatic metabolic capacity is already markedly compromised preoperatively may trigger a HE. Within this context many factors, such as hypovolemia, dehydration and infections play a role.

*Fluid* and *electrolyte disturbances*, especially reduced intravasal volume (blood loss, over-diuresis, large volume paracentesis without adequate volume replacement, profuse diarrhea, vomiting) may lead to decreased

liver perfusion with hypoxic hepatocellular injury that further compromises the already impaired hepatic metabolic activity. Hypokalemia, hyponatremia and metabolic acidosis increase renal production of ammonia and reduce liver and kidney blood flow. In addition, acidosis increases insulin resistance. Metabolic alkalosis is much more common in liver cirrhosis than metabolic acidosis. It often is due to impaired hepatic urea synthesis. Alkalosis increases the fraction of non-ionized ammonia and enhances the passage of $NH_3$ through the blood–brain barrier into the brain.

*Drugs*, especially narcotics and analgesics, such as benzodiazepines and barbiturates, may directly precipitate HE or perpetuate encephalopathy by enhancing the effects of endogenous neurotoxins. Overuse of diuretics may cause hypokalemia, hypovolemia and renal insufficiency that stimulate ammonia generation by the kidney with consequent HE. In addition, some diuretics inhibit hepatic urea synthesis (detoxification mechanism).

*Toxins*, primarily ethanol, already in low doses may damage the cirrhotic liver (and also the brain) and trigger HE.

The *pathogenesis* of HE is still poorly understood and largely hypothetical. Our understanding for the most part is based on the interpretation of cell culture and animal experiments. HE is caused by the interplay of various factors that alter metabolic and neurophysiological pathways and that finally result in impairment of astroglial-neuronal communication, and in an imbalance between excitatory and inhibitory neuronal activity [10, 15, 24]. Important factors involved in the development of HE are

- Neurotoxins
- Impaired astrocyte function
- Dysfunction of the blood–brain barrier
- Imbalance of amino acids
- False neurotransmitters

## Neurotoxins and Impaired Astrocyte Function

More than 100 years ago it was postulated that *ammonia* which cannot be effectively detoxified by the diseased liver is the most important neurotoxin involved in the development of HE [54]. Approximately 90% of patients with HE have increased arterial ammonia levels, and *ammonia remains the most important factor in the pathogenesis of HE* [8, 12]. Hyperammonemia in patients with liver cirrhosis results from increased intestinal production of ammonia, decreased ammonia removal by the liver (reduced hepatic urea and glutamine synthesis) and from a reduced skeletal muscle mass (decreased absolute glutamine synthesis despite increased activity of glutamine synthetase) [47]. Therapeutic measures that reduce serum ammonia concentration often lead to clinical improvement. However, there is no close correlation between severity of HE and blood ammonia levels [34].

Ammonia is mainly produced by the gut and by the kidney. Ammonia generated in the intestines derives from different sources, such as nitrogenous components of the diet, deamination of glutamine, and breakdown of urea by urease present in colonic flora [12]. The kidney determines blood ammonia by excreting urea and generating ammonia. Only small amounts of ammonia are generated by the kidney under normal conditions. The kidney, however, is an important source of ammonia after gastrointestinal bleeding. Ammonia produced in the liver itself by hepatic metabolism of amino acids and proteins does not reach the general circulation, but is metabolized within the liver. Under normal physiological conditions synthesis and degradation of ammonia are in equilibrium and the arterial ammonia concentration is kept constant around 30 µmol/L. At physiological blood and intracellular pH values approximately 99% of $NH_3$ exist as $NH_4^+$-ion (ammonia ion). Small changes in pH have effects on the equilibrium and affect the amount of non-ionized ammonia ($NH_3$), which is the form that passes the blood–brain barrier by diffusion. It has been estimated that at least 20% of ammonia may pass the blood–brain barrier ionized ($NH_4$) through an active transport. Cerebral blood flow and the permeability of the blood–brain barrier are other elements that may determine the delivery of ammonia to the brain (see below).

The liver plays a central role in the detoxification of ammonia by production of urea and glutamine. The daily production of urea in a healthy person weighing 70 kg on a balanced diet is approximately 30 g. Approximately 70–90% of ammonia is used for urea synthesis by the urea cycle in periportal hepatocytes. The remainder of ammonia that escapes periportal urea synthesis is trapped by perivenous scavenger cells and is transformed into glutamine by glutamine synthetase

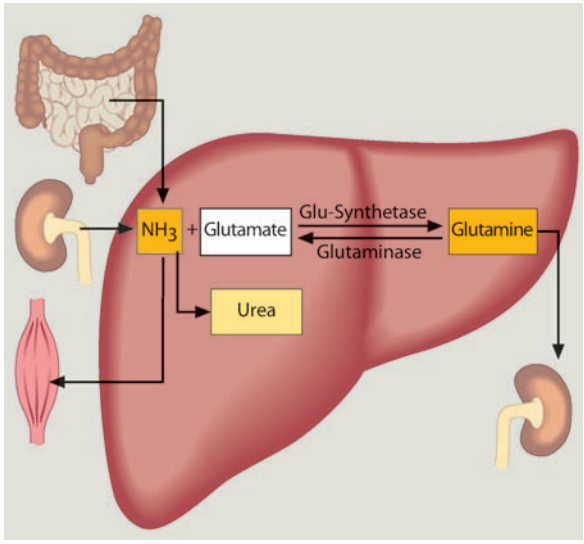


**Fig. 80.14** Ammonia derived from gut and kidney is detoxified by the liver (urea synthesis in periportal hepatocytes and glutamine synthesis in perivenous hepatocytes) and skeletal muscle (glutamine synthesis)

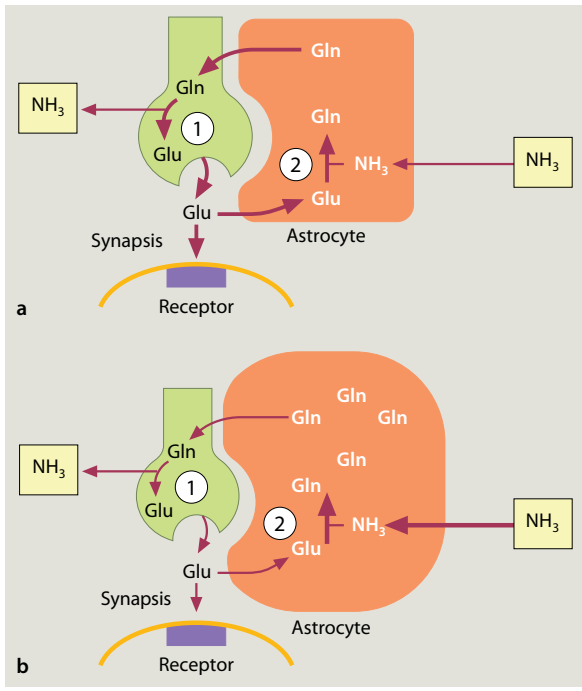


**Fig. 80.15** Cerebral ammonia metabolism under (**a**) normal conditions and (**b**) in hyperammonemia. Astrocyte swelling occurs due to increased ammonia and glutamine levels. The excitatory glutamatergic transmission is impaired. *1* Glutaminase, *2* Glutamine synthetase, *Gln* Glutamine, *Glu* Glutamate

(Fig. 80.14; see Chapters 6 and 9). Glutamine then reaches the general circulation, is filtrated by the glomeruli and absorbed by renal tubular cells. Approximately 60% of urinary ammonia derives from metabolism of glutamine. The remaining 40% are provided by other non-essential amino acids.

Both urea and glutamine synthesis are reduced in the cirrhotic liver to approximately 20% of its normal capacity. If portosystemic shunts are present, gut derived ammonia bypasses the liver and large amounts of ammonia reach the brain. Skeletal muscle has an important role in buffering ammonia that is produced in the intestines and not metabolized in the liver [17]. It is devoid of an effective urea cycle and contributes to the detoxification of ammonia by metabolizing ammonia to glutamine by glutamine synthetase. Chronic hyperammonemia results in increased muscular glutamine synthetase activity, and in chronic hepatic insufficiency muscle becomes the major route for ammonia detoxification. However, due to muscle wasting in most cirrhotic patients the synthesis of glutamine is insufficient to effectively detoxify ammonia.

Under physiological metabolic conditions ammonia crosses the blood-brain barrier and enters into the astrocytes. Within the astrocytes ammonia is detoxified by binding to α-ketoglutarate, thereby generating glutamic acid. Astrocytes also contain glutamine synthetase that catalyzes the formation of glutamine from ammonia and glutamic acid [36, 37]. In contrast to skeletal muscle, the brain is not able to adapt its ammonia removal capacity by induction of glutamine synthetase. Since under normal circumstances the blood–brain barrier is impermeable to amino acids, glutamine is exported from astrocytes by a specific transporter. It reaches the presynaptic neuronal region where it is transformed by glutaminase to glutamic acid. Upon neuronal stimulation glutamic acid is liberated into the synaptic cleft, it binds to its postsynaptic receptor and functions as an excitatory transmitter (Fig. 80.15a).

In patients with HE the permeability of the blood–brain barrier for ammonia, the cerebral extraction of ammonia and the cerebral ammonia metabolism are increased [58]. In advanced cirrhosis a reduction in the brain concentration of many organic osmolytes has been described. Dilutional hyponatremia in cirrhosis is associated with remarkable reductions in brain

organic osmolytes, particularly myo-inositol that probably reflect compensatory osmoregulatory mechanisms against cell swelling [43]. In hyperammonemia increased amounts of ammonia enter into the astrocytes and enhance glutamine synthesis. These unphysiologically high intracellular glutamine concentrations exceed the export capacity of the astrocyte. Since intracellular glutamine is active osmotically, astrocytes swell because of the increased inflow of water (Fig. 80.15b). The change in the state of cellular hydration causes impairment of several metabolic pathways and has been suggested as being responsible for brain edema and for the neurological manifestations of HE [21]. In addition, ammonia has been shown to evoke oxidative stress, inducing the generation of free radicals, which may contribute to the induction of swelling and failure of normal neurotransmission [7, 38].

Increased levels of glutamine can be demonstrated in the cerebrospinal fluid of patients with HE. Moreover, increased glutamine synthesis results in consumption of glutamic acid and in an impairment of excitatory glutamatergic neurotransmission (Fig. 80.15b) [7]. As a consequence inhibitory impulses prevail which significantly contribute to HE symptoms. Thus, *hyperammonemia leads to astrocyte dysfunction with impaired astroglial-neuronal communication* which forms the basis of neuropsychiatric alterations in HE.

*Mercaptanes* (*methanthiol, dimethyl-disulfide-* and *ethylmercaptane*), formed by bacterial action on sulfur containing aminoacids are also neurotoxic (inhibition of activity of $Na^+/K^+$-ATPase) and possibly increase ammonia-induced neurotoxicity. Their pathophysiological significance in HE is unclear. They are probably responsible for fetor hepaticus.

*Fatty acids* may inhibit $Na^+/K^+$-ATPase of cerebral microsomes. Increased serum concentrations of short- and medium-chained fatty acids ($C_4$–$C_8$), derived from bacterial metabolism have been demonstrated in patients with HE. A correlation with the HE-grade, however, has not been found.

Alterations of *cerebral blood flow*, of brain *glucose utilization* and of glucose metabolic pathways are consistent findings in patients with liver cirrhosis and HE. It is, however, unknown if they represent primary pathophysiologic alterations or are secondary to the above-mentioned factors.

The significance of *phenoles*, derivatives of the aromatic amino acids phenylalanine and tyrosine in the pathogenesis of HE is not clear.

## Amino Acid Imbalance and False Neurotransmitters

The ratio between branched chained amino acids (leucine, isoleucine, valine) and aromatic amino acids (phenylalanine, tyrosine, tryptophane) under normal physiological conditions is 3.5 and is reduced to 1 in patients with liver cirrhosis. The *decrease in branched chained amino acids* probably bears consequences for neurotransmission. Under physiological conditions glutamine is exported from astrocytes by exchange for aromatic and branched chained amino acids. With the ratio of amino acids altered and the permeability of the blood–brain barrier impaired in the cirrhotic patient, increased amounts of aromatic amino acids reach the astrocytes. Elevated cerebral aromatic amino acids, by inhibiting tyrosine-3-monooxygenase, stimulate the synthesis of the false neurotransmitters *octopamine* and *phenylethanolamine*. These false transmitters compete with the physiological transmitters dopamine and norepinephrine for receptor binding and result in impaired excitatory dopaminergic neurotransmission. However attractive, this old "false neurotransmitter hypothesis" still is unproven.

Elevated cerebral tryptophan levels result in increased synthesis of *serotonin* in the brain. Serotonin is a depressory transmitter and induces sleep. The pathophysiologic role of serotonin in HE is unknown.

*γ-Aminobutyric acid (GABA)* derives from the gut, is metabolized inadequately by the diseased liver and elevated concentrations reach the brain due to increased permeability of the blood–brain barrier. GABA binds to the cerebral GABA–benzodiazepine–barbiturate receptor complex that is coupled to a chloride channel, and acts a potent inhibitory neurotransmitter [5]. After binding to its receptor the chloride channel is opened. The increased influx of chloride ions leads to the polarization of the postsynaptic neuron and to the generation of an inhibitory potential. Patients with liver cirrhosis exhibit an increased sensitivity to benzodiazepines [4]. Benzodiazepines and benzodiazepine-like compounds (gut derived or of plant origin) potentiate inhibitory GABAergic neurotransmission and may elicit and perpetuate HE. Administration of the benzodiazepine antagonist flumazenil has been noted to ameliorate at least temporarily the neurodepressory status of patients with HE, which would support the pathophysiological role of GABA in the development of HE [20, 41]. However, there is no correlation

between blood and cerebrospinal fluid levels of GABA and the severity of HE. Moreover, many patients who respond well to flumazenil have increased serum concentrations of exogenous benzodiazepines. Thus, the classical GABA hypothesis has been abandoned and new endogenous GABA-receptor agonists and potent inhibitory agents, such as neurosteroids (e.g. allopregnanolone), are currently at the center of interest [1].

## Pathology

The histopathological brain changes seen in HE are nonspecific. While in Type A HE glial swelling and brain edema predominate, in Type C HE the principal neuropathological finding is altered astrocyte morphology in the basal ganglia and in the cerebral cortex. Astrocytes are increased in number and enlarged. Their nuclei are large and pale (containing glycogen inclusions), with margination of chromatin, and prominent nucleoli. The cytoplasm is scant, and cytoskeletal proteins are diminished. The changes resemble those seen in Alzheimer type II degeneration and the term Alzheimer type II astrocyte is used to describe the morphological features manifested by astrocytes in type C HE.

## Diagnosis

### *Clinical Manifestations*

The diagnosis of overt HE is clinical and rests on the careful mental status examination. Laboratory findings, neuroimaging, neuropsychological and neurophysiological examinations usually complement the diagnosis of HE but are not mandatory. HE is much more commonly seen in patients with chronic liver disease, where it usually begins insidiously with a slowly progressive undulating course. Initially, the mental changes are often unnoticed by the patient and the physician.

#### Minimal Hepatic Encephalopathy

Minimal hepatic encephalopathy (MHE) – formerly called latent, subclinical or stage 0 HE – is present in approximately 30–70% of patients with cirrhosis if specialized tests are used to detect minor changes in mental status. The patient with MHE feels well and neither he or she or his/her environment is aware of any mental deficit. Verbal ability is intact and during a normal conversation there are no clues as to the presence of mental dysfunction. Despite these findings patients with MHE have already deficits in attention, visual perception, memory function, and learning impairment. Simple activities of daily life are impaired, such as the ability to drive a car with consequent increased risk of travel accidents [3, 19, 39, 59, 60]. Therefore, patients with liver cirrhosis should be tested for MHE and informed in the case of abnormal test results. Only sophisticated tests such as EEG, visual evoked potentials and psychometric tests (see below) can detect mild forms of HE and MHE.

#### Overt Hepatic Encephalopathy

Clinically overt HE may be subdivided into different grades of severity based on changes in consciousness, vigilance, intellectual functions, alterations of personality, behavior and neuromuscular functions (Table 80.18). The clinical picture ranges from an attention deficit, forgetfulness, mood changes, disturbances of sleep with changes in day-night rhythm, to disorientation with regard to time and space, and finally a deep coma from which the patient is unarousable. Often an inadequate euphoric behavior is recognized retrospectively as an early grade HE.

*Flapping tremor (asterixis; "liver flap")* is commonly seen in grade II and III HE. It is a rapid, arrhythmic, flexion- and extension-tremor of the wrist and metacarpophalangeal joints, and rarely may also affect the elbows, shoulders and feet. It may be elicited by having the patient hold his arms outstretched, with the wrist dorsiflexed and the fingers extended. It is characteristic, but not pathognomonic for HE, and may also occur in $CO_2$-intoxication, hypokalemia, hypoglycemia, uremia, severe heart insufficiency and during treatment with phenytoin.

*Fetor hepaticus* refers to a musty, ammoniacal, sweet breath odor that probably is caused by mercaptanes. Its presence does not correlate with the severity of HE or with the prognosis. Signs of intracranial hypertension are rare in chronic HE (type C HE), but the rule in fulminant liver failure (type A HE see Chapter 78).

**Table 80.18** Clinical grading of hepatic encephalopathy

| Grade | Consciousness/vigilance | Personality and intellect | Neuromuscular and neurophysiological disturbances |
|---|---|---|---|
| **0** | Normal | Clinically no abnormality detected | Psychometric tests and visual evoked potentials pathologic |
| **I** | Trivial lack of awareness; shortened attention span; sleep abnormalities (disturbance of day-night rhythm) | Mood changes (depression, euphoria, irritability); forgetfulness | Tremor; apraxia; impaired coordination; impaired writing ability |
| **II** | Lethargy | Obvious personality changes with inappropriate behavior (e.g. disinhibition, yawning, grimacing); disorientation for time | Flapping tremor (asterixis); dysarthria; ataxia; diminished reflexes |
| **III** | Somnolence to semistupor, but responsive to stimuli | Confused; disoriented for time an place; aggressive; bizarre behavior; delusions | Flapping tremor (asterixis); inarticulate speech; Babinski positive; rigor; nystagmus |
| **IV** | Coma | No personality or intellect present anymore | Decerebrated; wide pupils; test of mental state not possible |

Repeated evaluation of the patient's handwriting is a simple method to follow the clinical course of HE.

## *Technical Investigations*

90% of patients with overt HE have elevated blood ammonia levels. Despite a lack of a close correlation between the ammonia level and the stage of HE, repeatedly normal blood ammonia levels with a high probability exclude HE, while hyperammonemia in a comatose patient makes HE highly probable. The level of hyperammonemia has no prognostic significance. Thus, repeated measurements of elevated ammonia levels during the course of HE are not meaningful.

As an expression of advanced liver disease hyponatremia, hypokalemia and hypophosphatemia often occur in patients with Type C HE.

Concentrations of α-ketoglutarate and glutamine in the cerebrospinal fluid are elevated in patients with HE, and are thought to be more specific for HE than ammonia. Nonetheless a lumbar puncture is neither required for the diagnosis nor for evaluation of the course of HE. Determining cerebrospinal fluid levels of α-ketoglutarate and glutamine may have a role in the differential diagnosis of a comatose patient of unknown cause, but is rarely done clinically.

A battery of *psychometric tests* which examine cognitive functions and visual-motor-spatial coordination have been designed over the last decades. These tests quantify neurological impairment and are able to detect mild or minimal HE. Among them is the number connection test, the paper pencil test, and the line tracing test to mention just a few. The sensitivity of psychometric tests in evaluating cognitive activities is high; their specificity, however, with regard to HE is low since they are also pathologic in encephalopathies of nonhepatic origin.

Sophisticated neurological techniques, such as *EEG* and analysis of *evoked responses* to auditory and visual stimuli are more sensitive (but also nonspecific) in detecting cerebral dysfunction in patients at risk for HE than pysochometric tests [30, 32]. *Critical flicker frequency* in patients with MHE is a relatively simple, reliable, and accurate method without any age or literacy dependence for the diagnosis of MHE [31, 48–50]. These tests are still rarely used in clinical routine, but are increasingly employed in clinical studies.

*Neuroimaging* with CT, MRI, and positron emission tomography (PET) is not required for the diagnosis of HE. These methods may be helpful in excluding other causes of coma, such as intracerebral tumors or infections, or may visualize brain edema in fulminant liver failure. In addition, PET allows investigating brain ammonia metabolism or in vivo imaging of cerebral benzodiazepine binding sites [9, 28].

Magnetic resonance spectroscopy may detect changes of cerebral metabolite pattern, and recently the *bispectral index* was reported to be a useful measure for grading and monitoring the degree of involvement of the central nervous system in patients with chronic liver disease [13, 22]. However, these advanced techniques

are usually limited to clinical studies and not part of routine clinical evaluation of patients with type C HE.

## Differential Diagnosis

The differential diagnosis includes encephalopathies and coma of different etiologies. If liver cirrhosis is known to exist, the assignment of encephalopathy as hepatic in origin usually is easy. If, however, the underlying illness is not known and no precipitating factors can be found, the differential diagnosis may be complex. It should include metabolic-toxic and infectious causes, intracranial masses, meningeal pathology and psychiatric illnesses (Table 80.19).

## Course and Prognosis

Type A HE develops rapidly, usually within a few hours or 1–2 days, and is rapidly progressive. Mortality rates of patients in stage IV (without liver transplantation) are very high and reach up to 80%. Death is frequently due to brain herniation as a result of brain edema and intracranial hypertension.

Type C HE usually develops slowly over longer periods of time, and often has an undulating course with recurrent HE episodes. However, in rare cases HE in cirrhotic patients may have an acute onset with frequent recurrences. The neuropsychiatric manifestations of Type C HE are usually reversible. But HE can also lead to permanent damage with dementia, extrapyramidal signs, cerebellar degeneration, myelopathy with spastic paraplegia, and peripheral polyneuropathy [26]. These changes may also be reversible in single patients after liver transplantation.

**Table 80.19** Differential diagnosis of hepatic encephalopathy

| |
|---|
| *Metabolic-toxic encephalopathies* |
| Hypoxia |
| Hypoglycemia |
| Ethanol |
| Acute intoxication |
| Withdrawal syndrome |
| Wernicke–Korsakoff syndrome |
| Electrolyte disturbances |
| Ketoacidosis |
| $CO_2$-narcosis |
| Psychoactive drugs |
| Salicylate and heavy metal intoxication |
| Wilson's disease |
| *Intracranial lesions* |
| Bleeding |
| Subarachnoidal |
| Subdural |
| Intracerebral |
| Tumor |
| Abscess |
| Vascular accident |
| *Meningeal irritation* |
| Meningitis |
| Encephalitis |
| Seizure disorders or postictal encephalopathy |
| *Psychiatric diseases* |

## Therapy

The goal of treatment of HE is the normalization of neurophysiological functions. According to its multifaceted pathogenesis, therapy of HE remains a challenge and rests on several pillars. The most important therapeutic strategies are based on the

- Elimination of precipitating factors, and on
- Lowering of ammonia levels [45]

If this is achieved, cerebral neurotransmission and astroglial function often improves and is accompanied by improvement of the clinical picture. Since the underlying disease is not affected by these measures, even after successful therapy patients remain at risk of encephalopathy. Only liver transplantation is able to change this course of events. Liver transplantation, however, is rarely performed in Type C HE, but becomes increasingly important in Type A HE.

### *General Measures and Elimination of Precipitating Factors*

Since HE usually is elicited by precipitating factors these must be identified and eliminated. This approach leads to a marked clinical improvement in many cases of early HE.

*Fluid losses* from vomiting, diarrhea or gastrointestinal bleeding and *electrolyte disturbances* have to be corrected. The site of origin of gastrointestinal bleeding

must be identified promptly and treated accordingly. A reduced intravascular volume must be carefully replenished though an overly vigorous fluid supply should be avoided, since variceal bleeding, pulmonary and brain edema may be elicited.

*Hypokalemia* must be corrected by careful oral or, in severe cases by intravenous administration of potassium. Patients who take aldosterone antagonists are at increased risk of hyperkalemia during administration of $K^+$. *Hyponatremia,* which nearly always is present in patients with liver cirrhosis, should not be corrected with NaCl-solutions since this furthers the development of ascites and edema. Moreover, rapid administration of $Na^+$ may result in central pontine myelinolysis. A hyponatremia should be met by restriction of daily fluid intake to approximately 1L. Measurements of blood glucose in patients with hepatic coma should be made every 4–6 h and *hypoglycemia* must be corrected rapidly by glucose infusions. Patients with liver cirrhosis are at an increased risk of infections. A *metabolic acidosis* may be an expression of sepsis or of renal insufficiency. Appropriate antibiotic treatment is indicated. *Pulmonary dysfunction* may lead to hypoxic liver injury. Adequate ventilation, including administration of oxygen should be assured.

All *drugs* that potentially may precipitate or perpetuate HE must be withdrawn. Among these are diuretics (hypokalemia, dehydration, hepato-renal syndrome, inhibition of urea synthesis), tranquilizers and centrally acting analgesics. Edema and ascites formation due to withdrawal of diuretics should be temporarily accepted. If a patient must be sedated a trial with antihistamines is warranted.

*Nutritional measures* are of paramount importance in the management of patients with HE. Table 80.20 summarizes the most important dietary measures. The *total caloric support* should amount to approximately 30 kcal/kg body weight/day, which occasionally is difficult to achieve in view of the concomitant necessity of daily fluid restriction to 1–1.5 L. Severe restriction of dietary *protein* is not recommended, and diets with a normal content of proteins may be administered safely to cirrhotic patients with episodic HE [11]. In patients with grade II and III HE, protein intake should be reduced to 20–30 g/day during the first 3–5 days. Then protein should be increased to 1–2 g/kg/day, in order to counteract the catabolic state. A positive nitrogen balance promotes liver regeneration and increases the capacity for ammonia removal by skeletal muscle. There is no fundamental difference between protein of animal origin compared to vegetable protein in the treatment of HE. However, due to its higher fiber content and its laxative action the latter might be preferred.

**Table 80.20** Nutritional measures in patients with acute hepatic encephalopathy

| |
|---|
| *Sufficient caloric supply* |
| 30 kcal/kg body weight/day |
| *Restriction of dietary protein*[a] |
| Day 1–5: 20–30 g/day |
| Then: 1–2 g/kg body weight/day |
| *Increase of glucose (lipid) calories* |
| 10% glucose 1–2 L/day |
| *Branched chained amino acids (BCAA)* |
| 0.2–1.2 g/kg body weight i.v./day[b] |
| *Replacement of vitamins and trace elements* |
| Vitamin B-complex |
| Vitamin K |
| Zinc |

[a]Patients with episodic HE may receive a normal protein diet

[b]Long-term oral administration of BCAA is possible in patients with minimal HE

The restriction in protein should be compensated by an *increase in glucose* and *lipid calories*. During the first 1–2 days, 1–1.5 (–2) L of a 10% glucose solution may be administered. Thereafter the proportion of glucose must be reduced.

*Branched chained amino acids* (BCAA; valine, leucine, isoleucine), 0.2–1.2 g/kg body weight/day, inhibit protein catabolism in liver and skeletal muscle, and stimulate protein synthesis. They are supposed to "protect" the astroglia and reduce the formation of false neurotransmitters by correcting the amino acid imbalance present in patients with Type C HE. Infusion of BCAA may indeed improve mental status, however, it does not increase the survival probability. Long-term oral administration of BCAA-enriched diets may improve cognitive function in some patients with minimal or persistent HE [14, 35, 40]. The high costs of this therapy and the lack of definitive evidence of the efficacy of BCAA, however, are reasons against their general recommendation in the treatment of patients with HE.

*Probiotic supplementation*, for example in the form of a probiotic yoghurt, may have a beneficial effect and lead to reversal of MHE in patients with nonalcoholic cirrhosis [3a].

Patients with liver cirrhosis nearly always are malnourished and deficient in *vitamins* and *trace elements*. Often B vitamins, vitamin K and zinc must be substituted. The latter is part of carbamoyl-phosphate synthetase, one of the rate limiting enzymes of urea synthesis.

## Ammonia-Lowering Strategies

As mentioned above, ammonia still is regarded as the most important factor in the pathogenesis of HE. Lowering ammonia blood levels is accompanied by clinical improvement in most patients. This goal may be reached by both

- Decreasing ammonia production and
- Stimulation of ammonia detoxification

Reduction of ammonia production may be accomplished by nutritional protein restriction, by lowering of ammoniagenic substrates in the gut by stimulating frequent bowel movements, and by inhibiting intestinal ammonia synthesis by nonabsorbable disaccharides (lactulose and lactitol) and antibiotics (neomycin and rifaximin).

*Lactulose*, a synthetic disaccharide (1,4-β-galactoside-fructose), is not absorbed in the gut due to a lack in brush border enzymes and has a cathartic effect (osmotic laxative). The substance reaches the colon where it is metabolized by bacteria to lactic, acetic and formic acid. The acidification of gut contents favors the formation of the less absorbable $NH_4^+$ from $NH_3^+$. In addition, the bacterial metabolism of ammonia is enhanced and urease forming bacteria are inhibited. The combined effect of these actions leads to a lowering of ammonia blood levels. The dose of lactulose (20–30 g tid) is titrated to achieve two to three soft stools per day. Unpleasant side effects in the initial phase of treatment may be flatulence and abdominal cramps. A long-term treatment with lactulose in clinically stable patients is not indicated.

*Lactitol* (β-galactoside-sorbitol) may be used as an alternative to lactulose in patients who are annoyed by the sweetish taste of lactulose. Its effects correspond to those of lactulose.

*Acidifying enemas* using 1–3 L of a 20% lactulose or lactitol solution may be used in patients who cannot tolerate oral disaccharides or in comatose patients [57].

Reliable data on the efficacy of nonabsorbable disaccharides in patients with HE are scant. Most clinicians agree that lactulose improves cognitive functions and health-related quality of life in patients with cirrhosis who have minimal HE [42]. A review of randomized trials concluded that there is insufficient evidence to support or refute the use of nonabsorbable disaccharides for HE [2].

In patients with known lactose intolerance, *lactose* may be used to induce soft stools. Lactic-acid producing *probiotics* reduce gut ammonia absorption and may be used as an adjunct to nonabsorbable disaccharides [33].

The oral hypoglycemic agent *acarbose* (100 mg p.o. tid) has been reported to be effective in lowering of blood ammonia and improving neuropsychiatric status in patients with grade I–II HE. The suggested mechanism of action is by the promotion of intestinal saccharolytic bacterial flora at the expense of proteolytic flora responsible for gut ammonia production [18].

*Nonabsorbable antibiotics* alter the gut flora and inhibit bacterial ammonia production, thereby reducing intestinal ammonia absorption. *Neomycin* (0.5–2 g p.o. tid to qid) is absorbed in small quantities and may be associated with significant side effects, such as oto- and nephrotoxicity. It should not be administered for more than 7–10 days. Alternatively, *rifaximin* (200 mg p.o. tid) may be given. The efficacy of antibiotics in patients with HE is comparable to that of nonabsorbable disaccharides. Additive and synergistic effects are not observed. All antibiotics may contribute to bacterial overgrowth syndromes, and their use should be limited to patients who cannot tolerate or are resistant to disaccharides.

Pharmacological prophylaxis with nonabsorbable disaccharides or antibiotics is not effective in the prophylaxis of HE after transjugular intrahepatic portosystemic shunt [44].

The therapeutic strategies to *increase ammonia detoxification* range from pharmacological stimulation of urea cycle activity and of glutamine synthesis to the use of extracorporael liver assist devices, such as human and porcine hepatocytes and extracorporeal albumin dialysis. The dipeptide *L-ornithine-L-aspartate* (LOLA) improves ammonia detoxification by providing aspartate for glutamine synthesis by perivenous scavenger cells and by skeletal muscle. Ornithine stimulates the urea cycle in periportal hepatocytes. LOLA at a dose of 20 g i.v. qd or 6 g p.o. tid lowers blood ammonia and improves neuropsychiatric status in cirrhotic patients [29, 53, 55].

*Sodium benzoate* combines with glycine to form hippurate and lowers blood ammonia levels in children

with inborn errors of urea cycle. Five gram p.o. bid has been reported to be as effective as lactulose [56].

*Extracorporeal albumin dialysis* may temporarily improve severe Type C HE via the removal of protein or non-protein-bound toxins but has no effect on survival [23]. Liver assist devices are still experimental and their clinical use in severe HE currently is not advised.

Improvement of neurotransmission by L-DOPA, bromocriptine (a long-acting dopamine receptor agonist), and bezodiazepine antagonists, such as flumazenil has been observed in a few patients with HE. The effects, however, were short-lived and these drugs have no place in the treatment of patients with liver cirrhsois and HE.

## *Orthotopic Liver Transplantation*

Patients with cirrhosis and HE should be evaluated for liver transplantation, since it is the only procedure that corrects the majority of alterations related to HE. Liver transplantation also is indicated in the small group of patients with severe, refractory hepatic encephalopathy, including such syndromes as dementia, spastic paraparesis, cerebellar degeneration, and extrapyramidal disorders [45]. For liver transplantation in acute liver failure see Chapter 78.

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## 80.5 Pulmonary Complications

Functional and anatomical lung changes are often encountered in patients with liver cirrhosis, even if they are not always evident clinically [9, 13, 14]. Pleural effusion and tense ascites may impair respiration. Mild arterial hypoxemia occurs in approximately every third patient with cirrhosis. On chest X-ray diminished lung volume, pleural effusions, interstitial opacifications and increased vascular profiles may be seen. Approximately 50% of patients with advanced cirrhosis awaiting liver transplantation exhibit a diminished CO-diffusion capacity.

Among the diseases that may affect liver and lungs concomitantly are $\alpha_1$-antitrypsin deficiency and cystic fibrosis. Both may lead to pulmonary fibrosis and emphysema. In sarcoidosis noncaseating epithelioid granulomas may be found in lungs and liver.

Fibrosing immune alveolitis is a rare extrahepatic manifestation of chronic hepatitis C, autoimmune hepatitis and primary biliary cirrhosis.

In this chapter the characteristic pulmonary complications of advanced liver cirrhosis are discussed, that is

- Hepatopulmonary syndrome
- Portopulmonary hypertension and
- Hepatic hydrothorax

## Hepatopulmonary Syndrome

### *Definition*

The hepatopulmonary syndrome (HPS) is characterized by the triad of (1) advanced chronic liver disease, (2) impairment of pulmonary gas exchange with arterial hypoxemia ($pO_2 < 70$ mmHg), and (3) reduced pulmonary vascular resistance (due to intrapulmonary vascular dilatations) in the absence of primary cardiopulmonary diseases [18, 30, 31].

### *Epidemiology*

Four to 47% of patients with cirrhosis have HPS [31].

### *Etiology and Pathogenesis*

HPS most often occurs in patients with advanced cirrhosis, but it can also be associated rarely with acute disease, such as ischemic hepatitis [10]. HPS describes a spectrum of gas exchange disorders due to impaired ventilation-perfusion, intrapulmonary vascular dilatations (IPVD) and shunts and reduction of oxygen diffusion. *The hallmark of HPS is impaired arterial oxygenation,* and *IPVD is the primary structural abnormality in HPS*. The exact mechanisms underlying impaired gas exchange are unknown. Pulmonary vasodilation and intrapulmonary arteriovenous shunts, impaired alveolar-capillary diffusion and ventilation-perfusion mismatch are considered important in the pathophysiology of HPS.

Intrapulmonary arteriovenous shunts through which non-arterialized blood directly flows into the pulmonary veins are deemed important in the development of hypoxemia. This hypothesis is corroborated by the fact that radiolabeled albumin macroaggregates (>25 μ in diameter), which under normal conditions are trapped in the pulmonary vasculature, pass through the pulmonary vasculature and reach the systemic circulation in patients with HPS [1]. *Orthodeoxia* (decreased partial pressure of arterial oxygen [$PaO_2$] from supine to upright position) is typically present in patients with HPS. It is believed to be due to altered pulmonary vascular tone inducing heterogeneous blood flow redistribution mainly to basal lung zones with prominent IPVD while standing [11]. Moreover, the increased cardiac output that often is found in patients with liver cirrhosis diminishes the transit time through the pulmonary vasculature and reduces the time available for oxygen diffusion, thereby contributing to impaired oxygenation. Carboxyhemoglobin levels are increased in cirrhotic patients with HPS and correlate with gas exchange abnormalities [4].

The mechanisms of IPVD formation are not clear. Vasodilating prostaglandins (prostacyclin, prostaglandin $E_1$ and $I_2$), vasoactive intestinal polypeptide, calcitonin, glucagon, substance P, atrial natriuretic factor, platelet activating factor and nitric oxide are regarded as possible humoral mediators of pulmonary vasodilatation. Currently nitric oxide, the strong local vasodilator of endothelial origin, is ascribed a major role in the development of IPVD [8]. An increased pulmonary expression of endothelial NO-synthase in liver disease

has been documented in animal experiments. In addition, the sensitivity of pulmonary vessels to angiotensin II is reduced and the vasoconstriction is attenuated in patients with liver cirrhosis and both contribute to pulmonary vasodilation. Finally, gas exchange disturbances in HPS may be also be related to pulmonary vascular remodeling [12]. There is no direct correlation between the degree of intrapulmonary vascular changes in HPS and the severity of liver disease, the grade of portal hypertension, and the presence of ascites or splenomegaly.

## Pathology

Pathologically small intrapulmonary arteriovenous communications at the precapillary level, capillary dilation in the basal lung fields, and pleural spider angiomas may be observed. HPS has been categorized into two groups on the basis of the distribution of vascular anomalies. Type I is the most common pattern and is characterized by more diffuse disease, while the type II pattern is more discrete and is characterized by localized arteriovenous shunts [17].

## Clinical Manifestations and Diagnosis

The clinical picture is characterized by the presence of *cirrhosis*, arterial desaturation when assuming upright position (*orthodeoxia*; when the patient is upright, perfusion is greatest at the lung bases, further exacerbating the ventilation-perfusion mismatch in HPS) and worsening dyspnea when assuming upright position (*platypnea*). Cyanosis and clubbing are quite frequent findings.

Arterial blood gas analysis in the supine and upright position yields important information. A further fall of the already low arterial $pO_2$ (<70 mmHg) upon assuming the upright position corroborates the diagnosis of HPS. Currently, the best screening tool for hypoxemia in the setting of liver disease is measurement of alveolar-arterial oxygen gradient ($PAO_2$–$PaO_2$). Changes in the alveolar-arterial oxygen gradient are often seen before arterial $pO_2$ ($PaO_2$) becomes abnormally low. $PAO_2$-$PaO_2$ has a diagnostic accuracy for HPS of 91% [25, 31]. Marked hypoxemia during sleep may occur in HPS patients who, according to wake-time oxygen values, have only mild to moderate hypoxemia [28]. Pulsoximetry is a simple, low cost, and widely available technique that reliably predicts the presence and severity of hypoxemia in patients with HPS [5].

Although patients with HPS often have a low diffusion capacity their lung volumes and expiratory flow rates may remain normal.

Cutaneous spider angiomas may be indicators of intrapulmonary vasodilation. There are data indicating that spider angiomas may be associated with marked systemic and pulmonary vasodilation, with more pronounced gas exchange disturbances and reduced hypoxia-induced vasoconstriction. Patients with HPS usually have a hyperdynamic circulation with systemic vasodilation and increased cardiac output, often greater than 7 L/min.

Intrapulmonary vascular changes in patients with HPS may be demonstrated by the following imaging techniques: (1) transthoracic contrast-enhanced echocardiography (CEE), (2) technetium 99m-labeled macroaggregated albumin scanning, and (3) pulmonary angiography. These techniques can assess atrial and ventricular intracardiac shunts and in addition, transthoracic CEE and angiography allow differentiating them from intrapulmonary shunts. Shunt volume can be quantified by scanning techniques. Technetium 99m-labeled macroaggregated albumin scanning is less sensitive than transthoracic CEE in detecting IPVD. *Currently, the preferred diagnostic modality for detecting IPVD is transthoracic CEE* [31]. Transthoracic CEE, however, is not able to differentiate between IPVD and arteriovenous communications. Pathologic results of transthoracic CEE are a frequent finding in patients with liver cirrhosis and not specific for HPS. HPS is frequently present in patients with left atrial enlargement, and in the context of liver cirrhosis, left atrial volume ≥50 mL is a simple and feasible parameter to detect HPS [38].

If breathing 100% oxygen does not lead to a rise in arterial $pO_2$ of greater than 150 mmHg, pulmonary angiography should be considered to exclude arteriovenous shunts. Pulmonary angiography is generally used to exclude other causes of hypoxemia (e.g., pulmonary embolism, pulmonary hypertension). Conventional CT and high-resolution CT are not used to

**Table 80.21** Diagnostic criteria for the hepatopulmonary syndrome

| Variable | Criterion |
|---|---|
| **Oxygenation defect** | Partial pressure of oxygen < 80 mmHg or alveolar–arterial oxygen gradient ≥ 15 mmHg while breathing ambient air |
| **Pulmonary vascular dilatation** | Positive findings on transthoracic contrast-enhanced echocardiography or abnormal uptake in the brain (>6%) with radioactive lung-perfusion scanning |
| **Liver disease** | Portal hypertension (most common) with or without cirrhosis |

Source: Adapted from [33].

**Table 80.22** Grading of severity of hepatopulmonary syndrome

| Stage | $PAO_2$-$PaO_2$ [a-d] (mmHg) | $PaO_2$ [d,e] (mmHg) |
|---|---|---|
| **Mild** | ≥15 | ≥80 |
| **Moderate** | ≥15 | <80–≥60 |
| **Severe** | ≥15 | <60–≥50 |
| **Very severe** | ≥15 | <50 |

[a]$PAO_2$-$PaO_2$ = alveolar-arterial gradient in partial pressure of oxygen
[b]All with positive transthoracic contrast-enhanced echocardiography
[c]Reference range, 4–8 mmHg
[d]For patients >64 years, a $PAO_2$-$PaO_2$ of >20 mmHg and a $PaO_2$ of <70 mmHg can be substituted as cutoffs
[e]Reference range, 80–100 mmHg (breathing room air at rest and at sea level)
Source: Adapted from [31].

diagnose HPS, but play a role in evaluating for comorbid respiratory conditions.

The diagnostic criteria of HPS are summarized in Table 80.21.

Grading the severity of HPS is important because severity affects survival. Patients with liver disease and detectable IPVD but with normal arterial oxygenation are classified as having subclinical HPS and should have blood gas levels checked annually to monitor for HPS (Table 80.22) [31].

## Differential Diagnosis

The differential diagnosis encompasses all lung diseases with arterial hypoxemia in patients with liver cirrhosis (Table 80.23).

**Table 80.23** Differential diagnosis of chronic hypoxemia in patients with liver diseases

*Frequent causes*
- Restrictive lung diseases
- Obstructive lung diseases
- Pleural effusion
- Impaired diffusion
- Left heart insufficiency

*Rare causes*
- Hepatopulmonary syndrome
- Portopulmonary hypertension
- Hyperdynamic circulation
- Extracardiac and extrapulmonary right–left shunts
- Treatment with estrogens
- Fibrosing immune alveolitis (chronic hepatitis C, primary biliary cirrhosis, autoimmune hepatitis)

## Course and Prognosis

The natural course of HPS has not been studied systematically. The prognosis of patients with HPS who do not undergo liver transplant is poor with a 3.8-fold lower median survival time compared with patients without HPS [36].

## Therapy

The therapeutic options in HPS are very limited. No medical therapy has been able to improve oxygenation consistently or to reverse pulmonary vascular alterations. Methylene blue, a potent inhibitor of guanylate cyclase, was reported to improve arterial hypoxemia and hyperdynamic circulation [35]. These effects, however, are only short-lived. Paracentesis can temporarily improve respiratory mechanics and provide symptomatic relief.

Currently, liver transplantation offers the only proven cure for HPS. Liver transplantation for HPS, however, may be associated with considerable mortality. Post-transplant mortality rates of up to 30% have been reported in patients whose arterial $pO_2$ before transplantation was ≤50 mmHg compared to only 4% in patients with a preoperative arterial $pO_2$ of greater than 50 mmHg [3, 21, 36]. Change of arterial $pO_2$ in the supine position while breathing 100% oxygen is a useful parameter to predict success of liver transplantation. Patients with a

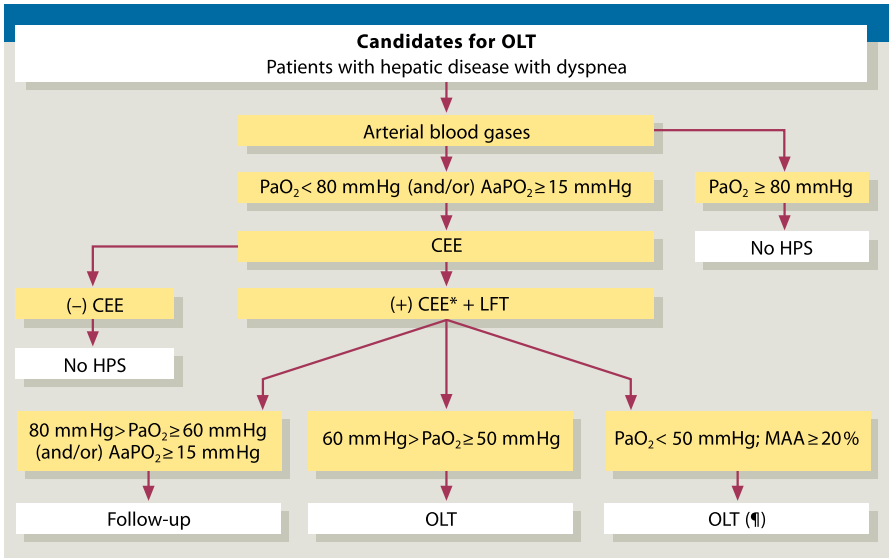


**Fig. 80.16** Screening, diagnostic and therapeutic work-up for HPS. If an increased $AaPO_2$ is demonstrated, with or without hypoxemia, transthoracic CEE and LFT are carried out. A positive transthoracic CEE confirms HPS; a negative transthoracic CEE excludes HPS (Reproduced from [32]. With permission)

*LFT* Lung function tests, *OLT* orthotopic liver transplantation, [*] high resolution thoracic CT scanning, *CEE* contrast-enchanged echocardiography, *MAA* macro-aggregates albumin [¶] high risk post-OLT mortality

rise of arterial $pO_2$ to ≥400 mmHg have a significantly better posttransplant prognosis compared to patients with a lower increase [19, 27]. Overall, transplantion nowadays is followed by resolution of HPS in approximately 80% of carefully selected patients. The 5-year survival is 76% compared with 23% of those who do not undergo transplant [37].

Screening, diagnostic and therapeutic work-up for HPS is summarized in Fig. 80.16.

## Portopulmonary Hypertension

### *Definition*

Portopulmonary hypertension (POPH; synonym: portopulmonary syndrome) is a secondary form of pulmonary hypertension characterized by an increased pulmonary vascular resistance with a normal pulmonary arterial occlusion pressure that develops in the setting of portal hypertension (cirrhotic and non-cirrhotic).

### *Epidemiology*

10% of all cases of secondary pulmonary hypertension are associated with portal hypertension. The risk of POPH increases with the duration of portal hypertension. POPH is less frequent than HPS. In retrospective clinical and autopsy studies the prevalence in patients with portal hypertension was estimated to be 0.6% and 0.25–0.7%, respectively [26]. Recently 16% of patients with decompensated cirrhosis with refractory ascites have been reported to have POPH [6]. Right heart catheterization studies have demonstrated the prevalence of POPH to be 4–15% [32].

### *Etiology and Pathogenesis*

Pulmonary hypertension (mean pulmonary arterial pressure > 25 mmHg) occurs in approximately 20% of patients with advanced cirrhosis and portal hypertension. Various mechanisms can contribute to the development of POPH, such as a hyperdynamic high flow circulatory state, cirrhosis-induced cardiac changes or pulmonary vasoconstriction. One to 4% of patients with POPH have evidence of an anatomically fixed pulmonary vasoconstriction (some investigators consider only these patients to have POPH).

The mechanisms responsible for the increased pulmonary vascular resistance in POPH are: (1) vasoconstriction, (2) remodeling of muscular pulmonary arteries, and (3) the formation of microthrombi. What exactly causes vasoconstriction is not clear. Gut derived

serotonin causes pulmonary vasoconstriction and may stimulate pulmonary arterial smooth muscle cells to proliferate. Circulating mediators, such as endothelin-1 may possibly mediate pulmonary arterial vasoconstriction. Interleukin-1, hepatocyte growth factor, vascular endothelial growth factor, members of the transforming growth factor-β family and bone morphogenetic protein receptor have all been associated with remodeling of pulmonary arteries [7].

Female sex and autoimmune hepatitis were associated with an increased risk of POPH compared to chronic hepatitis C in patients with advanced liver disease [16].

## Pathology

The pulmonary vascular pathology in patients with POPH is indistinguishable from that in primary pulmonary hypertension. The rise in pulmonary vascular resistance begins with an elastosis of the intima and smooth muscle proliferation of the media leading to concentric vascular obliterations. During the further course a *plexogenic arteriopathy* develops. The term denotes proliferation of capillaries with organized thrombi (plexiform changes) and angioma like growths (angiomatoid changes) that lead to a thickening of the entire arterial wall which finally becomes destroyed by fibrinoid necrosis. The lesions are distributed irregularly throughout the lungs and the severity of histopathological changes does not correlate with the degree of pulmonary hypertension.

## Clinical Manifestations and Diagnosis

In the early stages of POPH the patients are asymptomatic with regard to the lungs and POPH can only be diagnosed by invasive techniques. During the further course exertional dyspnea supervenes and patients may complain of thoracic pain, syncope and hemoptysis.

Doppler echocardiography allows screening of patients with portal hypertension for the presence of POPH. An increased right atrial pressure of ≥14 mmHg has a positive predictive value for POPH of 83% [6]. Right heart catheterization is necessary to confirm POPH and frequently identifies other causes of pulmonary hypertension (for example, high flow and increased central volume) in liver transplantation candidates [22].

The diagnostic criteria for POPH are threefold:

- Mean pulmonary arterial pressure (mPAP) > 25 mmHg
- Mean pulmonary arterial occlusion pressure (mPAOP) < 15 mmHg
- Pulmonary vascular resistance (PVR) > 240 dynes/s/cm$^{-5}$

Some patients may have increased mPAOP and increased PVR. If these patients have an increased transpulmonary gradient (mPAP–mPAOP > 15 mmHg), they should be considered to have POPH [32].

## Differential Diagnosis

The differential diagnosis includes all forms of primary and secondary pulmonary hypertension. All diseases that may lead to pulmonary hypertension must be excluded.

## Course and Prognosis

Prognosis of POPH is poor. Patients die from liver failure or due to right heart insufficiency. Survival rates after 1, 2, and 5 years are 76%, 72% and 50%, respectively [26].

## Therapy

Currently, there is no effective therapy for POPH. Improvement in pulmonary hemodynamics has been documented with 24 h continuous intravenous epoprostenol (prostacyclin), but the proof of long-term survival benefit is lacking [20]. β-Adrenergic blockers are associated with a significant worsening in exercise capacity and pulmonary hemodynamics, and are therefore contraindicated in patients with moderate to severe POPH [29].

There is no role for TIPS in the management of POPH.

Liver transplantation has been less well studied than in HPS, but transplantation does not seem to be a valid option for patients with severe POPH. Highly selected POPH patients who experience improved pulmonary

hemodynamics with prostacyclins and endothelin antagonists may benefit from liver transplantation [21, 23, 31]. A well functioning right heart ventricle is an absolute precondition for a successful liver transplantation. A combined liver–lung-transplantation has been performed in a few desperate cases, but without evident benefit.

## Hepatic Hydrothorax

### Definition

Hepatic hydrothorax (HH) denotes a pleural effusion that results from a free communication between the peritoneal and pleural cavity in patients with liver cirrhosis and ascites [2, 15].

### Epidemiology

Most patients with HH have decompensated cirrhosis and refractory ascites. Exact data regarding incidence and prevalence are not available. HH seems to occur in less than 10% of patients with refractory ascites.

### Pathogenesis

HH is caused by overstretching and rupture of the membranous part of the diaphragm due to tense ascites with high intraabdominal pressure. The relative negative pressure in the pleural cavity and the valve-like effect of the diaphragmatic lesion causes abdominal fluid to flow into the thoracic cavity.

### Clinical Manifestations and Diagnosis

HH occurs preferentially on the right side. In the vast majority of patients ascites is present at the time of diagnosis. With large diaphragmatic lesions and/or mild ascites production HH may be present in the absence of concomitant ascites.

A diagnostic tap discloses the noninflammatory nature of the effusion with results resembling those in ascites.

Intraperitoneal injection of methylene blue or scanning with $^{99m}$technetium-labeled colloidal sulfate demonstrates the movement of fluid from the peritoneal to the pleural cavity [24].

### Course and Prognosis

The prognosis of HH is poor with a high morbidity and mortality rate. Spontaneous bacterial pleurisy (neutrophil count > 400/mL; culture), empyema or a tension hydrothorax through the valve like effect of the diaphragmatic lesion may complicate HH.

### Therapy

HH usually is a major therapeutic challenge that requires careful planning of therapy and the timely application of all methods. Simply draining HH while marked ascites is present is futile since HH rapidly redevelops. Therapeutic thoracentesis, combined with intensive therapy of ascites (large volume paracentesis with intravenous albumin infusion, diuretics) should be performed in severely symptomatic patients. Patients who require repeated thoracentesis should receive a TIPS (provided that there are no contraindications). The response rates of HH to TIPS are 45–80% [34]. If HH reappears a pleurodesis, combined with surgical repair of the diaphragmatic lesion may be attempted. In refractory HH, orthotopic liver transplantation is the only remaining therapeutic option.

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## 80.6 Cardiovascular Complications

Heart and liver diseases may be associated in many ways:

- Hepatic complications of heart failure
- Cardiac complications of liver disease
- Cardiac and hepatic lesions of common etiology [16]

A pericardial effusion, for example may develop in the setting of fluid retention (ascites, edema) in cirrhosis [1]. It may be demonstrated by transthoracic echocardiography in approximately two thirds of patients with decompensated liver cirrhosis, and usually disappears after successful treatment of ascites.

Rarely a tense ascites may increase intraabdominal pressure, elevate the diaphragm, impair cardiac filling and result in a decreased cardiac output. In these cases a large volume paracentesis improves cardiac function.

Treatment of ascites by a large volume paracentesis without adequate volume replacement may lead to cardiocirculatory dysfunction with a fall in right atrial and pulmonary occlusion pressure, and a consecutive fall in cardiac output (see Section 80.1) [17].

Spontaneous bacterial peritonitis in cirrhotic patients may be associated with severe systemic, renal and hepatic hemodynamic derangement [20]. Hepatic complications of heart failure primarily include liver lesions due to acute or chronic vascular congestion (see Chapter 59).

In this chapter the cardiovascular changes typical of cirrhosis are discussed, i.e. hyperdynamic circulation and cirrhotic cardiomyopathy.

### Hyperdynamic Circulation

A hyperdynamic (hyperkinetic) circulation denotes cardiovascular alterations in patients with liver cirrhosis that are characterized by a decrease in systemic peripheral vascular resistance, peripheral vasodilatation, low arterial blood pressure and a compensatory increase of cardiac output per minute in the presence of a low arterio-venous oxygen gradient. These cardiovascular changes only rarely lead to heart insufficiency.

#### *Pathogenesis*

The pathophysiology of hyperdynamic circulation is still understood incompletely, but involves many factors that are also operative in the development of ascites. Vascular endothelial dysfunction and progressive vasodilation are central to the pathogenesis of hyperdynamic circulation in cirrhosis and to the detrimental effects observed in multiple organs with nitric oxide being the primary (but not the only) vasodilator molecule in these effects [10, 11]. Recent data also suggest that in cirrhosis-induced vasodilation, the angiotensin (1)-receptor is desensitized [8]. Vasodilation of muscle districts contributes to the reduced peripheral vascular resistance in advanced cirrhosis [5]. The consequent decrease of effective arterial volume activates the sympatho-adrenergic mechanisms and the renin-angiotensin-aldosterone system (see Chapter 54 and Section 80.1). Experiments in portal hypertensive rats show that central cardiovascular regulatory nuclei initiate hyperdynamic circulation in response to a gut signal associated with portal hypertension via capsaicin-sensitive vagal afferent nerves [13].

Patients with liver cirrhosis may also have arterial hypertension. In common with their normotensive counterparts, hypertensive cirrhotic patients are hyperkinetic and central hypovolemic, but vasodilation is reduced and regulation of arterial blood pressure may be less deranged [9].

#### *Clinical Manifestations*

Tachycardia, low arterial blood pressure with a high amplitude, dry, reddened, warm skin, bouncing peripheral pulses with capillary pulsations at the fingertips, a broadened cardiac apex beat, and occasionally a loud mid-systolic heart murmur at the cardiac base are the clinical signs of hyperdynamic circulation.

Delayed repolarisation of the myocardium occurs in a substantial fraction of patients with liver cirrhosis (not only of alcoholic origin) already at a stage with a mild increase in portal pressure. Compared to non-cirrhotic patients the QT-interval is prolonged [23]. It correlates with the Child-Pugh stage. Prolongation to greater than 440 ms seems to correlate with a shortened survival [3].

#### *Differential Diagnosis*

The hyperkinetic heart syndrome in cirrhosis has to be differentiated from cardiac changes in thiamine

(vitamin $B_1$) deficiency ("beri beri heart") and from similar conditions with decreased peripheral vascular resistance, such as extrahepatic arterio-venous communications, severe anemia, hyperthyroidism, Paget's disease, alcoholic cardiomyopathy and hyperdynamic circulation in late pregnancy.

A heart insufficiency with increased cardiac output may be caused by intrahepatic arteriovenous shunts in a non-cirrhotic liver [4]. Giant hemangiomas and hemangioendotheliomas, especially in children may cause a hyperkinetic heart failure due to a significant arteriovenous shunt load. Rarely, a similar clinical picture has been described in adults with Osler-Weber-Rendu syndrome. Long-term administration of estrogens may aggravate a preexisting vascular disease or lead to peliosis hepatis (see Chapter 60), which in rare cases may cause or precipitate heart failure.

### *Therapy*

Specific treatment of hyperdynamic circulation usually is not available. Interestingly, selective intestinal decontamination with norfloxacin, 400 mg p.o. bid for 4 weeks, may partially reverse the hyperdynamic circulatory state in cirrhotic patients [19]. If tachycardia is problematic, non-selective β-adrenergic blockers may be administered. They concomitantly lower portal hypertension. Cardiac failure is treated according to guidelines for non-cirrhotic patients.

Following liver transplantation the cardiovascular changes are reversible [21].

## Cirrhotic Cardiomyopathy

Cirrhotic cardiomyopathy (CC) implies left ventricular systolic and diastolic dysfunction and electrophysiological abnormalities under physiological conditions or under pharmacological stress in patients with liver cirrhosis [12, 14]. Nearly every third cirrhotic patient has elevated serum levels of troponin I as an expression of a subclinical myocardial injury. Being clinically latent CC can be unmasked by physical or pharmacological strain and by stressful procedures, such as large volume paracentesis without adequate plasma volume expansion, transjugular intrahepatic portosystemic shunt (TIPS) insertion, peritoneovenous shunting and surgery [6, 7, 15]. Diastolic dysfunction does not only occur in cirrhosis but may also be present in non-cirrhotic portal fibrosis. It indicates that portal hypertension is an important factor in the genesis of cardiac dysfunction [7]. Diastolic dysfunction is associated with poor survival in patients with cirrhosis and TIPS [6].

The pathophysiology of CC is multifactorial and includes diminished β-adrenergic receptor signal transduction, cardiomyocyte plasma membrane dysfunction, and increased activity or levels of cardiodepressant substances such as cytokines, endogenous cannabinoids, and nitric oxide [2]. However, if CC is indeed a discrete disease entity or if it just represents left ventricular abnormalities due to an increased work load in hyperkinetic circulation is currently under investigation [18, 22].

Treatment is mainly supportive. Orthotopic liver transplantation appears to improve or normalize the condition, generally after a period of several months.

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## 80.7 Endocrine Alterations

### Sexual Dysfunction

Sexual dysfunction, especially in patients with alcoholic liver cirrhosis occurs frequently. It is more pronounced in men and commonly leads to feminization, while signs of ovarian insufficiency predominate in women.

Eighty percent of male alcoholic cirrhotics complain of loss of libido, erectile dysfunction or infertility. Hypogonadism manifests with atrophic, small and soft testes. Terminal hair diminishes, and gynecomastia and a female body habitus develop (the most common cause of gynecomastia in patients with liver cirrhosis is spironolactone treatment). Prostatic hyperplasia is less common in patients with alcoholic cirrhosis.

Women often have menstrual irregularities, such as amenorrhea and metrorrhagia in advanced cirrhosis with coagulation defects. Libido and sexual behaviour are not altered in women with nonalcoholic liver cirrhosis [1].

The pathophysiology of sexual alteration is complex and multifactorial. The liver plays a major role in metabolism of sex hormones which is usually disturbed in advanced stages of cirrhosis. The lack of a secondary rise in gonadotropins in alcoholic testicular injury hints at a dysfunction of hypothalamic–hypophyseal–gonadal axis. Ethanol induces the enzyme aromatase that catalyzes the transformation of androgens to estrogens, it leads to an increased extragonadal synthesis of estrogens and to an upregulation of estrogen receptors [2].

In hereditary hemochromatosis, hypophyseal iron deposits may lead to secondary hypogonadism which, in contrast to that of patients with alcoholic cirrhosis can be treated by testosterone injections.

A reversal of sexual dysfunction following liver transplantation has been reported [5].

### Thyroid Dysfunction

Patients with liver cirrhosis are usually clinically euthyroid. Laboratory findings of a low-$T_3$ syndrome may be present. The hepatic transformation of $T_4$ in $T_3$ is diminished, while extrahepatic generation of reverse-$T_3$ from

$T_4$ is increased. Diminished levels of $T_3$ and increased reverse $T_3$-levels are found in plasma. TSH is within normal limits. Frequently $T_4$ levels also fall in the final stages of liver cirrhosis. Thyroid hormone replacement therapy is not indicated [4].

Hypothyroidism in patients with autoimmune liver diseases, for example primary biliary cirrhosis, autoimmune hepatitis or in hereditary hemochromatosis usually is due to immune thyroiditis (Hashimoto thyroiditis).

## Alterations of Glucose Homeostasis

Disturbed glucose tolerance, overt diabetes mellitus and hypoglycemia may be observed in patients with liver cirrhosis [7].

Sixty to 80% of patients with liver cirrhosis have disturbed glucose tolerance and approximately 20–30% have diabetes mellitus. The fraction of patients with overt diabetes mellitus is particularly high in patients with hemochromatotic cirrhosis. Increased serum insulin levels in patients with liver cirrhosis are primarily due to decreased hepatic degradation of insulin.

The so-called hepatogenous diabetes mellitus is due to insulin resistance with inadequately high serum insulin levels [8, 9]. Cirrhotic patients with still normal glucose tolerance already exhibit peripheral (i.e. muscular) insulin resistance which initially is compensated by increased secretion of insulin by the pancreatic β-cells. Insulin resistance also plays a pivotal pathophysiologic role in nonalcoholic fatty liver disease (see Chapter 89). Liver transplantation may improve or normalize both glucose tolerance and insulin sensitivity [6].

The prevalence of insulin resistance and type 2 diabetes mellitus in patients with chronic hepatitis C and HCV-cirrhosis is above average [3].

The liver has an extraordinary high capacity to produce glucose. Therefore, hypoglycemia in patients with liver cirrhosis is rare and occurs only with extensive loss of hepatic parenchyma. Hypoglycemia seen in patients with alcoholic cirrhosis is usually due to ethanol-induced inhibition of hepatic gluconeogenesis.

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## 80.8 Hematologic Alterations and Hepatic Coagulopathy

### Hematologic Alterations

#### *Red Blood Cells*

Erythrocytic alterations in patients with liver cirrhosis primarily regard changes in cell shape and anemias.

*Macrocytosis* is the most common variant and is especially pronounced in patients with alcoholic liver disease where it may often be aggravated by concomitant vitamin $B_{12}$ and/or folate deficiency.

The *target cell* which has a central knob of hemoglobin surrounded by an area of pallor and then a peripheral area of hemoglobin (Mexican hat cell; bull's-eye appearance) is also typical of liver disease.

Acanthocytes are erythrocytes that have lost their smooth oval shape and display cytoplasmic projections. *Acanthocytosis* is primarily observed in approximately 5% of decompensated alcohol induced cirrhosis and provides an indication of a grave prognosis. It is due to an altered lipid composition of the red cell membrane with an abnormally low density lipoprotein and an increase in cholesterol and phospholipids.

An increase in plasma volume in patients with decompensated cirrhosis with marked ascites may lead to *dilutional anemia*.

Bleeding esophago-gastric varices, portal hypertensive gastropathy or peptic ulcers may lead to *acute* or *chronic iron deficiency anemia*. Dysfunctional hemostasis in cirrhosis promotes this bleeding tendency.

A *megaloblastic anemia* in cirrhotic patients is mostly due to folate deficiency. It occurs primarily in alcoholics.

Occasionally a *sideroblastic anemia* occurs in patients with alcoholic cirrhosis which is due to an alcohol-induced defect of heme synthesis. These patients show a hypochromic anemia with normal or elevated serum iron concentrations, increased transferrin saturation and an increased iron deposition in the bone marrow.

An *aplastic anemia* is usually not part of liver cirrhosis, but may rarely (<1%) occur in patients with acute virus hepatitis B.

Hemolysis occurs in every other patient with liver cirrhosis. However, only a minority of patients develop *hemolytic anemia*. Due to copper overload of erythrocytes, an intracorpuscular hemolytic anemia may be the initial symptom in patients with an acute exacerbation of Wilson's disease.

*Zieve's syndrome* denotes the occurrence of an intracorpuscular hemolytic anemia and hyperlipoproteinemia Type V in patients with marked alcoholic fatty liver or cirrhosis.

#### *White Blood Cells*

*Leukopenia* in patients with liver cirrhosis is due to an increased sequestration of leukocytes within the spleen.

*Granulocyte dysfunction* manifests itself by an impaired chemotaxis and a diminished ability to kill intracellular organisms. In concert with immunologic disturbances it forms the basis for the increased occurrence of bacterial infections in patients with liver cirrhosis (see Section 80.2).

#### *Platelets*

*Thrombocytopenia* (platelet count < 150,000/μL) is a common complication in patients with chronic liver disease that has been observed in up to 76% of patients [1]. It is a consistent finding in patients with liver cirrhosis and portal hypertension. Up to 90% of the platelet pool is sequestered within the spleen. In addition, diminished production of thrombopoietin or its increased degradation by platelets sequestered in the spleen may contribute to reduced platelet counts [9]. A platelet count of 30,000–40,000/$mm^3$ in patients with decompensated cirrhosis is quite common. In addition to their numerical reduction, platelets display functional disturbances. Extensive hematomas after a minor trauma are common. Interestingly, marked spontaneous hematomas occur quite rarely in cirrhotics. Possibly elevation of von Willebrand factor described in patients with cirrhosis might compensate for defects in platelet number and function [6].

Thrombocytopenia associated with hepatitis C virus-related cirrhosis may be a limiting factor in the antiviral treatment of these patients. Eltrombopag, a new orally active thrombopoietin-receptor agonist that stimulates thrombopoiesis has been shown to increase platelet counts in patients with thrombocytopenia due

to HCV-related cirrhosis, thereby facilitating initiation of antiviral therapy [8].

## Hepatic Coagulopathy

Due to the close relationship between the liver and the coagulation system, liver diseases are regularly accompanied by hemostatic changes of various severities. Simple screening coagulation tests, such as activated partial prothrombin time, prothrombin time–INR are used clinically as though generally predictive of clinical bleeding, although they only poorly reflect the complexity of hemostatic changes in patients with cirrhosis. Among the factors responsible for impaired coagulation in cirrhotic patients are

- Deficiency of vitamin K dependent factors
- Activation of fibrinolysis
- Disseminated intravascular coagulation
- Thrombocytopenias and – pathias

Usually the disturbances are very complex and multifactorial [3–5, 7]. They represent a mixture of pro- and anticoagulant forces that are themselves further subject to change with altered physiological stress such as super-imposed infection or renal failure [2].

The liver synthesizes nearly all plasmatic factors of coagulation and fibrinolysis, whose levels are considerably reduced in advanced cirrhosis. The vitamin K dependent coagulation factors II, VII, IX and X and the anticoagulant proteins C and S are metabolized and activated by the liver with vitamin K dependent γ-carboxylation being the essential step in the activation of these factors. Diminished intestinal vitamin K absorption in chronic cholestatic liver diseases and loss of hepatic parenchyma in liver cirrhosis or in acute liver failure may lead to a *deficiency of clotting factors*. Due to its short half life, concentration of factor VII is the first to decrease. In addition, severe thrombocytopenia may limit thrombin generation in patients with cirrhosis [10].

The pathogenesis of *disseminated intravascular coagulation* in liver cirrhosis is not clear. Loss of liver parenchyma and an impaired endotoxin-clearance are being discussed.

The plasmatic factors of coagulation and fibrinolysis are cleared by the reticuloendothelial system of the liver. The reduced synthesis of inhibitors of fibrinolysis and/or the diminished hepatic clearance of activators of fibrinolysis may result in the activation of the fibrinolytic system. This *primary hyperfibrinolysis* may come along with life threatening bleeding [11].

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## 80.9 Hepatic Osteodystrophy

### Definition

The term *hepatic osteodystrophy* (HO, hepatic osteopathy, hepatic osteopenia) denotes the metabolic bone disease that occurs in patients with chronic liver disease.

### Epidemiology

HO occurs in 16–23% of patients with advanced liver cirrhosis, irrespective of etiology. The prevalence of vertebral and peripheral fracture rates among a group of patients with mixed liver disorders is approximately twice the rate of matched controls [1]. Patients with primary biliary cirrhosis are at a modestly increased osteoporosis and fracture risk due to predominant female sex and older age, but cholestatic disease per se does not differ significantly from noncholestatic disorders in terms of osteoporosis and fracture risk [1, 8].

### Pathogenesis

The pathogenesis of HO is unsettled. HO encompasses both osteoporosis and osteomalacia, with the former largely prevailing. In addition to the usual factors operating in osteoporosis, such as older age, female gender, immobility, prolonged corticosteroid use, alcohol abuse, and cigarette smoking, additional factors relating to vitamin D receptor polymorphisms, osteoblast trophic factors (e.g., insulin-like growth factor type I) and osteoclast regulating factors (e.g. receptor activator of nuclear factor kappaB ligand and osteoprotegerin) are being discussed in cirrhotic patients [1, 6, 7]. Overall reduced bone formation rate due to impaired osteobast function is the leading pathogenetic mechanism, rather than increased osteoclastic bone degradation. Hypogonadism often present in cirrhotic patients is an important promoting factor. Despite altered vitamin D and calcium metabolism (e.g. diminished intestinal absorption of fat soluble vitamins; 25-hydroxylation of cholecalciferol remains intact even in advanced stages of cirrhosis), bone mineralization appears unaffected even in advanced cholestatic liver disease [2, 4].

The rate of bone loss increases rapidly in the first 6 months after orthotopic liver transplantation. The single most important factor in the development of *posttransplant bone disease* is the degree of osteopenia at the time of liver transplantation. The use of high-dose corticosteroids and other immunosuppressive agents, immobility, and poor nutrition are believed to contribute to the excessive bone loss after liver transplantation.

### Clinical Manifestations and Diagnosis

Emerging HO is asymptomatic. Manifest HO with vertebral and nonvertebral fractures leads to pain and immobility [3, 5]. The diagnosis is made by bone mineral density assessment using dual-energy X-ray absorptiometry (DEXA) and should be performed in all patients with long-standing liver disease and cirrhosis.

### Therapy

Treatment of HO is basically the same as for osteoporosis in the general population, but the level of evidence for HO is usually less strong compared to the evidence level for therapeutic recommendations in the general population (D versus A, respectively) [1].

Treatment is based on lifestyle changes (e.g., regular exercise, smoking cessation) as well as *vitamin D* (400–800 IU daily) and *calcium* (1,000–1,200 mg daily) supplementation. Vitamin D deficiency should be corrected by increasing serum 25-hydroxyvitamin D levels to at least 25–30 ng/mL.

If female hypogonadism is present or early menopause (before age 45 years) is evident, hormone replacement therapy via the transdermal route is begun.

*Raloxifene*, a selective estrogen receptor modulator, may be considered in women who cannot take bisphosphonates.

*Bisphosphonates* are first-line drug therapy for osteoporosis. By inhibiting bone resorption, they preserve bone mass and can decrease vertebral and hip fractures. They should be administered to patients with known HO, for example alendronate 70 mg p.o. qw or risedronate 35 mg p.o. qw. Zoledronate (5 mg i.v. once per year) may be an alternative to oral bisphosphonates.

However, studies in patients with liver disease are not available.

Nasal (spray dose 200 IU qd in alternating nostrils) or subcutaneous *calcitonin* (100 IU qd or q2d) can be considered as an alternative when bisphosphonates are contraindicated or poorly tolerated. However, calcitonin is usually less effective than bisphosphonates for treating osteoporosis.

*Parathyroid hormone* (synthetic PTH 1–34: teriparatide) is reserved for patients with severe osteoporosis (e.g. DEXA T score < 3.5) who cannot tolerate, have contraindications or fail to respond to the previously mentioned drugs. Parathyroid treatment should be guided by a specialist in bone disease

In organ transplant recipients therapy of HO should begin already before the operation.

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## 80.10 Muscular Complications

Patients with liver cirrhosis have both a *diminished fat* and *muscle mass* (see Fig. 79.5). Muscle wasting is due to metabolic and hormonal factors that lead to an enhanced catabolism of muscle proteins and the provision of amino acids for hepatic gluconeogenesis [2]. Activation of the sympatho-adrenergic nervous system that often is present in cirrhotic patients results in elevated levels of catecholamines which promote muscle degradation. In addition, synthesis of insulin-like growth factor I is reduced which stimulates muscle protein synthesis. Ethanol also enhances muscle catabolism.

Compared to the general population, patients with cirrhosis more often complain about *muscle cramps*, especially in the calves and at night. The frequency and severity of cramps increase with the duration of cirrhosis and the degree of hepatic dysfunction. Ascites, a low mean arterial pressure and a high plasma renin activity are independent predictors for the occurrence of muscle cramps [1]. The pathophysiologic mechanisms are incompletely understood. Intramuscular movements of electrolytes and trace elements are discussed, but their significance has yet to be demonstrated. Weekly intravenous albumin infusions may reduce the incidence of cramps. This argues for the reduction of effective circulating plasma volume playing a role in the pathogenesis of muscle cramps.

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# Wilson's Disease

# 81

Uta Merle and Wolfgang Stremmel

## Chapter Outline



## Definition and Epidemiology

Wilson's disease is a rare autosomal-recessive disorder of copper metabolism with a prevalence of ~1:30,000 and a frequency of heterozygotes of ~1:90. Wilson's disease is characterized by hepatic and/or neurological symptoms and is assumed to be progressive and fatal without treatment.

## Etiology and Pathogenesis

Approximately 50% of dietary copper (~0.8–2 mg/day) is absorbed in the upper small intestine. After uptake by hepatocytes, biliary excretion is the main pathway for elimination of excess copper. In Wilson's disease export of copper into bile is impaired and leads to a pathological copper accumulation primarily within the liver and subsequently in the brain (particularly in basal ganglia) and other tissues (e.g. kidneys and cornea) [11]. Wilson's disease is caused by mutations of the Wilson's disease gene ATP7B which codes for a copper-transporting, transmembrane P-type ATPase that is primarily expressed in the liver (Fig. 81.1) [4, 22]. Approximately 300 mutations of the ATP7B gene have been described and most Wilson's disease patients are compound heterozygotes, possessing alleles with different mutations in both parental genes. Whether the mutation variability affects the phenotypic expression is subject to speculation as current data remain somewhat controversial due to the small size of most population studies [13].

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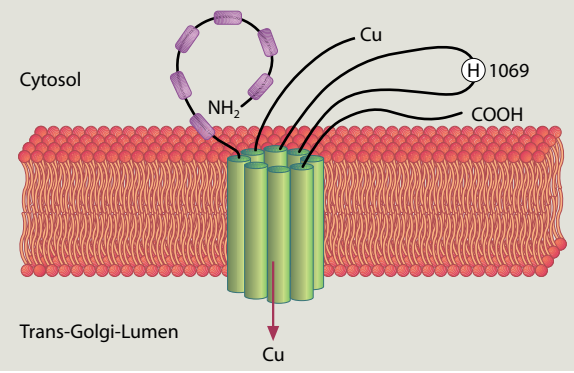


**Fig. 81.1** Structural model of the Wilson protein ATP7B illustrating copper transport across the lipid bilayer in the trans-Golgi network membrane. Displayed are the six copper-binding domains at the N-terminus of the protein and its eight transmembrane domains, forming a channel for the ATP-dependent copper transport. Highlighted is the histidine at position 1069, the most common missense mutation in Wilson's disease patients (H1069Q)

Due to the fact that Wilson's disease is reversed after liver transplantation, it is believed that loss of function in hepatocytes is the major reason for copper overload. In addition to reduced biliary copper excretion, impaired ATP7B function leads to a disturbed incorporation of copper into ceruloplasmin. Failure to incorporate copper during ceruloplasmin biosynthesis results in the secretion of an apoprotein that is devoid of enzymatic activity and rapidly degraded. The resulting decrease in serum ceruloplasmin concentration is a diagnostic hallmark of Wilson's disease. Because ceruloplasmin accounts for most of serum copper, total serum copper is most often reduced in Wilson's disease.

## Diagnosis

### *Clinical Presentation*

Although the biochemical defect that leads to copper accumulation in Wilson's disease is already present at birth, clinical symptoms are rarely observed before the age of 5 years [17]. The most common age at presentation is between the 10 and 24 years old. At that time Wilson's disease is predominantly a hepatic disorder, while neurologic or psychiatric symptoms typically appear later in life (Fig. 81.2) [14]. Although the majority of patients manifest disease-related symptoms before the age of 35, evaluation for Wilson's disease should also be carried out in older individuals [10]. All forms of clinical presentations occur, including clinically asymptomatic states with only biochemical abnormalities, steatosis, splenomegaly, and hepatomegaly [18, 21]. Symptomatic liver disease may manifest itself as fulminant hepatic failure associated with hemolysis, compensated or decompensated cirrhosis, or as acute or chronic hepatitis. The features of hepatitis can be similar to other causes of hepatitis, such as chronic viral hepatitis, chronic autoimmune hepatitis,

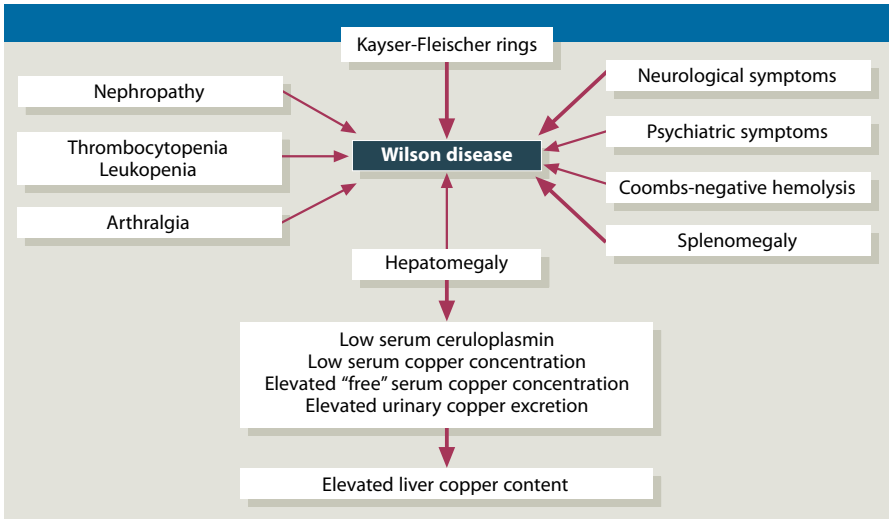


**Fig. 81.2** Clinical and laboratory parameters and their relevance for diagnosis of Wilson's disease. Forty-two percent of clinical symptoms are hepatic, 34% are neurological, 10% are psychiatric, and fewer than 10% are hematological, endocrinological, or renal abnormalities

chronic steatohepatitis, or drug-related hepatitis. In some patients transient episodes of hemolysis presenting as intermittent jaundice can occur.

Approximately 5% of patients present with fulminant hepatic failure. They have a poor prognosis without liver transplantation. Though this may be the first manifestation of the disease, these patients are typically already cirrhotic. Neurological symptoms usually develop later than liver disease, most often in the third decade of life. The initial symptoms may be very subtle, such as changes in behavior, deterioration in practical performances, or speech and writing problems with micrographia. Other common findings are tremor, lack of motor coordination, drooling, dysarthria, dystonia, and spasticity. Approximately one-third of patients present with psychiatric abnormalities. These include depression, anxiety, labile mood, and even frank psychosis. Many of the individuals with neuropsychiatric symptoms may have concomitant liver disease that is frequently asymptomatic.

Kayser-Fleischer rings represent the corneal deposition of copper within Descemet's membrane and have a golden-brown appearance (Fig. 81.3). They are present in nearly all patients with neurological symptoms. Approximately 50% of Wilson's disease patients with liver disease lack Kayser-Fleischer rings. Additionally, in early stages of the disease and in asymptomatic siblings they are commonly absent. Sunflower cataracts, representing deposits of copper in the lens, can be found by slit-lamp examination in some Wilson's disease patients only. They appear brilliantly multicolored and do not impair vision.

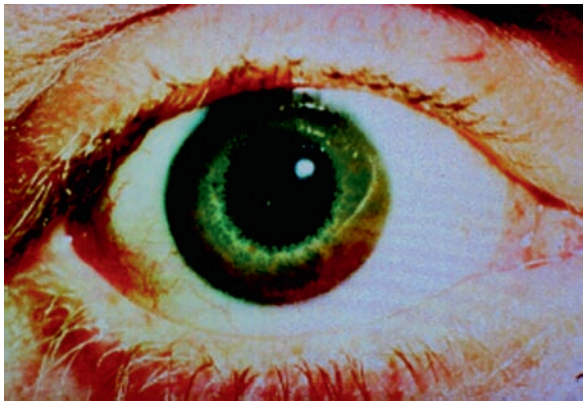

**Fig. 81.3** Kayser-Fleischer rings, visible as a golden-brown discoloration at the outer margin of the cornea

In addition to the common hepatic and neuropsychiatric presentations, signs and symptoms of Wilson's disease may arise as a result of the dysfunction of any organ in which excess copper is deposited. Clinical manifestations may include abnormalities of the kidney (aminoaciduria and nephrolithiasis), the endocrine system (hypoparathyroidism, infertility, secondary amenorrhea, and repeated miscarriages), the heart (cardiac arrhythmias and cardiomyopathy), and the skeleton (premature osteoporosis and arthritis).

Because of the variability in clinical presentation, correct clinical diagnosis is often difficult and sometimes delayed.

## *Diagnostic Findings*

Diagnosis is usually established on the basis of clinical findings and laboratory abnormalities. Based on Sternlieb's criteria, diagnosis is straightforward if two or more of the following symptoms are present: Kayser-Fleischer rings, typical neurologic symptoms, low serum ceruloplasmin levels (<20 mg/dL), and increased hepatic copper content (>250 μg/g dry weight) [20]. Diagnosis is far more complex in patients presenting with liver disease. In most patients a combination of various parameters is necessary to firmly establish the diagnosis as no one single finding is adequate for diagnosis of Wilson's disease [7].

*Kayser-Fleischer rings* are found in most neurological Wilson's disease patients and in ~70–80% of hepatic patients. Although sometimes they can be visible, slit lamp examination is necessary to confirm the presence or absence of Kayser-Fleischer rings. They are not pathognomonic for Wilson's disease, and can also be found in cholestatic liver disease, such as primary biliary cirrhosis or intrahepatic cholestasis associated with prolonged parenteral nutrition.

*Serum ceruloplasmin* is typically decreased below 20 mg/dL in patients with Wilson's disease. However, serum ceruloplasmin concentration has its limitations as approximately 20–30% of Wilson's disease patients with hepatic symptoms have serum ceruloplasmin levels in the normal range [19]. In addition, ceruloplasmin is an acute-phase reactant and may be elevated in inflammatory stages, during pregnancy, and in response to exogenous administration of estrogens. In some Wilson's disease patients these stimuli can increase

levels above the lower limit of normal. Conversely, a reduced serum ceruloplasmin concentration can be seen in about 20% of individuals heterozygous for Wilson's disease, in hypoproteinemia due to renal or enteric protein loss, in severe end-stage liver disease of any etiology, and in the rare condition of aceruloplasminemia.

In contrast to what would seem intuitive for a disorder of copper overload, total *serum copper* is often reduced (<70 μg/dL) in Wilson's disease due to the lowered level of serum ceruloplasmin. However, serum copper levels vary and can be elevated in the setting of fulminant Wilson's disease. The copper that is not part of ceruloplasmin is known as the serum non-ceruloplasmin bound copper concentration and is elevated above 10 μg/dL in most untreated patients. The non-ceruloplasmin bound "free" copper concentration can be calculated using the estimation that approximately 0.3 μg of copper are associated per mg ceruloplasmin:

Serum copper (μg/dL)–3 × serum ceruloplasmin (mg/dL) = non-ceruloplasmin bound copper (μg/dL)

*Urinary copper excretion* is commonly increased in patients with Wilson's disease and reflects the amount of non-ceruloplasmin copper in the circulation. While a daily urinary copper excretion of 40 μg (0.6 μmol) is the upper limit of normal, the conventional level taken as diagnostic of Wilson's disease is 100 μg (1.6 μmol). Urinary copper excretion, however, can also be increased in any disease with extensive hepatocellular necrosis, cirrhosis with cholestasis, and nephrotic syndrome.

Urinary copper excretion after provocation with D-penicillamine may be a utilized as an adjunctive diagnostic test to establish the diagnosis of Wilson's disease, but has only been standardized in children. Commonly 500 mg of oral D-penicillamine is administered to untreated patients at time zero and 12 h later during a 24-h urine collection. An increase of urinary copper excretion to >1,600 μg/24 h is considered as diagnostic for Wilson's disease.

*Liver biopsy* with determination of *hepatic copper content* remains the gold standard for diagnosing Wilson's disease. Normal hepatic copper content is less than 40 μg/g dry weight, while hepatic copper in Wilson's disease typically exceeds 250 μg/g dry weight [8]. A slightly elevated hepatic copper content can also be associated with cholestatic liver diseases, such as primary biliary cirrhosis and primary sclerosing cholangitis. The major problem with hepatic parenchymal copper concentration is that in later stages of Wilson's disease the distribution of copper within the liver is often not homogeneous. Thus the concentration can be underestimated due to sampling error. As the accuracy of measurement is improved with adequate specimen size, at least 1 cm of biopsy core length should be submitted for analysis.

Regarding *liver histology*, there is no one single feature pathognomonic for the diagnosis of Wilson's disease. Intracellular fat accumulation, hepatitis-like features, Mallory-Denk bodies, focal necrosis, fibrosis, and cirrhosis can often be found. The pathology can be similar to an ethanol-induced steatohepatitis, while other patients may show histological signs resembling autoimmune hepatitis. Although histological findings are often not helpful for the diagnosis of Wilson's disease, the exclusion of other etiologies by liver biopsy may be equally important. The presence of copper staining in histological sections by rhodanine or by other means can provide supportive evidence for Wilson's disease (Fig. 81.4). In early stages of Wilson's disease, however, a negative histochemical staining for copper does not rule out increased hepatic copper content and should not be considered sufficient for the exclusion of Wilson's disease. Indeed, hepatic copper concentration can be particularly high under this condition.

De novo diagnosis by *molecular studies* remains difficult due to the large number of disease-specific mutations scattered across the coding region ATP7B. Depending on the population tested, few mutations can be prevalent and can facilitate the otherwise cumbersome diagnostic mutation analysis [9]. In northern Europeans, the H1069Q mutation accounts for 60–70% of the disease alleles and in Asians the A778L mutation occurs in 30% of affected individuals.

By *ultrasound imaging* signs of liver steatosis or cirrhosis as well as hepato- and splenomegaly can help establish the diagnosis of Wilson's disease.

*Radiologic imaging* of the brain, especially magnetic resonance imaging (MRI) may show increased signaling on T2 weighed images in the region of the basal ganglia and other regions, as well as cortical atrophy. MRI changes are most often seen in Wilson's disease patients with neurological manifestations, but may also be found in Wilson's disease patients without neurological symptoms or completely asymptomatic patients.

As bone density is frequently decreased in Wilson's disease patients, performing osteodensitometry (e.g. dual energy x-ray absorbitometry,- DEXA) can be helpful.

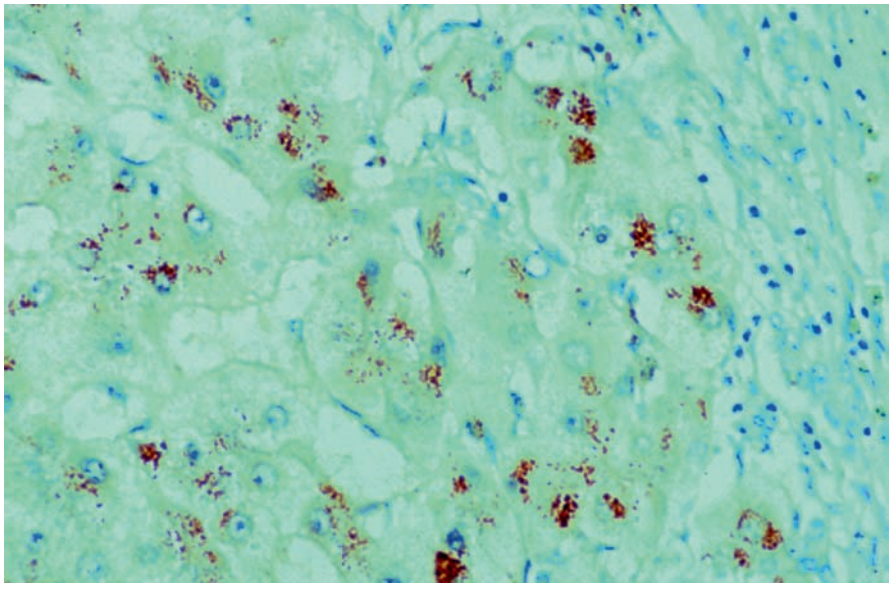

**Fig. 81.4** Liver histology in Wilson's disease. Deposition of copper as golden-brown granules is shown in the rhodanine-stained section (×400)

Diagnosis of Wilson's disease presenting as *fulminant hepatic failure* can be challenging (see Chapter 78). In these predominantly young and female patients coagulopathy, hyperbilirubinemia, Coombs-negative hemolytic anemia (due to massive release of copper from dying hepatocytes), and elevated serum and urinary copper concentrations as well as the combination of all of the above are characteristic features. Despite massive hepatic necrosis, which is responsible for the deleterious clinical course especially in the presence of hepatic encephalopathy, serum aminotransferases are usually less than 10 times normal and, thus, much lower than the values commonly encountered in fulminant hepatitis of other etiologies. It was reported that a particularly low level of alkaline phosphatase is sometimes indicative of fuminant Wilson's disease, but there are other reports that do not confirm this finding [6, 16]. Renal insufficiency is frequently present but is usually reversible. It is a result of tubular injury from copper and of the multiorgan failure that can occur during acute liver failure.

## Family Screening

Screening should be performed in every first-degree relative of any Wilson's disease patient. The probability of finding a homozygote in siblings is 25% and in parents or children is 0.5%. There is no one single biochemical test that accurately discriminates between homozygous patients and heterozygote carriers. Kayser-Fleischer rings may be absent at early stages of the disease. Determination of ceruloplasmin levels and 24-h urinary copper excretion can be misleading because heterozygotes can have borderline pathological values. The serum ceruloplasmin concentration as a screening tool has poor diagnostic strength due to an inadequate predictive value as a single test. Thus, individuals without Kayser-Fleischer rings who have subnormal ceruloplasmin and abnormal liver function should undergo a liver biopsy for quantitative copper determination. Mutation analysis for screening the family of an index patient with known mutations is a very reliable tool; otherwise, haplotype analysis may be used.

## Differential Diagnosis

Wilson's disease can mimic a variety of liver diseases and an elevated hepatic copper content can also be seen in chronic cholestatic liver diseases, such as primary biliary cirrhosis and primary sclerosing cholangitis.

## Prognosis

The long-term outcome of Wilson's disease depends on adherence to an effective treatment, without which the disease is often fatal. With lifelong medical treatment a normal life-expectancy can be achieved [21]. If therapy

is started in pre-symptomatic patients, development of symptoms is rarely seen and prognosis is very good [23]. Symptomatic patients usually stabilize or improve on treatment. This is especially true for hepatic symptoms, whereas neurologic symptoms can persist and sometimes even worsen despite treatment.

## Treatment

### *Medical Treatment*

Once the diagnosis of Wilson's disease is established, lifelong treatment is recommended because copper accumulation is progressive and ultimately fatal without specific therapy. An effective treatment offers patients an excellent long-term survival for this otherwise fatal illness.

As drug treatment, copper chelating agents and zinc salts are used (Table 81.1). Under treatment most Wilson's disease patients improve their liver function within 6–12 months (Fig. 81.5). The rarity of Wilson's disease makes it difficult to conduct double-blind placebo-controlled studies of sufficient statistical power to compare one therapeutic regimen with another.

D-penicillamine and trientine are chelating agents that remove copper by enhancing its urinary excretion.

*D-penicillamine* was introduced in 1956 as the first oral treatment for Wilson's disease. In addition to its action as a copper chelator, it is able to induce hepatic metallothionein synthesis which is capable of sequestering copper in a non-toxic form within cells. There is a large body of published evidence that D-penicillamine can effectively ameliorate hepatic and neurological symptoms. In addition, it can prevent the onset of disease in asymptomatic patients detected by family screening. The usual dosage of D-penicillamine for initial treatment is 900–2,400 mg/day divided in two to four doses. For maintenance treatment a reduced dose of 600–900 mg/day is recommended. Because absorption can be impaired if taken with a meal, D-penicillamine should be taken 1 h before or 2 h after a meal. The disadvantage of D-penicillamine is its serious toxicity profile; side-effects occur in up to 25–30% of patients. These adverse events can be divided into short- and long-term adverse effects. In the first 1–3 weeks after starting D-penicillamine therapy hypersensitivity reactions such as rash, fever, neutropenia, thrombocytopenia, proteinuria, and lymphadenopathy can occur. These early side effects may be managed by stopping D-penicillamine treatment and using an alternative drug, or by reintroducing D-penicillamine treatment in a lower dose that is then gradually increased over 10 days with or without concomitant corticosteroid administration. An early serious side-effect is neurological worsening that is seen especially in patients with a neurological presentation and that recovers only in approximately half of the patients. Long-term effects that are dose-dependent and due to interference with collagen and elastin formation include skin lesions like cutis laxa and elastosis perforans serpiginosa. Those may be caused by Vitamin B6 deficiency. Thus a substitution with 50 mg Vitamin B6 weekly is advisable. Immunologically induced long-term effects require immediate cessation of D-penicillamine treatment. They include systemic lupus erythematosus, immune complex nephritis, Goodpasture syndrome, arthritis, and bone marrow depression with leukopenia and thrombocytopenia.

**Table 81.1** Medical therapy of Wilson's disease

| Treatment | Recommended for | | Dosage | Side effects | Monitoring |
|---|---|---|---|---|---|
| | Initial therapy | Maintenance therapy | | | |
| **D-Penicillamine** | Yes | Yes | 900–2,400 mg/day | Hypersensitivity reactions, autoimmune reactions | 24-h urinary copper excretion, non-ceruloplasmin bound copper |
| **Trientine** | Yes | Yes | 1,200–2,700 mg/day | Iron deficiency anemia | 24-h urinary copper excretion, non-ceruloplasmin bound copper |
| **Zinc salts** (zinc acetate, zinc sulphate) | No | Yes | 150 mg elementary zinc/day | Epigastric discomfort, elevated amylase and lipase | 24-h urinary copper and zinc excretion, non-ceruloplasmin bound copper |

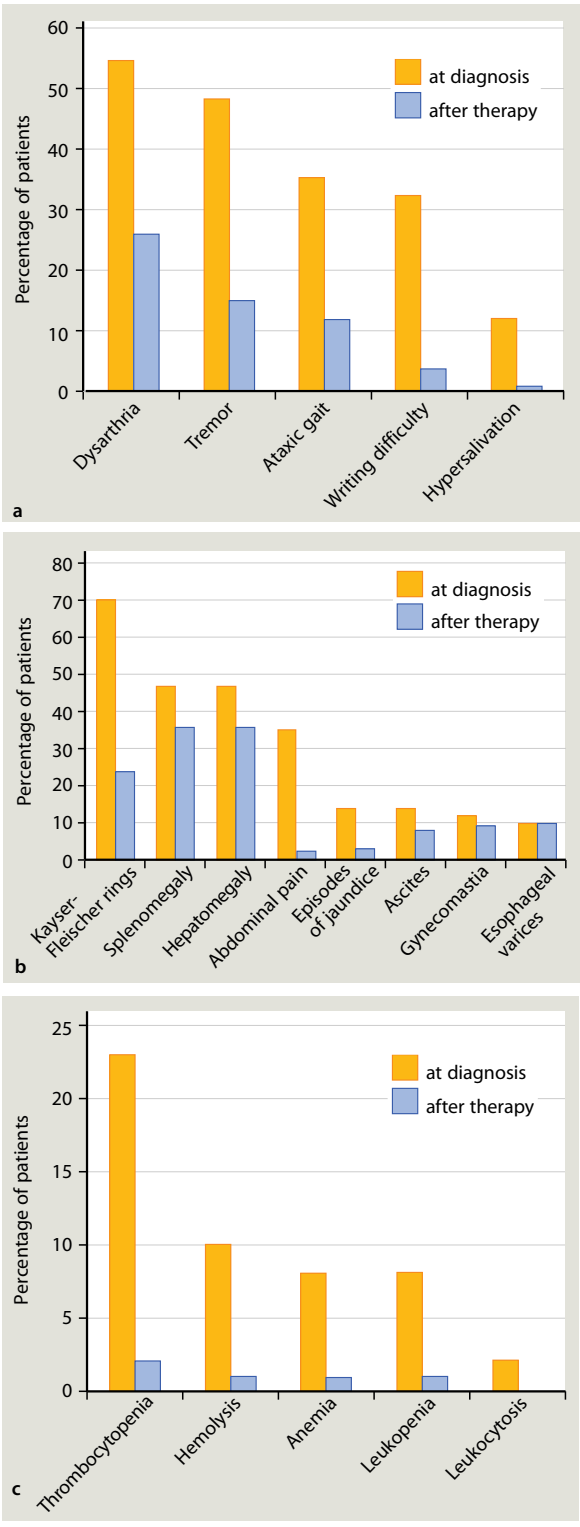


**Fig. 81.5** Comparison of clinical signs and symptoms before and after long-term treatment with D-penicillamine. The percentage of patients with the clinical manifestation is shown. (**a**) Neurological symptoms, (**b**) clinical symptoms, (**c**) hematological symptoms

*Trientine* is a chelating drug that was introduced in 1969 as an alternative to D-penicillamine. It is effective by promoting renal copper excretion like D-penicillamine does, but seems to have fewer side-effects [1]. The initial neurological worsening in particular is thought to be less frequent than under D-penicillamine treatment. Reported side effects are few but include iron deficiency anemia, sideroblasitc anemia, and pancytopenia. The initial dose for trientine is 1,200–2,700 mg/day given in two to three divided doses. For maintenance therapy a reduced dose of 900–1,200 mg/day is generally sufficient. Because absorption can be impaired by parallel intake of food, trientine, like D-penicillamine, should be administered separately from meals.

*Zinc salts* were first used as treatment for Wilson's disease in the early 1960s. In contrast to chelating agents zinc blocks copper absorption in the gastrointestinal tract by inducing metallothionein synthesis in enterocytes. Copper is bound to metallothionein with a high affinity and subsequently is lost when enterocytes are shed during normal cell turnover. The dose of elemental zinc is 150 mg/day, given in three divided doses. Effectiveness of treatment is dependent on the strict administration separately from meals, because food and even milk can interfere with zinc absorption. A major advantage of zinc therapy is its safety with no serious side-effects reported [2, 12]. The frequently occurring zinc-related dyspepsia can sometimes be overcome by changing the zinc formulation (to acetate, sulphate, or gluconate), but sometimes may cause the need for a change to another treatment.

*Tetrathiomolybdate* is another chelating agent which complexes protein-bound copper. When taken together with meals, it complexes copper in the food and prevents its absorption. Taken separately from food, tetrathiomolybdate is absorbed and complexes copper with albumin, rendering it unavailable for cellular uptake. Despite a recently published randomized controlled study (with 48 patients enrolled) comparing tetrathiomolybdate with trientine, the experience with this drug is very limited [3]. It appears to be useful for the initial treatment of neurological patients. However, tetrathiomolybdate has not yet been released by the authorities for general clinical use, and clinical trials continue to determine if chronic use will be effective and well-tolerated.

According to the recent AASLD practice guidelines on Wilson's disease, initial treatment for symptomatic patients should include a chelating agent [15]. Although

the larger body of published evidence exists for penicillamine, trientine seems to have a more favorable safety profile, especially in patients with neurological symptoms. Treatment of pre-symptomatic patients and lifelong maintenance therapy of successfully treated symptomatic patients can be accomplished with zinc salts or chelating agents in a reduced dosage. Nevertheless, definitive recommendations are difficult as randomized studies are lacking, and most data for the different treatment options are from clinical series or case reports or are based on expert opinions only.

Treatment must be monitored to determine its efficacy, to ensure patient compliance, and to identify adverse events. Patients should be monitored at least twice a year. Frequent monitoring is recommended during the initial stages of treatment. To confirm clinical and biochemical improvement, liver function tests and neurological assessment should be performed.

Adequacy of chelating treatment can best be assessed by measuring 24-h urinary copper excretion. We recommend to measure 24-h urinary copper excretion after stopping chelating therapy for 48 h, with collection of urine during the third day off-treatment. An adequate long-term treatment can be predicted if the 24-h urinary copper excretion (measured in the above described way) is below 100 μg/day (1.6 μmol/day). In addition, the efficacy of therapy can be estimated from the non-ceruloplasmin bound copper concentration in serum that should be below 10 μg/dL (<1.6 μmol/L). For monitoring zinc therapy we recommend collecting 24-h urine during active zinc treatment. Efficacy of zinc treatment can be confirmed if 24-h urinary copper excretion is below 100 μg/day (1.6 μmol/day), while compliance with zinc therapy and adequate absorption of zinc is reflected in a 24-h urinary zinc excretion of more than 2 mg/day.

### *Liver Transplantation*

Liver transplantation corrects the underlying metabolic defect and therefore cures the disease. However, the great shortage of donor organs and the need for immunosuppression post-transplantation render liver transplantation a practical option only in fulminant Wilson's disease or in patients unresponsive to medical therapy. The outcome of liver transplantation is excellent, with 1-year survival rates of 80–90%. Neurological symptoms can improve after liver transplantation, but the outcome of patients with neurological symptoms is inferior to patients without [5]. Thus, the indication for liver transplantation in patients with neurological symptoms should be evaluated carefully.

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# Hereditary Hemochromatosis and Iron Overload

# 82

Claus Niederau

## Chapter Outline



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## Definition and Classification of Iron Overload Diseases

Genetic hemochromatosis is today classified into four subtypes of which only type 1 is of clinical importance in North-America, central Europe, and Germany (Table 82.1). Type 1 is the well known form of iron overload due to an autosomal-recessive inborn error of metabolism; the homozygous C282Y mutation of the HFE gene on chromosome 6 accounts for more than 90% of the clinical phenotype in populations of celtic origin [64]. The mutation leads to an inadequately high intestinal iron absorption which, after some decades, may ultimately cause iron overload in, and damage to, various organs (Fig. 82.1). Types 2a and 2b of genetic hemochromatosis are juvenile forms of iron overload which lead to a severe phenotype prior to age 30, with cardiomyopathy and hypogonadism. The corresponding mutations are located in the hemojuvelin and hepcidin genes, respectively [167]. Type 3 has mainly been described in Italian families and refers to a mutation in the transferrin receptor 2 gene [77]. Histopathological and clinical consequences of type 3 hemochromatosis are similar to those seen in type 1. Types 2 and 3 are autosomal-recessive traits. Type 4 hemochromatosis follows an autosomal-dominant trait; the corresponding mutation is located in the basolateral iron carrier ferroportin 1 [135]. In contrast to the other types, in type 4 iron is accumulated mainly in macrophages; ferritin values are markedly elevated although transferrin saturation is only slightly increased.

Some other diseases which are partly ill-defined are also associated with iron overload: *aceruloplasminemia* is characterized by iron accumulation in brain and visceral organs which may lead to retinal degeneration, diabetes mellitus and neurological disease including movement disorders and ataxia in adults [173]. Iron chelation therapy can reduce iron overload and corresponding symptoms. Autosomal recessive aceruloplasminemia is caused by the complete lack of ceruloplasmin ferroxidase activity resulting from mutations in the gene that encodes ceruloplasmin [173]. *African iron overload* was originally described in Africans who drink a traditional beer brewed in steel drums. The disease does not, however, develop in all beer drinkers, suggesting the existence of a genetic susceptibility. A missense change (Q248H) in the gene encoding ferroportin has been reported in Africans and African-Americans with iron overload by independent groups,

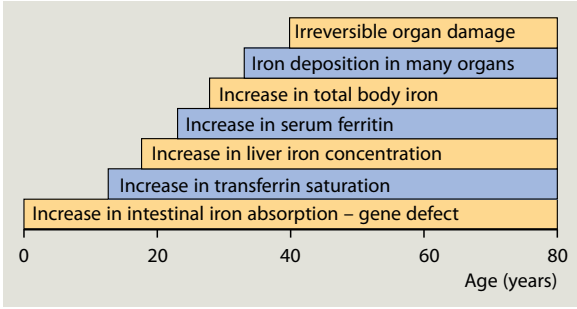


**Fig. 82.1** Schematic representation of the natural course of type 1 genetic hemochromatosis

**Table 82.1** Classification of hemochromatosis

| Types | Gene defect on | Affected gene | Inheritance | High prevalence |
|---|---|---|---|---|
| **Genetic Hemochromatosis** | | | | |
| **Type 1** | Chromosome 6 | HFE | Autosomal-recessive | Celtic inheritance |
| **Type 2a** | Chromosome 1 | Hemojuvelin | Autosomal-recessive | Juvenile form |
| **Type 2b** | Chromosome 19 | Hepcidin | Autosomal-recessive | Juvenile form |
| **Type 3** | Chromosome 7 | Transferrin receptor 2 | Autosomal-recessive | Italy |
| **Type 4** | Chromosome 2 | Ferroportin 1 | Autosomal-dominant | Italy |
| **Neonatal** | Unknown | Unknown | Unknown | Very rare |
| **Others?** | Unknown | Unknown | Unknown | Non-celtic origin |
| **Secondary Hemochromatosis** | | | | |
| (a) Chronic anemias (thalassemia, sickle cell disease, MDS, other rare hemolytic anemias) | | | | |
| (b) Multiple blood transfusions in general | | | | |
| (c) Longterm oral intake of high dietary or i.v. iron amounts | | | | |
| **Non-classified, ill-defined iron overload syndromes** | | | | |
| (a) Iron overload in the Bantu Africans | | | | |
| (b) Iron overload in aceruloplasminemia | | | | |

but the functional effect of this mutation is questionable [14, 54, 79]. *Neonatal hemochromatosis* already occurs in the fetus and presents with liver failure at birth or shortly thereafter. Its cause and inheritance are as yet not exactly known. The rare disease is often fatal, unless liver transplantation is performed. Recently an immunological pathogenesis has been proposed since treatment of at-risk pregnancies with immunoglobulins improved the prognosis [207].

*Secondary hemochromatosis* is usually caused by multiple blood transfusions in hemolytic anemias such as thalassemia, sickle cell anemia and myelodysplastic syndrome (MDS). In secondary hemochromatosis iron first accumulates in RES macrophages and later is transferred to parenchymal cells. In the presence of frequent blood transfusions iron may accumulate much faster when compared to genetic hemochromatosis; thus, iron overload often leads to severe cardiomyopathy and liver cirrhosis, limiting the patients' prognosis. Therapy consists of iron chelators because phlebotomies cannot be done due to the underlying anemia.

The following review will focus on type 1 HFE hemochromatosis which is the most prevalent genetic form. Most consequences of iron overload are similar for the various causes. Thus the pathophysiology of tissue and organ damage by iron excess is discussed in detail only for HFE hemochromatosis.

## Type 1 HFE Hemochromatosis

### History

The association between liver cirrhosis, pigment deposits in the liver, and diabetes mellitus was already recognized over a century ago [82, 192, 193]. The early descriptions reflected the various beliefs as to whether the disease was primarily hepatic, pancreatic, or hematologic in origin and as to whether it was a separate disease entity or a special form of alcoholic liver cirrhosis. The term "hemochromatosis" was first introduced by Recklinghausen in 1889, but was not generally accepted until the term was used by Sheldon as the title of his classic monograph in 1935 [163, 177]. His assumption that hemochromatosis was an inborn error of metabolism, however, has been proven and generally accepted only 50 years later. The controversy whether hemochromatosis is merely a form of alcoholic liver cirrhosis or an inborn error of iron metabolism lasted almost a century until Simon described the association between special HLA haplotypes and hemochromatosis which allowed the detailed analysis of the genetic nature of the disease [42, 112, 177, 180]. The mode of inheritance was identified as an autosomal recessive disorder [181]. Finally, Feder et al. identified the major mutation on the HFE gene associated with the clinical phenotype of genetic hemochromatosis [64].

### Epidemiology

Type 1 hemochromatosis probably is the most prevalent inborn error of metabolism in celtic populations [4]. The prevalence of C282Y/C282Y homozygotes is approximately 0.5% in central Europe and in the white population of northern America; the prevalence of C282Y and H63D heterozygotes approaches 40% in such white populations [4]. The phenotypic expression also depends on several non-genetic factors such as dietary iron contents and blood losses. For example, females develop clinical consequences of iron overload 5–8 times less frequent and 10–20 years later than males because of their menstrual blood losses. It is now also widely accepted that not all C282Y/C282Y homozygous men will develop the full clinical phenotype of hemochromatosis. It is, however, unknown whether 5% or 50% will show clinical disease during their life time and which other factors determine the phenotype. The homozygous C282Y/C282Y mutation accounts for more than 90% of the clinical phenotype in populations of celtic origin (Table 82.2) [4, 64]. A point mutation H63D is also frequently identified in the HFE gene as well as other less frequent mutations. Neither one of these mutations which are found in up to 40% of subjects with a celtic background correlates

**Table 82.2** Genotype/phenotype correlation in hemochromatosis

| Mutations/ polymorphisms | Prevalence in celtic populations | Risk of advanced clinical phenotype |
|---|---|---|
| C282Y/C282Y | 85–95% | Low if ferritin is < 1,000 ng/mL |
| H63D/C282Y | 3–8% | Very low |
| C282Y/wild type | – | None |
| H63D/wild type | – | None |
| Others | 1% | Unknown |

with the phenotype. In genetics a gene variation with a prevalence of more than 1% in the general population should not be called "mutation" but rather a "polymorphism" or "variation". A subject with a C282Y variation on one allele and a H63D variation on the other allele is named "compound heterozygote". Only a small percentage of such compound heterozygotes are at risk for clinical consequences of iron overload. C282Y and H63D heterozygotes are at no risk for iron overload (Table 82.2). In populations without a celtic background other genes may be involved in causing iron overload which is not infrequent (e.g., parts of Africa). It is unknown, however, which genes are involved in non-celtic genetic hemochromatosis. As of yet, the hemochromatosis types 2–4 are not thought to cause the majority of non-celtic genetic iron overload. Recent evidence suggests that genetic factors involved in the uptake of heme and heme-bound iron may also affect the phenotype of HFE hemochromatosis.

## *Etiology and Pathogenesis*

(See also Chapter 6 "Hepatic Metabolism: Heme and Non-heme Iron".)

Intestinal iron absorption and iron losses are finely balanced under physiological conditions. Approximately 10% of the total daily intake (10–20 mg) are finally absorbed from the small intestine (1–2 mg). Subjects with homozygous C282Y mutations, however, may absorb up to 20% of iron intake, which may amount to 2–4 mg/day. Thus homozygotes have an excess iron intake of approximately 1 mg/day (relates to approximately 350 mg/year or 3.5 g per 10 years. It may therefore take several decades until iron stores approach 10 g above which organ damage is thought to be induced. Many patients with the clinical end stage of hemochromatosis including liver cirrhosis and diabetes mellitus have total body iron stores of 20–30 g.

The intestinal iron absorption is downregulated when iron stores increase, also in patients with genetic hemochromatosis. This regulation, however, occurs on an increased level when compared with subjects without a HFE gene mutation. Correspondingly, intestinal iron absorption is massively increased in patients with hemochromatosis when iron stores have been depleted by phlebotomy. Therefore, phlebotomy treatment needs to be continued after iron depletion in order to prevent reaccumulation of iron. These regulatory processes however do not explain how HFE gene mutations cause the increase in intestinal iron absorption since the HFE gene product is neither an iron carrier nor an iron reductase or oxidase. Only recently many carriers and regulators of cellular iron uptake and release have been identified [69, 71, 145, 194]. It has also become increasingly evident that some of them interact with the HFE gene product in regulation of intestinal iron absorption. Recent studies have shown that the Nramp2 protein is the luminal iron carrier. Shortly thereafter the luminal iron reductase was identified as the Dcytb protein (duodenal cytochrome B) [69, 71, 145, 194]. At the same time the basolateral iron transporter ferroportin 1 (also named IREG1 or MTP1) was identified as well as the basolateral iron oxidase hephaestin [1, 52, 115, 116, 200].

Mutations in some of these proteins are responsible for rare types 2–4 of genetic hemochromatosis, but none of these genes is altered in type 1 hemochromatosis. Very recently, two further proteins were shown to act as important iron regulating proteins, transferrin receptor 2 and hepcidin [70, 71, 145]. Mutations in the transferrin receptor 2 gene may lead to the rare type 3 hemochromatosis, and mutations in the ferroportin 1 gene to type 4 hemochromatosis. Hepcidin may be the most important regulator of iron metabolism, involved in iron deficiency and overload, and has been shown to downregulate the basolateral iron carrier ferroportin. It has also been demonstrated that hepcidin itself is upregulated by HFE. Thus, an HFE mutation may reduce the upregulation of hepcidin which then does not downregulate ferroportin; the corresponding increase in ferroportin expression finally causes the increase in intestinal iron uptake [43, 44]. There may be further interactions between HFE, transferrin receptor 2, Nramp2, Dcytb, ferroportin, hephaestin and hepcidin, all of which are currently studied.

## *Diagnosis*

### Laboratory Tests

Any increase in serum iron should initiate the exclusion of hemochromatosis in order not to overlook early disease. Normal serum iron, however, does not exclude hemochromatosis and increased serum iron often occurs in the absence of hemochromatosis. Serum iron

values are highly variable and should be used neither for diagnosis nor for screening of hemochromatosis. The determination of transferrin saturation is a much better indicator of iron overload than serum iron values. The increase in serum transferrin saturation usually precedes the increase in ferritin for many years in hemochromatosis (Fig. 82.1). Transferrin saturation is more sensitive and more specific for detection of hemochromatosis when compared to serum ferritin. For screening of genetic hemochromatosis a threshold of approximately 50% for transferrin saturation may be optimal. It must be kept in mind however that the value of transferrin for diagnosis and screening of hemochromatosis is only good when one obtains fasting values. Ferritin on the other hand is a good indicator of largely increased iron stores and reliably indicates iron deficiency. It has less value for early detection of hemochromatosis. In genetic hemochromatosis a slightly increased serum ferritin (such as values of 300–500 ng/mL) is usually accompanied by transferrin saturations exceeding 80–90%. Unfortunately serum ferritin is also increased quite often in the presence of all kinds of infections and malignancies and thus has a low specificity for indicating hemochromatosis. In most instances increases in ferritin values are not caused by hemochromatosis but by a variety of other diseases [130]. Typically, ferritin increases which are not due to genetic hemochromatosis are accompanied by normal or only slightly elevated transferrin saturation. Therefore, transferrin saturation should be measured in order to enable the physician to correctly interpret increases in serum ferritin.

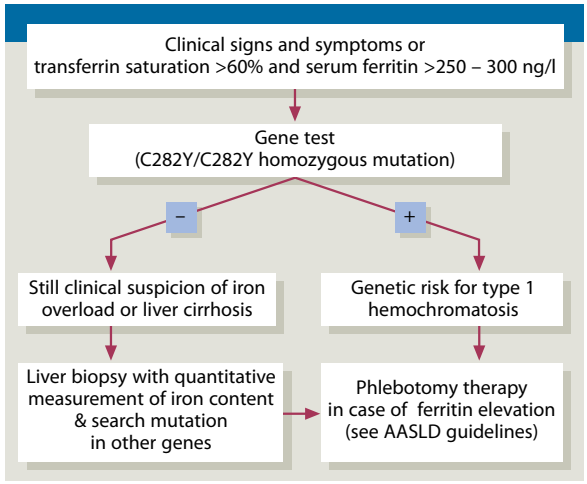


**Fig. 82.2** Diagnosis and treatment algorithm for type 1 hemochromatosis

## Liver Biopsy and Determination of Liver Iron Concentration

Although simultaneous increases of both serum ferritin and transferrin saturation strongly indicate a risk for hemochromatosis, diagnosis needs to be confirmed by genetic tests or by liver biopsy with determination of liver iron content. The iron concentration in the liver increases during life also in subjects with a HFE gene mutation. Thus, it is recommended to divide the liver iron concentrations measured in the liver biopsy by the patient's age in order to obtain the "liver-iron-index", which is more specific than the raw data of liver iron concentration [187]. The semiquantitative estimation of liver iron stores by Prussion blue staining is less sensitive and specific than the chemical quantification of the liver iron concentration. In case of a homozygous C282Y gene test, liver biopsy is no longer required for the diagnosis of genetic hemochromatosis (Fig. 82.2). There may, however, be other reasons to perform a liver biopsy in cases of suspected iron overload: (1) subjects with biochemical or clinical evidence of iron overload in the absence of the homozygous C282Y mutation should usually have a liver biopsy to prove iron overload; (2) in C282Y homozygotes the risk for liver fibrosis and cirrhosis is only increased at ferritin values > 1,000 ng/mL [110]. In those patients liver biopsy is recommended because the presence of liver cirrhosis markedly increases the later HCC risk. Thus, all cirrhotic patients should be screened for HCC with ultrasound and measurement of serum $\alpha_1$-fetoprotein (AFP) levels twice a year.

## Deferoxamine Testing and Ferrokinetic Measurements

Determination of urinary excretion of iron after administration of deferoxamine allows some estimation of total body iron stores. The deferoxamine test, however, often only shows pathological results when serum ferritin and transferrin saturation are markedly increased and does not allow for diagnosis of early disease. Ferrokinetic measurements today are only done for scientific reasons or difficult diagnostic problems.

### Computed Tomography (CT), Magnetic Resonance Tomography (MRT) and Biomagnetometry

CT density measurements of liver tissue allow a semi-quantitative estimation of liver iron concentrations. This method however is associated with radiation and therefore not allowed in many countries when alternative methods are available which do not expose the patient to radiation. MRT in contrast even allows a quite reliable measurement of liver iron content, provided that a special software is used and provided that the equipment is calibrated for such measurement. In clinical practice most MRT machines do not fulfill the criteria required for quantitative iron determination and diagnosis of early iron overload. Biomagnetometry allows the most accurate non-invasive measurement of liver iron concentration. However, this equipment is very expensive and only allows the measurements of iron concentration. Consequently biomagnetometry is done only at a few places worldwide and just useful for scientific studies but not for daily clinical practice. With availability of a reliable and inexpensive genetic testing, CT, MRT, and biomagnetometry do not need to be done for most patients any longer.

### Genetic Tests

In populations with a celtic origin, the homozygous C282Y mutation accounts for more than 90% of patients with the clinical phenotype of type-1 hemochromatosis [4, 61]. Approximately 5% of patients with the clinical phenotype are C282Y/H63D compound heterozygotes; the prevalence of C282Y or H63D heterozygosity in patients with the clinical phenotype of hemochromatosis is considerably lower than in the general population. Thus, a subject who is heterozygous for C282Y or H63D per se has no risk of iron overload. In subjects homozygous for C282Y, both serum ferritin and transferrin saturation are frequently increased; however, only male subjects had an increased risk for liver disease when compared to subjects without HFE gene alterations in a recent large screening study. It is as yet unknown how many C282Y homozygotes will later develop clinical signs and symptoms due to iron overload. It has become increasingly evident that only a minority of C282Y homozygotes progress to end stage iron overload with liver cirrhosis and diabetes mellitus. In subjects who are not C282Y homozygotes but have laboratory, histological or clinical evidence of iron overload, further genes may be analyzed for mutations such as hemojuvelin, transferrin receptor 2, ferroportin 1 and hepcidin. The types 2–4 of hemochromatosis probably occur mainly in populations without a strong celtic background (many of the rare genetic types have been described in Italy). African subjects may well develop the clinical phenotype of hemochromatosis although they almost never have a C282Y HFE mutation; in the latter subjects the gene responsible for iron overload is as yet unknown.

## *Early Diagnosis and Screening*

The prevalence of C282Y homozygotes is 0.5% in celtic populations [4, 61]. The phenotypic expression however is variable and also depends on several non-genetic factors such as dietary iron intake and blood losses. Until 1980 most patients with hemochromatosis were detected with late irreversible complications such as liver cirrhosis and diabetes mellitus. With the better understanding of the disease, the broad use of ferritin and transferrin saturation measurements and the availability of reliable gene tests, diagnostic efforts have concentrated on the detection of early disease in the absence of liver cirrhosis and diabetes mellitus. Several studies have shown that iron removal by phlebotomy is associated with a normal life expectancy in patients diagnosed at such early stages (Fig. 82.3) [63, 128, 129]. Thus, several further studies have focused on screening procedures in order to diagnose more subjects with early disease [57]. These studies include populations with special risks, family members as well as the general population (Table 82.3) (for further literature see [133]). It has also been shown that an increasing number of patients are now diagnosed in early stages and that this trend increases survival (Figs. 82.4 and 82.5).

A large number of studies have shown that screening is useful for detection of asymptomatic C282Y homozygotes by using transferrin saturation and serum ferritin as well a genetic test for the C282Y mutation [57, 130, 142]. A broad screening of the general population however is as yet not recommended by WHO and CDC mainly because it is unknown how many of the asymptomatic C282Y homozygotes will later develop clinical disease (for further literature see [195]). The largest

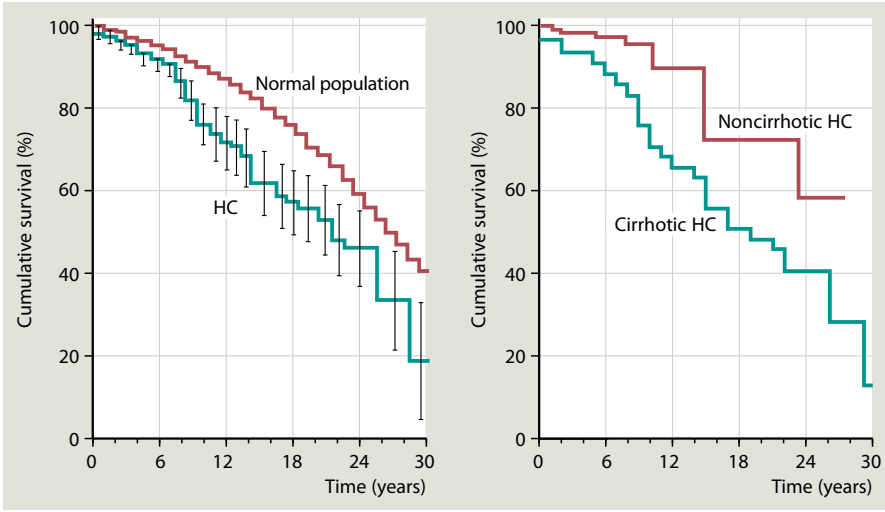


**Fig. 82.3** Survival of 251 patients with genetic hemochromatosis (HC) (with and without cirrhosis) in comparison with a matched general population (Modified from [129])

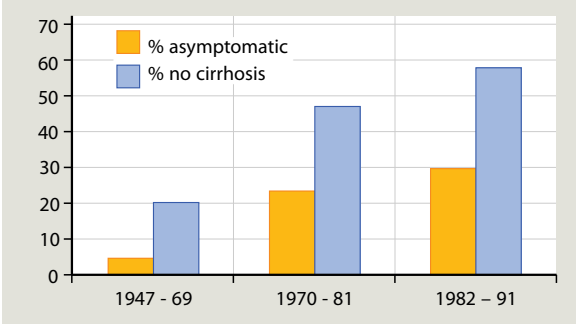


**Fig. 82.4** Prevalence of early diagnosis in 251 patients with genetic hemochromatosis during three time periods. Early diagnosis was defined either as diagnosis without symptoms or as diagnosis without evidence of liver cirrhosis (Modified from [129])

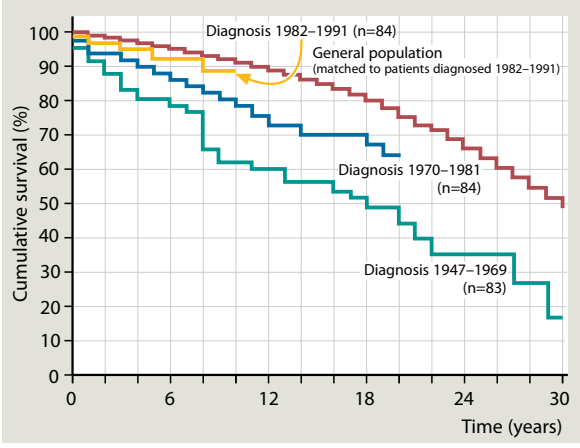


**Fig. 82.5** Cumulative survival in patients with genetic hemochromatosis according to the time of diagnosis (Modified from [129])

**Table 82.3** Methods for early diagnosis of hemochromatosis

**Screening in the general population not recommended**

A screening of HFE gene alterationsis is not recommended for the general population because it remains unknown how many of the C282Y homozygotes will develop the clinical phenotype. Such screening would be meaningful only in populations of celtic origin.

**Family screening**

Genetic testing can reliably determine who of the first degree relatives of a hemochromatotic patient is a heterozygote or homozygote. Heterozygotes are healthy and do not need follow-up. C282Y homozygotes should be followed and treated by phlebotomy if ferritin increases > 300 ng/mL in men and > 200 ng/mL in women.

**Hemochromatosis should be excluded in patients with**

- Newly diagnosed diabetes mellitus
- Chronic liver disease of unknown etiology
- Elevation of iron, transferrin saturation or serum ferritin
- Cardiomyopathy of unknown etiology
- Arthropathy of unknown etiology
- Loss of potency/libido and amennorrhea of unknown etiology

**Every liver biopsy needs to be checked for iron deposits**

screening study analyzed HFE gene mutations in almost 100,000 subjects in North America. In white subjects, C282Y homozygosity was found in 0.44%, a value similar to many previous studies in other populations with a celtic/caucasian background. Asian or black people in contrast almost never had a HFE gene mutation. Among the white C282Y homozygotes only males had a significant increase in liver disease when compared to subjects without a HFE gene variation [4]. Only further

prospective follow-up studies will determine how many asymptomatic C282Y homozygotes will develop clinical consequences of iron overload.

It is also unknown at which ferritin values phlebotomy treatment should be initiated in asymptomatic C282Y homozygotes (see chapter on treatment for details). The values recommended by the American Association for the Study of Liver Diseases (AASLD) are based more on the judgment of experts than on solid data. The only solid data show that the risk for liver fibrosis and cirrhosis increases above the threshold of 1,000 ng/mL serum ferritin [110]. The value of screening family members is obvious when a first-degree relative has clinical hemochromatosis. Such family screening is easy to do with the gene test. Heterozygous family members are not at risk for hemochromatosis unless they have additional risk factors.

The clinical phenotype of hemochromatosis is detected in 1–2% of patients with newly diagnosed diabetes mellitus und in 3–15% of patients with liver cirrhosis [131]. The latter patients should be screened for iron overload although elevated ferritin levels are common in cirrhosis in the absence of hemochromatosis. Little is known as yet about the prevalence of hemochromatosis in patients with arthropathy or cardiomyopathy of unclear etiology. Several smaller studies indicate that arthropathy may be a rather early clinical sign of iron overload, whereas cardiomyopathy usually only occurs when there is massive iron overload.

### *Differential Diagnosis*

Symptoms and clinical signs determine the differential diagnosis of hemochromatosis. Most often signs of liver disease, diabetes mellitus, and arthropathy lead to suspicion of hemochromatosis. In other patients elevations of serum iron or ferritin initiate a further work-up. The use of serum ferritin, transferrin saturation, and genetic testing today enable the diagnosis of hemochromatosis in celtic populations in > 90% of subjects. Previously it was sometimes difficult to distinguish hemochromatosis from alcoholic hepatitis which may also be associated with ferritin elevations and some iron deposits in liver biopsy; today, the determination of the C282Y mutation allows this differentiation to be made without major problems.

## Complications of Iron Overload

Liver cirrhosis, diabetes mellitus, and increased skin pigmentation are the classic triad of genetic hemochromatosis. Cardiomyopathy, cardiac arrhythmias, and impotence are also typical complications of advanced iron overload. Arthropathy, in contrast, may be an early sign of hemochromatosis which may enable the diagnosis in the precirrhotic stage [129].

### *Liver cirrhosis*

The liver is the organ which is affected by genetic iron overload most early and heavily. At early stages, excess iron stores are mainly found in periportal parenchymal cells as ferritin and haemosiderin. When iron excess further increases, there is development of perilobular fibrosis and iron stores are also found in bile ducts and Kupffer cells. Septal fibrosis finally progresses towards complete cirrhosis. The stage of fibrosis is closely associated with the degree of iron excess [110, 129]. In many affected symptomatic patients there are signs of liver disease at the time of diagnosis (Fig. 82.6). Many non-specific symptoms may as well be a consequence of liver involvement such as abdominal discomfort and

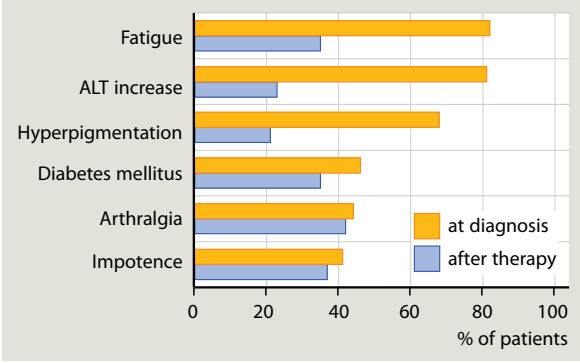


**Fig. 82.6** Signs and symptoms in 185 patients with genetic hemochromatosis prior and after iron removal (Modified from [129])

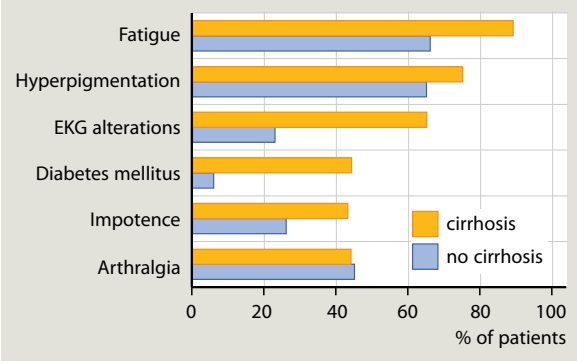


**Fig. 82.7** Signs and symptoms in 251 patients with genetic hemochromatosis with and without liver cirrhosis (Modified from [129])

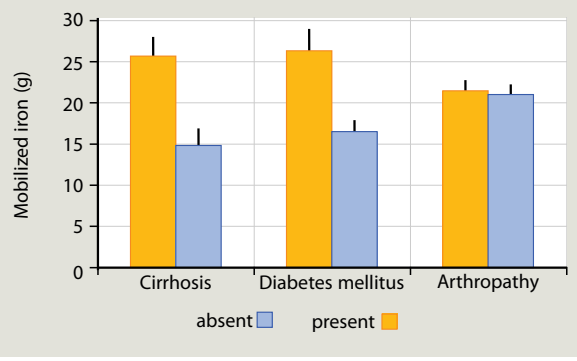


**Fig. 82.8** Mobilized iron by phlebotomies in 185 patients with documented complete iron removal (Modified from [129])

fatigue. In asymptomatic patients who are often diagnosed by some screening procedure, signs of liver disease are infrequent. Complications due to cirrhosis such as ascites, jaundice and portal hypertension are seen rarely and only in advanced iron overload [128, 129]. Many further complications of hemochromatosis are seen more frequently in patients with cirrhosis when compared to patients without cirrhosis (Fig. 82.7); this reflects the fact that cirrhotic patients have a higher iron burden than noncirrhotic patients (Fig. 82.8). The risk for liver cirrhosis usually increases only at ferritin values > 1,000 ng/mL [110]. Similar to insulin-dependent diabetes, liver cirrhosis cannot be reversed by removal of iron [129]. However, less advanced stages respond well to removal of iron. Survival is significantly reduced in the presence of liver cirrhosis while patients diagnosed in the precirrhotic stage have a normal life expectancy when treated with phlebotomies (Fig. 82.3) [129].

## *Liver Carcinoma*

Liver carcinoma develops in approximately 30% of patients with hemochromatosis and liver cirrhosis independent of iron depletion [129]. The interval between complete iron depletion and diagnosis of liver cancer was approximately 9 years in large cohorts of German patients [128, 129]. The risk of dying from liver cancer is increased in patients with hemochromatosis by 100–200 times when compared to the general population (Fig. 82.9). Among the liver cancers there were hepatocellular carcinomas (HCC) as well as cholangiocellular carcinomas. In the own cohort all liver cancers developed in patients with liver cirrhosis. Thus cancer screening by ultrasound and measurement of serum AFP levels (twice a year) is only recommended for cirrhotic patients although in the literature there are a few published case reports about HCC in patients with hemochromatosis in a noncirrhotic stage. The degree and duration of iron overload are probably the decisive risk factors for the development of liver cancer in hemochromatosis. Patients who develop a liver cancer usually have the largest amount of mobilizable iron among various subgroups [129, 131].

## *Association of Hemochromatosis with other Liver Diseases*

Some studies indicate that C282Y heterozygosity may aggravate the progression of other concomitant liver

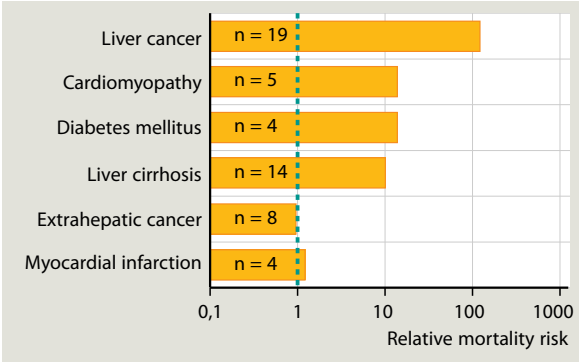


**Fig. 82.9** Relative mortality risk in 251 patients with genetic hemochromatosis in comparison to the general population (Modified from [129])

diseases such as porphyria cutanea tarda, chronic hepatitis C, alcoholic hepatitis, and non-alcoholic steatohepatitis. In the latter patients, one might find slightly elevated liver iron concentrations and serum ferritin levels when they are C282Y heterozygotes (for review see [62]). Many studies however suggest that these associations are of minor importance for the clinical prognosis. Phlebotomy as yet has only been proven meaningful in porphyria cutanea tarda where it also ameliorates the cutaneous manifestations.

## *Diabetes mellitus*

"Diabetes mellitus" was actually the most frequent term used to denominate hereditary hemochromatosis in the early descriptions. It reflected the various beliefs as to whether the disease was primarily pancreatic, hepatic, or hematological in origin or whether it was a separate disease entity [82, 163, 192, 193]. Liver cirrhosis, damage to pancreatic beta-cells, and genetic predisposition have been proposed as the major factors causing impaired glucose tolerance and diabetes mellitus in hemochromatosis [126–128, 174, 186]. Early studies reported an almost 80% prevalence of diabetes mellitus in hemochromatosis [25, 66, 177, 208]. In more recent studies the prevalence is reported to be < 50% [129]. In a large series of 474 patients with hemochromatosis from France, 191 patients (40%) had diabetes at the time of diagnosis. The prevalence and stage of diabetes was related to the degree of iron deposition in the pancreas. Patients with diabetes had a twofold higher mobilizable iron content than non-diabetics [209]. In another cohort study which included mostly precirrhotic patients, the prevalence of diabetes was only 21% [2]. Thus, the decrease in the prevalence of diabetes during the last decades is probably due to the fact that many patients are now diagnosed at an early stage when glucose metabolism is still normal or only slightly abnormal. Investigations into the prevalence of unrecognized hemochromatosis in diabetic patients showed some variations in Europe and overseas. Screening revealed a prevalence of 5–8 per 1,000 unrecognized cases in Europe and 9.6 per 1,000 in Australia [143, 182]. Therefore diabetics should always be carefully investigated for iron overload.

Diabetes mellitus and impaired glucose tolerance are frequent features in several chronic liver diseases [17, 41]. Because insulin resistance has been established as a major factor causing impairment of glucose metabolism in chronic liver disease, it might also be responsible for impaired glucose metabolism associated with hemochromatosis [159]. Our own studies showed hyperinsulinemia and hence insulin resistance without impaired glucose tolerance in the noncirrhotic stage of hemochromatosis [127]. In contrast to increased insulin concentrations, circulating concentrations of C-peptide were normal in noncirrhotic patients, indicating a normal insulin secretion by pancreatic beta-cells. Circulating glucagon in response to oral glucose were also normal. The latter results are in accordance with two other studies demonstrating normal glucagon following arginine stimulation in patients with cirrhotic and noncirrhotic hemochromatosis. The observation of normal glucagon concentration also in cirrhotic patients is further substantiated by histological studies showing that the iron depositions in hemochromatosis are almost exclusively found in beta-cells and not in alpha-cells [83]. Since the circulating gastric inhibitory peptide (GIP) in response to oral glucose remained unchanged, insulin secretion by beta-cells, glucagon secretion by alpha-cells, and the enteroinsulinar axis (GIP) are not impaired in noncirrhotic hemochromatosis. The increase in circulating insulin is likely to be due to a decrease in diminished hepatic extraction of insulin. Iron accumulation in hepatocytes may be responsible for the impaired hepatic degradation of insulin. There is a close correlation between the progression of alterations of glucose metabolism and the development of liver cirrhosis. Thus, a part of diabetes may be due to the development of liver cirrhosis. On the other hand, the high frequency of diabetes in advanced iron overload also reflects the advancement of iron deposition in beta-cells. Insulin secretion by pancreatic beta-cells is normal in precirrhotic stages [127]. With the progression of iron overload and destruction of beta-cells in combination with the development of liver cirrhosis, pancreatic insulin secretion becomes impaired [16, 56]. In the end-stage of hemochromatosis, insulin deficiency is associated with severe reduction in the mass of immunoreactive beta-cells [161]. Along with the component of impaired insulin secretion by damaged beta cells, there is an almost obligatory insulin resistance observed in iron overload disease. This is due to an insulin receptor/postreceptor defect in the iron loaded liver, which is, in contrast to the destruction of the beta-cells, partially reversible after completion of the

**Table 82.4** Changes in clinical features during initial treatment period in 183 patients with biopsy proven iron depletion[a] (Modified from [129])

| Feature | At time of diagnosis | After depletion or iron[b] | | |
|---|---|---|---|---|
| | | Improved | Unchanged | Worsened |
| | Percent of group (no. of patients) | | | |
| Diabetes mellitus (n = 81) | 44 (81) | 41 (33) | 53 (43) | 6 (5) |
| Insulin-dependent | 25 (46) | 41 (19)[c] | 50 (23) | 10 (4) |
| Non-insulin dependent | 19 (35) | 40 (14) | 57 (20) | 3 (1) |
| Glucose tolerance (n = 101) | 56 (101) | 10 (10) | 87 (88) | 3 (3) |
| Impaired | 15 (27) | 37 (10) | 56 (15) | 7 (2) |
| Normal | 40 (74) | – | 99 (73) | 1 (1) |

[a]From 2 of the 185 patients with biopsy proven iron depletion sufficient follow-up information could not be obtained

[b]Data obtained from a 6-months period after the end of the initial phlebotomy period

[c]The daily insulin dose could be reduced in 19 of the 46 insulin-dependent patients, but insulin dependency could be abolished in none of the patients

phlebotomy therapy [128, 129]. The analysis of patients in whom complete iron depletion could be achieved showed that therapy did not eliminate insulin-dependency (Table 82.4) [129]. However, in 19 of the 46 insulin-dependent patients (41%) in whom complete iron depletion had been achieved, the insulin dose could be reduced. Statistically, hemochromatotic patients without diabetes showed significantly less mobilizable iron compared with patients who had already developed diabetes (Fig. 82.8). Thus, the degree of iron overload reflects the degree of alterations in glucose metabolism. Survival was significantly reduced in patients with diabetes mellitus at entry compared to patients without diabetes [129]. The survival curve for non-diabetic patients was virtually identical to that of a matched normal population.

## *Heart Disease*

Cardiomyopathy and cardiac arrhythmias are specific complications of hemochromatosis caused by iron deposition in the heart [24, 178, 199]. Clinical or electrocardiographic signs of heart disease may be found in 20–35% of patients with HFE hemochromatosis [128]. Arrhythmias usually respond well to iron removal [129, 178]. In type 1 hemochromatosis cardiomyopathy is rare and usually associated with advanced iron overload at higher ages. However, in particular in young patients who present with cardiac disease, cardiomyopathy is a frequent cause of death [66, 178]. Only recently has it become clear that young patients with severe cardiomyopathy may be affected by juvenile type 2 hemochromatosis. Severe iron overload, hypogonadism, cardiomyopathy, liver cirrhosis, and amenorrhea can be seen at age 15–24 years of age. The type 2 associated cardiomyopathy is often irreversible despite initiation of phlebotomy or chelation therapy and may require a rapid transplantation of the heart and potentially also of the liver [86, 90]. It has been reported that venesection therapy may aggravate cardiomyopathy. In these rare cases therapy with iron-chelating agents may be performed instead of or in addition to a cautious phlebotomy treatment. Phlebotomies usually have not to be stopped completely, but their frequency has to be reduced as well as the amount of blood withdrawn with each phlebotomy.

## *Arthropathy*

Joint changes in genetic hemochromatosis may occur in two different types [56, 128, 129, 176]. The most prevalent changes are seen in the metacarpophalangeal joints II and III in the form of cystic and sclerotic changes, cartilage damage, and narrowing of intraarticular spaces. Sometimes other joints of the hands and feet are also affected. The large joints (knees and hips) can be affected in form of chondrocalcinosis. The pathogenesis of joint changes in hemochromatosis remains unclear. The arthropathy is one of the few complications which is not associated with the degree of iron overload. It has been speculated that iron may inhibit pyrophosphatase and may thereby lead to

crystallization of calcium pyrophosphates. Alternatively, iron may have direct toxic effects on the joints. Arthropathy may be an early sign of hemochromatosis and may help to make the diagnosis in a precirrhotic stage [129]. Hemochromatosis should therefore be considered in all patients with an arthropathy of unknown etiology.

### Endocrine Abnormalities

In contrast to arthropathic changes, endocrine abnormalities are a late consequence of iron overload. Sexual impotence and loss of libido may occur in up to 40% of male patients [128]. Interestingly, gynecomastia is not frequently associated with the other endocrine abnormalities in contrast to what is seen in alcoholic cirrhosis [97, 98, 128]. The endocrine abnormalities in hemochromatosis have been attributed to primary testicular failure, to secondary pituitary hypogonadism, to impaired metabolism of sex hormones due to liver failure, and to diabetes mellitus which is often associated with alterations of libido and potency [33, 98, 108, 179, 184, 185, 201, 205, 206]. Most studies have demonstrated decreased testosterone concentration in impotent male patients potentially caused by primary gonadal failure or by secondary pituitary failure. Iron deposits have been shown both in testes and pituitary [112, 177]. Later studies have clearly demonstrated that hypogonadism in hemochromatosis is mainly, if not exclusively due to pituitary failure in contrast to alcoholic cirrhosis where testicular failure is predominant [97, 98]. In contrast to alcoholic cirrhosis, where estrogen levels are increased, estrogen levels were found decreased in hemochromatosis [97]. Most endocrine changes are late and irreversible complications of iron overload and do not respond well to phlebotomy treatment [129]. Iron overload only infrequently affects other endocrine organs such as thyroid and adrenal glands. A severe hypogonadism with amennorrhea in young women and impotence in young men is today thought to be due to type 2 hemochromatosis [101].

### Skin

The increased skin pigmentation is mainly seen in light exposed areas. To a large degree the pigmentation is thought to be due to an increase in melanin and not due to iron excess itself. The increase in skin pigmentation is reversible after iron removal.

### Other Potential Complications

Iron overload has been speculated to aggravate atherosclerosis; however, the evidence for that speculation is rather weak (for review see [132]). In some reports extrahepatic malignancies were increased in HFE hemochromatosis while other studies did not find such an association [8, 11, 19, 58, 72, 129]. It is not yet clear whether HFE gene mutations are involved in the pathogenesis of porphyria cutanea tarda since the prevalence and association of both risk factors greatly vary in different parts of the world; associations between HFE gene mutations and porphyria have been described in particular for southern Europe but not for northern Europe (for literature see [191]).

## Therapy

### Phlebotomy

Phlebotomy treatment is the standard care to remove iron in genetic hemochromatosis. One phlebotomy removes approximately 200–250 mg iron from the body. Since patients with the classical clinical phenotype may have excess iron between 10–30 g, it may take 12–24 months to remove the iron overload when phlebotomies of 500 mL blood are done weekly (Table 82.5). Phlebotomy treatment is generally well tolerated and hemoglobin usually does not drop below 12 g/dL. Complete iron removal usually is seen when hemoglobin becomes decreased; at this time serum ferritin is often reduced to less than 20 ng/mL. Several studies have shown that liver iron is completely removed at such ferritin values; thus the effect of therapy can be checked by following ferritin measurements rather than by liver biopsy. After complete removal of excess iron the intervals of phlebotomies may be increased to 2–3 months; serum ferritin should be kept in the lower normal range between 20–50 ng/mL. Phlebotomy should not be interrupted for longer intervals because otherwise there is a risk of reaccumulation of iron to the genetic defect.

**Table 82.5** Therapy of iron overload

| | |
|---|---|
| **Phlebotomy** | |
| *In symptomatic genetic hemochromatosis* | |
| Aims: | Complete iron depletion in 12–24 months |
| Treatment: | 1–2 phlebotomies of 500 mL each week until serum ferritin is in the range of 20–50 ng/ mL |
| | Long-term therapy with 4–8 phlebotomies per year to keep ferritin between 20–50 ng/mL and thus to prevent reaccumulation of iron |
| *In asymptomatic C828Y homozygotes therapy should be initiated above the following ferritin values:* | |
| Subjects < 18 years: | >200 ng/mL |
| Men: | >300 ng/mL |
| Women (not pregnant): | >200 ng/mL |
| Women (pregnant): | >500 ng/mL |
| **Therapy with iron chelators in secondary hemochromatosis and anemia** | |
| Aims: | Removal of iron overload by increase of iron excretion in feces and urine |
| | In case of further blood transfusions at high frequency stabilization of iron balance and reduction of further iron accumulation are aimed at |
| Treatment: | Until recently 25–50 mg deferoxamine/kg as s.c. infusion for 10–12 h daily; today deferoxamine is largely replaced by the oral chelator deferasirox |
| | 20 mg/kg deferasirox once daily prevents iron accumulation up to 800 mL erythrocytes concentrates/month |
| | Long-term treatment necessary; normalization of ferritin and liver iron concentration is often not possible |
| **Diet** | |
| Recommended: | Avoidance of food with very high iron content (e.g. liver) and food with iron supplement |
| | A further strict iron-depleted diet is very difficult to perform and not recommended |
| | A single phlebotomy of 500 mL blood is as effective for iron removal as a very strict iron-depleted diet for a whole year |

## Iron Removal by Chelators

Therapy of genetic hemochromatosis with *deferoxamine* is not recommended because phlebotomy is more effective with fewer side-effects and costs. In secondary hemochromatosis, however, phlebotomy treatment is not possible because of the underlying anemia. Until recently, these patients had to be treated by continuous s.c. or i.v. infusions of the chelator deferoxamine for 8–12 h per day (Table 82.5). Deferoxamine chelates iron by increasing its excretion in the urine. Because of its short half-life and low oral bioavailability continuous parenteral application is necessary; these problems reduce the compliance in many patients. Just recently *deferasirox*, the first orally effective iron chelator, has been approved for secondary hemochromatosis, e.g. caused by poly-transfusions in thalassemia (see page 1064). Deferasirox (20 mg/kg once daily) can assure a neutral iron balance in patients who need up to 800 mL erythrocyte concentrates/month; it can even reduce iron stores when fewer transfusions are required (Table 82.6). Gastrointestinal and cutaneous side-effects of deferasirox are usually mild and reversible. Recently, a phase 2 study has started to look at the safety and efficacy of deferasirox in genetic hemochromatosis. As yet, deferasirox is only approved for secondary hemochromatosis.

**Table 82.6** Dose of deferasirox for secondary hemochromatosis

| | Aims of chelation therapy | |
|---|---|---|
| Transfusion rate (unit of erythrocyte pack) | Balance of iron metabolism | Reduction of iron overload |
| Low: < 2 units/ months | 10 mg/kg | 20 mg/kg |
| Moderate: 2–4 units/months | 20 mg/kg | 20 mg/kg |
| High: > 4 units/ months | 20 mg/kg | 30 mg/kg |

## Diet

An iron-depleted diet is not recommended for patients with genetic hemochromatosis. One phlebotomy of 500 mL blood removes approximately 250 mg iron. A

strict dietary iron restriction, which is difficult to observe, for a whole year would just have the effect of a single phlebotomy. It is thus only recommended that patients do not eat larger amounts of food with very high iron content (such as liver) and that they do not eat food to which iron has been added (Table 82.5).

### *Liver Transplantation*

Advanced liver cirrhosis and carcinoma may be indications for liver transplantation in hemochromatosis [20, 99]. The prognosis of patients with a liver transplant for hemochromatosis is markedly worse than that for patients with other liver diseases; a considerable number of patients with hemochromatosis died after transplantation from infectious complications or heart failure [20]. Liver transplantation does not cure the genetic defect.

## Prognosis

Untreated hemochromatosis often has a lethal prognosis in the presence of liver cirrhosis and diabetes mellitus. The prognosis is markedly worse in patients with cirrhosis when compared to those without cirrhosis at diagnosis; the same is true for diabetes mellitus. It is generally accepted that phlebotomy therapy improves the prognosis. *Patients diagnosed and treated in the early noncirrhotic stage have a normal life expectancy* (Fig. 82.3) [128, 129]. Thus, early diagnosis markedly improves the prognosis (Figs. 82.4 and 82.5). Iron removal by phlebotomy also improves the outcome in patients with liver cirrhosis. The prognosis of liver cirrhosis due to hemochromatosis is markedly better than in other types of cirrhosis [158]. Hepatomegaly and elevation of aminotransferases often regress after iron removal [128, 129]. Phlebotomy also improves the general well being. Insulin-dependent diabetes mellitus and hypogonadism are irreversible complications despite complete iron removal [129]. Earlier changes in glucose and insulin metabolism, however, may be ameliorated after iron removal (Table 82.4). For unknown reasons arthropathy does not respond well to phlebotomy treatment although it may be a rather early sign of iron overload. The prognosis of asymptomatic C282Y homozygotes is not exactly known. The AASLD consensus guideline recommends starting phlebotomy treatment when serum ferritin values exceed 300 ng/mL in men and 200 ng/mL in women. The risk for liver fibrosis and cirrhosis is increased at ferritin levels exceeding 1,000 ng/mL. Further studies need to determine whether asymptomatic C282Y homozygotes with ferritin values between 300 and 1,000 ng/mL should be treated or followed expectantly with serial ferritin measurements.

## Juvenile Hereditary Hemochromatosis

### *Prevalence*

Juvenile hemochromatosis is very rare. There is probably no specific ethnic background for juvenile hemochromatosis; subjects with such mutations have been identified in most parts of the world. Some clustering of HJV mutations has been identified in Italy and Greece although only a few families account for this phenomenon. Mutations in HJV represent the majority of worldwide cases of juvenile hemochromatosis. Only a small number of patients have been identified with HAMP-related juvenile hemochromatosis (see below).

### *Pathophysiology*

Juvenile hemochromatosis is characterized by onset of severe iron overload occurring typically in the first to third decades of life. Both sexes are equally affected since juvenile hemochromatosis is inherited in an autosomal recessive manner. Prominent clinical features include hypogonadotropic hypogonadism, cardiomyopathy, and liver cirrhosis. The main cause of death is cardiomyopathy. Two genes have been shown to be associated with juvenile hemochromatosis: (1) 90% of cases are associated with mutations in hemojuvelin (HJV) (locus name HFE2A) which encodes HJV; (2) 10% of cases are associated with HAMP (locus name HFE2B) which encodes hepcidin.

Despite the nomenclature of HFE2A and HFE2B, juvenile hemochromatosis is not associated with HFE mutations. In order to avoid confusion most physicians use the terms type *2A (hemojuvelin mutations)* and type 2B *(HAMP mutations)*.

The hemojuvelin protein has 425 amino acids with a size of 41 kd. The hydrophobic signal peptide is located at the N-terminal. At the C-terminal, there is a transmembrane domain and a glycophosphatidyl inositol sequence. The function of hemojuvelin protein is not fully understood, but it likely regulates the hepcidin pathway and is thought to be upstream of hepcidin [139]. Mutations in hemojuvelin are associated with low levels of hepcidin in urine suggesting that hemojuvelin regulates hepcidin. Twenty-six different HJV mutations, all in the coding region, have been thus far identified in homozygous or compound heterozygous forms. The mutation G320V is the most prevalent mutation (almost 60% of all cases) and has been reported in most Greek individuals with juvenile hemochromatosis, in all individuals with juvenile hemochromatosis from Quebec as well as in Canadian, French, Dutch, Italian, American, Albanian, Romanian, and Chinese families [102, 103, 106, 107, 153].

HAMP encodes the peptide hepcidin. The small 25-amino-acid peptide hepcidin is predominately expressed in liver and excreted by the kidney. Hepcidin is the key regulator of intestinal iron absorption and iron release from macrophages. Hepcidin facilitates ferroportin internalization and degradation. Hepcidin mutations may thereby lead to an increase in ferroportin and thus iron uptake from the intestine. Five different mutations in HAMP causing juvenile hemochromatosis in five independent families have been reported as yet. These individuals had an Italian, Greek, Arab or Portuguese origin [46, 113, 171, 172].

## Natural History

Juvenile hemochromatosis is characterized by early onset of severe iron overload. In contrast to HFE type 1 hemochromatosis, both sexes are equally affected. Juvenile hemochromatosis most often presents in the first to third decades. In clinical practice, individuals with juvenile hemochromatosis are rarely diagnosed before significant iron overload occurs. This problem will continue to exist unless there is some genetic screening during childhood. Typical features include hypogonadotropic hypogonadism, cardiomyopathy, and liver cirrhosis [51, 196]. The clinical course is severe and many patients die from cardiomyopathy [30, 45]. Severe cardiac failure may be the presenting finding [65]. Hepatocellular cancer has not been reported in juvenile hemochromatosis, probably because many patients have died prematurely in the past due to cardiac complications [26, 27]. Mortality can, however, be reduced also in juvenile hemochromatosis when it is diagnosed early and treated properly. Phlebotomy in asymptomatic patients can prevent organ damage.

The phenotype may be less progressive and severe in individuals with digenic inheritance of heterozygous mutations in HAMP and HFE or heterozygous mutations in HJV and HFE. However, digenic inheritance accounts for only a small proportion of genetic hemochromatosis. The presence of heterozygous HJV or HAMP mutations does not fully explain the variability in the penetrance of HFE-related hemochromatosis. In order to identify other genetic modifiers one needs to further study the effect of HJV, HAMP, HFE, and heme-related alleles on the phenotype [102, 103, 105, 111, 118, 146].

## Diagnosis

Data are minimal as documented cases are rare; however, the diagnostic procedures may follow the advice given for type 1 HFE hemochromatosis. Serum ferritin concentration in juvenile hemochromatosis is often high and has been reported to range from 1,000 to 7,000 ng/mL. Transferrin saturation often approaches 100%. Mutation scanning will detect most mutations in HJV-related juvenile hemochromatosis. Mutation scanning would only be unable to detect an extensive deletion. To date, the most frequent mutation in HJV is G320, accounting for more than 60% of all mutations.

## Treatment

Phlebotomy is also the standard therapy in juvenile hemochromatosis and is done similarly as in HFE hemochromatosis. The patients should have 1–2 phlebotomies of 500 mL blood each week because they usually have a large iron excess [188]. In patients with juvenile hemochromatosis and anemia or severe cardiac failure, administration of chelators such as

deferoxamine have been used in attempts to reduce mortality; some case reports suggest that such therapy might improve left ventricular ejection fraction [96].

## Transferrin Receptor 2 (TFR2)-Related Type 3 Hemochromatosis

### Prevalence

TFR2-related hereditary hemochromatosis is very rare with about 20 patients reported worldwide who came from Japan, Italy, France and Portugal [103, 114]. Screening for the TFR2 mutation Y250X among Italian blood donors with increased transferrin saturation identified a single Y250X heterozygote, resulting in a carrier frequency of 0.9% in this selected small cohort [45]. In contrast, screening of individuals with non-HFE-associated hemochromatosis worldwide did not show any Y250X mutation [5, 6, 12, 104].

### Pathophysiology

TFR2-related hemochromatosis is defined as type 3 and has also been named HFE3; however, the term HFE3 should not be used because the HFE gene is not affected. TFR2-related hemochromatosis is inherited in an autosomal recessive manner. TFR2 is a type II transmembrane glycoprotein; its full length transcript originates an 801-amino-acid transmembrane protein. TFR2 is mainly expressed in hepatocytes and at lower levels also in Kupffer cells [210]. TFR2 binds and internalizes transferrin. However, binding occurs at a 25- to 30-fold lower affinity as compared to that of the transferrin receptor 1 (TFR1) [94]. TFR2 protein shows significant amino acid homology with TFR1 [93]. A finely regulated interaction between TFR2, TFR1 and HFE is now thought to affect the hepcidin pathway [70]. TFR2 may also play an essential role for sensing of the iron status. Hepcidin concentrations in urine are low in TFR2 hemochromatosis [123]. In mice with a TFR2-Y245X targeted mutation and in TFR2-knockout mice hepatic mRNA of hepcidin was also down-regulated [95, 204]. The TfR2-deficient mouse homozygous for Y245X (orthologous to human Y250) shows iron overload in liver and other organs similar to human iron overload [27, 68, 69]. The TFR2 knockout mouse also shows iron overload and is unable to express hepcidin in response to iron [204].

TFR2 is 21 kb long and consists of 18 exons. Several polymorphisms are either silent or missense mutations [104]. A single nucleotide polymorphism in the non-coding region of TFR2 has also been identified [119]. Nine disease-causing mutations (E60X, R105X, M172K, Y250X, Q317X, L490R, V561X, AVAQ621–624del, Q690P) have been reported; most are rare or private [170]. The most frequent mutation is Y250X, identified in several Italian families [27, 170]. The Q317X mutation was associated with HFE C282Y/H63D compound heterozygosity in two siblings with a severe juvenile phenotype and in association with wild-type HFE in a brother with a type 1 disease [148]. Some pathological variants produce a truncated protein (E60X, R105X, Y250X, Q317X, V561X) while others produce an abnormally structured protein (AVAQ motif deletion, L490R, Q690P) [168].

### Natural History

Age of onset in TFR2-related type 3 hemochromatosis is earlier than in HFE type 1 form [77, 84, 152]. Progression is, however, slower than in juvenile type 2 hemochromatosis [45, 77, 168]. The phenotype is similar to type 1 disease with iron accumulation in liver, heart, pancreas and endocrine organs. Many patients present with fatigue, arthralgia, abdominal pain, decreased libido or with biochemical signs of iron oveload [27, 77, 84, 100, 168]. Complications of type 3 hemochromatosis include cirrhosis, hypogonadism, and arthropathy. Cardiomyopathy and diabetes mellitus appear to be rather rare. Hepatocellular carcinoma has not been observed in the small number of cases diagnosed as yet. Most individuals with type 3 hemochromatosis were of Italian or Japanese origin. Some of the Japanese male subjects affected already had liver cirrhosis at diagnosis [84, 100].

Similar to type 1 hemochromatosis, the penetrance of type 3 hemochromatosis is lower than 100%. One middle-aged woman who was homozygous for the E60X mutation had no evidence of clinical disease; a second female even had iron deficiency [168]. The presence of a H63D allele of HFE has been described in individuals with type 3 hemochromatosis [27, 152].

However, its contribution to the phenotype of iron overload remains unclear. In contrast, inheritance of compound heterozygosity for the HFE mutations C282Y and H63D and homozygosity for the TFR2 mutation Q317X produced a phenotype of severe juvenile hemochromatosis in one family [148].

### Diagnosis

TFR2-related hemochromatosis may be suspected in patients who present with biochemical or clinical evidence of iron overload but do not have HFE gene mutations. Diagnosis of TFR2-related hemochromatosis is similar to that in type 1 including liver histology and iron content [77]. Only a few mutations have been described in single families [27, 77, 84, 100, 102, 114, 168]. Mutations are spread over the entire gene. A panel of four mutations (E60X, M172K, Y250X, AVAQ621–624del) account for about 50% of mutations in individuals with TFR2-hemochromatosis. Mutation scanning followed by sequencing of variant fragments allows rapid screening of coding sequences and splice junctions and detects almost all mutations [15].

### Therapy

Standard therapy is iron removal by weekly phlebotomies similar to the management of type 1 disease. Individuals with increased ferritin should be treated in the same manner as HFE hemochromatosis.

## Type 4 Hemochromatosis–Ferroportin Disease

### Prevalence and Pathophysiology

Ferroportin-associated iron overload (also named ferroportin disease) was first recognized by Pietrangelo et al. in 1999 who described an Italian family with an autosomal dominant form of non-HFE hemochromatosis [144]. Many family members had iron overload resulting in liver fibrosis, diabetes, impotence, and cardiac arrhythmias. In addition to autosomal dominant inheritance, features distinguishing this entity from HFE hemochromatosis included early iron accumulation in reticuloendothelial cells and a marked increase in ferritin prior to that in transferrin saturation. Several patients showed a reduced tolerance to phlebotomy and became anemic despite elevated ferritin. In 2001 this form of non HFE hemochromatosis was linked to mutations of ferroportin which had just been identified as the basolateral iron transporter (also termed iron-regulated transporter 1/metal transporter protein 1–IREG-1/MTP-1) simultaneously by three groups [1, 52, 115, 121, 135]. Thereafter, numerous ferroportin mutations have been implicated in patients from diverse ethnic origins with previously unexplained hemochromatosis. Iron overload due to ferroportin mutations has been defined as type 4 hemochromatosis or ferroportin disease (for review see [147]).

Ferroportin shows a high level of expression in human placenta, liver, spleen, and kidney [52]. It is also expressed at the basolateral surface of duodenal enterocytes and required for intestinal iron absorption [52, 115, 116]. Ferroportin mRNA and protein expression are increased when iron absorption is high. In addition, ferroportin stimulated iron efflux following expression in Xenopus oocytes [115]. Thus, ferroportin is the intestinal iron exporter which is upregulated in hemochromatosis. Later it was shown that hepcidin binds to ferroportin in vitro [122]. Following that binding, ferroportin was internalized and degraded, leading to a reduction of the export of iron. Thus, posttranslational regulation of ferroportin by hepcidin completes a feedback loop regulating circulating iron and its tissue distribution [122]. Ferroportin was mapped to human chromosome 2q32 [81]. Then genetic analysis of ferroportin in a Dutch family with hemochromatosis type 4 identified a heterozygous A-to-C transversion at nucleotide 734 in exon 5 in all affected individuals [135]. At the same time Italian groups also mapped the disease locus responsible for autosomal dominant hemochromatosis type 4 to 2q32 and recognized ferroportin as the positional candidate [121, 144]. Several studies corroborated that the distinguishing features of this disorder, in addition to autosomal dominant inheritance, is early iron accumulation in reticuloendothelial cells and a marked increase in serum ferritin before that in transferrin saturation [67].

The iron export is tightly regulated because both iron deficiency and iron excess are harmful. The main

regulator of this mechanism is the peptide hepcidin which binds to ferroportin, induces its internalization and degradation, thereby reducing iron export [122]. Plasma iron levels, in turn, regulate hepcidin production, thus completing the feedback loop. Increase in iron absorption may be caused either by hepcidin deficiency or its ineffective interaction with ferroportin. All recent studies have shown that hepcidin deficiency appears to be the common characteristic of most types of genetic hemochromatosis (mutations in HFE, transferrin receptor 2, hemojuvelin, or hepcidin itself). The remaining cases of genetic iron overload are due to heterozygous mutations in the hepcidin target ferroportin. This phenotype was consistent with a loss-of-function hypothesis [67].

A second model for how mutations could cause disease is a gain of function model whereby overactive ferroportin leads to inappropriately high transport of iron from the diet through enterocytes, resulting in an iron overload phenotype, particularly observed in patients who have mutations at N144 or C326Y [166, 202]. In contrast to patients with loss of function, these patients have high transferrin saturation and serum ferritin, and liver biopsy shows that iron is located also in hepatocytes and not only in macrophages [203]. Ferroportin mutants associated with type 4 hemochromatosis thus fall into two categories, those that traffic poorly to the cell surface and loose iron export function (A77D, V162, and G490D) and those that retain cell-surface expression and export function (Y64N, N144D, N144H, Q248H, C326Y). The loss-of-function mutations probably cause disease by withholding iron from the bone marrow through sequestration within macrophages, subsequently leading to a signal for increased dietary iron uptake being sent from the erythron to the intestine. The second set of mutations may cause disease by resisting negative feedback mechanisms that normally operate to maintain iron homeostasis [175]. Recent studies further support the central role of the hepcidin-ferroportin interaction in regulating extracellular iron concentrations and to the concept that most iron overload diseases are caused by mutations affecting the production or function of either the ligand hepcidin or its target ferroportin [123].

Ferroportin disease or type 4 hemochromatosis is the most prevalent non-HFE type of hemochromatosis. Type 4 hemochromatosis does not result from deficient hepcidin production (as most other forms) but rather from mutations in ferroportin. In contrast to other forms of hemochromatosis, type 4 (ferroportin disease), is inherited dominantly. All known ferroportin mutations are missense mutations leading to amino acid substitutions or deletions. The phenotype can be classified into two groups, varying in both the severity of tissue iron loading and in the type of tissue affected. One group is characterized by an early rise in ferritin levels with low to normal transferrin saturation and iron accumulation predominantly in macrophages [31]. The other group is more similar to classical hemochromatosis, with high transferrin saturation and at least some parenchymal iron loading. The reason for the phenotypic variability is not completely clear, but it has been speculated that mutant ferroportin can multimerize with normal ferroportin and thus affect its function. Depending on the type of the genetic variation, mutant ferroportin may affect the cellular location of normal ferroportin or its responsiveness to hepcidin [44].

Patients with ferroportin disease often have high serum ferritin despite normal transferrin saturation (at least in early stages of disease) and iron deposits predominantly in the liver macrophages and to a lesser degree also in some hepatocytes [10, 13, 79, 121, 135, 144, 164, 169, 202, 203]. Patients often have a borderline anemia, and these cases do not respond well to phlebotomy [91, 144]. The ferroportin Q248H polymorphism has been associated with increased ferritin also in sub-Saharan Africans and in African Americans [165]. African Americans participants of the HEIRS study who did not have HFE C282Y or H63D but had elevated ferritin were matched to participants with normal ferritin to investigate the association of the Q248H with elevated ferritin. The frequency of Q248H was higher among men with elevated ferritin when compared to those with normal ferritin [165]. At this point the pathophysiology of iron overload in Africans remains rather unclear.

## *Natural History*

Little is known about the natural history of ferroportin-associated iron overload. In the first Italian families with ferroportin disease, some family members had clinical signs of iron overload resulting in liver fibrosis, diabetes, impotence, and cardiac arrhythmias [144]. Later it became clear that the clinical penetrance of the genetic defect is rather mild. It has recently been

reported that patients carrying the A77D mutation of ferroportin may develop relevant iron overload with organ damage and liver cancer [40]. It remains however unclear whether there is a good genotype-phenotype correlation in ferroportin disease.

### Diagnosis

Increase in serum ferritin is a common laboratory finding also in the absence of iron overload. In the presence of early-onset bilateral cataracts hereditary hyperferritinemia-cataract syndrome may be suspected; this disease results from a heterozygous mutation in the L ferritin IRE sequence [88]. An analysis of 52 DNA samples from patients referred for suspected hyperferritinemia-cataract syndrome identified 24 samples with a point mutation or deletion in the IRE. The 28 samples without an IRE mutation were then genotyped for mutations in HFE, H ferritin and ferroportin genes showing an increased frequency (12 of 28) of heterozygotes for the H63D mutation but no H ferritin mutations. In addition, three novel ferroportin mutations were identified suggesting that these patients had type 4 hemochromatosis. Thus both L ferritin IRE and ferroportin mutations can cause hyperferritinemia. The knockout of the ferroportin gene in mice results in prenatal death [53]. Heterozygous animals showed mild abnormalities of iron homeostasis. Mice with ferroportin deletion restricted to the intestines developed severe iron deficiency anemia. Thus ferroportin is essential for prenatal and postnatal iron homeostasis [53]. Disease-associated ferroportin mutations include Y64N, A77D, N144D and N144H, N144T, V162, Q248H, C326Y, and G490D in patients with iron overload and N174I, Q182H, and G323V in patients with hyperferritinaemia [10, 12, 49, 79, 88, 91, 121, 135, 164, 166, 169, 202].

### Therapy

Because of the mild clinical penetrance of the genetic defect there had been doubts about the rationale for iron-removal therapy. However, a recent study shows that there may be clinically relevant iron overload with organ damage and liver cancer in patients carrying the A77D mutation of ferroportin [40]. Treatment schemes are similar to those described for other types of genetic hemochromatosis.

## Secondary Hemochromatosis

### Pathophysiology

Most forms of secondary hemochromatosis are due to hemolytic anemia associated with multiple transfusions, such as thalassemia, sickle cell disease, and myelodysplastic syndrome. Most of these patients need blood transfusion on a regular basis for survival. The transfusions have markedly improved the prognosis and quality of life in patients with thalassemia major [137, 154, 156]. However, in the long run multiple blood transfusions often lead to iron overload if patients are not treated with iron chelators. Each pack of erythrocytes contains 200–250 mg iron, which is approximately 100-fold of the dietary iron daily absorbed from the intestine [137]. Iron from degraded red blood cells is primarily taken up by macrophages; thereafter, iron can be bound by transferrin and may reach various organs. In general, iron overload due to blood transfusions is similar to genetic hemochromatosis; however secondary iron overload develops much faster than the genetic forms, sometimes already after 10–12 blood transfusions [117, 156]. Subsequently, secondary iron overload also results in a more rapid organ damage when compared with genetic hemochromatosis. Secondary iron overload can obviously not be treated by phlebotomies because a marked anemia is the clinical landmark of the disease. Secondary iron overload often limits the prognosis of patients with thalassemia; life expectancy deteriorates with increasing iron concentrations in the liver [190]. Therapy with iron chelator may reduce the transfusional iron burden if the frequency of transfusion is not too high. In severe hemolytic anemia with frequent transfusions, iron chelation may sometimes only reduce the further increase in iron overload. It has also been speculated that iron chelation exerts some beneficial effects by reducing the labile iron pools and thus the non-transferrin bound iron (NTBI) [141, 155]. NTBI is thought to be very toxic and to predominantly cause the damage to cardiomyocytes in secondary hemochromatosis. The development of HFE versus secondary hemochromatosis does not

only differ in terms of the speed of iron accumulation but also in the type of organ damage; in secondary hemochromatosis cardiomyopathy is often the complication which determines the prognosis [109]. It is interesting that heart disease is also very frequent in juvenile genetic hemochromatosis in which there is also a rapid iron accumulation.

## Diagnosis

In general, serum ferritin values closely reflect liver iron concentration and may be used to determine the indication and timing of therapy and to check the effects of iron chelation.

## Therapy

Until recently *deferoxamine* was the only iron chelator available in most countries. In some countries the drug deferiprone was also approved for patients who did not tolerate deferoxamine [89]. The clinical use of deferiprone was limited due to its side-effects, such as agranulocytosis and neutropenia [7]. Long-term data prove that deferoxamine can reduce iron overload and its organ complications [35, 48, 120, 137]. Deferoxamine, however, needs to be given daily by s.c. route or by i.v. infusion for several hours. Thus, patients with thalassemia often experienced the deferoxamine treatment worse than thalassemia itself [78]. Although deferoxamine in general may improve patients' prognosis, minor compliance problems often limit the beneficial effects of this iron chelator [23, 36, 156].

Without iron chelation, children with thalassemia often develop severe cardiomyopathy prior to age 15 [37, 60]. After age 15 liver cirrhosis is also a significant complication in secondary iron overload due to thalassemia, while transfusion-associated hepatitis C also plays a major role for the development of liver disease in addition to iron toxicity [34, 211]. Theoretically one might be able to normalize liver iron in patients with thalassemia [22]. In clinical practice, however, the doses of deferoaxmine needed are so high that the chelator has toxic side-effect on nerves and eyes. Thus, in most patients one would tolerate slightly elevated liver iron concentrations [138]. The optimal age at which chelation therapy should be started in patients with severe thalassemia is unclear. In general chelation therapy should start early enough to prevent complications of iron overload. Very early chelation by deferoxamine before age 3 however was associated with metaphysal dysplasia [50, 136, 150]. Already between ages 3–5, liver iron concentration may reach values which are associated with a significant risk for liver fibrosis in severe thalassemia [9]. Children who are younger than 5 years should therefore be cautiously be treated with chelators if they have received transfusions for more than one year in the past [138]. Deferoxamine can reduce the incidence and ameliorate the course of iron associated cardiomyopathy [23, 120, 137, 162].

*Deferasirox* is a new oral iron chelator with high selectivity for iron III [124]. Deferasirox binds iron in a 2:1 proportion with a high affinity and increases the biliary iron excretion. This chelator is able to reduce iron overload in hepatocytes and cardiomyocytes [87, 124, 125]. Due to its half-time of 11–18 h it needs to be taken only once daily [74, 134]. Deferasirox exerts similar iron chelation when compared with deferoxamine in patients with thalassemia; the effect of 40 mg/ kg deferoxamine was similar to that of 20 mg/ kg deferasirox [151]. In adults with thalassemia and iron overload deferasirox at daily doses of 10, 20 and 40 mg/ kg resulted in a daily loss of 0.12, 0.33, and 0.45 mg iron/ kg body weight. A large phase 3 study analyzed the clinical effects of deferasirox in 287 adults patients (>16 years) and in 299 pediatric patients (2–16 years) with secondary iron overload [29]. Both in adults and children 20–30 mg/kg/day deferasirox reduced liver iron concentration during a 1-year treatment by 0.4 and 8.9 mg Fe/g, respectively, and also reduced serum ferritin by 36 and 926 ng/mL, respectively. At the 20 mg/ kg dose iron balance was neutral despite further transfusions while the 30 mg/kg dose resulted in a net loss of iron. MRT showed that 10–30 mg/day deferasirox reduced iron concentration in the heart after one year of therapy. Deferasirox may cause minor increases in serum creatinine as well as mild gastrointestinal discomfort and skin exanthema which are usually self limiting. The compliance is better for deferasirox when compared with deferoxamine [29]. The chelating effects of deferasirox do not depend on the underlying type of hemolytic or myelodysplastic disease [75, 157, 189, 198]. Considering the compliance problems with deferoxamine, deferasirox also has a better cost-effectiveness [47, 92, 197]. Thus, according to international guidelines deferasirox is now the standard therapy of secondary iron overload diseases [76, 80]. Data pooled from

several studies show that the dose of deferasirox should be chosen according to the iron load already documented and according to the rate of transfusions (Table 82.6) [38, 75, 80]. Currently a phase 2 study is analyzing the safety and efficacy of various doses of deferasirox in patients with HFE genetic hemochromatosis.

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# $\alpha_1$ - Antitrypsin Deficiency

# 83

Henryk Dancygier

## Chapter Outline



$\alpha_1$-antitrypsin is a 56 kd-glycoprotein ($\beta_1$-globulin; 394 amino acids with 3 carbohydrate side chains) belonging to the family of serine protease inhibitors also called "serpines". It is secreted into the circulation primarily by the liver and to a lesser extent by macrophages. Serpines regulate proteolytic cascades, for example in inflammatory processes, blood coagulation and complement activation.

$\alpha_1$-antitrypsin usually occurs in lacrimal and nasal secretions, saliva, duodenal juice, bronchial secretion, cerebrospinal fluid and breast milk. The concentration of $\alpha_1$-antitrypsin in amniotic fluid commonly corresponds to approximately 10% of its normal serum level. Inflammation, infections, malignant diseases, pregnancy or estrogen treatment may lead to a two- to threefold increase in serum concentration. Patients with $\alpha_1$-antitrypsin deficiency, however, do not show a noteworthy induction by these stimuli.

The antiproteolytic activity of $\alpha_1$-antitrypsin (half life 4–5 days) is not only directed against trypsin, but also against many serine proteases (for example chymotrypsin, pancreatic elastase, collagenase, renin, urokinase, clotting factor XII) and neutral proteases of polymorphonuclear leukocytes. Therefore, the old term $\alpha_1$-antitrypsin is often replaced by the more inclusive term $\alpha_1$-protease inhibitor. The antiproteolytically active center of $\alpha_1$-antitrypsin is located near the carboxyterminus of the polypeptide. Here the peptide chain forms a loop whose amino acid sequence keeps the molecule in a metastabile form (Fig. 83.1). This loop with methionine in position 358 forms the structural basis for the inhibition of serine proteases. Oxidation of methionine abolishes the antiproteolytic effect. The most important substrate for $\alpha_1$-antitrypsin is neutrophil elastase. Protease inhibition occurs by forming a 1:1 complex of $\alpha_1$-antitrypsin and its target protease.

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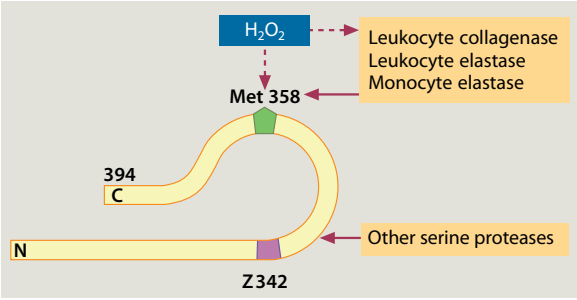


**Fig. 83.1** Structure of the active center of $\alpha_1$-antitrypsin. The molecule forms a loop as a prerequisite for its antiproteolytic effect. The amino acid at position 358 determines the substrate specificity. For example, methionine: inhibition of leukocyte elastase; arginine: inhibition of thrombin. Oxidation of methionine leads to the loss of enzymatic activity. Z mutation results in an exchange of amino acids at position 342

## Definition

The classical form of $\alpha_1$-antitrypsin deficiency is an autosomal-codominant disorder that was described for the first time in 1963. Reduced serum levels of $\alpha_1$-antitrypsin or its complete absence may lead to liver disease and to the development of pulmonary emphysema.

The changes in the liver are characterized by a secretory defect of mutant $\alpha_1$-antitrypsin which accumulates in the endoplasmic reticulum of hepatocytes forming globular inclusions. In the pediatric patient, liver disease may manifest as neonatal cholestasis or juvenile cirrhosis, in the adult as chronic liver disease leading to cirrhosis and hepatocellular carcinoma. Most patients die of respiratory failure (50–70%; not discussed in this chapter), 10–15% of decompensated liver cirrhosis [8].

## Epidemiology

$\alpha_1$-antitrypsin deficiency is a relatively frequent autosomal-codominant disorder caused by the mutation of two alleles. It occurs in approximately 1:1,800 live births and is the most common genetic cause of liver disease in children.

## Etiology and Pathogenesis

The gene coding for $\alpha_1$-antitrypsin is located on chromosome 14 close to the genes for the immunoglobulin heavy chains. It consists of five exons. While exon I regulates gene expression by binding nuclear proteins, exon V is coding for the active center of $\alpha_1$-antitrypsin. Several point mutations of $\alpha_1$-antitrypsin cause a perturbation in protein structure ($\alpha_1$-antitrypsin Z or $\alpha_1$-ATZ) with consequent polymerization and intracellular accumulation. The retention of polymers of $\alpha_1$-antitrypsin within hepatocytes results in protein overload [8].

The reduction of serum levels of $\alpha_1$-antitrypsin is not caused by an impairment of protein synthesis at the translational or transcriptional level, but is due to a *transport defect of the mutant protein across the endoplasmic reticulum to the Golgi apparatus*. The translated protein contains a sequence of 24 amino acids at its N-terminus, signaling its location in the endoplasmic reticulum where carbohydrate side chains are attached to three asparagine residues. Usually a modification of these carbohydrate chains takes place in the Golgi apparatus with transformation of the molecule from the "high mannose" to the "complex type" (processing). This transformation is the prerequisite for the secretion of the protein from the liver into the extracellular space. The mutant Z-gene leads to an amino acid substitution (lysine for glutamic acid at position 342) resulting in an unstable molecule. The ensuing "aggregate" of $\alpha_1$-antitrypsin molecules, due to its new physical properties cannot be transported through the tubular system of the endoplasmic reticulum. Structural processing in the Golgi apparatus cannot occur, thus preventing exocytosis of $\alpha_1$-ATZ proteins into the blood. However, single proteins may separate from the aggregates, cross the Golgi apparatus and appear in the circulation. These $\alpha_1$-ATZ monomers exhibit partial protease inhibitor activity (see below) and explain residual $\alpha_1$-antitrypsin levels in serum.

The complete causal chain that leads to liver damage remains elusive. The retention of $\alpha_1$-ATZ in the endoplasmic reticulum, hepatocyte damage induced by uninhibited leukocyte proteases, and stimulation of cellular autoimmune reactions are currently discussed. A correlation between $\alpha_1$-antitrypsin deficiency in serum, its retention in the endoplasmic reticulum and the development of liver damage could be experimentally demonstrated in phenotype PiZZ animals.

The $\alpha_1$-antitrypsin phenotype is extremely variable. Currently, more than 100 allelic variants of the $\alpha_1$-antitrypsin gene are known. Two alleles determine an individual's phenotype. They may be differentiated by their electrophoretic mobility on isoelectric focussing

and are designated with capital letters. Fast moving proteins receive the first letters of the alphabet, while the most slowly moving protein is denominated with letter Z. The most common phenotype is PiMM (Pi = protease inhibitor) is associated with normal serum levels of $\alpha_1$-antitrypsin. In Europe, in addition to the most prevalent M allele, S and Z alleles occur at significantly lower frequencies. Cryptic genetic variants within the $\alpha_1$-antitrypsin gene may contribute to susceptibility to liver disease. Single nucleotide polymorphisms that confer a significant risk for liver disease have been identified [2]. In Table 83.1 the most important $\alpha_1$-antitrypsin phenotypes, their prevalence, their impact on $\alpha_1$-antitrypsin serum levels and the development of liver disease is reported.

## Pathology

The microscopical changes are characterized by numerous distinct globular eosinophilic intracytoplasmic inclusions. They occur predominantly in periportal or periseptal hepatocytes, but may occasionally be seen in the cytoplasm of bile duct epithelial cells. Concomitant steatosis is often present.

On PAS staining after diastase digestion the diastase resistant inclusions are bright red to purple (Fig. 83.2). The diagnosis can be confirmed by specific immunohistochemical staining (Fig. 83.3). An antibody that specifically recognizes a conformation-dependent neoepitope on polymerized $\alpha_1$-antitrypsin has also been described [7].

In the heterozygous state inclusions are present without associated liver disease. In the adult with homozygous $\alpha_1$-antitrypsin deficiency, the disease may progress to micronodular or mixed macro- and micronodular type cirrhosis with little inflammatory changes in the fibrous septa and parenchyma. In neonates and infants biliary lesions predominate. Neonatal cholestasis, ductular reaction and fibrosis occur. Occasionally liver disease progresses to biliary type cirrhosis.

## Clinical Manifestations

The clinical manifestations of patients with homozygous PiZZ $\alpha_1$-antitrypsin deficiency are highly variable. They depend on the age at first manifestation, on the organs involved and the rate of progression. The major organs involved are lungs and liver. Pulmonary disease is characterized by early developing pulmonary emphysema. Extrahepatic manifestations may rarely occur and encompass pancreatic fibrosis, membranoproliferative glomerulonephritis, panniculitis,

**Table 83.1** $\alpha_1$-antitrypsin phenotypes

| Allelic pattern | Amino acid position | Amino acid | Homo-/heterozygous | $\alpha_1$-antitrypsin concentration in serum (N = 100–300 mg/dL) | Significance |
|---|---|---|---|---|---|
| Pi**MM** | 264<br>342 | Glutamate<br>Glutamate | Homozygous | 100% | Normal phenotype |
| Pi**ZZ** | 342 | Lysine | Homozygous | 10–15% of N | Marked configurational change with secretory defect.<br>Hepatic and pulmonary changes. |
| PiM**Z** | 342 | Lysine | Heterozygous | 60% of N | 3% of population.<br>No liver damage. |
| Pi**SS** | 264 | Valine | Homozygous | 60% of N | Configurational change.<br>Rapid protein metabolism by accelerated mRNA-degradation (no secretory dysfunction). |
| PiM**S** | 264 | Valine | Heterozygous | 60% of N | 7% of population.<br>No liver damage. |
| Pi**SZ** | 264<br>342 | Valine<br>Lysine | Heterozygous | 40% of N | Liver damage less severe than in PiZZ. |

*N* normal

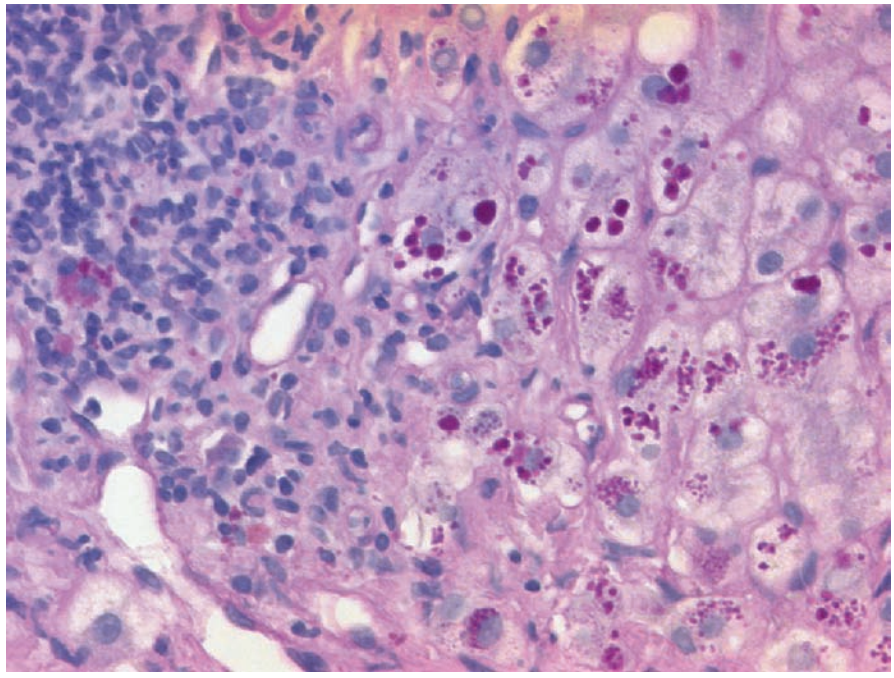

**Fig. 83.2** $a_1$-antitrypsin deficiency. PAS positive, diastase resistant intracellular globules are present in periportal repatocytes. (PAS staining after diastase digestion; x 400)

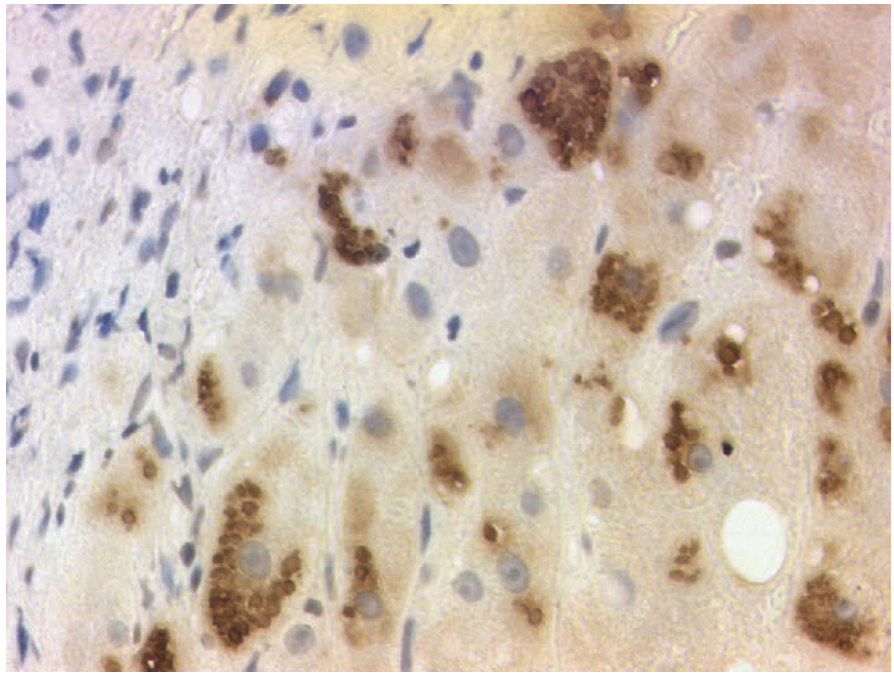

**Fig. 83.3** $\alpha_1$-antitrypsin immunoreactive hepatocytes. Note the accentuation in staining of the outer border of the globules. Avidin-Biotin Peroxidase

systemic vasculitis, and vascular aneurysms [4, 11]. The following discussion is confined to liver disease.

In the *newborn,* neonatal cholestasis with persistent jaundice is often noticed in the first or second month. The liver may be enlarged and palpable. Pruritus, hypercholesterolemia, dark urine, steatorrhea with acholic stools and deficiency of fat soluble vitamins may occur. The clinical picture resembles extrahepatic biliary atresia (see Chapters 56 and 109). Some children show growth retardation. Occasionally the

disease may manifest with gastrointestinal bleeding, bleeding from the umbilical cord stump or by an increased propensity to develop hematomas. Only approximately 10% of children with $\alpha_1$-antitrypsin deficiency exhibit a hepatosplenomegaly with ascites and impaired synthetic liver function in early childhood. The prevalence of acute liver failure in childhood is even less.

The disease may also first manifest in *juveniles* or in *early adulthood* with an increase in abdominal girth due to hepatosplenomegaly or ascites or by bleeding esophageal varices. In some of these patients an accurate history may reveal a prolonged neonatal jaundice of unknown cause.

$\alpha_1$-antitrypsin deficiency is also a rare cause of hepatic disease in *adults* presenting with signs of chronic liver disease, portal hypertension or with a hepatocellular carcinoma.

## Laboratory Findings

In addition to an elaborate family history (diseases of liver and lungs?) and a history of the perinatal period the diagnosis of $\alpha_1$-antitrypsin deficiency is based on common laboratory findings (serum electrophoresis [$\alpha_1$-antitrypsin is an $\alpha_1$-globulin], aminotransferases, parameters of cholestasis and bilirubin, synthetic liver function). Serum electrophoresis should be used as the initial screening test. *An $\alpha_1$-antitrypsin deficiency often may already be recognized by a reduced or absent $\alpha_1$-globulin fraction on serum electrophoresis*. Additionally, $\alpha_1$-antitrypsin concentration should be measured directly. Serum levels of $\alpha_1$-antitrypsin less than 20% of normal hint towards a homozygous type PiZZ, while a decrease to 40–70% of normal is caused by the heterozygous types PiSZ or PiMZ. $\alpha_1$-ATZ is demonstrated by isoelectric focussing, where it causes an atypical band, due to its altered mobility in the electrical field. $\alpha_1$-antitrypsin phenotype should be determined in all cases of neonatal hepatitis or chronic liver disease of unknown origin in child- and early adulthood. A monoclonal antibody is available for identification and quantitative measurement of type PiZZ that may also be used in prenatal diagnosis of $\alpha_1$-antitrypsin deficiency. Since the gene has been cloned and sequenced, diagnosis at the genetic level is also possible [12].

The histological findings (see above) complement the diagnosis.

## Differential Diagnosis

The differential diagnosis has to include cholestatic syndromes in childhood, especially extrahepatic biliary atresia and viral infections. For further discussion see Chapters 56, 85 and 109.

## Course and Prognosis

A national screening study in Sweden showed that of 200,000 newborns screened 120 had PiZ [9]. Fourteen of them had prolonged obstructive jaundice, nine with severe clinical and laboratory evidence of liver disease. Five had only laboratory evidence of liver disease. Eight other PiZ infants had minimal abnormalities in serum bilirubin and hepatic enzyme activity and variable hepatosplenomegaly. All PiZ infants with hepatic abnormalities appeared healthy at 6 months of age.

During further follow-up liver cirrhosis may develop in early childhood. It is unknown whether genetic or exogenous factors abet progression. Possibly patients in whom $\alpha_1$-ATZ cannot be adequately degraded within the endoplasmic reticulum are at particular risk of disease progression. Ultimately only about 10–15% of patients with $\alpha_1$-antitrypsin deficiency develop a clinically significant disease within the first 20 years of life. It may be assumed that in a significant portion of the remaining patients histological changes are present that, however, will lead to overt liver disease later in life [10, 13].

Fifteen percent of male adults 50–60 years old with $\alpha_1$-antitrypsin deficiency of the PiZZ type have cirrhosis. It is currently unknown why only a small proportion of PiZZ individuals progress to clinically significant disease. Men with $\alpha_1$-antitrypsin deficiency and cirrhosis are also at a significantly higher risk for primary liver cancer [5].

Currently there is no evidence that the heterozygous phenotype (PiMZ or PiSZ) predisposes children or adults for clinically relevant liver disease.

## Therapy

A causal treatment of liver disease induced by $\alpha_1$-antitrypsin deficiency is not available.

The administration of chemical chaperones that mediate increased secretion of mutant $\alpha_1$-ATZ as a potential pharmacological strategy for prevention of liver injury and emphysema has generated encouraging results in animal experiments but did not live up to its expectations in humans [1].

Gene therapy influencing synthesis and secretion of $\alpha_1$-ATZ by introducing viral vectors is in its initial experimental stages and not ready for clinical use [3].

Orthotopic liver transplantation is the treatment of choice in advanced liver cirrhosis [6].

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# Porphyrias

# 84

Ulrich Stölzel and Manfred O. Doss

## Chapter Outline



## Abbreviations

| | |
|---|---|
| AIP | Acute intermittent porphyria |
| ALA | δ-Aminolevulinic acid |
| ALAD | δ-Aminolevulinic acid dehydratase porphyria dehydratase |
| ALADP | δ-Aminolevulinic acid dehydratase porphyria (Doss porphyria) |
| ALAS | δ-Aminolevulinic acid synthase |
| CEP | Congenital erythropoietic porphyria (Morbus Günther) |
| CHP | Chronic hepatic porphyria |
| COPRO | Coproporphyrin |
| CPO | Coproporphyrinogen oxidase |
| EPP | Erythropoietic protoporphyria |
| FECH | Ferrochelatase |
| HCC | Hepatocellular carcinoma |
| HCP | Hereditary coproporphyria |
| HEP | Hepatoerythropoietic porphyria |
| HEPTA | Heptaporphyrin |
| PBG | Porphobilinogen |
| PBGD | Porphobilinogen deaminase |
| PPO | Protoporphyrinogen oxydase |
| PROTO | Protoporphyrin |
| URO | Uroporphyrin |
| UROD | Uroporphyrinogen decarboxylase |
| UROS | Uroporphyrinogen III synthase |
| VP | Variegate porphyria |
| XLPP | X-linked Protoporphyria |

## Definition

Porphyrias are a heterogeneous group of metabolic disorders, which are based on a genetic deficiency along the heme synthesis (Table 84.1) [2]. Only the

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**Table 84.1** Enzyme defects along the heme synthesis in porphyrias, regulated induction of ALAS1, and major clinical manifestations

| Porphyria | Enzyme | Induction of ALAS1 | Major clinical manifestation | | | |
|---|---|---|---|---|---|---|
| | | | Neurovisceral | Cutaneous | Anemia | Liver |
| ALADP (Doss) | ALAD | + | ++ | – | –/+ | – |
| Lead intoxication | ALAD | + | + | – | +[a] | +[b] |
| AIP | PBGD | + | ++ | – | – | – |
| CEP (Günther) | UROS | – | – | ++ | ++ | –/+ |
| PCT/HEP | UROD | – | – | + | – | |
| HCP | CPO | + | + | –/+ | – | + |
| VP | PPO | + | + | –/+ | – | – |
| EPP | FECH | – | – | –/+ | –/+ | – |

[a]Lead also inhibits the enzymes CPO and FECH
[b]"Lead hepatitis"

biochemical results of the metabolic disorders and not mutations or enzyme deficiencies per se lead to clinical manifestations, in which interoperating endogenous and exogenous factors are triggered off.

Clinically they can be divided into acute and non-acute porphyrias, as well as pathogenetically into hepatic and erythropoietic porphyrias [20]. Apart from the recessive congenital erythropoietic porphyria (Günther's disease) and the exclusively cutaneous type of protoporphyria, all other porphyrias are classified as hepatic.

The clinical presentation of acute hepatic porphyrias includes an abdominal–neuropsychiatric–cardiovascular syndrome, whereas chronic hepatic, erythropoietic porphyrias present with dermatological symptoms due to photodermatosis. The symptomatic phase of acute hepatic porphyria is characterized by excessive accumulation and excretion of the porphyrin precursors δ-aminolevulinic acid (ALA), porphobilinogen (PBG) and porphyrins. This includes the intermittent acute variations, which are distinguished via the localisation of the genetically disturbed enzyme sequence. Among acute porphyrias, the acute intermittent porphyria (AIP) is most common, followed by the variegate porphyria (VP), the hereditary coproporphyria (HCP) and a rare recessive acute hepatic porphyria with δ-aminolevulinic acid-dehydratase deficiency (ALADP, synonym: porphobilinogen-synthase-defect porphyria, Doss-porphyria), which is biochemically similar to lead poisoning. Therefore lead poisoning as toxic and toxo-genetic acute porphyria can be integrated into the acute types.

The opposite of acute porphyrias are the porphyria cutanea tarda (PCT) as a chronic hepatic porphyria (CHP) and the erythropoietic porphyria (EPP) with likewise chronic course.

The diagnosis of clinical symptomatic porphyrias is based on the biochemical analysis of the metabolic excesses, in which each porphyria expresses its own specific diagnostic (excretory) constellation.

Porphyrias are characterised by a huge molecular heterogeneity. They can be caused by a multitude of different mutations. Genetic defects of homozygous porphyrias show that there is quite often a compound heterozygosity. This means that both alleles are differently mutated. Rare homozygous forms can also be found among dominant autosomal hereditary porphyrias. Molecular testing is helpful to screen asymptomatic carriers. However, since asymptomatic gene carriers of porphyric disorders are common in the normal population, genetic testing is not recommended for clinically diagnosing porphyrias. Although there is a considerable interplay between gene defect and environmental factors, only a minority of cases may occur as predominantly acquired disease.

Dual porphyrias have been diagnosed in rare cases with clinical and biochemical findings of two porphyrias. For example, a double heterozygous condition of coexistent AIP and PCT genes in the same individual was described.

Apart from the genetically predisposed erythropoietic and hepatic porphyrias, clinically asymptomatic secondary porphyrinurias and porphyrinemias can be detected. The latter appear reactive among a number of different diseases and dysfunctions.

## Epidemiology

The three most important porphyrias are the acute intermittent porphyria (AIP), the Porphyria cutanea tarda (PCT) and the erythropoietic protoporphyria (EPP).

The observed frequencies among different porphyrias diagnosed in the German Competence Center for Porphyria Diagnosis and Consultation (www.porphyria.com) between 1965 and 2006 are shown in Table 84.2. The proportion among acute porphyrias in that period was AIP:VP:HCP:ALADP = 71:11:6:0.3.

Hepatic types occur much more often compared to erythropoietic porphyrias.

Data recorded from surveys of porphyria centres in Europe listed in Table 84.3 show the prevalence of the genetic defects.

The secondary (asymptomatic) dysfunctions of porphyrin metabolism caused by other different diseases outweigh by far the "primary" porphyrias.

The asymptomatic secondary coproporphyrinuria is in the first place of all hepatic porphyrin metabolic disorders, before the primary porphyrias.

Recently in Germany some patients were diagnosed with lead induced acute porphyria due to lead contaminated marijuana.

## Etiology and Pathogenesis

### *Acute Porphyrias*

The pathogenesis of acute hepatic porphyrias (AIP, VP, HCP, ALADP, lead poisoning) is multifactorial. The hereditary enzyme defect destabilizes the normally impeccably regulated hepatic porphyrin biosynthesis. This defect can persist without any clinical effects for a lifetime. The majority of the gene carriers (circa 80%) never develop a clinical porphyria manifestation with increased urinary excretion of porphyrin precursors and porphyrins. Moreover, in some individuals typical biochemical signs of a latent state can be seen in the absence of complaints and clinical symptoms.

The final product heme itself regulates, via repression of the key enzyme ALAS 1, the production of porphyrin precursors and porphyrins in the liver [6].

**Table 84.2** Number and sex ratio (f/m) of different porphyrias diagnosed in the German Competence Center for Porphyria Diagnosis and Consultation (www.porphyria.com) (1965–2006)

| Porphyrias/acute hepatic porphyrias | | |
|---|---|---|
| Porphyrias | Number of cases | (f/m) |
| Porphyria cutanea tarda (chronic hepatic porphyria) | ~2,000 | ~1/2 |
| Protoporphyria (erythropoietic and erythrohepatic) | 198 | ~1/1 |
| Congenital erythropoietic porphyria (Günther's disease) | 32 | ~1/2 |
| Acute intermittent porphyria | 706 | ~2/1 |
| Variegate porphyria | 105 | ~1/1 |
| Hereditary coproporphyria | 61 | ~2/1 |
| Doss-porphyria | 3 | –/3 |
| Total | 3,105 | ~1/1.3 |

**Table 84.3** Gene mutations and prevalence of various porphyrias

| Porphyria | Chromosome location | Number of mutations | Prevalence* |
|---|---|---|---|
| ALADP (Doss) | 9q34 | 8 | Rare** |
| AIP | 11q23.3 | >200 | 5–10 |
| CEP (Günther) | 10q25.2–q26.3 | >20 | 0.1 |
| PCT/HEP**** | 1p34 | >50 | 20–50 |
| HCP | 3q12 | >30 | 0.5 |
| VP | 1q22 | >100 | 1 |
| EPP | 18q21 | >100 | 0.5*** |

*Prevalence (cases/100,000) reported from various centers in Europe
**So far six cases described
***High prevalence in South Africa (founder effect)
****Only 20 cases of HEP so far described

Exogenous and endogenous influences such as drugs, fasting, stress, alcohol or hormones may cause consumption of regulatory heme (e.g. for cytochromes) in the liver con-sequenced by de-repressing ALAS 1.

This induction is the starting phase of the hepatic porphyria process leading to excessively stimulated porphyrin synthesis and expansion of porphyrin precursors and porphyrin metabolites in tissue and in blood. Both porphyrin precursors ALA and PBG are considered to exhibit neuropharmacological effects.

The metabolic explosive character of the increase in porphyrin precursors masks the underlying hereditary enzyme deficiency and leads, in terms of overcompensation, to an excessive porphyrin synthesis. The increase of metabolites correlates with symptoms of the acute clinical porphyria crisis. The pathophysiological links between metabolic derangement and expression of clinical symptoms have yet to be elucidated.

A clinical manifestation without an increase of ALA and PBG does not exist. The deficiency of the enzyme activity remains constant for life and is not an indicator of the pathogenetic, metabolic and clinical process of the disease. The disease is triggered by the induction of hepatic ALAS and corresponding metabolic reactions.

The molecular genetics of acute porphyrias is shown in Table 84.3.

### Acute Intermittent Porphyria

Mutations in the porphobilinogen deaminase (PBGD) gene are without any doubt associated with the manifestation of AIP. It is transmitted as an autosomal dominant trait. The activity of the enzyme can be reduced with diminished (cross-reactive immunological material (CRIM) negative) (Type I) or normal protein content of the PBGD (CRIM-positive) (Type III).

The human gene is located on the large arm of chromosome 11 splitting into 15 exons including 10 kb of DNA. Two different promoters produce either an erythroid or a non-tissue-specific isoenzyme. Rare mutations in areas, which are reserved exclusively for the non-specific enzyme, do not lead to alterations of the erythroid form of the isoenzyme. As a result, a subtype of the acute intermittent porphyria exists with normal PBGD activity in the erythrocytes (Type II). The rate of such patients in Germany is 5%. Whereas more than 200 different mutations were found for the classical type of the acute intermittent porphyria, only a few mutations are known for the non-erythroid subtype of acute intermittent porphyria.

### Variegate Porphyria

Mutations in the protoporphyrinogen oxidase (PPOX) gene are associated with the manifestation of the variegate porphyria (VP). The complete genomic sequence of the gene is clarified [38]. In the meantime various mutations in the PPOX gene were found worldwide. The gene for human PPOX is located on the long arm of the chromosome 1. Rare homozygous carriers for VP have been described. The founder gene mutation R59W was first described by Meissner et al. and corresponds to the high incidence of VP in South Africa [37]. Approximately 20,000 individuals in South Africa carry the R59W mutation derived from a family that emigrated from The Netherlands in the seventeenth century.

### Hereditary Coproporphyria

The gene of the human coproporphyrinogen oxidase (CPO) is localized on the long arm of chromosome 3 locus 12 with seven exons and six introns containing 14 kb DNA. Different mutations have been described in heterozygous patients. Furthermore, homozygous and compound-heterozygous gene carriers show the molecular heterogeneity of porphyrias. Almost each family seems to have its own mutation.

### Doss-Porphyria (ALADP) (Delta-Aminolevulinic Acid-Dehydratase-Defect)

ALADP is a result of an inherited autosomal recessive disorder. The first two patients were described by Doss et al. in Germany displaying two separate point mutations of the ALAD gene, one in each allele [15, 41]. The expression of one mutation results in an almost inactive enzyme. The other mutation leads to an unstable protein with a shorter half-life period, indicating that a compound heterozygote constellation accounts for the near-total deficiency in patients and the half-normal activity in their parents and other family members. Heterozygotes with 50% ALAD activity or individuals who carry a gene with one aberrant allele

are more sensitive to lead and can develop symptoms under low lead exposure [13, 14]. The prevalence of heterozygotes is 2% in the asymptomatic population. Therefore, most instances of compound heterozygosity in ALAD result in spontaneous abortion. ALADP is the first example of a morphine-based conformational disease [26].

## *Non-acute Porphyrias*

### Porphyria Cutanea Tarda

Porphyria cutanea tarda (PCT) is associated with impaired function of the enzyme uroporphyrinogen decarboxylase (UROD) in the liver, leading to characteristic alterations of urinary heme precursors and to typical lesions of sun-exposed skin (Table 84.4). [9, 34]. The UROD gene is composed by ten exons with 3 kb DNA in length and is located on the short arm chromosome 1 locus 34. According to Elder et al. three types of PCT are distinguished [8, 16].

**Type I (Sporadic Type).** The UROD activity is diminished in overt disease and returns to normal in clinical remission. This type is not related to mutations within the URO-D Locus. Manifestation seems to be modulated by other mutated genes, e.g. HFE gene, by viral infections or environmentally triggered.

**Type II (Familial, Hereditary Type).** Approximately half of all cases with PCT Type II are genetically predisposed by about 50% reduced UROD activity in all tissues. So far more than 50 different mutations of UROD have been described (Table 84.3).

**Type III.** This type is characterized by normal activity and concentration of the erythroid UROD. The reduced UROD-activity was found to be limited to the liver.

Many asymptomatic gene carriers indicate a less distinctive phenotypic penetrance of the defect and the role of further exo- and endogenous conditions is emphasized.

Thus, further genetic modifications responsible for the development of a clinical manifestation have to be taken in account. More detailed information concerning the molecular background has been stepwise elucidated within the last years.

Manifestation of PCT in women aged between 20 and 40 related to oral contraceptives is not rare. The estrogen induced PCT is genetically predisposed in about 80% (diminished UROD).

Hepatoerythropoietic porphyria (HEP) is a rare porphyria with clinical manifestation mostly in childhood. The HEP is characterized by homozygous or compound heterozygous mutations of UROD (activity below 10%) and accumulation of porphyrins in liver and blood associated with high urinary excretion.

**Porphyria Cutanea Tarda and Iron.** Iron overload is common in PCT and much evidence exists that iron is an inhibitory cofactor of UROD activity in hepatocytes. Elevated serum iron markers point towards an association with hemochromatosis, which has been found in 2–27% of patients with PCT. Compared to healthy controls, a high frequency of the C282Y mutation (ranging from 11% to 47%) in the HFE gene, the major genetic alteration in genetic hemochromatosis, has been found in patients with PCT from the UK, Germany, The Netherlands, Southern Italy, Spain, Australia and the USA. In a study from Northern Italy, however, C282Y occurred as frequently in PCT patients as in controls, while another much lesser predictive HFE mutation (H63D) was significantly increased in PCT patients.

**Table 84.4** Pathobiochemical progression and clinical expression of chronic hepatic porphyria to PCT

| Condition | Urinary porphyrins | | Liver porphyrin accumulation | Liver injury | Cutaneous symptoms |
|---|---|---|---|---|---|
| | Total (µmol/24 h) | Pattern | | | |
| Normal | <0.2 | COPRO >> URO >> HEPTA | – | – | – |
| Secondary coproporphyrinuria | <1.0 | COPRO >> URO > HEPTA | – | + | – |
| CHP A | <1.0 | COPRO > URO > HEPTA | (+) | + | – |
| CHP B | <1.5 | URO > COPRO > HEPTA | + | + | – |
| CHP C | <2.0 | URO > HEPTA > COPRO | ++ | + | –/+ |
| CHP D (PCT) | >2.5 | URO > HEPTA >> COPRO | +++ | + | +/++ |

Sixty-one percent of the PCT patients in Saxony (eastern part of Germany) carry HFE mutations [48]. The patients with homozygous mutations C282Y (5%) show serological and histological significant signs of increased iron storage.

The HFE gene codes for a HLA antigen (MHC class I) a very similar molecule (HFE-protein) that is expressed on the external membrane of hepatocytes and a multitude of cell types. The HFE gene mutations C282Y and much less H63D affect iron uptake via transferring receptor into the cell and lead to an unnecessarily high iron absorption in the small intestine. Patients with hereditary hemochromatosis and excessive iron storage are mostly homozygous for the mutations C282Y. In contrast, the heterozygous or compound heterozygous occurrence of the mutations C282Y or H63D lead at the most to a clinically insignificant increased iron accumulation.

Accordingly, based on clinical studies, iron removal is an efficient treatment of patients with PCT, resulting in improvement of hepatic UROD activities in Type I. Patients with PCT and homozygous mutation C282Y should be treated with phlebotomy similar to patients with hemochromatosis. For patients with PCT and heterozygous mutations of the HFE-gene a treatment with chloroquine seems sufficient, as they do not develop a heavy iron overload [48].

The data about a direct influence of iron on UROD activity are contradictory and findings of direct inhibition of the enzyme via iron, could not be reproduced by others. Uroporphyinogen could be converted into porphomethene by oxidation of a single bridge carbon between adjacent pyrrole rings, which inhibit the UROD.

Recently discovered mutations of the hemochromatosis-HFE-gene (C282Y, H63D) were significantly more often found homozygous and heterozygous in patients with PCT [39]. The HFE-protein modulates the intake of transferrin bound iron into cells such as hepato- and enterocyte, and HFE Mutations can amplify intestinal iron uptake.

A worldwide number of studies strongly suggest heterozygous, compound heterozygous and homozygous HFE genotypes as an essential predisposition for a siderosis preceding the clinical manifestation of PCT. Ethanol-treated HFE (−/−) mice seem to be an excellent model for studies of alcohol-mediated PCT [46].

### Erythropoietic and X-linked Protoporphyria

A partial deficiency of the ferrochelatase (FECH) activity forms the basis of the erythropoietic protoporphyria (EPP). The genetic modus seems to follow in most cases an autosomal dominant (d-EPP) trait with a different degree of penetrance. However, gene polymorphisms located on the non mutated chromosome in trans position (so called hypomorphic FECH allele IVS3-48C) contribute to clinical expression [19]. The FECH gene is located on chromosome 18 and consists of 45 kb including 11 exons. The first de novo mutation, two deletions and two-point mutations were found in Finnish families with erythropoietic protoporphyria. Sarkany et al. described a double heterozygous, recessive autosomal form (r-EPP) with heavy liver involvement [40]. In approximately 7% of families FECH mutations are not detected by current methods. These findings indicate that EPP may rarely be caused by mutations at a locus other than FECH. Recently S. Whatley described eight families with ALAS-2 gene mutations (51). These gain of function mutations casuse a previously unrecognized X-linked protoporphyria (XLPP) characterized by high protoporphyrin concentrations in erythrocytes.

### Congenital Erythropoietic Porphyria (Guenther's Disease)

The congenital erythropoietic porphyria (CEP) is a rare autosomal recessive disease of the heme biosynthesis characterised by considerably reduced activity of the enzyme uroporphyrinogen III synthase (UROS) [21]. Hitherto discovered mutations in the UROS gene show a great heterogeneity and the majority of patients with CEP display compound heterozygosity. An exception represents a single "missense-mutation" C37R, which can be found in up to one third of all UROS alleles. The disease is characterised by accumulation of isomer Type I porphyrins in the bone marrow, spleen and other organs.

## Pathology

Despite slightly elevated liver enzymes and reports of increased risk of hepatocellular carcinoma in acute porphyrias, progressive liver disease is not characteristic.

In contrast, significant pathology of the liver occurs in PCT and EPP.

## *Porphyria Cutanea Tarda*

The liver biopsy cylinder emits typical red fluorescence due to porphyrin accumulation when exposed to long wave UV light (Woods light, 366 nm) that can precede the skin manifestation of the disease (Fig. 84.1). With a urinary excretion of approximately 2 µmol/24 h, the liver is already full of accumulated porphyrins. The red fluorescence is caused by uro- and heptaporphyrins. Computed tomography and ultrasound sometimes detect round lesions in the liver, which have been shown to corresponded histologically to focal steatosis. These lesions disappear after therapy of PCT with phlebotomy or chloroquine [36]. Focal nodular hyperplasia (FNH) has also been described and partly traced back to an intake of estrogens.

In a large cohort including more than 200 patients with PCT, Köstler et al. found histological proven liver cirrhosis in 13% [28, 29]. Geographically, different prevalences of liver cirrhosis could correlate with a regionally varying occurrence of cofactors such as chronic hepatitis C or certain hemochromatosis HFE mutations [17, 39]. In general, each underlying liver disease characterizes the histological picture of the liver. While some authors describe the histological picture of the liver in PCT with periportal-accentuated siderosis, portal inflammation and steatosis, others point out characteristic needle-shaped intracytoplasmic crystals (Fig. 84.2) [28, 35]. These water-soluble crystal structures showing double refraction in polarized light were considered to be deposited accumulated porphyrins. The proof of such crystals located very close to the intracellular ferritin-containing iron points towards interactions between iron and porphyrin synthesis [44].

Since elevated aminotransferase (ALT and AST) levels occur in more than half of the patients without markers of HCV and/or HBV infection, liver damage in PCT may occur in the absence of these viruses [47]. This is supported by the finding that in patients without HCV and/or HBV infection, higher serum ALT and AST activities were found in patients with overt disease or relapse, whereas patients in remission displayed significantly lower serum enzyme activities.

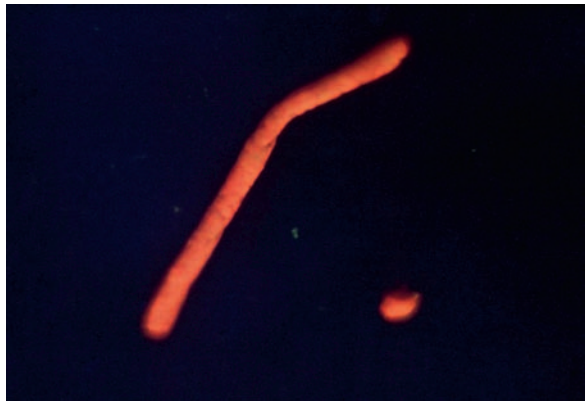

**Fig. 84.1** The liver biopsy cylinder emits a typical red fluorescence in PCT when exposed to long wave UV light (Woods light, 366 nm)

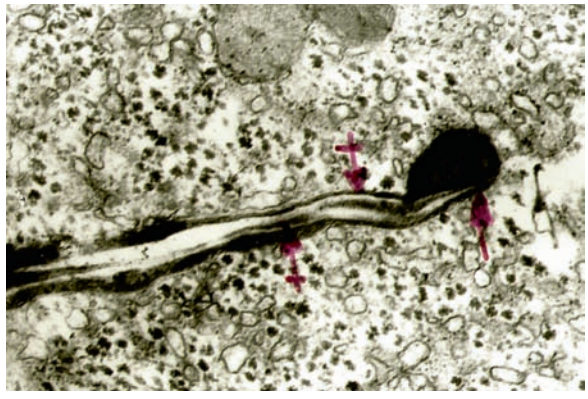

**Fig. 84.2** Accumulated porphyrins form characteristic needle shaped intra-cytoplasmic crystals (*arrows*) in hepatocytes (Kindly provided by Professor Dr. med. E. Köstler, Department of Dermatology, Klinikum Dresden Friedrichstadt, Germany)

Depending on the associated liver damage, patients with PCT display elevated enzyme activities of ALT, AST and γ-GT in serum. Since most patients show higher activities of ALT compared to AST in serum, this pattern corresponds more to viral hepatitis and to non-alcoholic rather than to alcoholic liver disease.

A differentiation and revision of liver changes in PCT have become possible because of the serological capability to prove the HCV infection since 1989. Molecular genetic procedures have been available to recognize mutations of the HFE gene responsible for hemochromatosis since 1996 [18, 33]. Therefore, older reports on coincidence between PCT and hepatocellular carcinoma (HCC) should be clarified since the role of HCV in PCT is established and the incidence of

HCC in HCV liver cirrhosis is high with an incidence of 3–10% per year [45]. Additionally, neoplasia is common in hemochromatosis and risks can be additive if both diseases are associated with each other. The predominating injuries can induce chronic hepatitis, steatosis, a more or less intensified siderosis, liver fibrosis or, in case of an advanced or combined damage, liver cirrhosis. The hepatocellular accumulation of porphyrin and the dimension of the porphyrinuria correlate with the activity and amplify liver damage.

### *Erythropoietic Protoporphyria*

The hepatocellular uptake and excretion of the lipophile protoporphyrin into the bile is the most important pathophysiological aspect for liver pathology. One quarter of all patients with EPP have an associated liver damage that can progress to cirrhosis. 10% of all patients develop gallstones; these gallstones consist mainly of protoporphyrin. The capacity of the liver for the clearance of the protoporphyrin is limited. In case of an uncomplicated course with only cutaneous symptoms, the deposition of protoporphyrin in the liver is insignificant. In contrast, overtaxing the liver excretory capacity leads to a crystalline storage of protoporphyrin in "Maltese Cross" structures that show a clear birefringence in polarized light (Fig. 84.3). Protoporphyrin crystallizes in a high concentration and is probably hepatotoxic in concentrations greater than 500 µg/g (normal approximately 1 µg/g). The protoporphyrin induced liver damage can progress into fibrosis and cholestatic cirrhosis. Irreversible liver damage is characterized by decreasing fecal protoporphyrin excretion as a "signum malum ominis". Enterohepatic reuptake and circulation of protoporphyrin further contributes to retention.

## Clinical Manifestations

### *Acute Porphyrias*

The description "acute hepatic porphyrias" points towards the potentially acute character of the clinical manifestation. The age of onset is mostly after puberty up to the third decade of life. Females are affected more than males. Initial manifestation in children and teenagers as well as in elderly people is very rare. The acute porphyria syndrome develops similarly in all four types of the acute hepatic porphyria and cannot be differentiated clinically. This applies also to the VP and the HCP in which skin symptoms

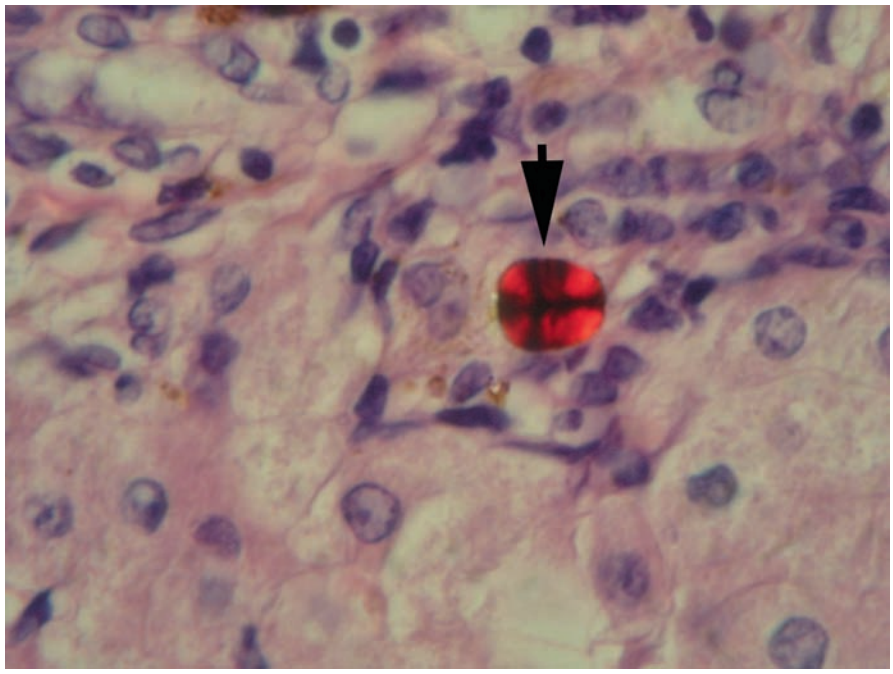

**Fig. 84.3** Hepatic "Maltese Cross" structures (*arrow*) in EPP that show a clear birefringence in polarized light (Kindly provided by PD Dr. med. J.O. Habeck, Department of Pathology, Klinikum Chemnitz, Germany)

(photodermatosis on face and hands) are mostly missing in central Europe. Abdominal pain, intermittent and colicky, is the initial and most frequent symptom [23]. At the same time or later, back pain and extremity pain as well as paresthesias can occur. Nausea and vomiting, constipation and ileus symptoms can follow. Tachycardia and dark-reddish urine are important diagnostic clues. The characteristic color of the urine increases overnight (Fig. 84.4a and b). If a porphyria is not diagnosed and the porphyria progresses uncontrolled, peripheral motor and sensory neuropathy are the most frequent complications. First the extensor muscles of hands and arms are affected. Some patients go on to develop reduced tendon reflexes, sensory abnormalities, ocular symptoms and cranial nerve dysfunction. Nerve conduction studies and needle EMG demonstrate a mixed axonal-demyelization polyneuropathy in AIP patients. Thirty percent of all patients display hyponatremia and hypokalemia as well as an increase of creatinine and urea in serum. Twenty percent show hyperbilirubinemia, hypercholesterolemia, proteinuria, leukocytosis, anemia and an increase of thyroid hormone concentrations in serum. Liver changes are usually mild in acute hepatic porphyrias. Mild elevated liver enzymes in serum (ALT, AST) can be observed, histologically accompanied with slightly elevated liver fat and iron content. Stein and Tschudy demonstrated hypercholesterolemia as well as an increase of aminotransferases in serum in all patients [49]. Observations from Scandinavia in a larger cohort of patients showed that patients with acute hepatic porphyrias have a significantly higher risk of developing liver cirrhosis and hepatocellular carcinoma (HCC). Kauppinen and Mustajoki analyzed 82 deceased Finnish porphyria patients (63 patients with acute intermittent porphyria and 19 patients with variegate porphyria, 42 men and 40 women with an average age of 57 years) [27]. HCC was found as the cause of death in seven patients (six patients with acute intermittent porphyria and one patient with variegate porphyria). Bjersing et al. reported on HCC in 17 Swedish patients with acute intermittent porphyria. At the Marburg Porphyria Center (Germany) one case of pancreatic cancer and three cases of hepatocellular cancer in patients with acute intermittent porphyria were observed. Usually the development of an acute porphyria crisis is closely related to the intake of porphyrinogenic medication, poor dietary intake or fasting, physical stress, alcohol and infections. Moreover, premenstrual manifestations are not rare in female patients and difficult to distinguish from non-porphyria induced complaints [5]. In male patients smoking was found to be associated with the number of clinical manifestations. In lead intoxication microcytic anemia, basophilic stippling on blood smear, and a "lead line" (bluish pigmentation on the gum-tooth line) can additionally be found. The course of an acute hepatic porphyria follows a certain sequence: latent stage, manifestation as clinical

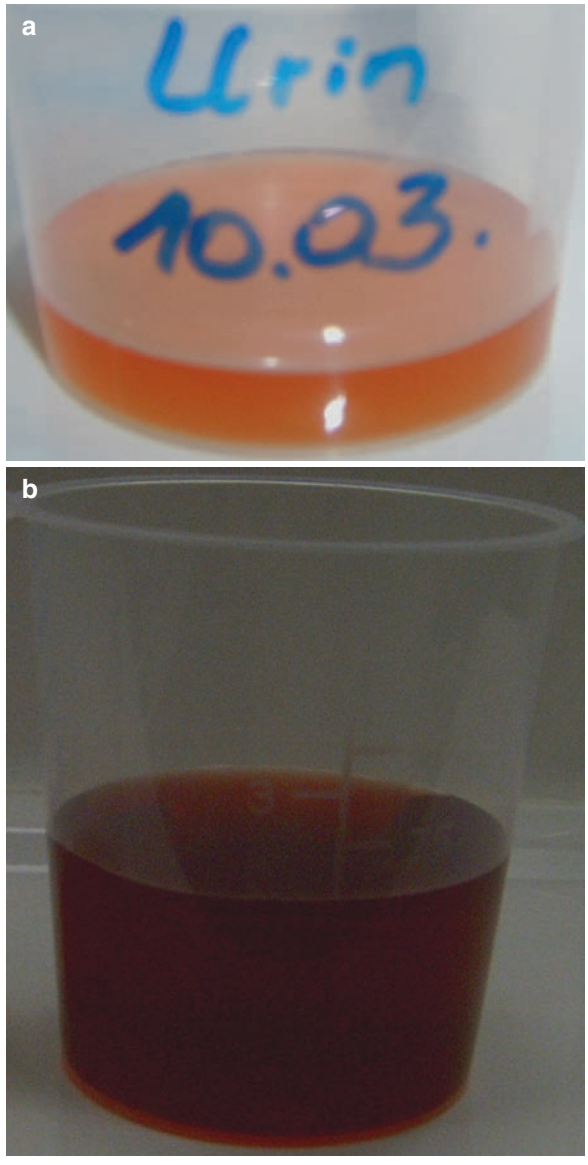


**Fig. 84.4** The characteristic color of the urine (**a**) in AIP increases overnight (**b**)

exacerbation, remission, latency in sense of a clinical cure, and potentially renewed clinical manifestation.

## *Non-acute Porphyrias*

### Porphyria Cutanea Tarda

The porphyria cutanea tarda (PCT) derives its name from the cutaneous lesions that develop on sun light exposed areas. The clinical onset of the disease is usually in adult life (Fig. 84.5). Blisters and scars develop predominantly on the hands and face, often associated with easy vulnerability of the skin and a characteristic hypertrichosis periorbitally close to the temples and to the cheekbones occurring periorbitally. The fluid-filled vesicles can rupture easily. Additionally, some patients display hyperpigmentation of the skin.

Darkly colored urine only occurs in cases of excessively high porphyrin concentrations (above 10 µmol/24 h, normally less than 0.2). The development of chronic hepatic porphyria is almost identical to the sequence of the subclinical stage: porphyrin storage in the liver and urinary excretion, acceleration of porphyrin accumulation with help of manifestation factors, clinical stage of the cutaneous symptoms, remission with decline of metabolites and stabilization in the latent subclinical stage.

### Hepatoerythropoietic Porphyria

HEP presents in childhood with severe skin sensitivity, hypertrichosis and scarring often associated with hemolytic anemia and splenomegaly. Manifestations may also be observed in adults.

### Erythropoietic Protoporphyria

Cutaneous photosensitivity occurs already in early childhood and leads to burning, itching, pain, edema and erythema on sunlight-exposed areas (Fig. 84.6). One quarter of all patients display mild anemia with hypochromia and microcytosis. It is not rare that protoporphyria is manifested in adulthood as hepatobiliary cholestatic disease since photosensitivity can be absent or hidden because of subjective habituation to avoid sunlight.

10% of all patients develop gallstones, which consist mainly of protoporphyrin. The liver complication is the most important clinical aspect of the protoporphyria in adulthood. From a clinical point of view, in patients with unexplained jaundice, hepatomegaly and abdominal pain this disease should be taken into account and light sensitivity should be asked for. Patients mostly consult a physician because of skin symptoms but the liver complication is the most

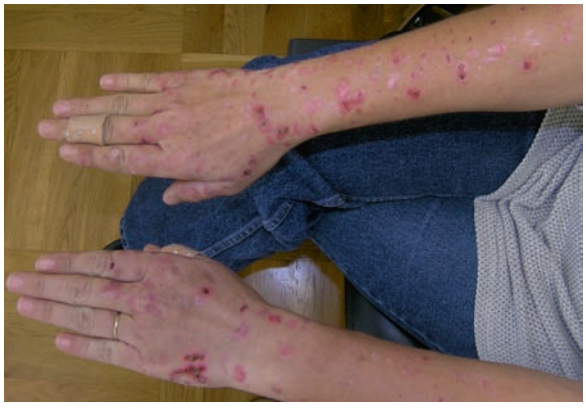

**Fig. 84.5** Cutaneous lesions in PCT on sunlight-exposed areas

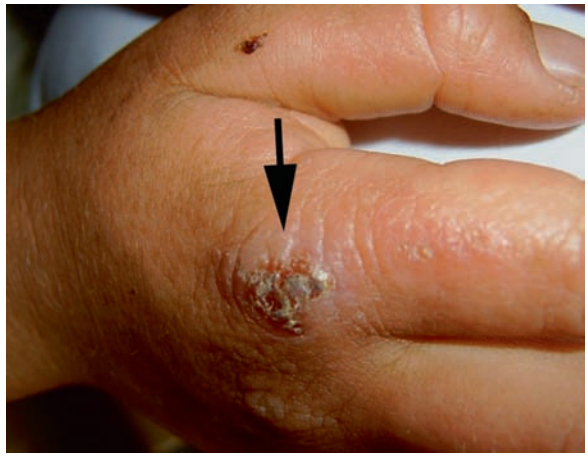

**Fig. 84.6** Cutaneous photosensitivity in EPP can occur already in early childhood and leads to burning, itching, pain, oedema and erythema on sunlight-exposed areas

important and limiting clinical aspect of advanced EPP. Motor neuropathy was reported in end-stage protoporphyric liver disease.

### Congenital Erythropoietic Porphyria (Günther's Disease)

Besides serious photosensitivity with blisters, erosions, ulcerations, hirsutism and mutilations, a characteristic reddish-brown discoloration of the teeth containing high concentrations of porphyrin (erythrodontia) may appear. They fluoresce brightly red under long-wave UV-light. The disease becomes mainly apparent in childhood but can also occur in adulthood. Important findings are anemia, haemolysis and a hepatosplenomegaly. The urine is often reddish-brown coloured because of an excessively high excretion of porphyrins of the isomer I series, mainly uro- and coproporphyrin I.

## Diagnosis

### *Acute Porphyrias*

It should be emphasized that it is clinically more important to differentiate between an acute hepatic porphyria and a chronic hepatic porphyria than it is to differentiate between the four acute hepatic porphyrias (AIP, HCP, VP, ALADP). The diagnosis of each type of clinical symptomatic acute hepatic porphyria is based on an increased excretion of both porphyrin precursors ALA, PBG and porphyrins in the urine. Simultaneously, depending on each underlying hereditary enzyme deficiency, a typical constellation of elevated porphyrins in urine and stool allows distinguishing among the acute porphyrias (Table 84.5). Suspecting acute hepatic porphyria, 20 mL of urine should be analyzed for elevated excretion of ALA and PBG and total porphyrins. This turns out to be almost always positive in cases of an acute hepatic porphyria and therefore an immediate diagnostic and therapeutic orientation is possible.

The diagnosis of an acute hepatic porphyria, including considerable therapeutic and long term prophylactic consequences, should not be based on screening procedures as these can turn out to be incorrect (false-positives or false-negatives). Further special diagnostic tools help to sub-classify acute hepatic porphyrias using criteria listed in the Table 84.5, including measuring the activity of porphobilinogen-deaminase in erythrocytes.

In the absence of an increase of both porphyrin precursors (ALS and PBG), an acute porphyria syndrome cannot be diagnosed even in the presence of abdominal, neurological and cardiovascular symptoms that point towards an acute hepatic porphyria. A normal or only limited excretion of ALA and PBG excludes de facto the diagnosis of acute hepatic porphyria. Measuring enzyme activities confirms the genetic disorders. However, the enzyme defect itself does not necessarily lead to clinical symptoms and can remain a sub-clinical variant lifelong. Thus, enzyme examinations as well as molecular genetic analysis for the diagnosis of a clinical acute hepatic porphyria are not relevant. Polysymptomatic patients suspected of having acute porphyria but displaying normal urinary excretion of porphyrin precursors and porphyrins do not suffer from a porphyria even in case of a lowered porphobilinogen-deaminase in the erythrocytes.

Enzyme activities at the edge of the statistically normal distribution are not criteria for a porphyia tendency, when the urinary metabolic excretion in a clinical porphyria-like syndrome is normal.

**Table 84.5** Differential diagnosis of acute porphyrias and lead intoxication

| Porphyria | Urine | | | | Stool | | Erythrocytes |
|---|---|---|---|---|---|---|---|
| | ALA | PBG | URO | COPRO | COPRO | PROTO | PROTO |
| ALADP (Doss) | ↑↑ | (↑) | ↑ | ↑↑ | n | n | ↑ |
| AIP | ↑↑ | ↑↑ | ↑↑ | ↑↑ | v | v | v |
| HCP | ↑↑ | ↑↑ | ↑ | ↑↑ | ↑↑ | ↑ | v |
| VP | ↑↑ | ↑↑ | ↑ | ↑↑ | ↑ | ↑↑ | v |
| Lead intoxication | ↑↑ | (↑) | ↑ | ↑↑ | n | n | ↑ |

↑ = elevated, n = normal, v = variable

## Non-acute Potphyrias

### Chronic Hepatic Porphyria and Porphyria Cutanea Tarda

The diagnosis of a PCT or a chronic hepatic porphyria is based on an excessive increase of uro- and heptacarboxyporphyrin up to a proportion of 80% of total urinary porphyrin excretion [10]. The urinary excretion of the remaining porphyrins can also rise. The excretion of PBG remains normal while urinary ALS can be slightly elevated especially under the influence of alcohol. The corresponding concentration of plasma uro- and heptacarboxyporphyrin and of stool isocoproporphyrin is increased.

Characteristic red porphyrin fluorescence of the liver biopsy, if processed to long wave UV light (Woods light, 366 nm), points towards the diagnosis of PCT.

Reduced activity of the UROD in the liver, either because of exogenous-toxic (PCT Type I) or genetic (PCT Types II and III) reasons, is obligate for the development of CHP or PCT. Lower activity of the UROD in the erythrocytes is characteristic for a hereditary disposition (PCT Type II). UROD activity should not be measured routinely since hereditary UROD deficiency does not play a role in modulating demographic or clinical features of PCT.

Table 84.4 shows that the pattern of urinary porphyrin excretion as well as porphyrin storage in the liver is crucial for the differentiation of the subclinical stage. The process from latency up to the manifestation corresponds with a progressive increase of uro- and heptacarboxyporphyrin in liver, urine, plasma and stool. In overt PCT the urinary copro-/ uroporphyrin quotient drop to 0.1 (normally approximately 8).

The inversion of the physiological copro-/uroporphyrin relation starts off the chronic hepatic porphyria phase that does not show any cutaneous symptoms (Table 84.4) [13].

### Erythropoietic Protoporphyria

Whenever an increased concentration of free protoporphyrin is found in heparinized blood (erythrocytes and plasma) EPP should be suspected. The urinary porphyrin precursors ALA and PBG are not elevated. The simultaneous examination of heparinized blood, stool and urine is mandatory to question liver involvement.

## Differential Diagnosis

### Acute Hepatic Porphyrias

Diagnostically acute porphyrias should be differentiated from a multitude of diseases including abdominal-neurological, cardiovascular and physical symptoms. The differential diagnosis is ultimately linked to pathobiochemical examination and established criteria. An important differential diagnosis is acute and chronic lead poisoning, which occurs as toxic or toxo-genetically determined porphyria. The metabolic abnormalities in lead poisoning are very similar with the ALADP (Doss porphyria) (Table 84.5). The pathobiochemical demarcation includes different enzyme reactivations in lead poisoning but not in Doss porphyria. Clinically lead intoxication is characterized by very comparable symptoms. In addition it can be associated with microcytic anemia, basophilic stippling on blood smear and a “lead line” (bluish pigmentation on the gum-tooth line). Since lead affects heme synthase enzymes (ALAD, CPO and FECH), a significantly elevated urinary ALA concentration (more than fivefold the upper limit of normal) combined with normal or only slightly elevated urinary PBG as well as increased urinary COPRO and zinc complexed red cell protoporphyrin allow the differentiation of acute hepatic porphyrias from secondary coproporphyrinurias. Alcoholic liver disease can be accompanied by abdominal and neurological symptoms as well as an isolated (unspecific pattern) coproporphyrinuria, and consequently misinterpreted clinically as an acute hepatic porphyria [12]. Fecal porphyrin examination is very important in the differential diagnosis of porphyrias. HCP is characterized by a marked elevation of the coproporphyrin isomer III in stool and urine (isomer inversion), while VP and EPP are associated with increased fecal protoporphyrin concentration (Table 84.5).

### Chronic Hepatic Porphyria and Porphyria Cutanea Tarda

The differential diagnosis includes other skin diseases that are caused by exposure to sunlight and other porphyrias. Photocutaneous symptoms can occur in EPP, CEP, HEP, HCP and VP, but not in AIP and Doss

porphyria. All these diseases can be distinguished with the meticulous analysis of the urinary porphyrins and porphyrin precursors (ALA, PBG) in stool and blood.

The very rare homozygous type of the PCT, the hepatoerythropoetic porphyria (HEP) as well as Günther's disease (CEP) occur already in infancy or early adolescence. CEP can be distinguished from hepatoerythropoietic porphyria (HEP) by dominance of isomer I porphyrins in urine and the absence of high levels of isocoproporphyrin in feces.

Furthermore, in HEP the erythrocyte protoporphyrin is elevated and consists mainly of zinc protoporphyrin, whereas an increase of the erythrocyte protoporphyrin cannot be observed in the heterozygous form of the PCT.

An extremely low activity of the UROD (<10%) is typical for the HEP as homozygous type of PCT.

## Therapy and Prognosis

### *Acute Porphyrias*

General treatment strategies include admitting the patient to an intensive care unit, stopping porphyrinogenic medication, analgesic therapy and glucose infusion with electrolyte equalization. The most important therapeutic measures are summarized in Table 84.6. Glucose and, more importantly, intravenously administered human hemin down-regulates the chaotic metabolic derangement [1, 7]. Mild cases can be treated with glucose alone. Neurological symptoms should be immediately treated with heme in order to reverse and avoid progress or persistence. The so-called "Glucose effect" was recently confirmed by elucidating fascinating regulatory links between hepatic heme biosynthesis and glucose metabolism on the level of nuclear receptors through PGC-1α [11, 22]. Glucose combined with insulin is considered to be more effective than glucose alone. On one hand, fasting precipitates and worsens the symptoms and on the other hand glucose can help to treat mild clinical manifestations of acute porphyria [32]. If tolerated, a diet rich in carbohydrates or oral glucose can be added or replace intravenous application. Large volumes of 10% glucose may increase risk for hyponatremia. Intravenously infused human hemin (3 mg/kg body weight/on 4 consecutive days, Normosang®, Orphan Europe) fills up the hepatic free heme pool, improves the function of hepatic heme-proteins, strikingly represses ALA synthase, decreases the overproduction and the urinary excretion of ALA and PBG within days.

**Table 84.6** Therapy of acute porphyrias

| |
|---|
| 1. Withdraw unsafe medication and consider intensive care unit observation |
| 2. Regulatory treatment with glucose or together with hemin<br>Intravenous glucose (300–500 g/day) should be given for mild symptoms (moderate pain, no paresis or hyponatremia). Glucose combined with Insulin is considered to be more effective than glucose alone<br>CAVE: Large volumes of 10% glucose may increase risk for hyponatremia<br>Severe symptoms and neurological complications should be treated as soon as possible with intravenously given human hemearginate (3 mg/kg body weight/on 4 consecutive days. After infusion, preferably into large vein to reduce local toxicity, the vessel should be washed with saline for 15 min<br>In order to avoid vien damage, it is suggested to dilute the human hemearginate into 100 ml of human albumin (4–20 %, depending on country availability) instead of saline solution. Hemin as a lyophilized powder (Panhematin®; Abbot Laboratories) is available in the United States |
| 3. Monitoring and electrolyte equalization: sodium, magnesium<br>Analgesic therapy: aspirin, gabapentin, morphine derivatives<br>In case of tachycardia and hypertension: metoprolol, propranolol, enalapril, losartan<br>In case of unrest and vomiting: lorazepam, phenothiazines, odansetron<br>In case of ileus: neostigmine<br>In case of respiratory failure: mechanical ventilation<br>In case of infection: penicillin and derivatives, cephalosporins gentamycine<br>In case of paresis: physiotherapy<br>Monitoring: urinary excretion of ALA, PBG and porphyrins in AIP and additionally fecal excreted porphyrins in HCP and VP |

After infusion, preferably into a large vein to reduce local toxicity, the vessel should be washed with saline for 15 min. In order to avoid vien damage, it is suggested to dilute the human hemearginate into 100 ml of human albumin (4–20 %, depending on country availability) instead of saline solution. Hemin as a lyophilized powder (Panhematin®; Abbott Laboratories) is available in the United States whereas human hemin (Normosang®) does not have FDA approval. Frequent treatment with hemin can result in iron overload, as 100 mg of hemin contain 8 mg of iron.

Repeated clinical manifestations can be prevented by prophylactic hemin administration at intervals ranging from weekly to monthly.

Hemin therapy was found to be helpful in relieving symptoms in lead intoxication and ALADP.

Intravenously applied recombinant PBD reduced PBG levels but did not show any benefit on clinical endpoints.

Ultimately, in severe, unremitting complicated diseases liver transplantation may be considered [42]. Correction of the hepatic enzyme defect using adenoviral vectors or transplantation of hepatocytes may be options for the future.

In women with acute porphyria pregnancy is in general not at risk, although progesterone potently induces liver heme production. However, pregnancy associated vomiting and subsequent caloric deficiency should be corrected promptly by glucose infusion.

For those women suffering from frequent attacks related to menses, gonadotropin-releasing hormone analogues combined with low-dose estrogen patch to suppress menopausal symptoms, can be helpful [3]. Before treatment with LH-RH- agonist is considered, low dose hormonal oral contraceptives can be tried under strict monitoring of the urinary excretion of porphyrin precursors and porphyrins.

The H2 receptor antagonist cimetidine was successfully used in a few patients [24]. The reports on LH-RH-agonists and even on cimetidine are variable and controlled data has not been available so far. The H2 blocker cimetidine can be used safely for patients with acute porphyria [43]. Parenterally injected magnesium was reported to be helpful in case of epileptic seizures.

To avoid porphyrinogenic medication, alcohol and physical stress is of major importance, as well as a balanced diet with a high proportion of carbohydrates. The patients should be advised not to smoke. Infections and all other diseases are a special stress for patients with acute porphyria, and the porphyrin precursors ALA and PBG should be monitored in order to detect and to effectively treat a renewed porphyria manifestation as soon as possible.

Based on experimental work, pharmacological data and clinical reports of safe and unsafe drugs have been published. In Germany the main drug manual "Rote Liste" contains safe and unsafe drugs (www.rote-liste.de). Moreover, international guidelines are useful to individually handle problems (www.porphyria.uct.ac.zaown, www.drugs-porphyria.org, www.porphyria-europe.com) [48a].

## *Non-acute Porphyrias*

### Chronic Hepatic Porphyria and Porphyria Cutanea Tarda

Phlebotomy and treatment with low dose chloroquine are therapeutically effective steps [25, 31]. The therapeutic aim is the elimination of accumulated porphyrins from the liver and other organs. The patients should be advised to avoid known precipitation factors. For patients with advanced liver cirrhosis and reduced albumin synthesis phlebotomy is contra-indicated.

In most cases the dosage of 125 mg chloroquine twice a week leads to metabolic and clinical remission [30]. In case of excessive urinary porphyrin excretion of more than 10 mg/day the treatment can be started with weekly phlebotomy and after 1 month continued with chloroquine for several months up to 1 year.

The treatment stops when the urinary porphyrin excretion stabilizes in a sub-clinical level (<0.3 mg/day).

A complete normalization of the porphyrinuria is rare and in particular it cannot be expected in case of a genetic disposed form. Since a low to moderate increased porphyrinuria does not play a significant clinical role, further treatment is not recommended. Steatosis of liver cells and siderosis can regress after phlebotomy as well as after a therapy with chloroquine.

### Erythropoietic Protoporphyria

Light sensitivity is symptomatically treated with beta-carotene with a dosage of 50- up to 200 (300) mg/day. Monitoring of serum carotene levels is recommended (11–15 μmol/L). In contrast to acute porphyrias, for patients with EPP there is in general no restriction for porphyrinogenic substances or medication.

Cholestyramine and ursodeoxycholic acid are used for the treatment of EPP induced liver disease.

Cholestyramine and other absorbents such as activated charcoal interrupt the enterohepatic circulation of protoporphyrin. Ursodeoxycholic acid should help to slow the progress of cholestasis and to enhance the biliary elimination of protoporphyrin. Clinical benefit can obviously only be achieved in the early phase of hepatobiliary damage. As much as possible the liver should be protected from other factors that may further contribute to further liver damage such as alcohol or viral hepatitis. Inadequate exposure to sunlight may

require Vitamin D substitution. Although intravenous heme does not down-regulate ALAS 2 in bone marrow, clinical improvement of liver function was described in patients with EPP treated with heme. Plasmapheresis, and extracorporeal albumin dialysis (molecular adsorbents recirculation system (MARS), Prometheus) can be used for elimination of protoporphyrin. Liver transplantation is the therapy of choice in EPP patients with cholestatic liver cirrhosis. Recently, for the first time, bone marrow transplantation has been reported in EPP [50]. Iron substitution markedly improved protoporphyrin accumalation in a few cases with XLPP.

### Congenital Erythropoietic Porphyria

Therapeutic measures have been disappointing up to now. Light protection, blood transfusion in case of a severe anemia and splenectomy can be considered.

Bone marrow transplantation was performed in some cases. Two children were reported to be disease-free for 3 and 2 years post-transplantation. In a mouse model, repair of UROS mutations in bone marrow cells was demonstrated with the help of viral vectors. Advances in gene therapy could be a real hope for affected patients in the future.

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# Inherited Syndromes of Intrahepatic Cholestasis

# 85

Henryk Dancygier

## Chapter Outline



(See also Chapters 52, 56, and 86).

Genetically determined intrahepatic cholestasis may manifest as a deficiency of bile ducts (syndromatic [Alagille syndrome] or non-syndromatic) or as progressive familial intrahepatic cholestasis.

## Alagille's Syndrome (Arteriohepatic Dysplasia)

Alagille's syndrome (AGS) is an autosomal dominant inherited multisystem disorder caused by mutations in the Jagged-1 gene on chromosome 20p11.2 [7, 8]. Three of the following five major clinical criteria should be present for diagnosis.

- Chronic cholestasis
- Congenital heart disease
- "Butterfly" vertebrae
- Posterior embryotoxon of the eye
- Characteristic facies [1]

The *clinical spectrum* of AGS displays a great phenotypic variability [28]. Incomplete manifestations, but also complex disease of heart, kidneys and liver, including liver failure, have been described. Involvement of heart and liver are the most common and often the initial manifestations of AGS.

### *Liver*

Liver disease is diagnosed in 95% of patients in the first year of life. Most newborns develop jaundice within the first 6 months which may be intermittent, while

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biochemical signs of cholestasis (increased alkaline phosphatase and γ-glutamyl transpeptidase) are persistent. During the first years of life the complications of chronic cholestatic liver disease appear, such as xanthomas and pruritus. Histologically, ductular proliferation is observed in the first 3 months of life. Bile duct hypoplasia and paucity subsequently develops, which finally results in a complete loss of small intrahepatic bile ducts. The disease ultimately progresses to liver cirrhosis.

### Cardiovascular System

The cardiovascular anomalies are manifold. Peripheral hypoplasia of the pulmonary artery is characteristic. In addition, coarctation of the aorta, Fallot's tetralogy, atrial and ventricular septal defects, patent ductus arteriosus, ventricular and arterial hypoplasias (hepatic, renal, carotid, celiac arteries), hypoplasia of the portal vein, arterial stenoses (pulmonary, renal and subclavian arteries), Moyamoya disease and aneurysms of the carotid arteries are observed [18].

### Facies

Approximately 95% of patients with AGS have a peculiar face with a prominent forehead, deep-seated eyes, mild hypertelorism, straight nose and a small protruding chin.

### Ocular Changes

Posterior embryotoxon, irregularities of pigment cells, cataract, strabismus, and glaucoma are observed.

### Skeletal Anomalies

Finding a bifid spine, butterfly-like vertebral arch defects, short digital phalanges and metacarpal bones, clinodactyly, and shortening of the distal ulnar and radial bone are all hints to the possible presence of AGS and should prompt a search for this syndrome.

### Other Changes

In addition to the major clinical features, renal changes (50%), chronic otitis media (35%) with deafness and intracranial hemorrhages (12%) are observed. Intestinal anomalies (e.g., small intestinal atresia or stenosis), endocrine and exocrine pancreatic insufficiency, tracheal and bronchial stenoses, and laryngeal alterations with a falsetto voice have also been described [23, 24].

Mental defects with growth retardation are more likely the consequence of long hospitalizations with inadequate treatment of malnutrition, rather than distinct features of AGS.

The *prognosis* of AGS depends on its severity. The overall probability for survival into the third decade is approximately 75%. Indicators of an unfavorable prognosis are complex cardiac anomalies and a cholestatic jaundice that initiates soon after birth. Approximately one third of children with neonatal jaundice need a liver transplant, while children without neonatal cholestatic jaundice have a markedly better prognosis with less need of liver transplantation and longer survival times [23].

## Progressive Familial Intrahepatic Cholestasis

Progressive familial intrahepatic cholestasis (PFIC) encompasses a clinically heterogeneous group of autosomal recessive inherited syndromes caused by mutations of transporter genes (see Chapters 5 and 52; Fig. 52.3).

PFIC was first described in members of an Amish family who descended from Jacob Byler. PFIC patients with a genetic defect identical to the Byler family have *Byler's disease (PFIC 1)*, while children with similar clinical features but different genotypes suffer from *Byler's syndrome (PFIC 2)* [4, 5].

PFICs are characterized clinically by (1) a chronic, non-remittent hepatocellular cholestasis, and (2) a characteristic combination of clinical, biochemical and histological findings (Table 85.1). The pathopysiological

**Table 85.1** Classification, clinical and histological features of genetic transporter defects causing cholestasis and/or jaundice

| Syndrome | Mutated transporter, biochemical function | Gene symbol | Chromosome | Serum γ-GT | Serum bile acids | Hepatic manifestations | Extrahepatic manifestations |
|---|---|---|---|---|---|---|---|
| **PFIC 1 (Byler's disease)** | FIC1; aminophospholipid flippase[a]; maintaining phospholipid asymmetry of the canalicular membrane | *ATP8B1* | 18q21–q22 | ↔ ↓ | ↑ | Recurrent cholestatic episodes progressing to permanent cholestasis. Liver failure<br>Liver fibrosis, cirrhosis. Electron microscopy: course granular bile in canaliculi | Diarrhea, pancreatitis, hearing problems, elevated sweat chloride concentrations, wheezing |
| **BRIC 1 (Summerskill–Tygstrup–Walsh syndrome)** | FIC1; aminophospholipid flippase | *ATP8B1* | 18q21–q22 | ↔ ↓ | ↑ | Recurrent cholestasis. Histology: bland cholestasis typically not leading to cirrhosis | Steatorrhea, renal stones, diabetes mellitus |
| **PFIC 2 (Byler's syndrome)** | BSEP; Canalicular bile salt export pump | *ABCB11* | 2q24 | ↔ ↓ | ↑ | Neonatal giant cell hepatitis. Frequent cholelithiasis. Progressive cholestasis with jaundice rapidly lead to fibrosis and end-stage liver disease within the first decade of life | No extrahepatic manifestations |
| **BRIC 2** | BSEP; Canalicular bile salt export pump. | *ABCB11* | 2q24 | ↔ ↓ | ↑ | Recurrent cholestasis not leading to liver failure, often associated with cholelithiasis | No extrahepatic manifestations |
| **PFIC 3** | MDR3; Phosphatidylcholine floppase [22] | *ABCB4* | 7q21.1 | ↑ | ↑ | Presents in late infancy, or young adulthood. Progressive cholestasis leads to liver fibrosis and cirrhosis with its complications (particularly portal hypertension with gastrointestinal bleeding). Hepatic gallstone formation possible<br>Histology: ductular proliferation with portal and periportal inflammation, fibrosis, cirrhosis. | No extrahepatic manifestations |
| **LPAC syndrome** | MDR3; Phosphatidylcholine floppase | *ABCB4* | | ↑ | ↑ | Intrahepatic gallstones and/or biliary cirrhosis | No extrahepatic manifestations |

(*continued*)

**Table 85.1** (continued)

| Syndrome | Mutated transporter, biochemical function | Gene symbol | Chromosome | Serum γ-GT | Serum bile acids | Hepatic manifestations | Extrahepatic manifestations |
|---|---|---|---|---|---|---|---|
| **Cystic fibrosis** | CFTR | *ABCC7* | 7q | ↑ | ↑ | Elevated sweat chloride concentration, chronic lung disease, pancreatic insufficiency, prolonged neonatal cholestasis, cholecystolithiasis, secondary sclerosing cholangitis | See Chapter 86 |
| **Dubin–Johnson syndrome**[b] | MRP2; Canalicular organic anion conjugate export pump [20] | *ABCC2* | 10q24 | ↔ | ↔ | Conjugated hyperbilirubinemia (≤5 mg%). No pruritus or other signs of cholestasis. Increase of coproporphyrin I. May arise in the neonatal period with prolonged jaundice or later in life during pregnancy or oral contraceptive intake | No extrahepatic manifestations |
| | | | | | | Histology: deposition of course brown-black pigment in hepatocellular lysosomes ("black liver jaundice"). No typical cholestasis | See Chapter 52 |
| **Intrahepatic cholestasis of pregnancy** | MDR3; Phosphatidylcholine floppase | *ABCB4* | | ↓ (70%) or ↑(30%) | ↑ | Cholestasis and pruritus in the third trimester of pregnancy | See Chapter 98 |
| | In a minority of patients: Aminophospholipid flippase | *ATP8B1* | | | | | |

[a]*Flippase* is a transporter that translocates a lipid compound from the outer to the inner leaflet of the membrane bilayer. *Floppase* translocates a lipid compound from the inner to the outer leaflet of the membrane bilayer

[b]Dubin–Johnson syndrome is characterized by a hereditary transporter defect that causes jaundice, but it is not a cholestatic syndrome

*PFIC* Progressive familial intrahepatic cholestasis, *BSEP* bile salt export pump, *BRIC* benign recurrent intrahepatic cholestasis, *LPAC* low phospholipid associated cholelithiasis, *MRP* multidrug resistance protein, *CFTR* cystic fibrosis transmembrane conductance regulator, *γ-GT* γ-glutamyl transpeptidase. ↔ normal, ↑ increased, ↓ decreased

Source: Adapted from [11, 27, 31]

basis for PFIC is a defect in one or several genes that code for important transport proteins involved in bile secretion, resulting in deranged hepatocellular bile secretion with impaired bile flow. Nowadays, the identification, cloning and functional characterization of transport proteins allows for the exact characterization of several PFIC syndromes [2, 3, 11, 12–15, 17, 25, 26]. The therapeutic options, however, are very limited. These include ursodeoxycholic acid, partial external biliary diversion and orthotopic liver transplantation.

## PFIC 1

PFIC 1 (Byler's disease) and a related disease, benign recurrent intrahepatic cholestasis type 1 (BRIC 1), are caused by mutations of the same single gene (*FIC1; ATP8B1*) that is located on chromosome 18q21–22. Nonsense, frameshift, and deletional mutations cause PFIC 1, whereas missense and splice site mutations cause BRIC 1. However, identical mutations can cause both PFIC 1 and BRIC 1 [2]. FIC1 codes for a P-type ATPase that is involved in the ATP-dependent transport of aminophospholipids (energy dependent aminophospholipid translocase) across the canalicular membrane.

The process by which a defect of this protein leads to cholestasis is unknown. It most likely is involved in the transport of bile acids, and its activity affects the lipid composition of canalicular bile. In addition to its involvement in the canalicular liver cell membrane, FIC1 is expressed in biliary, intestinal, pancreatic and renal epithelia which might explain the multisystem nature of PFIC 1 [10, 33]. FIC1 protein expression in the intestine exceeds that in the liver, and therefore defects in both the intestine and liver (e.g. increased intestinal absorption of bile acids in addition to deranged hepatocanalicular bile secretion) may be involved in the pathogenesis of PFIC 1 and BRIC 1. Diminished nuclear translocation of ileal and hepatic farnesoid X receptor may lead to pathologic alterations in intestinal and hepatic bile acid transporter expression and thus contribute to the pathogenesis of cholestasis in PFIC 1 patients [6].

Clinically, the disease is characterized by cholestatic episodes progressing to permanent cholestasis with liver fibrosis and eventually cirrhosis, with liver failure usually occurring in the first 2 decades of life.

Liver transplantation, usually within the first decade, is the most effective treatment. Biliary diversion may be helpful in some patients without cirrhosis.

## Benign Recurrent Intrahepatic Cholestasis Type 1 (BRIC 1; Summerskill–Tygstrup–Walsh Syndrome)

The molecular defect of BRIC 1 is localized on the *FIC1 (ATP8B1)* gene [30, 32]. Typically the disease begins with recurrent episodes of jaundice in the first decade of life that continue into adult life. Cholestatic episodes often follow a viral infection of the upper respiratory tract, and are heralded by pruritis, loss of appetite, anorexia and nausea. Nearly every other patient complains of abdominal pain. The jaundice lasts for 3–4 months, then spontaneously subsides, and usually recurs in approximately yearly intervals. Asymptomatic periods of several years, however, are also well documented.

*Biochemically,* a marked hyperbilirubinemia, with a moderate elevation of alkaline phosphatase and *typically normal γ-glutamyl transpeptidase* and aminotransferase levels is observed (atypical cases without pruritus and with high serum γ-GT have been reported). On cholangiography (MRCP or ERCP) the bile ducts are radiographically normal.

*Histologically,* the liver architecture is normal. A bland cholestasis, i.e. without inflammatory changes, is present. There is no fibrosis and the disease does not progress to cirrhosis. During clinically asymptomatic periods the histological findings are entirely normal.

*Treatment* with corticosteroids, phenobarbitol, ursodeoxycholic acid, cholestyramine, low fat diet, and rifampin have all been tried and are ineffective. In patients with intense pruritis, plasmapheresis may lead to some improvement of symptoms and biochemical parameters. Although not readily understandable based on our current understanding of the pathophysiology of the disorder, a recent report describes complete and long-lasting resolution of pruritus as well as normalization of serum bile salt concentrations in cholestatic BRIC patients within 24 h after endoscopic biliary

drainage [29]. Patients with BRIC 1 should be reassured that their disease is benign and does not progress to chronic liver disease.

## PFIC 2 and BRIC 2

The molecular defect of PFIC 2 (Byler's syndrome) and BRIC 2 is localized on the *BSEP (ABCB11)* gene on chromosome 2q24 and leads to a deficient expression of the major canalicular bile acid transporter, the bile acid export pump (BSEP). The deranged canalicular bile salt excretion leads to the accumulation of bile acids within hepatocytes, with subsequent liver cell damage [16]. As in PFIC 1 and BRIC 1, serum γ-GT activity is not elevated despite the cholestasis. The clinical symptoms of PFIC 2 overlap with PFIC 1 but with no extrahepatic manifestations. Depending on the severity and number of mutations, variable degrees of intrahepatic cholestasis will result. Thus, while PFIC 2 usually progresses to end-stage liver disease within the first decade of life and may even be complicated by the development of hepatocellular carcinoma in children younger than 5 years of age, BRIC 2 is a relatively mild, benign disorder.

PFIC 2 may be reliably diagnosed with immunofluorescence, by demonstrating absence of BSEP immunoreactivity from the canalicular membrane. Immunohistochemical evidence of BSEP deficiency correlates well with a demonstrable mutation in the *ABCB11* gene [19, 21].

The most effective treatment of patients with PFIC 2 is liver transplantation or partial biliary diversion.

## PFIC 3

The molecular defect of PFIC 3 is localized on the *MDR3 (ABCB4)* gene on chromosome 7q21.1 and leads to MDR3 deficiency [25]. MDR3 serves as a phospholipid translocase that flips phosphatidylcholine from the inner to the outer layer of the canalicular membrane (flippase). Biliary phospholipids are ascribed a cytoprotective effect and mutations of the *MDR3* gene lead to the formation of phosphatidylcholine-poor bile with a normal bile acid concentration. The high content of bile acids in the absence of phospholipid may make this bile toxic to both hepatocytes and biliary epithelial cells [2].

PFIC 3 is phenotypically variable (possible hepatic gallstone formation) but clinically resembles PFIC 1 and 2. The characteristic difference is an elevated γ-GT in serum in patients with PFIC 3 in contrast to PFIC 1 and 2 patients, and a ductular proliferation with portal and periportal inflammation and fibrosis in PFIC 3 patients [9, 15].

In contrast to PFIC 2, the diagnosis of PFIC 3 cannot be made reliably by immunofluorescence but rather requires gene-sequencing [19].

Liver transplantation is the primary treatment. However, ursodeoxycholic acid, as an initial therapy (possibly by shifting biliary bile salt composition to the more hydrophilic, less toxic bile acid) leads to some success in approximately 30% of patients [11]. Patients who do not respond to ursodeoxycholic acid should be considered for liver transplantation.

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# Cystic Fibrosis

# 86

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## Chapter Outline



## Definition

Cystic fibrosis (CF) is an autosomal-recessive disorder caused by a genetic defect on the long arm of chromosome 7 with consequent absence or functional impairment of **C**ystic **F**ibrosis **T**ransmembrane **R**egulator (CFTR) which results in an impaired exocrine secretion of a highly viscous, thick mucus.

## Epidemiology

The incidence of CF is 1:2,000–4,500 births. It is the most common, potentially lethal genetic disorder of the white population. The sweat glands, respiratory tract and the pancreas are primarily affected. CF related-liver disease occurs mainly in the first decade of life with a prevalence of 41% of patients at 12 years of age [10]. In a cohort study of 177 patients with CF the overall incidence of liver disease was 1.8 cases per 100 patient years, but was higher (2.5%) in the first decade. The incidence of cirrhosis in patients with CF related-liver disease was 4.5 cases per 100 patient years. In the majority of cases liver cirrhosis develops before adulthood [7, 16].

Coinheritance of Gilbert's syndrome-associated UGT1A1 mutation increases the risk of gallstone formation in CF patients [17].

## Etiology and Pathogenesis

The CF gene was detected in 1989. Its product, *CFTR, is a cAMP-dependent chloride channel in the apical membrane of secretory epithelial cells*. In concert with various anion-exchangers CFTR controls the transmembranous

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flow of chloride and other ions (see Chapters 5, 52, and 85). CFTR is involved in many epithelial transport functions, such as $HCO_3^-$-secretion, $Na^+$-resorption, exocytosis and mucin secretion. In the liver, CFTR is expressed exclusively on the apical domain of the intra- and extrahepatic bile duct and gall bladder epithelial cells. It does not occur in hepatocytes or in other cells in the liver [2].

CFTR significantly affects ductular secretion. Its absence results in defective transmembranous chloride transport across biliary epithelial cells, a process required for the generation of an intraluminal electrical potential, the paracellular flow of $Na^+$ and water, and $HCO_3^-$-secretion. Thus, ultimately CFTR is required for diluting and alkalinizing of bile. A less alkaline, hyperviscous bile leads to cholestasis with consequent chronic liver disease. Additionally, the biliary epithelium may be damaged by toxic substances, such as hydrophobic bile acids, further aggravating cholestasis. During the further course of the disease additional pathogenic mechanisms may supervene, such as stenosis of the common bile duct by pancreatic fibrosis, development of sclerosing cholangitis and cholangiocyte injury by bacterial microorganisms within the context of ascending cholangitis.

Which patients with CF, at what time and to what extent will develop liver disease cannot be predicted. But a history of meconium ileus and pancreatic insufficiency have been reported to be predictive of liver disease [3, 10]. More than 400 mutations of the CFTR-gene are known. A relation between liver disease and specific CFTR-mutants, however, could not yet be established.

## Pathology

The characteristic hepatic lesion in CF is *focal biliary fibrosis* due to a viscous eosinophilic secretion occluding the ductuli and damaging biliary epithelial cells [12]. The portal tracts are enlarged, the cholangioles proliferate and are dilated, containing amorphous secretion. There is relatively little inflammatory reaction. Cholangiolar fibrosis may progress to biliary cirrhosis. In autopsy studies the incidence of liver cirrhosis in CF is approximately 40% [6]. Alongside these typical changes perivenous steatosis up to a marked fatty liver with steatohepatitis and parenchymal siderosis may occur. A hypoplastic gallbladder filled with thickened bile is found in approximately 30% of patients, and 20% have gallstones [4, 5].

## Clinical Manifestations

The clinical picture may be deduced from the specific secretory defect of epithelial cells in various organs and is characterized by chronic obstructive lung disease, exocrine pancreatic insufficiency and hepatobiliary manifestations. Compared to the pancreas and the lungs, a clinically significant involvement of the liver is far less common. However, with increasing life expectancy of CF patients hepatic problems become more relevant. The liver is enlarged and solid. On ultrasound a homogeneously increased echotexture is seen. Liver fibrosis may be assessed roughly by serum markers, such as tissue inhibitor of matrix metalloproteinase 1, collagen IV, and prolyl hydroxylase, by transient elastography or by biopsy [15].

Liver function remains preserved for long periods of time. Once cirrhosis develops signs of portal hypertension may appear. The hepatic manifestations of CF are summarized in Table 86.1.

## Course and Prognosis

The mean survival of patients with CF has improved significantly in the last decades. The prognosis primarily depends on lung disease and the colonization of the respiratory tract by Pseudomonas aeruginosa. More than 90% of fatalities are due to progressive pulmonary disease. Liver disease has no impact on the development of lung disease.

The natural course of liver disease in CF has not yet been studied sufficiently. If liver disease develops it usually occurs in childhood and then hepatic involvement progresses relatively fast. The mean age at diagnosis of liver cirrhosis is 9 years. Nearly 90% of these children have already marked esophageal varices, and approximately one half of them bleed at the beginning

**Table 86.1** Hepatobiliary manifestations in cystic fibrosis [3, 5, 6]

| Manifestation | Incidence (%) |
|---|---|
| Asymptomatic elevation of liver enzymes | 10–35 |
| Neonatal cholestasis | <2% |
| Steatosis, steatohepatitis | 20–60 |
| Focal biliary cirrhosis | 11–70 |
| Biliary cirrhosis | 5–15 |

of the second decade. If a patient has reached adulthood without hepatobiliary manifestations, the risk of developing a clinically relevant liver involvement in CF is low [8, 9, 14].

## Therapy

A causal treatment of CF is not available. A genetic approach enabling the introduction of the normal CFTR-gene into epithelial cells would be desirable.

Ursodeoxycholic acid (UDCA), 20 mg/kg body weight daily may be associated with improvement of hepatobiliary symptoms and parameters of cholestasis. Its effect on long-term prognosis is not established. At best it may be classified as of "borderline significance", and there is no compelling evidence for routine use of UDCA in CF [1, 4, 7, 9, 11, 13].

Liver transplantation remains the only therapeutic option in patients with advanced liver cirrhosis. Coexistent pulmonary insufficiency often hampers liver transplantation, and combined liver-lung transplantation should be considered.

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# Amyloidosis

# 87

Henryk Dancygier

## Chapter Outline



## Definition

The term amyloidosis encompasses diseases that are characterized by the extracellular deposition of a homogeneous, eosinophilic, proteinaceaous material called amyloid. The name was coined by Rudolf Virchow in 1854 to describe its starch-like characteristics when stained with iodine containing solutions. Starch, however, is not a component of amyloid and various complex carbohydrates contained in amyloid are responsible for these tinctorial properties.

Amyloid deposits consist of at least three components

- Fibril proteins
- Amyloid P component
- Glycosaminoglycan

Approximately 95% of the amyloid material consists of *fibril proteins*, whose characteristics depend on the underlying disease. In multiple myeloma it consists of immunoglobulin light chains which are synthesized by the myeloma cells. In chronic inflammatory diseases the protein derives from the aminoterminal two thirds of an acute phase protein produced by the liver, *serum amyloid A (SAA)*.

The *amyloid P component* (AP-protein) occurs in all types of amyloid. The AP-protein is identical with *serum amyloid P (SAP)*, a protein that is present in normal serum.

The *glycosaminoglycan* of amyloid is usually heparin sulfate, which probably is responsible for the iodine-tinctorial properties.

Congo red staining with a yellow-green birefringence when observed by polarizing microscope is specific for all amyloids. Despite their different protein composition, all amyloids have a similar ultrastructural appearance. On electron microscopy they consist

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of variably long, partially parallel running, 7–10 µm thick fibrils. Each fibril is composed of paired filaments wounding around each other. AP-component runs perpendicular to the amyloid fibrils.

## Classification

Amyloidosis may be classified according to diverse criteria. Although the chemical classification according to the predominant constituent fibril protein (designated by various letters) is becoming increasingly important, the classical *clinical classification* is still useful [1, 13].

Clinically, primary, secondary (reactive), familial, and localized amyloidosis may be distinguished. The first three forms are generalized systemic diseases with amyloid deposits involving almost any organ, for example, kidney, heart, liver, spleen, gastrointestinal tract, tongue and subcutaneous tissue. There is considerable overlap between the various protein fibrils.

In *primary amyloidosis* (AL) no underlying disease is present. However, during follow-up in approximately one third of patients a B-cell neoplasm develops. Thus, amyloidosis may be a precursor of, for example, multiple myeloma. *Systemic senile amyloidosis* is characterized by Asc-amyloid.

*Secondary amyloidosis* (AA, AL) occurs within the context of chronic inflammatory and neoplastic disease. Rheumatoid arthritis (methotrexate therapy), systemic lupus erythematosus, lung abscesses, osteomyelitis, tuberculosis, chronic recurrent subcutaneous abscesses in i.v. drug abusers, chronic peritoneal or hemodialysis, malignant disease (especially renal carcinoma and malignant lymphomas) are among the diseases that may lead to reactive amyloidosis. Secondary amyloidosis is seen in 0.9% of patients with Crohn's disease and in 0.07% with ulcerative colitis [5]. While in industrialized countries neoplastic and chronic inflammatory diseases predominate, in industrially underdeveloped countries tuberculosis and leprosy prevail.

*Familial amyloidosis* (AA, AF) is very rare and may occur in familial Mediterranean fever (AA), familial amyloidotic polyneuropathy (AF) with predilection of peripheral and autonomic nerves and in familial islandic amyloid angiopathy (HccAA). Several families with deletion/insertion mutation in the apolipoprotein AI gene as the cause of hereditary amyloidosis with hepatic presentation have been described [2, 8].

**Table 87.1** Localized amyloidoses

| Organ | Disease | Protein |
|---|---|---|
| Lung | Nodular pulmonary amyloidosis | AL |
| Heart | Senile cardiac amyloidosis | Asc |
| | Isolated atrial amyloidosis | IAA |
| Brain | Alzheimer's disease | β-amyloid |
| | Down's syndrome | β-amyloid |
| | Creutzfeld–Jakob disease | Prion-amyloid |
| | Kuru | Prion-amyloid |
| | Scrapie | Prion-amyloid |
| | Gerstmann–Sträussler syndrome | Prion-amyloid |
| Thyroid | Medullary carcinoma | AE |
| Pancreatic islets | Type 2 diabetes mellitus | AE |
| | Islet cell tumors | AE |

An *isolated amyloidosis* is localized to single organs (Table 87.1).

## Development and Deposition of Amyloid

### AL-Amyloid

AL-amyloidosis is caused by a deranged immunoglobulin synthesis with a surplus of intact immunoglobulins. The light chains of these immunoglobulins are partially degraded in the lysosomes of macrophages, endothelial cells and other cells of the reticuloendothelial system and secreted into the extracellular space. Here glycosaminoglycans and serum amyloid P-component are attached to the variable regions of the light chains and deposited as AL-amyloid. The amino acid sequence of AL-amyloid is identical in all organs in one and the same patient. However, due to the variability of light chains the amino acid sequence differs between patients. AL-amyloid occurs in primary amyloidosis and in amyloidosis associated with multiple myeloma or B-cell lymphoma.

### AA-Amyloid

AA-amyloid consists of 76 amino acids. The amino acid sequence in the aminoterminal two thirds of the

protein corresponds to a naturally occurring serum protein, SAA (see above). In contrast to AL-amyloid, the amino acid sequence of AA-amyloid is identical in all patients, irrespective of the underlying disease.

SAA is produced by the liver as an acute phase reactant in inflammatory disease. It has the features of an Apo-HDL-lipoprotein and initially exists as Apo-SAA. Hepatic Apo-SAA mRNA synthesis is stimulated by interleukin-1 and interleukin-6. The SAA released into the circulation is an amyloid precursor and is transformed to AA-amyloid in lysosomes of macrophages and other cells of the reticuloendothelial system.

## Hepatic Amyloidosis

The liver is commonly involved in systemic amyloidosis, both in the primary (AL) and secondary (AA) form.

### *Pathology*

Amyloid always is deposited extracellularly in the stroma of an organ. The hepatic deposits are along the portal arteries or central veins and may be combined with amyloid deposition in the perisinusoidal space of Disse (Fig. 87.1). In a series of 98 patients amyloid deposition was solely sinusoidal in 66%, solely vascular in 13%, and both sinusoidal and vascular in 21% [9]. Perisinusoidal amyloid deposits compress both the sinusoids and hepatocytes, and lead to pressure atrophy of liver parenchymal cells. Rarely, globular amyloid deposits are found in the space of Disse. Histologically AL-amyloidosis cannot be distinguished from AA-amyloidosis. Distinction between the various amyloids is made by immunohistochemistry for different amyloid proteins. With increasing amyloid deposition the organ becomes pale and solid.

### *Diagnosis*

#### Clinical Manifestations

The clinical picture depends on the amount of amyloid deposited in the liver and on the underlying disease in reactive amyloidosis. Hepatic amyloidosis is asymptomatic in its early stages. Symptomatic patients are usually older than 50 years and male. They complain of involuntary weight loss, fatigue, abdominal pain, anorexia, nausea, and dysgeusia (in declining order of frequency) [4, 9].

Hepatomegaly with or without signs of portal hypertension is usually present. Jaundice and hepatic insufficiency are signs of advanced disease [10, 12].

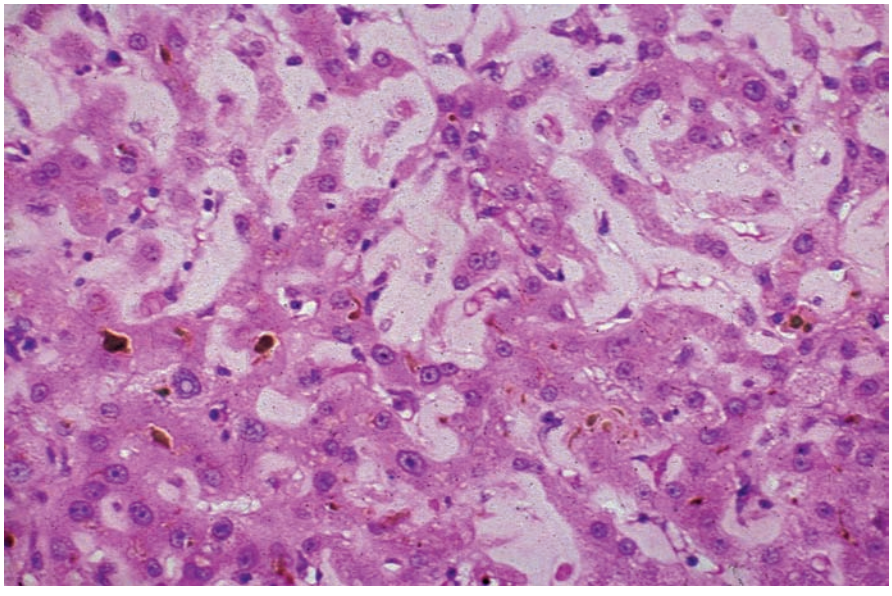

**Fig. 87.1** Amyloidosis of the liver. The hyaline material is deposited in the space of Disse and leads to pressure atrophy of hepatocytes. Additionally cholestasis is present with bilirubin containing material in dilated canaliculi. Hematoxylin & Eosin (×200)

Many patients have concurrent extrahepatic manifestations of amyloidosis and approximately 80% have amyloid detected in biopsies of nonhepatic tissue.

### Laboratory Investigations

No single laboratory test is specific for amyloidosis. Marked elevations of serum alkaline phosphatase levels occur in the majority of patients, while aminotransferase concentrations are only mildly increased. Impairment of coagulation is observed in advanced stages of hepatic amyloidosis. Factor X deficiency is common, possibly also due to binding of factor X to amyloid fibrils [3].

Ultrasound, CT, and MRI findings are nonspecific. $^{123}$I-SAP-scintiscanning is based on the rationale that since all amyloids contain SAP, labeled SAP will bind specifically to amyloid. Initial results demonstrated that the technique is specific and sensitive in detecting hepatic amyloid, but it is still not widely used clinically [7].

The definitive diagnostic test for amyloidosis is biopsy. Congo red staining has a specificity of 99% in demonstrating amyloid. For the first time in 1947, and often repeated since, it has been claimed that a liver biopsy is contraindicated in an amyloidotic liver, because the amyloid infiltrate props open the vessels which leads to an increased bleeding risk [15]. This "legend of the lardaceous liver" has been refuted [11]. Although there is an increased bleeding risk due to Factor X deficiency in some patients with amyloidosis, liver biopsy may be performed safely [3, 9]. Preferably, biopsy should be performed under laparoscopic guidance to minimize this risk.

## *Differential Diagnosis*

The differential diagnosis must include all diseases that are associated with hepatomegaly. Hepatic amyloidosis should be considered if hepatomegaly is accompanied by elevations of serum alkaline phosphatase levels, proteinuria, a monoclonal protein in serum or urine or Howell-Jolly bodies in the blood smear as a sign of hyposplenism.

A hepatomegaly in a patient with known AL-amyloidosis not necessarily is due to hepatic amyloid deposition. More often it is caused by right heart failure as a result of cardiac amyloidosis.

## *Course and Prognosis*

Deposition of amyloid in the liver always is associated with amyloid deposits in other organs. Clinically overt hepatic amyloidosis (jaundice, coagulopathy) characterizes the final stage of the disease and has a poor prognosis with a median survival of only 9 months (17% survive 5 years) [4, 9].

## *Therapy*

A specific therapy for hepatic amyloidosis is not available, and all therapeutic data is based on small case series. Corticosteroid-monotherapy is not effective. Some patients may show increased survival by treatment with prednisone (60 mg/kg body weight qd) combined with melphalan (1 mg/kg body weight qd) for 7 days every 6 weeks. Colchicine has been used to treat systemic amyloidosis. In some cases increased survival with autologous stem cell transplantation compared with conventional chemotherapy was observed.

Liver transplantation is usually not a therapeutic option, but offers an effective way to treat the rare transthyretin familial amyloidotic neuropathy [6, 14].

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# Alcoholic Liver Disease

# 88

Helmut Karl Seitz and Sebastian Mueller

## Chapter Outline



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## Introduction

Alcohol and alcoholic beverages have been consumed for thousands of years. The oldest evidence is probably a grape squeezer found near Damascus dating back to the time around 6000 BC. According to the Old Testament, God told Noah to plant a grapevine following the Great Flood around 4000 BC (and he drank of the wine, and was drunken) (Genesis 9.21). In old China, the benefits and dangers of alcohol were mentioned around 2700 BC. Another old witness of alcohol abuse dates back to the times of Echnaton in Upper Egypt. The hieroglyph is approximately 3,500 years old and is shown in Fig. 88.1. Homer, Ovid, and Plutarch all mentioned drinking of wine and the danger of this behaviour. The term "alcohol" was most likely introduced by Paracelsus around 1530 AC, from the Arabic word al-kuhl, meaning "the finest part of something". As in ancient times, alcoholic beverages were predominantly consumed by royalties and priests and were therefore only available to a small class of people; consequently, until recent times alcohol consumption has not been considered a public health problem.

Alcohol is a natural product which, consumed in small amounts, increases pleasurable feelings, and has euphoric, relaxing and sedative properties, similar to a psychopharmacon. On the other hand, alcohol has also toxic properties, particularly at high doses, and may lead to addiction and dependency.

In Europe, alcohol consumption decreased from the end of the 19th century to the Second World War. From 1950 to 1980, alcohol consumption then increased, as it did in the U.S. During the last 15 years, per capita alcohol consumption decreased mostly in Southern and Western European countries, whereas especially in Eastern European Countries alcohol consumption increased. Countries and their alcohol consumption are shown in Table 88.1 [35].

In this context it is interesting to note that in European countries alcohol consumption has an uneven

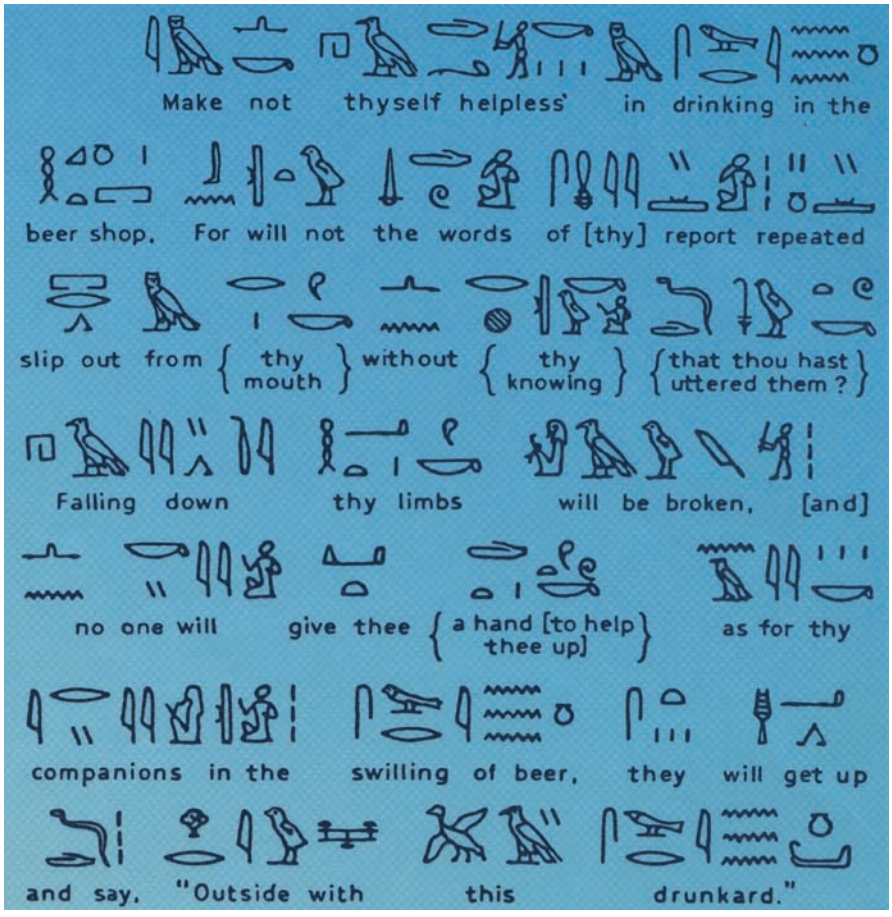



**Fig. 88.1** Evidence of excessive alcohol consumption in early Egypt. These hieroglyphics, with English subtitles, are taken from "The precept of Ani".-a papyrus of etiquette, which dates from about 1500 BC [14]

**Table 88.1** Total annual consumption of pure alcohol (in L) per capita in European Countries [35]

| Country | 1999 | 2003 | Changes between 1970–2003 in % |
|---|---|---|---|
| Luxembourg | 12.9 | 12.6 | 26.3 |
| Hungary | 10.6 | 11.4 | 25.1 |
| Czech Republic | 11.0 | 11.0 | 31.0 |
| Ireland | 9.6 | 10.8 | 83.6 |
| Germany | 10.6 | 10.2 | –0.5 |
| Spain | 9.9 | 10.0 | –13.8 |
| Great Britain | 8.4 | 9.6 | 80.1 |
| Portugal | 10.6 | 9.6 | –2.7 |
| Denmark | 9.5 | 9.5 | 39.7 |
| Austria | 9.3 | 9.3 | –11.3 |

distribution within the population. A small percentage of individuals drinks high quantities of alcohol with significant consequences. For example in Germany, the per capita consumption of pure alcohol in 2005 was 10.0 L [35]. That translates to an average of approximately 22 g of pure alcohol per day per person. According to the Addiction Report 2007, Germany has two million alcoholics, three million people with alcohol abuse and nine million people with a risky handling of alcoholic beverages who could easily enter into the first two categories [35]. Alcoholism and alcohol abuse has therefore become a major public health problem worldwide. The most comprehensive estimates of death rates caused by alcohol come from the World Health Organization (WHO) Global Burden of Disease Project, which concluded that alcohol accounts for approximately 1.8 million deaths per year (3.2% of all deaths) [69]. One of the most significant diseases caused by chronic alcohol consumption is cirrhosis of the liver.

Alcohol is toxic to almost every organ and every cell in the human body. A summary of alcohol-associated diseases is given in Table 88.2. The only positive effect of alcohol is its anti-arteriosclerotic effect which may prevent coronary heart disease and ischemic stroke in individuals with high risks for these diseases. Countless publications in the last 20 years have emphasized that small amounts of alcohol may be beneficial and may decrease mortality due to myocardial infarction. This decrease in mortality has been studied in huge populations with various risk factors, among different age groups and in both gender. In summary, the protective effect of alcohol may depend on the amount consumed and also on various other factors, particularly on age and additional risk factors for coronary heart disease. Elderly patients with a history of myocardial infarction or who have more than one risk factor for coronary heart disease except hypertension may benefit from small amounts of alcohol. However, with a daily consumption of 20 g in men and half of that in women its protective effect is almost completely saturated [93]. Younger individuals may not benefit from regular alcohol consumption. They may, on the contrary, increase their risk of other alcohol-associated diseases.

**Table 88.2** Alcohol associated diseases

**Acute intoxication** (apnea, aspiration of gastric content)
**Alcohol addiction**
**Gastrointestinal tract, pancreas and liver**
- Alcoholic liver disease
- Alcoholic pancreatitis
- Cancer of the upper digestive tract (oral cavity, pharynx, hypopharynx, pharynx, esophagus)
- Motility disorders (esophagus, gastroesophageal reflux, gastric emptying, diarrhea)
- Mucosal damage (including hemorrhagic gastritis)
- Lactose intolerance
- Colorectal cancer

**Metabolic disorders**
- Hypoglycemia
- Hyperlipoproteinemia
- Hyperuricemia (including gout)
- Porphyria

**Cardiovascular disease**
- Cardiomyopathy
- Arrhythmia (including atrial fibrillation)
- Arterial hypertension

**Alcoholic myopathy**
**Alcoholic osteopathy**
**Neurological and psychiatric disorders**
- Peripheral neuropathy
- Dementia
- Cerebellar atrophy with dyskinesia
- Depression (including suicide)

**Traumatic disorders**
- Bone fractures
- Subdural hematoma

**Breast cancer**
**Infections**
- Endocarditis
- Tuberculosis
- Viral hepatitis
- Sepsis

**Skin disease**
- Psoriasis
- Teleangiectasias
- Spider angiomas
- Palmar erythema
- Rhinophyma

## Metabolism of Alcohol

Serum ethanol concentrations reflect gastrointestinal absorption, diffusion, metabolism and unchanged excretion of ethanol. Thus, absorption of ethanol associated with increasing ethanol blood concentrations has to be distinguished from elimination with decreasing ethanol blood concentrations (Fig. 88.2). At the end of alcohol absorption a peak is detectable which may change to a plateau if alcohol is further continuously consumed.

Alcohol is absorbed from the upper gastrointestinal tract by simple diffusion. This is a fast process due to the small molecule of alcohol and its excellent solubility in water, but not in fat. Delayed gastric emptying and food in the upper gastrointestinal tract may lead to lower ethanol blood concentrations, while higher alcohol blood concentrations are observed after gastrointestinal bypass surgery and after consumption of highly concentrated alcoholic beverages such as liquors compared to low concentrated beverages like beer and wine. Gastrointestinal absorption of ethanol depends on various factors (Table 88.3).

Twenty percent of alcohol is absorbed from the stomach and 80% from the upper small intestine. In the gastric mucosa, alcohol can be metabolized by various alcohol dehydrogenases (ADHs). This is called gastric first pass metabolism of alcohol [16]. In the small intestine such metabolism has not been observed. The rest of the ethanol enters the liver via the portal vein. There is a partial metabolism of alcohol in the liver but also a release of alcohol without metabolism. Ninety percent of ethanol is metabolized in the liver

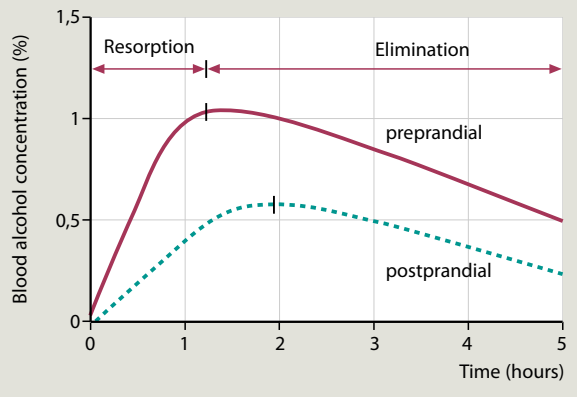



**Fig. 88.2** Alcohol absorption in the preprandial and postprandial state. Absorption is faster with an empty stomach [70]

**Table 88.3** Factors influencing gastrointestinal ethanol absorption

- Ethanol concentration of the beverage
- Blood perfusion of stomach and duodenum
- Simultaneous food intake
- Rate of gastric emptying
- Body temperature
- Menstrual cycle

after multiple passages, and 5–10% in the gastric mucosa. Approximately 3–5% of the orally absorbed ethanol is excreted unchanged through the lungs, the skin and the kidneys.

Ethanol is primarily metabolized through ADHs. Their kinetic parameters, their localization and substrate specificity is given in Table 88.4. In addition, ethanol can also be oxidized by a cytochrome P-450–2E1 (CYP2E1) dependent microsomal oxidizing system (MEOS) in various cells but predominantly in hepatocytes [40].

The ethanol distribution space (body water) is smaller in women than in men and smaller in the elderly than in younger individuals. As a consequence, the same ethanol consumption per kg body weight results in higher ethanol blood concentrations. This is also true for overweight or obese persons since ethanol is almost insoluble in fat. The ethanol elimination rate is also higher in men compared to women. A maximal amount of 150 mg ethanol per kg body weight per hour can be metabolized by a healthy man. This represents approximately 10 g ethanol in a 70 kg person per hour or 250 g ethanol in a 70 kg person per 24 h. The ethanol elimination rate is about 13 mg per 100 mL per hour and seems to be sex independent. Chronic ethanol consumption increases the ethanol elimination rate significantly mostly due to the induction of CYP2E1. It is noteworthy that no feedback mechanisms exist for ethanol metabolism. Organs with a high blood supply such as liver, kidney and brain have an increased uptake of ethanol in resting condition compared to skeletal muscles.

### *Gastric First Pass Metabolism of Ethanol*

As mentioned above, ethanol is metabolized in the stomach by various ADHs (Table 88.4). This so-called gastric first pass metabolism of alcohol is primarily due to σ-ADH encoded by ADH4 with a Km of 41 mM. However, also γADH encoded by ADH1C and χ-ADH

**Table 88.4** Characteristics of human alcohol dehydrogenases [34]

| Gene/ Locus | Allele | Protein subunit | $K_m$ ethanol (mM) | $V_{max}$ ($min^{-1}$) | Ethnic/nutritional distribution | Location |
|---|---|---|---|---|---|---|
| ADH1A | ADH1A | A | 4.0 | 30 | Europe, Africa | Liver, lung, stomach, ileum, colon, uterus, kidney, spleen, skin, testis, ovary, cervix, heart, skeletal muscle, pancreas, prostate, adrenal cortex and medulla, thyroid, blood vessels (intima and media: mainly ADH1B) |
| ADH1B | ADH1B*1 | β1 | 0.05 | 4 | Europe, Africa | |
| | ADH1B*2 | β2 | 0.9 | 350 | Asia | |
| | ADH1B*3 | β3 | 40 | 300 | Africa, Native American | |
| ADH1C | ADH1C*1 | γ1 | 1.0 | 87 | All | |
| | ADH1C*2 | γ2 | 0.63 | 40 | Europe | |
| | ADH1C*3 | | ? | ? | Native American | |
| ADH4 | ADH4 | π | 30 | 20 | All<br>Sweden | Liver, small intestine, pancreas, stomach, testis, kidney |
| ADH5 | | X | >100 | 100 | All | All tissues examined |
| ADH6 | | | ? | ? | All | Liver, small intestine, fetal liver highest of all |
| ADH7 | | σ, μ | 30.0 | 1,800 | All | Stomach (other epithelial tissues not examined) |

encoded by ADH3 contribute to gastric alcohol metabolism. Various factors affect gastric ADH activity and thus gastric ethanol metabolism [76]. They are summarized in Table 88.5.

Women have a lower gastric σ-ADH activity than men [27]. However with age, the ADH activity of men decreases and reaches the level of female activities at the age of 65 years or older [75]. Furthermore, some drugs bind to gastric σ-ADH and inhibit its activity, for example cimetidine. Other drugs such as [62] aspirin may injure gastric mucosa leading to a decrease in gastric ADH activity and thus to a decrease in gastric first pass metabolism of ethanol. Finally, ranitidine also decreases gastric first pass metabolism. However, this decrease is due to an enhanced gastric emptying with a decreased contact time of ethanol to gastric ADH. In all these situations gastric first pass metabolism is decreased which results in increased ethanol blood concentration following alcohol consumption. Gastric emptying is a modulator of gastric ethanol metabolism [62]. Delayed gastric emptying increases gastric first pass metabolism and enhanced gastric emptying decreases gastric first pass metabolism. This may be of relevance when alcohol is taken together with food, e.g. fat which delays gastric emptying or in diabetic patients with gastroparesis.

Gastric morphology also has an influence on gastric ethanol metabolism. Atrophic gastritis or gastric atrophy decreases gastric parietal cell mass and therefore the amount of ADH in the stomach, leading to a decreased gastric first pass metabolism. The presence of bacteria in the stomach also has an effect, for example helicobacter pylori possesses (HP) ADH activity [72] and produces acetaldehyde from ethanol as may do other microbes. Although HP metabolizes ethanol, the HP associated gastric mucosal damage counteracts this metabolism and therefore first pass metabolism of alcohol is decreased in the presence of HP. Gastric bacteria do not contribute much to overall alcohol metabolism,

**Table 88.5** Factors influencing gastric first-pass metabolism

- Concentration of alcohol consumed
- Polymorphism of ADH1C
- Ethnicity (a lack of sigma ADH expression has been observed in Asians)
- Gender
- Age
- Drugs (cimetidine, ranitidine, aspirin)
- Gastric mucosal injury (atrophic gastritis, H. pylori-associated gastritis)
- Rate of gastric emptying

however, they are capable to produce acetaldehyde from alcohol and acetaldehyde is a powerful toxin and carcinogen. This may be relevant with respect to gastric mucosal damage and the pathogenesis of upper aerodigestive tract cancer. In summary, gastric first pass metabolism of ethanol is modified by various factors (Table 88.5). Its overall contribution to alcohol metabolism is not more than 5–10% in vivo [62].

## *Hepatic Alcohol Metabolism*

### Alcohol Dehydrogenase

ADH is localized in the cytoplasm of the hepatocytes. ADH requires as cofactor $NAD^+$ which is reduced to $NADH + H^+$ during the metabolism of ethanol to acetaldehyde (Figs. 88.3 and 88.4). ADH is a zinc containing enzyme with a molecular weight of 80,000 and a predominant location around the central vein within the hepatic lobule. It is a dimer consisting of three poylpeptide chains (α, β, γ). The various ADH isozymes are described in Table 88.4 [34]. Class I ADH, the major ADH in the liver, has a Michaelis-Menten constant for ethanol of 0.5–1.0 mM. This equals 0.02–0.05 per mill ethanol. Thus, class I ADH reacts at a relatively low ethanol concentration. Ethanol metabolism via ADH can neither be increased with increasing ethanol concentrations nor after chronic alcohol consumption. ADH 2 which encodes for pi-ADH is only present in the human liver.

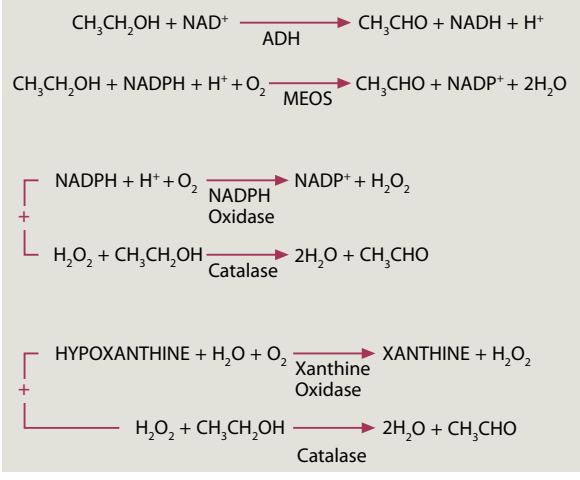



**Fig. 88.3** Ethanol metabolism: (1) Catalyzed by alcohol dehydrogenase (ADH). (2) Catalyzed by cytochrome P-450 and the microsomal ethanol oxidizing system (MEOS). (3 and 4) Catalyzed by catalase

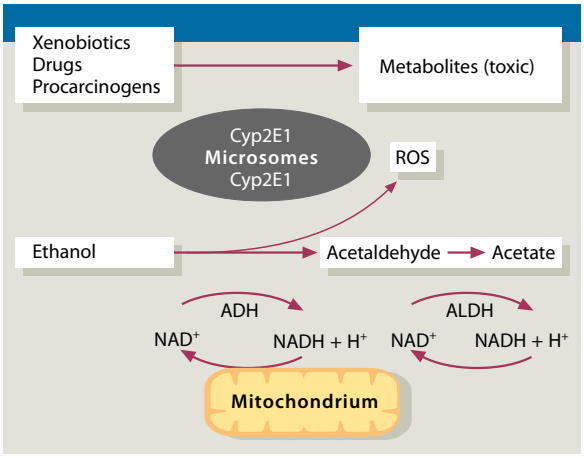



**Fig. 88.4** Ethanol metabolism via ADH and CYP2E1. Both reactions yield acetaldehyde. Additionally, the ADH reaction generates reducing equivalents as NADH, the CYP2E1, ROS. Acetaldehyde is further oxidized via ALDH to acetate. Again NADH is produced. NADH is reoxidized in the mitochondria. Since NADH cannot cross the mitochondrial membrane, shuttle mechanisms are needed to shift NADH into the mitochondria. Since some drugs, xenobiotics and procarcinogens also depend on the CYP2E1 to catalyze their metabolism, an interaction at the CYP2E1 binding site between ethanol and these compounds may occur. Ethanol metabolism is delayed with age, since ADH activity as well as CYP2E1 activity decreases with age and also the mitochondrial function ages. *ADH* alcohol dehydrogenase, *ALDH* acetaldehyde dehydrogenase, *CYP2E1* cytochrome P-4502E1, *NAD* nicotinamide adenine dinucleotide, *NADH* reduced NAD, *ROS* reactive oxygen species

ADH1B and ADH1C show polymorphism. While the ADH1B2 allele encodes for an enzyme which is approximately 40 times more active to produce acetaldehyde as compared to the enzyme encoded by the ADH1B1 allele, the ADH1C1 allele encodes for an enzyme with 2.5 times more acetaldehyde production as compared to the ADH1C2 allele. This has severe consequences with respect to ethanol associated cancer development [82]. With respect to liver disease, the presence of the ADH1B2 allele seems to be protective, since individuals with this gene produce enormous amounts of acetaldehyde following alcohol ingestion. Under these circumstances severe side effects of acetaldehyde such as tachycardia, sweating, flushing, nausea and vomiting occur (flush syndrome); therefore, these individuals avoid alcohol completely.

Metabolic consequences of the ADH reaction are either due to an increase in hepatic NADH or hepatic acetaldehyde. Production of NADH leads to a change in the hepatic redox potential and has a significant influence on hepatic intermediary metabolism [39, 41]. This includes:

- Stimulation of fatty acids and triglyceride synthesis and inhibition of ß-oxidation of fatty acids. As a result, fatty liver and also hyperlipoproteinemia type IV and V according to Fredricksen may occur.
- Decreased pyruvate- and increased lactate concentrations in the liver. As a consequence, an inhibition of gluconeogenesis with hypoglycemia and also lactacidosis with hyperuricemia may occur. The increase in lactate also stimulates hepatic stellate cells (HSCs) to produce collagen.
- Disturbed porphyrin metabolism with the occurrence of secondary porphyria.

The production of acetaldehyde leads to

- Mitochondrial damage with alteration of the respiratory chain and ATP production. As a morphological consequence, megamitochondria may occur.
- Damage of the microtubular system with an altered secretion of proteins such as albumin, transferrin and very low-density lipoproteins. As a morphological equivalent, ballooning of the hepatocyte may occur.
- A decrease in glutathione and thus an alteration of the detoxification of xenobiotics and reactive oxygen species (ROS).
- An inhibition of the nuclear repair systems with an enhancement of carcinogenesis.
- A disturbed methyl transfer with decreased levels of the active methyl donor S-adenosylmethionine (SAME) and an increase of homocysteine, which produces endoplasmic reticulum stress resulting in fatty liver, in a decrease in mitochondrial glutathione and in apoptosis. As a consequence, membrane damage and hypomethylation of DNA may occur. Aberrant methylation causes inflammatory response and tissue injury and DNA hypomethylation causes liver cancer.
- Binding of acetaldehyde to proteins with a generation of neoantigen, activation of the immune system and production of antibodies.
- Binding of acetaldehyde to DNA and generation of mutagenic DNA lesions.
- Stimulation of fibrogenesis.

**Table 88.6** Metabolic diseases favored by hepatic ethanol metabolism

| |
|---|
| • Hyperproteinemia |
| • Hyperhomocysteinemia |
| • Porphyria |
| • Hyperuricemia |
| • Hypoglycemia |
| • Hyperlactacedemia/acidosis |
| • Altered testosterone: estrogen ratio |

Since ethanol metabolism, primarily via ADH, affects hepatic intermediary metabolism, the occurrence of various metabolic diseases is favoured by chronic ethanol consumption (Table 88.6).

### Hepatic Microsomal Ethanol Oxidizing System

The hepatic microsomal ethanol oxidizing system (MEOS) is located in the endoplasmic reticulum of the hepatocytes [40]. MEOS requires molecular oxygen and NADPH as a cofactor (Fig. 88.3). It has an optimal activity at a pH 6.9–7.5, and a Michaelis-Menten constant of 7–11 mM for ethanol. MEOS metabolizes not only ethanol but also other primary aliphatic alcohols such as methanol, propanol, butanol and pentanol, as well as secondary alcohols such as isopropanol and tertiary alcohols such as t-butanol. The activity of MEOS is gender dependent with higher activities in the male gender. Castration, oopherectomy, and substitution with sex hormones affects MEOS activity. MEOS activity decreases with age and may depend on diets with higher activities following hypocaloric carbohydrate deficient diets and lower activities following protein malnutrition. MEOS activity can also be induced by certain drugs. Major components of MEOS are CYP2E1 and NADPH, cytochrome c reductase as well as phospholipids. MEOS is localized in the smooth endoplasmic reticulum of the hepatocyte which proliferates following chronic alcohol consumption associated with an increase in MEOS activity and an increase in CYP2E1. As a result, ethanol metabolism is increased, associated with an increased generation of acetaldehyde and with an increase of ROS (Fig. 88.4). This increased oxidative stress is of special importance as a pathogenetic mechanism of ALD and will be discussed separately. Various cytochrome P-450 species are involved in MEOS activity (Table 88.7). The isoenzyme form of CYP2E1 has the highest metabolic activity for ethanol. The genetic polymorphism for CYP 2E1 is well known, however, it may not play an

**Table 88.7** Cytochrome P450 species involved in ethanol metabolism [92]

| Cytochrome P 450 isoenzyme | MEOS-activity(nmolesacetaldehyde/min/n mole cytochrome P 450 |
|---|---|
| 1A2 | 10.90 |
| 2A6 | 3.75 |
| 2B6 | 2.89 |
| 2D6 | 0.70 |
| 2E1 | 11.51 |
| 3A4 | 3.38 |

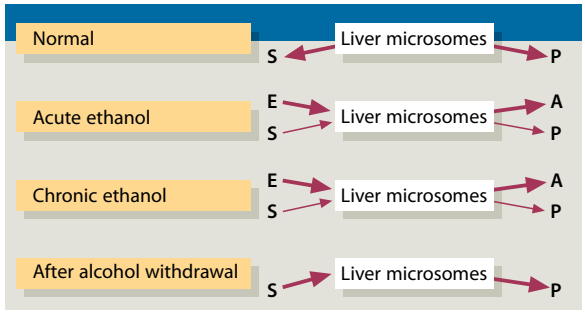



**Fig. 88.5** Interaction between ethanol metabolism and the metabolism of certain drugs. If the drug requires CYP2E1 for its metabolism, its metabolism is inhibited in the presence of ethanol and drug concentrations increase in the blood. This is especially dangerous for psychoactive drugs such as barbiturates, benzodiazepines or meprobamate which may then act at a higher dose for a longer period of time on their target organ and may lead to respiratory depression. When ethanol is consumed chronically, CYP2E1 is induced (characterized by the box increased in size). Again ethanol metabolism is preferred compared to drug metabolism. However more ethanol due to the induction may be oxidized leading not only to acetaldehyde but also to reactive oxygen species, which may initiate alcoholic liver disease. If alcohol is absent from the body in a CYP2E1 inductive state (for example in the morning following chronic ethanol consumption in the evening), the drug is metabolized much faster and drug blood level decrease leaving the therapeutic range. In this situation dose adaptation is necessary. In some situations, the drug may be metabolized to a toxic metabolite with hepatotoxicity (acetaminophen, isoniazide, vitamin A, see Table 88.8). *S* substrate, *E* ethanol, *P* product, *A* acetaldehyde

important role with respect to ALD. CYP2E1 is not only localized in the endoplasmic reticulum but also in the nucleus and in the plasma membranes. CYP2E1 and CYP3A4 are also found in the epithelium of the intra- and extrahepatic bile ducts. CYP2E1 is also present in Kupffer cells, but not in HSCs, where CYP3A4 has been described (Table 88.7).

Chronic ethanol ingestion even at relatively low doses, such as 40 g ethanol per day, and even after a short period of time such as 1 week, results in a significant induction of CYP2E1 [63]. However, this induction varies among individuals. Some individuals have a strong induction and some have no induction at all. The enhanced metabolism of ethanol after chronic alcohol consumption is due the induction of CYP2E1 and it is important to note that CYP2E1 activity needs NADPH and reutilizes reducing equivalents from the ADH reaction as NADPH from NADH. The metabolic and clinical consequences of methanol metabolism via MEOS are multiple. Major consequences are (Fig. 88.4):

- Production of hydroxyl-ethyl radical, superoxide anion, and hydroxy peroxide which contribute to liver damage.
- Interaction of the microsomal ethanol metabolism with the metabolism of various xenobiotics, drugs and carcinogens leading to increased toxicity and carcinogenesis.

Since a variety of xenobiotics are also metabolized via CYP2E1, a competition at the binding site of CYP2E1 occurs [39, 41, 77] (Fig. 88.5). In the presence of certain drugs, ethanol metabolism is preferred. In such a situation the metabolism of many drugs is inhibited resulting in an enhanced drug effect at the target site. This is predominantly relevant for psychoactive drugs such as benzodiazepanes or barbiturates with increasing blood concentrations in the presence of ethanol.

Chronic ethanol consumption results in a proliferation of the smooth endoplasmic reticulum and an induction of CYP2E1 associated with an enhanced metabolism of xenobiotics. This can lead to a decrease of therapeutic blood concentrations of drugs which therefore requires the prescribed dose to be adjusted. Various procarcinogens can also be activated and this enhanced activation may be important in ethanol associated carcinogenesis [77]. On the basis of this mechanism, chronic ethanol consumption enhances the toxicity of various xenobiotics, since some drugs and xenobiotics are metabolized to toxic intermediates by microsomal CYP2E1 metabolism. This is clinically relevant for a number of drugs, but also for carbon tetrachloride, various solvents and cocaine (Table 88.8).

In summary, individuals with chronic ethanol consumption may have a high risk drug induced liver injury and need to adjust their drug doses. A summary of the drugs, xenobiotics, and carcinogens which react with ethanol at the CYP2E1 site is listed in Table 88.8.

**Table 88.8** Interaction between alcohol and drug metabolism

| Via CYP2E1 | via ADH | via ALDH |
|---|---|---|
| Paracetamole | Cimetidine | Sulfonylureas |
| Barbiturates | Ranitidine | Sulfonamide |
| Isoniazide | Chlorpromazine | Metronidazole |
| Cyclophosphamide | Chloralhydrate | Griseofulvin |
| Halothane | | Tolazoline |
| Methadone | | Procarbazine |
| Phenylbutazone | | Quinacrin |
| Propranolol | | |
| Rifampicin | | |
| Warfarin | | |
| Tolbutamide | | |
| Tranquilizers | | |
| Vitamin A | | |

### Hepatic Catalase

Catalase is localized in the peroxisomes of the hepatocyte and is capable of oxidizing ethanol to acetaldehyde by using $H_2O_2$ (Fig. 88.3). However, catalase does not contribute significantly to ethanol metabolism.

### Hepatic Acetaldehyde Dehydrogenase

At least 4 different isozymes of hepatic acetaldehyde dehydrogenase (ALDH) exist. In the liver ALDH1 is present in the cytoplasm and does not participate much in the metabolism of acetaldehyde due to its high Km. In contrast, ALDH2 is located in the mitochondria and is responsible for the hepatic metabolism of acetaldehyde which is produced through ADH and MEOS. Since acetaldehyde injures mitochondria, mitochondrial function also decreases, as described above, including the activity of ALDH2. As a result, acetaldehyde further increases and a vicious cycle is induced.

It should be pointed out that acetaldehyde may probably not play an important role in the pathogenesis of ALD, since the capacity to remove it by intramitochondrial ALDH2 is high. A special situation exists in Asians. Fifty per cent of Asians have a mutation of the ALDH2 gene resulting in low activity of the ALDH2 enzyme [33]. In 10% of the Japanese this mutation is homozygous, associated with zero ALDH activity. These individuals cannot drink ethanol at all since they develop severe side effects such as flushing, tachycardia, nausea and vomiting. They are completely protected from alcohol drinking. Forty percent of Japanese however are heterozygotes. They may consume alcohol with an ALDH2 activity of approximately 10–15% compared to those of normal white. They also develop a flushing syndrome. However this can be tolerated, so that they continue to drink. As a result acetaldehyde levels increase in the blood, in the liver and in the saliva. Since acetaldehyde is a carcinogen, these individuals have a high risk for alcohol associated cancer development such as cancer of the upper alimentary tract and the colon [82]. With respect to the liver, contradictory results have been reported and the question is still open whether ALDH2 heterozygotes have an increased risk for ALD or not. ALDH 2 can also be inhibited by various drugs leading to a flush reaction (Table 88.8).

## *Bacterial Metabolism of Ethanol*

In recent years bacterial metabolism of ethanol became a major issue with respect to organ injury. Bacterial ethanol metabolism may take place in the oral cavity, in the stomach and in the large intestine. It has been shown that oral microbes are capable of oxidizing ethanol to acetaldehyde which plays an important role in upper alimentary tract carcinogenesis [82]. Also in the stomach, various microbes, especially HP [72] and Neisseria species are capable of oxidizing ethanol to acetaldehyde, and this may be of particular interest for the occurrence of gastritis. Finally, fecal bacteria also produce acetaldehyde from ethanol. This metabolism leads to the highest levels of acetaldehyde measured per gram of mucosal tissue when compared to the entire body on a per gram of tissue basis. Acetaldehyde production by gastrointestinal bacteria may contribute to alcohol associated carcinogenesis [82]. Since some of these bacteria are also capable of fermenting carbohydrates to ethanol, with the subsequent generation of acetaldehyde, the question still remains whether bacterial overgrowth, for example in the stomach due to chronic atrophic gastritis, contributes to gastric cancer in a population with high carbohydrate intake such as in Asian populations. With respect to more detailed information on bacterial ethanol metabolism, the interested reader is referred to a recent review article on this topic [71].

# Alcoholic Liver Disease

## Epidemiology

Chronic alcohol consumption is the major cause for chronic liver disease in Germany (40–50%), with a death rate of more than 18,000 cases per year [35]. Similarly, in the U.S. alcohol is implicated in more than 50% of liver related deaths, and alcoholic liver disease (ALD) is a major health care cost expenditure, accounting for nearly $3 billion annually [45, 47, 50]. At present, the country with the fastest increase in alcohol associated health problems is the Peoples Republic of China, with an annual per capita increase in alcohol consumption of 400% and even higher rates seen in some geographic regions [12].

The exact number of alcohol related deaths is difficult to obtain because of the inaccuracy of the reports. If the relationship between alcohol intake and ALD is examined on a population basis, risk of ALD starts at 30 g of ethanol per day. However, the absolute risk remains much smaller. Serious liver disease develops only in 1–2 out of 10 when more than 60 g of ethanol are consumed daily [47]. The severity of ALD does not depend on the amount of alcohol consumed only. Therefore hereditary factors in the development of ALD have to be considered.

## Natural Course

The natural course of ALD is given in Fig. 88.6. ALD consists of alcoholic fatty liver (AFL), alcoholic steatohepatitis (ASH), alcoholic cirrhosis (AC) and alcoholic hepatocellular cancer (HCC). ALD is the most important organ manifestation of chronic alcohol abuse. It is noteworthy that although 90–100% of chronic heavy alcohol consumers develop AFL, only 10–35% develop ASH and even fewer (8–20%) develop AC, which may be explained by the influence of genetic factors [81].

## Risk Factors

Risk factors for ALD are summarized in Table 88.9. Various risk factors for ALD exist. Classic studies by

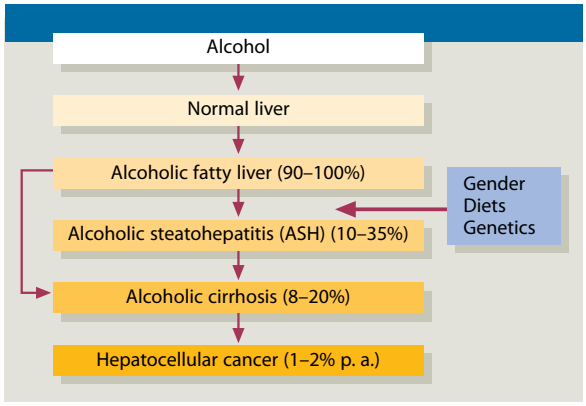



**Fig. 88.6** Natural course of alcohol liver disease. Gender, diets and genetic factors may modulate this course (see text) [80]

Lelbach as well as Pequinot clearly showed that the amount of alcohol consumed over a life time correlates significantly with cirrhosis prevalence [37, 65]. In these studies a clear cut threshold could not be defined. It is interesting, however, that even at a very high alcohol intake not everyone develops cirrhosis. According to the data available it was concluded that daily doses of more than 24 g of ethanol may be associated with an increased risk of cirrhosis in men, while in women this risk is seen at half of this dose.

Women have an increased risk for ALD at a lower dose and a shorter exposure time [15, 29]. The mechanisms of an increased risk for ALD in women is not clear. Various explanations exist including higher blood ethanol concentrations in women following the same ethanol intake calculated per kg body weight due to a decrease in body water, an estrogen dependent uptake of endotoxins from the gut to the liver and an increased sensibility to develop autoantibodies against acetaldehyde- or hydroxyethylradical derived epitopes. The gender dependent effect of gastric first pass metabolism may not be enough to explain the increased

**Table 88.9** Risk factors for alcoholic liver disease

| |
|---|
| • Alcohol intake (men > 40 g/day; women > 20g/day) |
| • Drinking habit (regularly vs. sporadic) |
| • Gender |
| • Genetics (gene polymorphisms) |
| • Nutritional status (malnutrition, overweight) |
| • Comedication (isoniazide, methotrexate, vitamin A, etc.) |
| • Other types of liver disease (hepatitis B/C, hemochromatosis, nonalcoholic fatty liver disease) |

susceptibility of women towards the toxic effect of ethanol on the liver.

Drinking habits and the pattern of alcohol consumption may influence cirrhosis risk [5]. Regular, continuous alcohol consumption as reported for Southern Europe, is associated with a higher risk of cirrhosis, while sporadic, heavy alcohol abuse as reported for Scandinavian countries results rather in cardiovascular events than in the development of AC.

Due to the fact that only a small percentage of drinkers develop cirrhosis of the liver, various laboratories have focused on genetic factors within the last decade. In this context a variety of gene polymorphisms coding for ethanol metabolizing enzymes such as ADH, ALDH, CYP2E1, and for antioxidative enzymes such as glutathione-S-tranferase (GST) and manganese superoxide dismutase (MnSOD) have been studied [65]. Also polymorphisms of genes coding for cytokines, immune- and fibrosis factors such as tumor necrosis factor α (TNFα), interleukin 10, interleukin 1β, CD14 endotoxin receptor, cytotoxic T-lymphocyte antigen 4 and transforming growth factor ß1 (TGFß1) and matrix metalloproteinases (MMPs) have been investigated [90]. In summary, although a variety of single nucleotide polymorphisms (SNPs) of genes that code for proteins involved in the pathogenesis of ALD have been tested, the results are contradictory and initial euphoria over seemingly identified genetic markers has faded since many results could not be reproduced [90].

The nutritional status is of considerable importance with respect to risk assessment. Both malnutrition as well as obesity are associated with an increased risk for AC [32, 57, 78]. This is especially relevant with the endemic occurrence of non-alcoholic fatty liver disease (NAFLD) in the Western World due to obesity, especially that associated with type 2 diabetes mellitus and peripheral insulin resistance. Since people not only eat too much, but also consume alcohol together with their meals, an additive effect of alcohol on the development of fatty liver and more advanced liver disease has to be considered. It is unquestionable that alcohol exacerbates NAFLD.

Other types of liver disease are also negatively affected by chronic alcohol consumption. The development of fibrosis in hepatitis C is strikingly enhanced by the concomitant use of alcohol. This has been shown in a great number of studies. A recent study from Sweden with sequential liver biopsies has clearly shown that this risk already starts at an alcohol dose of less than 40 g per day [99].

Alcohol is also harmful in hepatitis B. It has been shown that regular alcohol consumption even at a dose of 25 g per day or more accelerates hepatocarcinogenesis in patients with hepatitis B [61]. Thus, alcohol consumers develop HCC approximately 10 years earlier as compared to abstainers. Finally, since ethanol results in an enhanced uptake of iron from the upper gastrointestinal tract, hereditary hemochromatosis is negatively influenced by chronic alcohol consumption.

The simultaneous intake of certain drugs together with ethanol may also harm the liver (Table 88.8). This is well known for acetaminophen, which is activated to a toxic intermediate by CYP2E1 due to the induction by alcohol, and to a lesser degree detoxified due to a decrease in glutathione in the alcohol consumer [39, 85]. Other drugs with enhanced toxicity due to alcohol are metothrexate (frequently used in rheuma therapy) and isoniazide, a tuberculostatic drug [39].

Finally, it should be emphasized that chronic alcohol consumption increases the toxicity of retinoids (ß-carotin and vitamine A) [38]. Since retinol and retinoic acid are metabolized through CYP2E1, it is not surprising that their concentrations are low in the liver of a chronic drinker. This fact may be relevant in alcohol associated hepatocarcinogenesis [82]. However, although the chronic alcoholic needs vitamin A, there is only a small therapeutic window for vitamin A replacement therapy. The rapid metabolism of vitamin A via CYP2E1 results in the generation of polar metabolites with apoptotic properties which may damage the liver and finally lead to hepatic fibrosis [17].

## Alcoholic Fatty Liver

### *Definition*

A normal healthy liver weighs approximately 1.5 kg, while an alcoholic fatty liver (AFL) has a weight of approximately 2.0–2.5 kg. A normal liver has a fat content of 0.5–1.5% of its wet weight which is approximately 4–8% of its dry weight. This is significantly enhanced in AFL where 50% or more of the liver is fat. Histologically, a macrovesicular steatosis has to be

distinguished from microvesicular steatosis which is mostly associated with mitochondrial inability to oxidize fat. In addition to fat, proteins and water are retained in the hepatocyte, probably due to a disturbed microtubular function which leads to the ballooning of the hepatocytes [39].

## Epidemiology

Almost everybody develops AFL at a certain dose of chronic alcohol consumption which is individually different. It has been shown that overweight or obese individuals are more prone to alcohol liver toxicity and develop AFL at a higher rate. In an Italian study, for example, individuals with more than 60 g alcohol consumption per day showed steatosis at ultrasound in 46%, while obese patients with a body mass index of more than 25 kg/m$^2$ had steatosis in 76%, and alcohol consuming overweight patients in 95% [6].

Individuals with signs of alcohol dependency and alcohol abuse present with AFL at an early stage of ALD. The prevalence of AFL was estimated to be approximately six million in Germany, which is 7.3% of the total population, and these are more patients than those with diabetes mellitus [92]. Since AFL is easy to diagnose using ultrasound, an early intervention in these patients could take place if the general practitioner is aware of the severe consequences of this diagnosis.

## Pathogenesis

Various factors contribute to the occurrence of AFL [39, 47, 54]. A summary is given in Fig. 88.7. The liver is the predominant site of alcohol oxidation where alcohol is oxidized via ADH to acetaldehyde and further by ALDH to acetate (Figs. 88.3 and 88.4). This metabolism needs NAD $^+$ and generates NADH. The result is an overproduction of reducing equivalents in the liver and a striking change in the hepatic redox state. These reducing equivalents shift all redox partners into their reducing form and influences free fatty acid (FFA) and triglyceride metabolism significantly. NADH favours FFA and triglyceride synthesis and inhibits mitochondrial β-oxidation of FFA. The result is an accumulation of triglycerides in the liver. Excess NADH and an increased NADH/NAD ratio results in

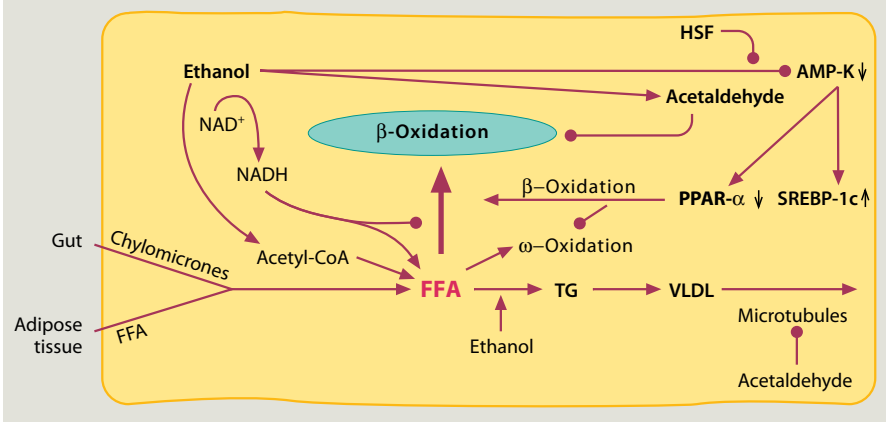



**Fig. 88.7** Development of alcoholic fatty liver (AFL). Various factors contribute to the fat accumulation in the liver: (1) Ethanol oxidation results in the generation of NADH which overfloods the hepatocytes and shifts all redox partners into their reducing intermediate, favouring fatty acid- and tryglyceride synthesis and inhibiting mitochondrial ß-oxidation of fatty acids. (2) The hepatic influx of free fatty acids from adipose tissue and of chylomicrons from the intestinal mucosa is enhanced by ethanol. (3) Ethanol inhibits adenosine monophosphate activated kinase (AMP-K) resulting in a decrease of peroxisome proliferating-activated receptor (PPAR)-α and an increase of sterol regulatory element binding protein (SREBP)-1c, stimulating lipogenesis and inhibiting lipolysis. The effect of ethanol on AMP-K is modulated by the type of fat consumed. High saturated fats counteract the effect of ethanol on AMP-K. 4) Acetaldehyde injures mitochondria and microtubules. The reduction of mitochondrial function results in a reduction of NADH oxidation and favours fat accumulation, the damage of microtubules interferes with the secretion of macromolecules from the cell to the blood. Thus, very low density lipoproteins (VLDL) are retained within the liver.

the activation of SIRT1 which favours fatty acid synthesis and histone acetylation.

Alcohol inhibits adenosine monophosphate-activated kinase (AMP-K). AMP-K leads to an activation of peroxisome proliferators activated receptor (PPARα), an important nuclear transfer factor for genes involved in lipolysis. It has been shown that PPARα protects against alcohol induced liver damage in mice. In addition, AMP-K results in a decreased activation of sterol regulatory element binding protein 1c (SREBP1c), a nuclear factor involved in lipogenesis. SREBP1c knock out mice are less susceptible to developing AFL. Thus, alcohol may induce AFL by both mechanisms via AMP-K. It is interesting that high unsaturated fat diets may counteract the effect of ethanol on AMP-K. Thus, in addition to chronic ethanol consumption other factors such as overweight and obesity as well as the quality of dietary fat (saturated versus polyunsaturated fatty acids) may modulate the quantity and quality of hepatic fat. Both render the liver more susceptible to a second hit resulting in lipid peroxidation which is more pronounced with polyunsaturated fat as compared to saturated fat.

In addition, alcohol consumption leads to an increased mobilization of FFA from fat tissue and also from the gut as chylomicroms. Theses FFA and chylomicroms are taken up by the liver and contribute to the development of AFL.

Furthermore, alcohol results in an inhibition of lipid secretion from the liver to the blood. Thus, acetaldehyde damages microtubules leading to morphological and functional changes of these important cell organelles which are responsible for the secretion of cellular proteins and macromolecules such as albumin, transferrin and lipoproteins. In addition, AA also damages mitochondria, interfering with the respiratory chain and the mitochondrial reoxidation of NADH to $NAD^+$, thereby further stimulating AFL. The results of a recent animal study comparing a NASH and an ASH mouse model by using micro array analysis demonstrate that the decrease of AMP-K observed in the alcohol model was not observed in the NASH model, while PPARα was found to be more decreased in NASH as compared to ASH.

## Histopathology

Early and mild steatosis is seen in zone 3 (perivenular) hepatocytes [8, 31] Fig. 88.8). However, when the liver injury is more severe it can affect zone 2 and zone 1 hepatocytes. The fact that steatosis starts around the central vein may be explained by the increased activity of ADH in this area, with an enhanced shift of the redox potential following ethanol metabolism (Fig. 88.8).

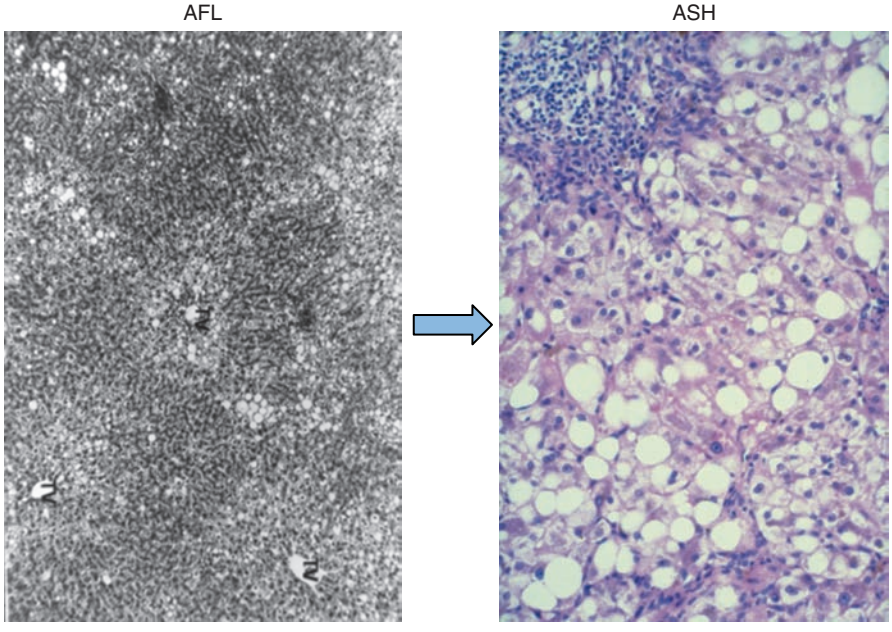



**Fig. 88.8** Histomorphology of AFL (*left*) and ASH (*right*). Fat droplets are predominantly found around the central vein (increased redox state following ethanol oxidation due to an the presence of more alcohol dehydrogenase). ASH is characterized primarily by fat, hepatic infiltration of granulocytes, ballooning of the hepatocytes, Mallory-Denk body formation and fibrosis (for more details see text). *AFL* alcoholic fatty liver, *ASH* alcoholic steatohepatitis

Although single, large macrovesicular fat droplets predominate, microvesicular fat droplets may also occur. At the simple fatty liver stage the only inflammatory changes are those seen in association with lipogranulomata, which are perivenular in location. Lipogranulomata comprise aggregated fat-laden macrophages with lymphocytes. Occasionally even true epitheliod granulomata can be seen [8, 31]. Lipogranulomata may either disappear or may end in focal perivenular fibrosis. It is interesting to note that at least in animal experiments, livers of older rats react more sensitively towards chronic ethanol consumption with enhanced fat deposition [74].

In addition, microvesicular steatosis, so-called alcoholic foamy degeneration, may also occur, which is of heterogeneous nature and also occurs in other hepatic diseases. An impaired mitochondrial ß-oxidation of fatty acids has been discussed as cause of this observation. Full blown alcoholic foamy degeneration is rare and found in only 1% of liver biopsies from alcoholics [31].

## Clinical and Laboratory Findings

AFL may develop within weeks, but may also regress completely within a few weeks or months after abstinence (Fig. 88.9). Subjective complaints are often absent or nonspecific, such as dyspepsia, bloating, meteorism, flatulence, anorexia, fatigue, intolerance of food, impotence or a sensation of pressure in the right upper abdomen. The most common finding is hepatomegaly. Jaundice is rare. Hepatic skin stigmata may be seen.

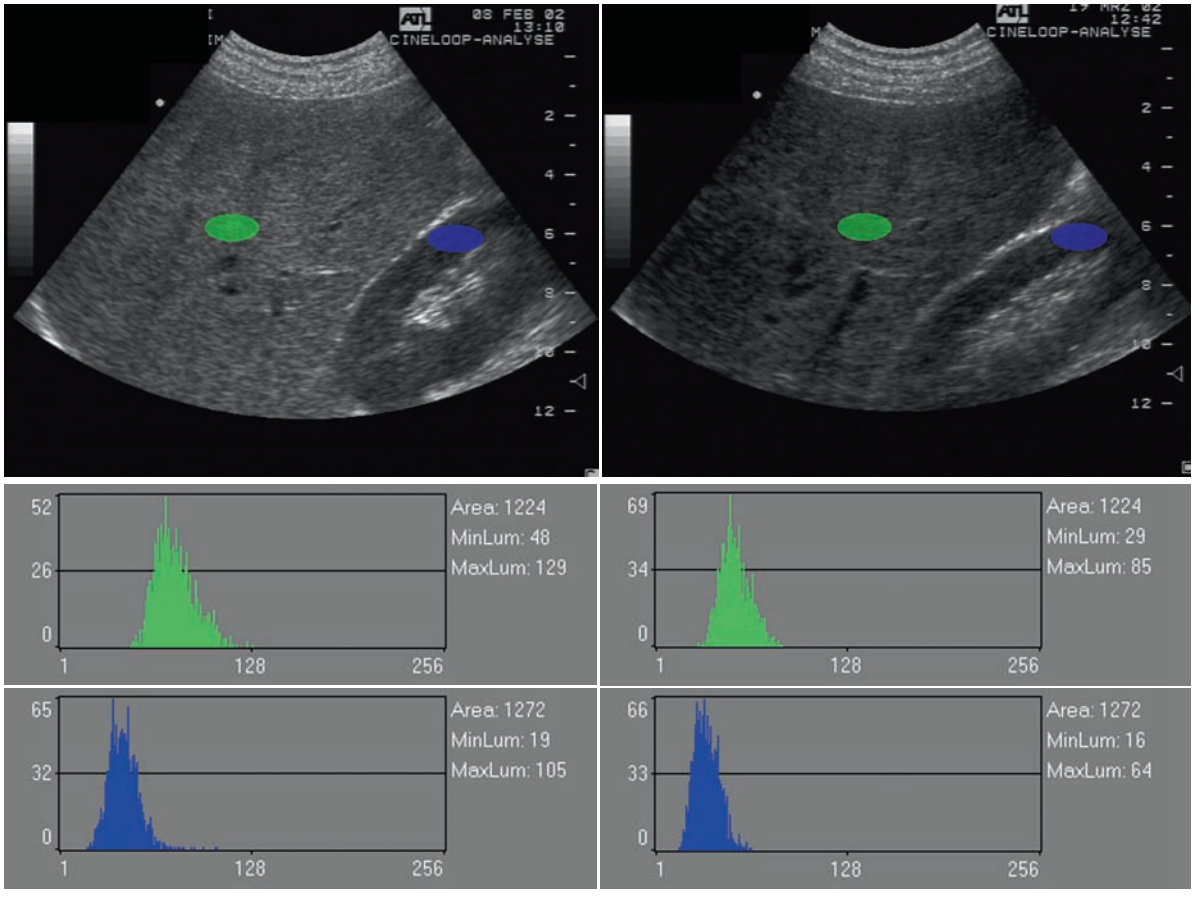



**Fig. 88.9** Sonographic course of alcoholic fatty liver (AFL) by digital evaluation of liver echogenicity. Pixel intensity analysis shows AFL of an alcoholic patient at the time of admission to the hospital (*left side*) and complete recovery of AFL of the same patient following 3 months of alcohol abstinence (*right side*) [84]

Routine laboratories demonstrate abnormalities attributed to chronic alcohol abuse. This includes an elevation of serum gamma-glutamyltrasnferase (γGT) activity [79]. The increase in serum γGT activity is a sign of adaptation rather than a sign of liver injury, and may also be seen in other pathological conditions (Table 88.10). This increase can be as high as 2,000–3,000 U/L without significant liver injury. An increase in γGT activity without an increase in serum transaminase activity has a sensitivity and specificity as an alcohol marker of more than 80%. However, in the presence of advanced liver disease associated with transaminase activities, γGT activity looses its specificity for alcohol completely since all types of liver disease are associated with an increase of serum γGT activity. The specificity of γGT as an alcohol marker in chronic liver disease is as low as 18% [4].

Other markers for chronic alcohol consumption are summarized in Table 88.11. It is interesting to note that bone fractures, such as those of the ribs and the vertebra, are frequently found in alcoholics due to the fact that alcoholics may frequently fall down and break their bones and may suffer from alcoholic osteopathy. Thus, the specificity of bone fractures seen in a routine chest X-ray is as high as 90% for alcoholism [55].

**Table 88.10** Differential diagnosis of an isolated increase in serum γGT activity

- Alcohol
- Drugs
- Acute pancreatitis
- Myocardial infarction/cardiac insufficiency
- Overweight, Diabetes mellitus
- Hypothyrodism
- Anorexia nervosa
- Myotonic muscular dystrophy
- Guillain-Barre syndrome
- Porphyria cutanea tarda
- Neurological diseases
- Malignant diseases/radiotherapy

**Table 88.11** Laboratory parameters identifying chronic ethanol consumption

| Short half-life (HL) | Intermediate HL | Long HL |
|---|---|---|
| Serum ethanol | γ-glutamyltransferase | Mean corpuscular volume of red blood cells |
| Ethanol-glucuronide | Carbohydrate deficient transferrin | |
| | Phosphatidyl ethanol | |

Alcohol serum markers may detect short or long term alcohol exposure depending on the half-life of these markers. For example, serum concentrations of ethanol-glucuronide may predict alcohol consumption during the past 24–48 h, while γGT activity and carbohydrate deficient transferring (CDT) concentrations have a half life of approximately 2 weeks, and the mean corpuscular volume (MCV) of the erythrocytes has a much longer half life of months. CDT is the most specific marker for chronic alcohol consumption and may be increased following 4–6 weeks of more than 60 g ethanol per day [36]. CDT measurement may be helpful in evaluating the following situations: relapse during withdrawal therapy, demonstration of alcohol abstinence to regain a driving license, and demonstration of alcohol abstinence after liver transplantation.

In AFL, AST may be slightly elevated (mostly due to its release from muscle) and ALT may be relatively low due to vitamin B6 deficiency. Thus, the AST/ALT ratio is above 1.

Sonography shows a typical "large, white liver" with diffusely pronounced echogenicity. Single reflexes are coarsened, and sound transmission is reduced. The degree of hepatic steatosis is difficult to assess by sonography. However, special software does allow for better quantification of hepatic fat (Fig. 88.9) [84].

## Differential Diagnosis

As pointed out, NAFLD also presents with fatty liver and needs to be ruled out. Other situations and diseases with fatty liver are: protein malnutrition, fasting, gastrojejunal bypass operation, parenteral nutrition, hypoxemia, infections, Cushing's disease, acromegaly, fatty liver of pregnancy, chronic inflammatory bowel disease, Reye's syndrome, side effects of drugs, and inborn errors of metabolism. Many drugs which induce fatty liver also result in steatohepatitis (Table 88.12).

## Complications

AFL is completely reversible with alcohol abstinence [84]. Complications are rare and may include intrahepatic

**Table 88.12** Drugs leading to hepatic steatosis and mimicking alcoholic steatohepatitis

- Perhexiline
- 4,4-Diethylaminoethoxyhexestrole
- Insulin
- Amiodarone
- Glucocorticoids
- Estrogens
- Nifedipin
- Tamoxifen

cholestasis. A special complication of AFL is *Zieve's syndrome* consisting of the triad jaundice, hyperlipidemia and hemolysis. The anemia resulting in reticulocytosis is responsible for enhanced erythropoesis and leads to lipid storing reticular cells, so-called foam cells in the bone marrow: The syndrome generally occurs following acute heavy alcohol misuse and manifests itself in the form of anorexia, nausea, vomiting, diarrhea, and often severe colic-like abdominal pain mimicking an acute abdomen. The pathogenesis is still not clear. The syndrome is reversible within weeks following alcohol abstinence.

Rupture of multiple fat loaded hepatocytes leading to pulmonary embolism has been published as case reports. Extremely rare is acute liver failure.

Patients who consume alcohol until admission to the hospital and who need emergency surgery may develop problems during initiation and monitoring of anesthesia. Intra- and postoperative complications are frequent and include withdrawal syndrome, bleeding, and delayed wound healing. Thus, operations should be performed if possible following alcohol detoxification therapy.

In addition, due to the induced CYP2E1, interactions with a variety of drugs occur as mentioned above. This may result in enhanced drug metabolism requiring dosage adaptation, or in the generation of toxic intermediates leading to drug-induced liver injury. CYP2E1 induction may also affect an increased activation of various xenobiotics used at the work place, including solvents or substances used in the dry cleaning industry. These compounds are highly activated via induced CYP2E1 to toxins and this may result in additional toxic liver disease.

If alcohol is consumed without adequate calories and hepatic glycogen levels are low, severe hypoglycemia may occur. This is due to the fact that ethanol metabolism generates NADH, resulting in a shift of pyruvate to lactate. Pyruvate is a major precursor of gluconeogenesis. If this compounds is missing, serum glucose levels cannot be obtained. As a practical consequence in the emergency room, unconsciousness alcoholics need to be checked for blood glucose. If it is low, glucose infusion should always be given together with vitamin B1, since alcoholics are deficient in vitamin B1 (thiamine) and glucose mobilizes the last traces of vitamin B1 when shifted as acetyl Co-A into the Krebs cycle. An acute deficiency of vitamin B1 results in Korsakow-Wernicke syndrome, a severe neurological disorder.

## Prognosis

Under normal conditions, AFL is reversible within weeks. Monitoring of the patient during alcohol abstinence is mandatory. If AFL still exists despite alcohol abstinence, other causes have to be evaluated such as type 2 diabetes or obesity. If alcohol is further consumed, AFL may persist or perivenular fibrosis associated with poor prognosis may occur. If more than 100 g ethanol per day is consumed, ASH develops within 4 years in 50% of cases. Independent of the drinking behavior, a 10 year survival of 46–72% in AFL has been reported.

## Therapy

Abstinence is the therapy of choice. If AFL has been detected during routine check up, the situation needs to be discussed with the patient. The patient should be seen by the general practitioner on a regular basis and serum markers for alcohol consumption should be measured. If the patient is alcohol dependent, an alcohol detoxification program should be prescribed in a center specialized in this form of treatment. Thereafter, talking therapy eventually supported by the use of anti-craving substances such as naltrexone or acamprosate should be continued. To monitor the course of AFL, sonography should be performed following 3 months of treatment.

The only compound with a positive effect on AFL seems to be methadoxine, a vitamin B6 derivative, which has been tested in a multicenter randomized placebo controlled trial with satisfactory results [9].

## Alcoholic Steatohepatitis

Alcoholic steatohepatitis (ASH) is one manifestation of ALD and is characterized by a specific histomorphological appearance associated with typical clinical syndromes. As pointed out, only 25–33% of patients with chronic alcohol misuse develop ASH. The severity of ASH may vary between mild, moderate and severe. ASH always develops from AFL and the pathogenetic mechanisms for the conversion from AFL to ASH are similar to those described for the development of NASH (Fig. 88.8). While mild ASH just requires alcohol abstinence as therapy, severe ASH is associated with a poor prognosis.

### *Definition*

ASH is a special form of toxic liver disease due to chronic excessive alcohol consumption and is defined by characteristic histomorphological criteria which include steatosis, necrosis, inflammation with infiltration of polymorphic granulocytes and fibrosis with the occurrence of Mallory-Denk bodies (MDBs) (Figs. 88.8 and 88.10) [8, 31]. MDBs are found intracellularly (Fig. 88.10). The histomorphologic appearance of ASH is indistinguishable from NASH (Table 88.13).

**Table 88.13** Comparison of histomorphology between ASH and NASH [22]

| | ASH (%) | NASH (%) |
|---|---|---|
| Macrovesicular steatosis | 57 | 85 |
| Microvesicular steatosis | 21 | 49 |
| Lobular hepatitis | 85 | 54 |
| Mallory-Denk Bodies | 16 | 3 |
| Nuclear vacuoles | 7 | 76 |
| Ductular proliferation | 96 | 53 |
| Perivenular fibrosis | 33 | 0 |
| Fibrosis/Cirrhosis | 63 | 38 |

### *Pathophysiology*

As pointed out, the prerequisite for the development of ASH is AFL. AFL is more sensitive to a second hit as compared to a normal liver. This second hit may vary interindividually with respect to quantity and quality, as in NAFLD.

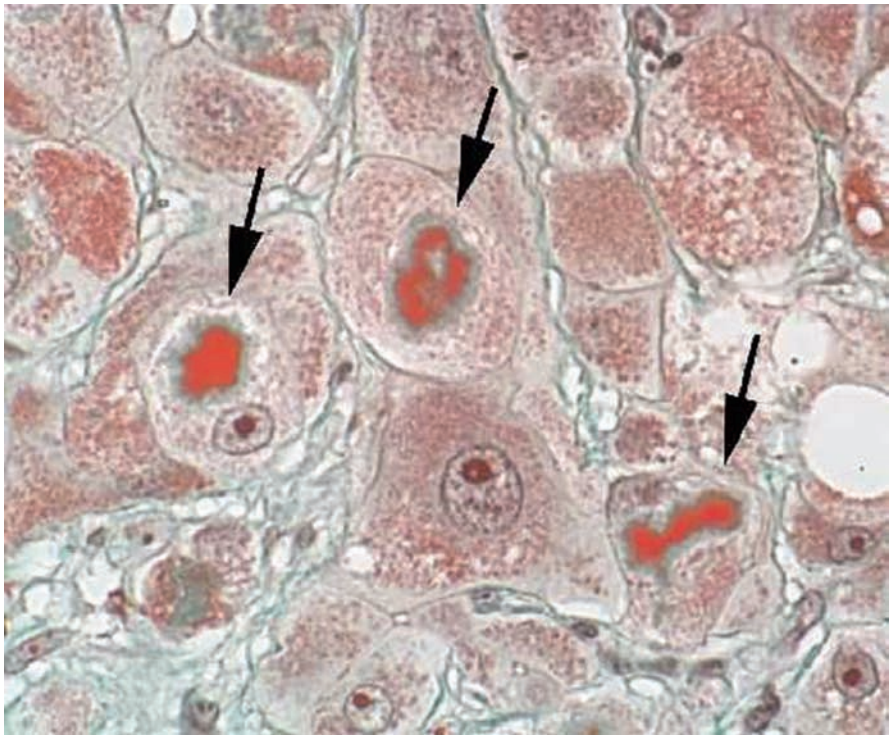


**Fig. 88.10** Mallory-Denk bodies (*arrows*) in alcoholic steatohepatitis. Hematoxylin & Eosin (kindly provided by Dr. Thomas Longerich, Insitute of Pathology, University of Heidelberg, Germany)

*The role of acetaldehyde*. Acetaldehyde is a toxic and carcinogenic agent. As discussed, acetaldehyde binds to proteins and DNA resulting in functional alterations of proteins and in the activation of the immune system [39, 59]. Acetaldehyde binds to mitochondria associated with histomorphological alterations (megamitochondria) and a decrease of mitochondrial function (fatty acid oxidation, ATP production and finally acetaldehyde metabolism) and to microtubules leading to a decreased secretion of macromolecules from the liver to the blood such as transferrin, albumin and lipoproteins (VLDL) (Fig. 88.11). Mitochondrial damage induces ROS and apoptosis, but also survival factors such as the nuclear factor kappa B (NFκB). Acetaldehyde also plays an important role in the inhibition of methyltranfer observed in heavy drinkers, which is associated with membrane injury and carcinogenesis [82]. The binding to glutathione decreases the activity of the antioxidative defense system and therefore oxidative stress prevails.

The binding of acetaldehyde to proteins results also in the generation of neoantigens with a consecutive antibody response (Fig. 88.12). Thus, a variety of autoantibodies occurs in ALD, which may play a role in ASH, especially in women (Fig. 88.13). A summary of the effects of acetaldehyde on the pathogenesis of ASH is given in Fig. 88.14.

*The role of reactive oxygen species (ROS)*. The production of free radicals that lead to lipid peroxidation seems to be of major importance in ALD [80]. With regard to ethanol metabolism several enzymatic systems are discussed as source of free radicals:

1. CYP2E1-dependent MEOS
2. Mitochondrial electron transport system of the respiratory chain
3. NADH-dependent cytochrome reductase
4. Xanthine oxidase and aldehyde oxidase
5. NADPH-oxidase in neutrophil leukocytes

The induction of CYP2E1 through chronic alcohol intake is of considerable significance.

ROS and hydroxyl-ethyl-radicals (HER) that cause an increased lipid peroxidation are produced through the cytochrome system. They bind covalent to microsomal proteins and lead to the production of specific antibodies against epitopes (Fig. 88.13) [2].

Several enzymatic systems, including the cytochrome P4502E1 (CYP2E1)-dependent microsomal monooxygenase system, the mitochondrial respiratory chain and the cytosolic enzymes xanthine oxidase and aldehyde oxidase, have been implicated as sources of $O_2$- and $H_2O_2$ in hepatocytes during ethanol oxidation. Alcohol-mediated free radical formation may be due to enhanced electron leakage from the mitochondrial

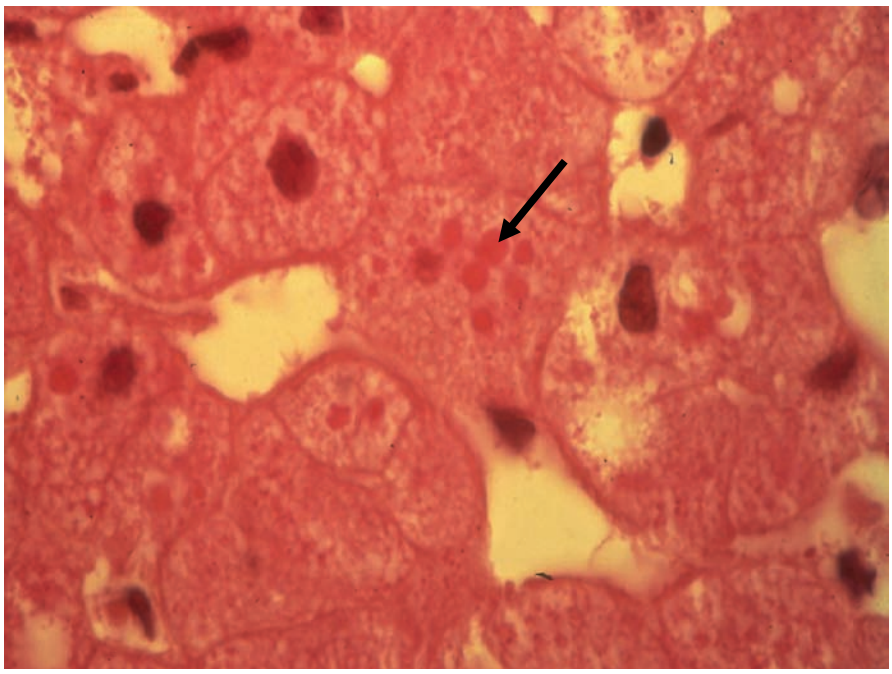


**Fig. 88.11** Megamitochondria (*arrow*) in alcoholic steatohepatitis. Hematoxylin & Eosin

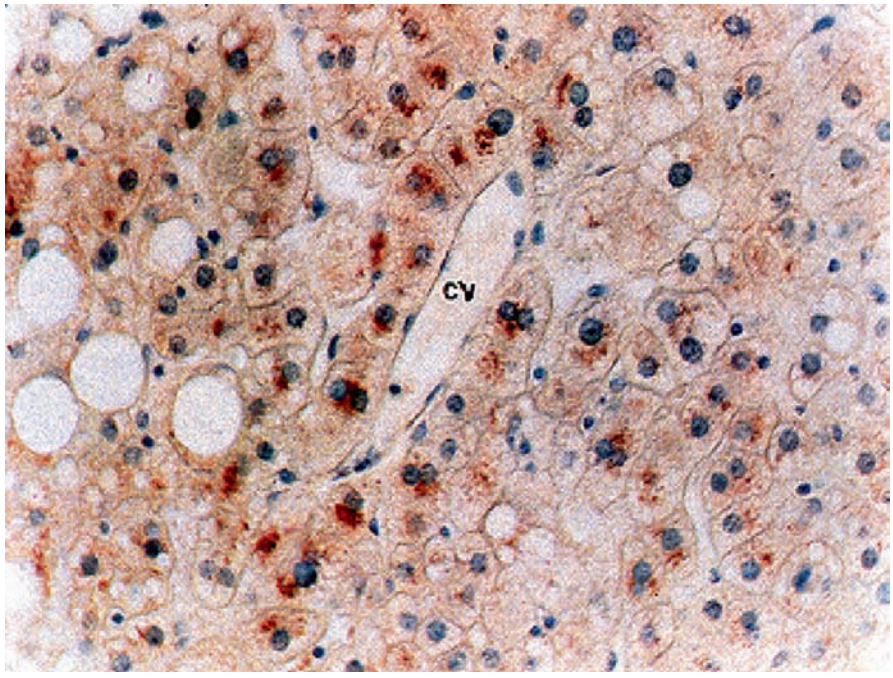



**Fig. 88.12** Immunohistochemistry of hepatic acetaldehyde-protein adducts in alcoholic liver disease (kindly provided by Dr. O.Niemela, Finland)

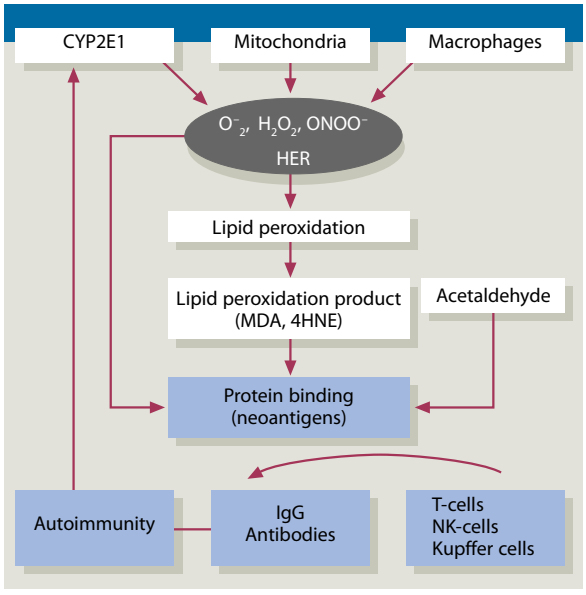



**Fig. 88.13** The effect of ethanol on the immune system. ROS and HER are generated through various pathways including CYP2E1, mitochondria or macrophages. These ROS result in lipid peroxidation, and lipid peroxidation products such as MDA and 4HNE bind to protein forming neoantigens. Similar neoantigens are formed when HER or acetaldehyde bind to protein. These antigens result in an activation of T-cells, NK cells or Kupffer cells resulting in antibody production, which may initiate autoimmunity. *CYP2E1* cytochrome P-4502E1, *HER* hydroxyethyl radicals, *4HNE* 4-hydroxynonenal, *MDA* malonedialdehyde, *NK* natural killer, *ROS* reactive oxygen species

respiratory chain, along with stimulation of NADH shuttling into mitochondria, to an interaction between N-acteylsphingosine (from TNFα) and mitochondria, to activated phagocytes in the liver, to hepatic iron overload, and to nitric oxide. The reaction of nitric oxide with $O_2$ results in the formation of peroxynitrite, which is highly reactive and impairs cell function. Inducible nitric oxide synthase (iNOS) is stimulated by ethanol and iNOS knockout mice are protected against alcohol-induced liver injury. Although all these factors may contribute to the generation of ROS following alcohol ingestion, animal experiments have convincingly demonstrated the important role of CYP2E1 in the production of ROS and ALD [30].

Chronic alcohol consumption leads to a 10- to 20-fold induction of CYP2E1, which metabolizes ethanol to acetaldehyde [39, 82]. This cytochrome is also involved in the activation of various co-carcinogens to their ultimate carcinogens, including nitrosamines, aflatoxins, and polycyclic hydrocarbons (Fig. 88.14) [77].

It has been shown that the concentration of CYP2E1 in the liver correlates with the generation of hydroxylethyl radicals (HER) and thus with lipid peroxidation. HERs bind to proteins and form neoantigens. Antibodies against HER-derived epitopes are detectable in the sera of patients with ALD. Induction of CYP2E1 resulted in ALD in animals, and inhibition of CYP2E1 by

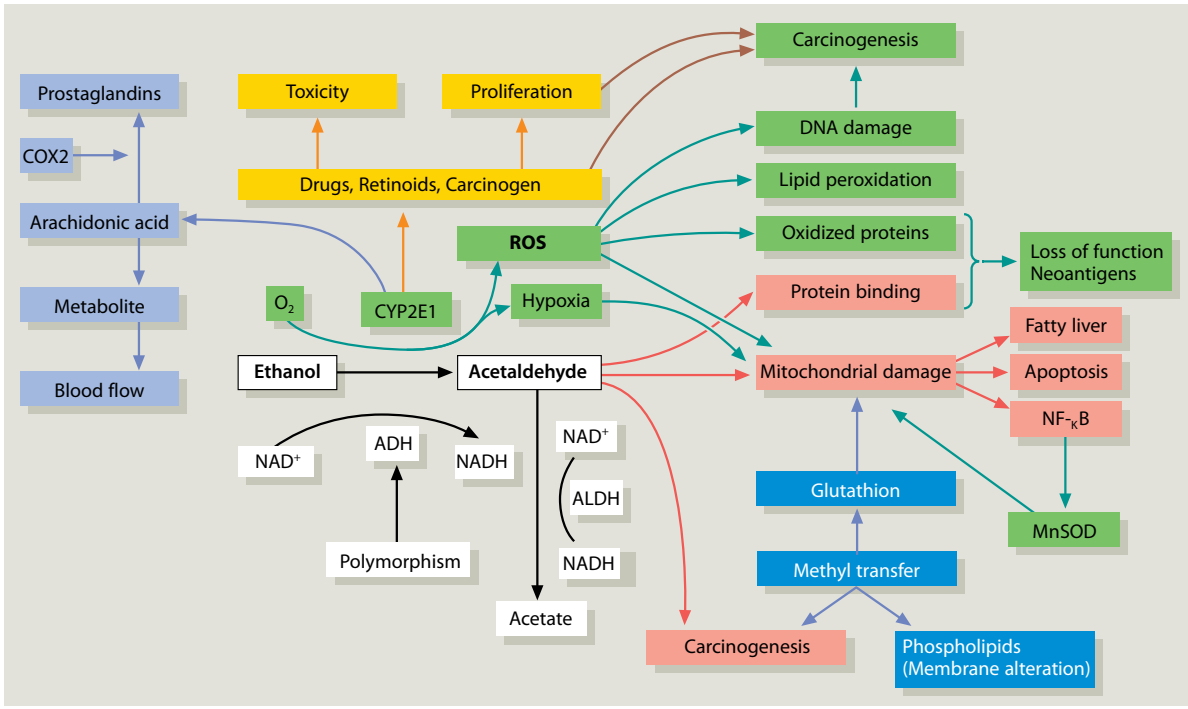



**Fig. 88.14** Ethanol metabolism and its consequences with respect to mechanisms involved in the pathogenesis of alcoholic liver disease: ethanol is metabolized to acetaldehyde via ADH or CYP2E1. The concentration of acetaldehyde depends on its production by ALD and its detoxification to acetate by ALDH. Both enzymes show polymorphism and therefore the amount of acetaldehyde produced is different among individuals. Acetaldehyde is a toxin and carcinogen, and binds to proteins and subcellular organelles such as mitochondria or microtubules. Mitochondrial function is decreased resulting in the occurrence of fatty liver, apoptosis and the activation of survival factors such as NFκB and in a further increase of acetaldehyde since ALDH is a mitochondrial enzyme. Also, methyl transfer is affected by acetaldehyde (see text) resulting in a decrease of phospholipids such as lecithin which is associated with membrane alterations and in DNA hypomethylation which is associated with enhanced carcinogenesis. In addition, acetaldehyde binds to glutathion which further injures mitochondria. Generally, the binding of acetaldehyde to proteins leads to the generation of neoantigens (see Fig. 88.13) and to a loss of various enzymatic functions. CYP2E1 mediated ethanol oxidation results in the generation of reactive oxygen species (ROS) and in hypoxia. ROS bind to proteins and DNA and initiate lipidperoxidation. Furthermore since a variety of drugs and xenobiotics share with ethanol the binding site at CYP2E1 an interaction between ethanol metabolism and their metabolism occurs leading to an increased toxification of drugs and carcinogens. Retinoic acid levels also decrease due to an increased metabolism of retinoic acid via induced CYP2E1 [80]. *ADH* alcohol dehydrogenase, *ALDH* acetaldehyde dehydrogenase, *CYP2E1* cytochrome P-4502E1, *ROS* reactive oxygen species

chlomethiazole was associated with an improvement in the damage. It has been concluded that this is mainly due to the stimulation and inhibition of free radical formation, respectively [30]. The role of CYP2E1 induction and cell injury has been studied in detail in the liver. For example, oxidized DNA products have been found to be lower in CYP2E1 knockout mice compared to wild-type mice, whereas more pronounced hepatic damage was observed in transgenic mice overexpressing CYP2E1 [7].

In humans, the extent of CYP2E1 induction is individually determined, but may be significant following the ingestion of 40 g alcohol per day, corresponding to 400 mL of 12.5 vol.% wine, for 1 week (Fig. 88.15) [63]. ROS produced by CYP2E1 lead to lipid peroxidation, with the generation of malondialdehyde and 4-HNE. 4-HNE causes the production of exocyclic DNA etheno adducts, which can be measured in urine and determined immunohistologically in the liver [25].These adducts have strong mutagenic and carcinogenic properties. It has been shown that these adducts are present in AFL, but are more pronounced in advanced ALD such as cirrhosis. Some evidence suggests that the degree of CYP2E1 induction in cell cultures can be correlate with the extent of exocyclic etheno-DNA adducts.

It should be pointed out that hepatic FFA may also induce CYP2E1 and may lead to ROS. Thus fatty liver enhances the production of ROS and is especially sensitive to these species, since increased lipid peroxidation may occur and increased lipid peroxidation products

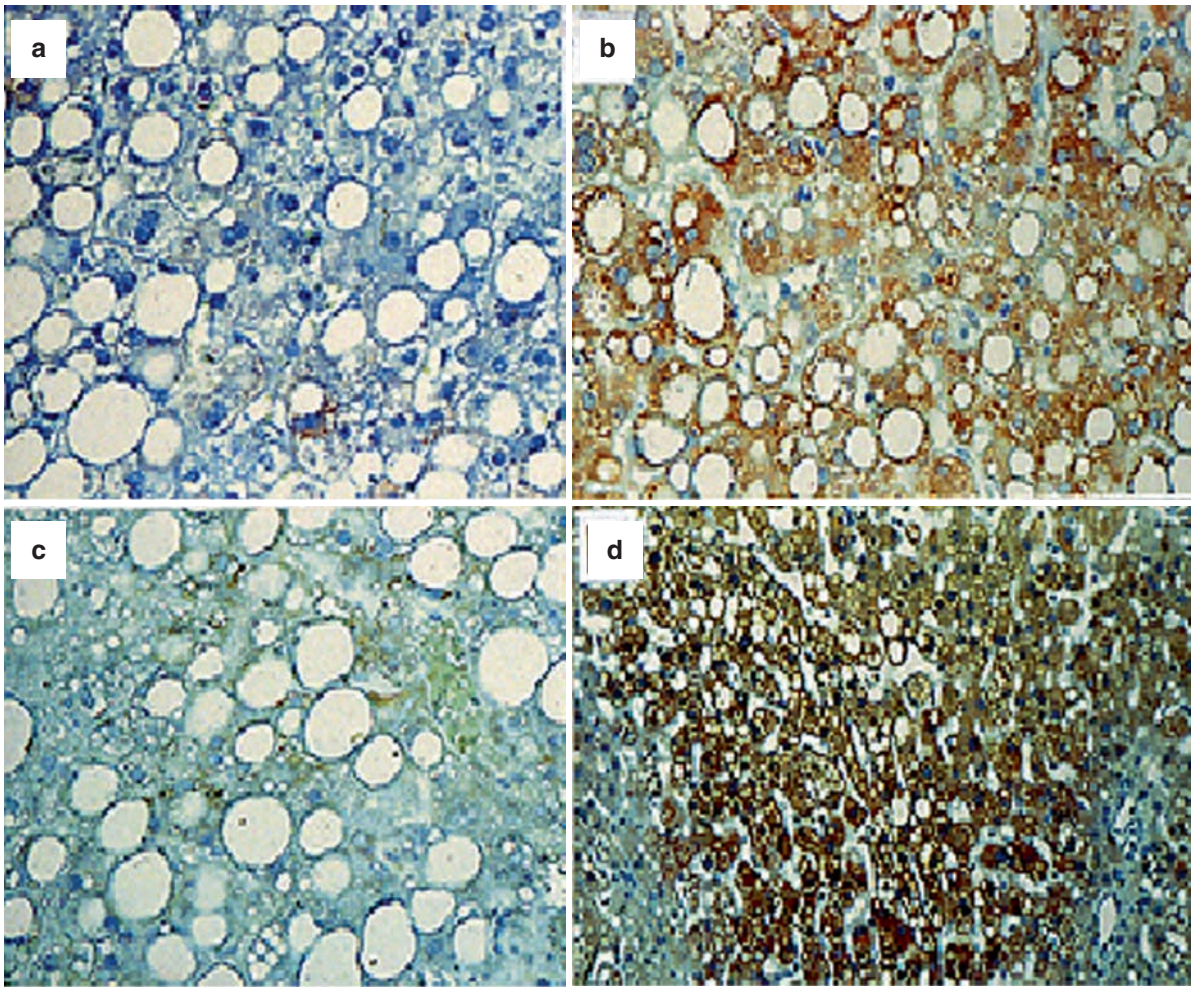



**Fig. 88.15** Immunohistochemistry of hepatic cytochrome P-4502E1 (CYP2E1) in liver biopsies from four different patients with alcoholic liver disease (ALD). The brown colour reflects CYP2E1. The intensity of CYP2E1 induction varies between almost none (a), mild (b), moderate (c) and strong (d)

such as 4-HNE and malondialdehyde are generated which may bind to protein resulting in protein adducts (with change in function and antibody production) and which also may bind to DNA as described above. Therefore it is not surprising that polyunsaturated fatty acid enriched diets such as fish oil enhance the liver injury observed following alcohol consumption as compared to unsaturated fatty acid enriched diets. A summary of the effects of CYP2E1 and ROS on the pathogenesis in ASH is illustrated in Figs. 88.14, 88.16 and 88.17.

**The role of tumor necrosis factor α and other cytokines.** In fatty liver, FFA can directly activate the IKK-β/NFκB pathway in hepatocytes via a lysosomal, cathepsin B-dependent mechanism. Hereby a translocation of Bax to lysosomes with lysosomal destabilization and release of cysteine protease (cathepsin B) is required resulting in an activation of NFκB via IKK-β and a consecutive increase in TNFα, and other interleukins including osteopontin. In addition, acetaldehyde also leads to an increase in NFκB. The degree of inflammation and fibrosis correlates with NFκB expression which in turn coincides with apoptosis albeit Bcl-2 and uncoupling protein 2 (UCP-2) [18].

Most of TNFα may, however, be secreted by Kupffer cells due to endotoxin stimulation. Chronic alcohol consumption may lead to intestinal bacterial overgrowth and to mucosal injury. As a result, lipopolysaccharides and bacterial products are entering the portal vein and these endotoxins activate hepatic Kupffer cells via CD14/ toll 4 receptor complex leading to intracellular signal transduction, with the release of

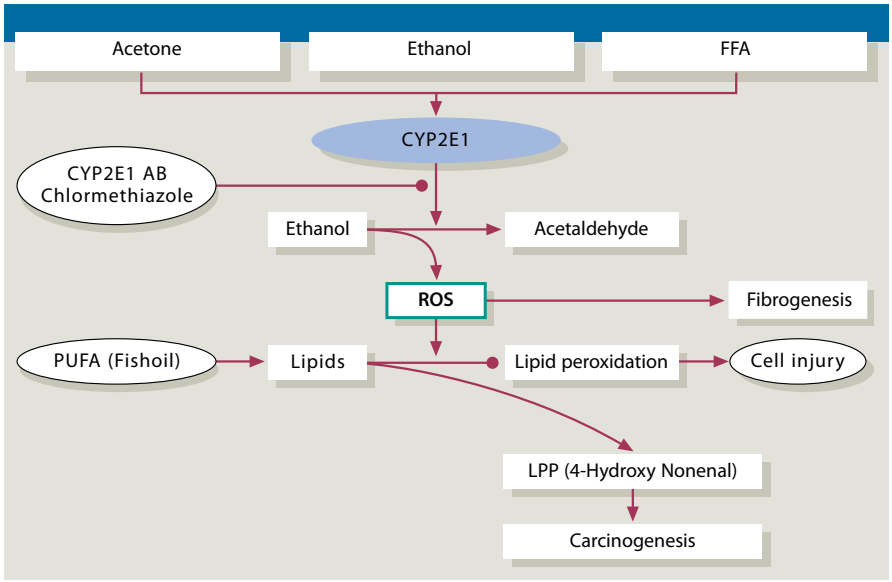



**Fig. 88.16** The potential role of CYP2E1 in the pathogenesis of ALD. CYP2E1 can be induced by ethanol, acetone and free fatty acids (important also in non-alcoholic fatty liver disease). CYP2E1 catalyzes ethanol oxidation to acetaldehyde and produces ROS. This reaction can be completely blocked by CYP2E1 antibodies or by chlomethiazole, a specific CYP2E1 inhibitor. Chlomethiazole indeed inhibits alcoholic liver disease in experimental animals. ROS initiates fibrogenesis and leads to lipid peroxidation which is favoured by the ingestion of PUFA (e.g. fishoil). LPP such as 4-hydroxynonenale not only contributes to ALD, but also to hepatocarcinogenesis by binding to DNA and forming exocyclic etheno-DNA adducts [80]. *ALD* alcoholic liver disease, *CYP2E1* cytochrome P4502E1, *FFA* free fatty acids, *LPP* lipid peroxidation products, *PUFA* polyunsaturated fatty acids, *ROS* reactive oxygen species

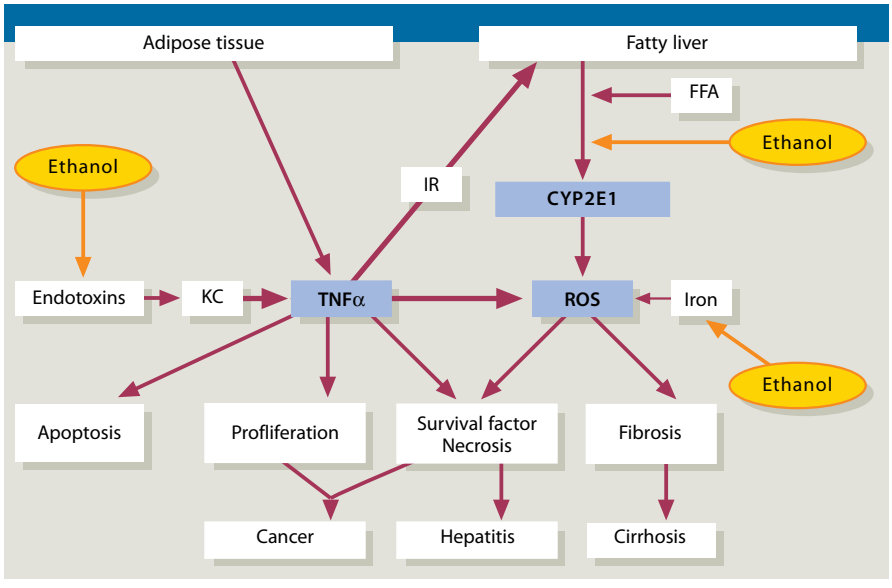



**Fig. 88.17** The central role of CYP2E1 and TNFα in the pathogenesis of alcoholic liver disease. TNFα is generated by Kupffer cells induced by gut derived endotoxins and from fatty tissue (primarily central fat). TNFα increases the generation of fatty liver via stimulation of IR resulting in hyperinsulinemia and fatty liver. FFA and ethanol induce CYP2E1 resulting in ROS. This is further enhanced by ethanol mediated iron uptake and iron accumulation in the liver. TNFα results in apoptosis, cell proliferation and necrosis. ROS leads to both fibrosis and necrosis. Both factors contribute to inflammation (hepatitis), fibrogenesis (cirrhosis) and carcinogenesis [80]. *CYP2E1* cytochrome P4502E1, *FFA* free fatty acids, *IR* insulin resistance, *ROS* reactive oxygen species, *TNFα* tumour necrosis factor α

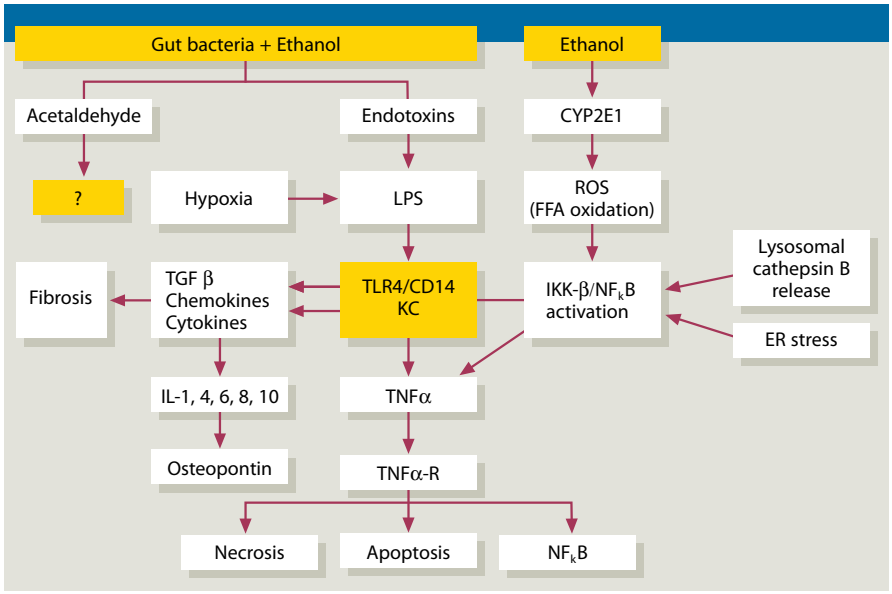



**Fig. 88.18** Pathophysiology of alcoholic steatohepatitis. Various factors contribute to the morphological feature observed. Endotoxins from the gut penetrate the mucosal barrier, which is altered by ethanol. The uptake of endotoxins is estrogen dependent. These endotoxins, primarily LPS bind to toll-like receptor TLR and to CD14 on the surface of Kupffer cells which release chemokines and cytokines including TGFβ-1) and TNFα. These factors lead to fibrosis, inflammation, necrosis and apoptosis. In addition, CYP2E1 induced by ethanol and/or FFA produces ROS. ROS may activate the IKK-β/NFκB pathway directly or via cathepsin B-dependent mechanisms, which further increase TNFα. Modified from reference #80. *CYP2E1* cytochrome P4502E1, *ERS* endoplasmic reticulum stress, *FFA* free fatty acids, *IL* interleukin, *LPS* lipopolysaccharides, *NFκB* nuclear factor kappa B, *ROS* reactive oxygen species, *TGFβ* transforming growth factor β, *TLR4* toll like receptor 4, *TNFα* tumour necrosis factor α

proinflammatory cytokines, particularly TNFα, eicosanoids, ROS and nitric oxide. These cytokines induce inflammation and fibrogenesis (Fig. 88.18). TNFα results after binding to its receptor in cell apoptosis. It has to be pointed out that Fas induced apoptosis is significantly enhanced in the presence of CYP2E1 [95].

Similar to how ALD can be abolished by the use of a CYP2E1 inhibitor, TNFα antibodies may also prevent ASH in animals. Furthermore, the application of antibiotics leading to a decrease in gut endotoxins, or the knock out of Kupffer cells by gadolinium-chloride, also improve hepatic histology in the Tsukamoto-French alcohol model. Subsequently, when TNFα receptors knockout mice were studied, ASH could also be prevented. All this supports the central role of TNF α in the pathophysiology of ASH [80, 94, 95]. A simplified scheme of the pathogenesis of ASH with TNFα and CYP2E1 at its center is shown in Fig. 88.16.

Besides other factors, interleukin-1 and 6 are significantly elevated in alcoholic patients with advanced liver disease. It has to be pointed out that interleukin 6 inhibits HOGG1, an important repair enzyme for 8-oxoguanosin adducts. Interleukin-6 is also anti-apoptotic through upregulation of the anti-apoptotic gene mcl-1.

Increased TNFα serum levels correlate significantly with a lower long term survival rate [23]. Also IL-6 activates hepatic stellate cells and correlates with the severity of ASH. Histologically, ASH is associated with necrosis and infiltration of leukocytes. In this regard the leukocyte adhesion molecules seem to be of major significance [98].

There is also some evidence that polymorphisms in the TNFα-promoter gene and polymorphisms of IL-10 play a modificatory role in the development of ASH [65].

More recently, intrahepatic gene expression was studied in ASH as compared to AFL and normal livers [83]. Gene expression was analysed by DNA microarray on RNA isolated from patients livers. The most important results confirmed by real time RT-PCR was an up regulation of IL-8, osteopontin and TNFRSF14, a member of the TNFα gene family. Osteopontin is probably the most interesting protein which has also been

found to be elevated in experimental alcohol induced liver injury, and may deserve further attention.

Most recently, it has been shown that genes involved in hepatic fibrogenesis, inflammatory response, and oxidative stress are overexpressed in ASH. Some candidate genes such as tissue inhibitor of metalloproteinases-1 (TIMP-1), growth-related oncogene (GRO-a), and several components of NADPH oxidase (dual oxidases 1 and 2) correlated with histology and parameters indicative of disease severity [13].

AC may develop from ASH. However it is not clear, whether ASH with enormous inflammation driven oxidative stress and hyperproliferation is an important prerequisite for cancer development some decades later in the state of cirrhosis. Therefore it would be important to know whether cirrhotic patients who went through ASH have an increased risk for hepatocellular cancer compared to those without ASH.

**Effect of ethanol on the ubiquitin-proteasome pathway.** Another mechanism, by which alcohol may contribute to the occurrence of ASH is the inhibition of ubiquitin-proteasome pathway [26]. The ubiquitin-proteasome pathway regulates protein digestion within the cell. Many liver cell functions are regulated by this mechanism of protein degradation. These include cell cycle check points and activation of transcription factors such as nuclear factor NFαB, and hypoxia inducible factor 1α (HIF1α). The loss of proteasomes or the inhibition of the ubiquitin-proteasome pathway can lead to hepatocellular injury including proliferation and apoptosis, and hepatic inclusions of aggregated cytokeratins. Liver cell gene expressions, dependent on transcription factor activation by the proteasome, could impede the inflammatory response of the liver and the response to hypoxic injury.

Proteasome function is bi-phasically regulated by ethanol metabolism. High ethanol levels suppress proteasome activity, while lower levels may have no or an activating effect on the enzyme. Ethanol stabilizes proteins normally degraded by the proteasomes. This may include among others CYP2E1, NFκB, and HIF1α. Thus, CYP2E1 toxicity is enhanced.

The inhibition does not occur after two weeks of ethanol feeding. This suggests that an oxidized product of CYP2E1 generated by ethanol CYP2E1 would be a likely mechanism of inhibition. Indeed, transgenic mice in which the gene of CYP2E1 had been knocked out, failed to develop proteasome activity inhibition even after one month of ethanol feeding. One candidate product is 4 HNE, an end product of lipid peroxidation which results from the HER generation by CYP2E1 during ethanol oxidation. Thus, oxidative stress leading to 4-HNE may further potentiate oxidative stress by inhibiting degradation of CYP2E1.

Another possible mechanism that could explain the loss of proteasome activity after alcohol feeding is phoshorylation of a regulatory subunit of the proteasome.

## *Histopathology*

Liver histology is characterized by the ballooning degeneration of the hepatocytes associated with necrosis, enhanced apoptosis and frequently, but not regularly the occurrence of MDBs (Fig. 88.10) [8, 31]. Ballooning of the hepatocyte represents a disturbed secretion of proteins and water due to an alteration of the microtubular system. Hepatocellular necrosis is an obligate histological finding which may occur as single or group necrosis. Most important is a polymorphonuclear cell infiltrate also containing T-lymphocytes, and natural killer (NK) cells.

The occurrence of MDBs may be linked to polyubiquinated proteins in the liver, especially cytokeratins (CK) 7, 8, 18, 19 [19]. Hyperphosphorylation of cytokeratin 8 has been found in MDBs and it is believed that hyperphosphorylation induces MDB formation. The turnover of cytokeratins is signalled by phophorylation followed by ubiquitination. Proteolysis of the cytokeratins then occurs by the proteasome. In addition, MDBs also stained positive with antibodies against heat shock protein as well as p62 (Z1P). MDBs are associated with a poor prognosis. In summary, proteasome inhibitors such as ethanol induce MDB formation by leading to an inhibition of proteolysis and an accumulation of protein in the cell. Since cytokeratins are major components of MDB they disappear from the cell (ghost cell formation). Cells containing MDB lose their bile canaliculi and cytokeratin in the plasma associated with an enlargement of the cell. The functional consequences are yet unclear. Lack of cytokeratins may make cells more vulnerable to injury, physical stress, may disturb the progression of the cell cycle and may develop apoptosis in response to FAS. MDB may also be found in other liver diseases, such as Wilson's disease, α1-antitrypsin deficiency, primary biliary cirrhosis, hepatitis C, hepatocellular cancer and NASH (for review see [19, 26, 31].

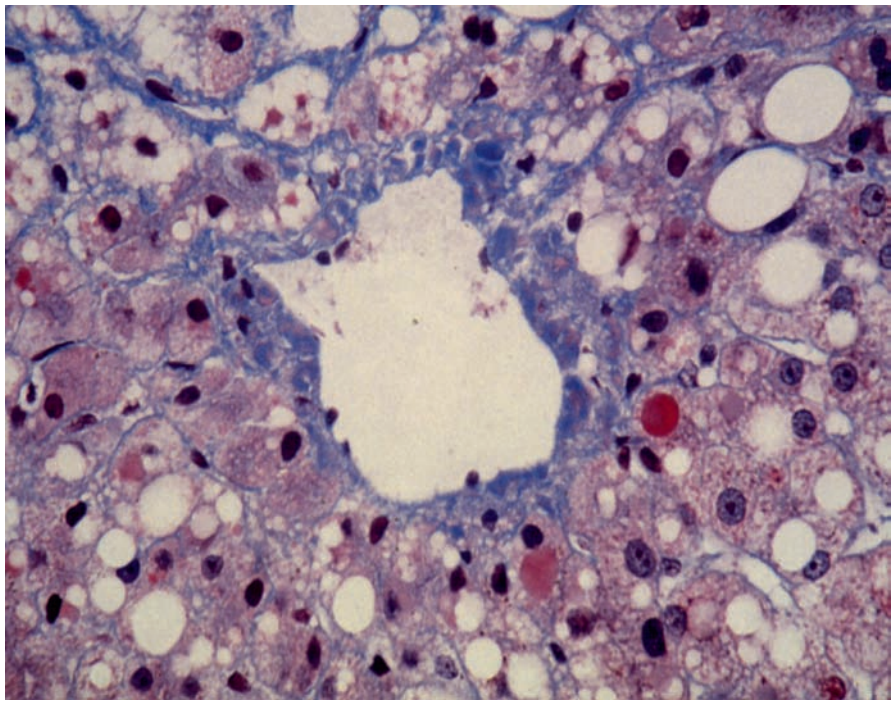


**Fig. 88.19** Histomorphology of alcoholic liver disease with perivenular sclerosis (collagen is stained in blue) as an early sign of fibrosis with poor prognosis if alcohol consumption is continued. Trichrome stain [31]

In addition to MDB also giant mitochondria are observed which represent morphological and functional altered mitochondria (Fig. 88.11). As for AFL the morphological alterations are most pronounced around the central vein where fibrosis starts (Fig. 88.19). Perivenular fibrosis at the fatty liver stage or in ASH is a poor prognostic sign which has a high probability for the development of advanced liver disease when alcohol is further consumed.

Cholestasis and bile duct proliferation may occur and cholestasis represents another independent poor prognostic marker associated with increased mortality [60]. Typical histomorphological features of ASH as compared to NASH are given in Table 88.13.

## *Clinical Findings*

Seventeen per cent of all liver biopsies of patients who are admitted for alcohol detoxification reveal ASH and 40% of patients with alcoholic cirrhosis also have ASH in a cirrhotic liver. ASH shows a wide variety of subjective and objective findings that can be classified into mild, moderate and severe forms due to their intensity and frequency. Twenty-five percent of the patients develop excessive liver necrosis with clinical signs of hepatic failure and hepatic encephalopathy. Though there has been shown a correlation between the degree of clinical manifestation and the severity of histological evaluation, the presence of ASH cannot be excluded due to the mere fact of few clinical symptoms. In most studies the acute mortality of severe ASH lies between 15% and 25% [47, 50].

Diagnosis of ASH is made by clinical symptoms, typical laboratory alterations and histology. Clinical symptoms are given in Fig. 88.20, and consist of nonspecific

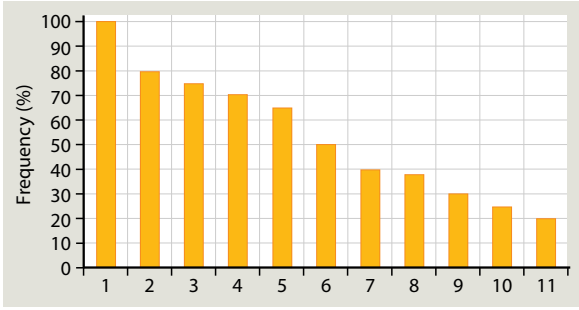



**Fig. 88.20** Clinical presentation and symptoms in alcoholic steatohepatitis. The numbers represent the following clinical symptoms: 1 = hepatomegaly; 2 = ascites; 3 = anorexia; 4 = encephalopathy; 5 = splenomegaly; 6 = weight loss; 7 = alcohol withdrawal syndrome; 8 = fever; 9 = pancreatitis; 10 = gastrointestinal bleeding; 11 = infection [51]

**Table 88.14** CAGE Test

- Have you ever tried to **CUT DOWN** your alcohol consumption?
- Has ever anyone been **ANNOYED** by your alcohol intake?
- Did you ever feel **GUILTY** due to your alcohol drinking behaviour?
- Did you ever need alcohol as an **EYE-OPENER** in the morning to start daily routine?

right upper quadrant pain, nausea, emesis and frequently associated with fever and jaundice. Signs of hepatic decompensation such as encephalopathy, ascites and hepatorenal syndrome are often observed in critically ill patients [51]. Patients may also present symptoms of alcohol withdrawal and signs of malnutrition. Approximately one third of all patients with ASH without cirrhosis show signs of feminization, e.g. gynecomastia and feminine hair-growth. Since ASH is often present in a cirrhotic liver, clinical symptoms of a decompensated hepatic cirrhosis may be found [56].

It is mandatory to ask for the amount and the duration of alcohol consumption. In some situations a questionnaire for alcohol dependence may be of help such as the CAGE test. Serological markers can be useful to identify alcoholism (Tables 88.11 and 88.14).

## Laboratory Findings

Laboratories show an increase in the activity of AST and ALT, always with higher values for AST but rarely of more than 300 U/l. The AST:ALT ratio is higher than 1 and in most cases 2 or more. In addition, serum γGT activity is significantly elevated occasionally up to more than 1,000 U/L. γGT elevation represents enzyme induction by ethanol and not liver injury, while an increase in aminotransferase activity represents liver cell damage. The predominant increase of AST may be explained by the fact that AST is partially located intramitochondrially and mitochondria are primary targets for alcohol. On the other hand alcohol is also a toxin for skeletal muscle cells which contain high concentrations of AST which may be liberated during excess alcohol consumption. The relative lower increase in ALT is probably due to a deficiency of pyridoxalphosphate. Also, an increase in GLDH represents mitochondrial damage. An elevation of the activity of alkaline phosphatase can be seen in 40–80% of cases dependent on the severity of the disease [47, 50, 56].

**Table 88.15** Laboratory parameters in alcoholic liver disease [92]

| Laboratory parameters | Evaluation |
|---|---|
| γ-GT-activity in the serum | Frequently elevated (sometimes extremely high values) |
| AST/ALT-ratio in the serum | > 1.5 (sometimes > 2) |
| Alcohol serum markers (MCV, CDT) | Increased |
| Uric acid, triglycerides | Sometimes increased |
| Bilirubin an anemia | Rare (hemolysis) |

In the presence of alcoholic cirrhosis, typical cirrhosis markers such as low platelet counts and decreased liver function (decreased serum albumin and coagulation factors) are also observed

In severe ASH β- and γ globulins are elevated. Thus immunoglobulin A is elevated since many of the neoantigens produced by acetaldehyde and ROS belong to this immunoglobulin fraction. Laboratory values of ASH and NASH are summarized in Table 88.15.

In addition, typical for ASH is an increase in the leukocyte count with toxic granulation together with fever due to a cytokine mediated inflammation. Typical hematologic abnormalities are macrocytotic hyperchromic anemia and thrombocytosis.

If ASH is severe, functional parameters such as prothrombin time, serum bilirubin, and serum albumin may deteriorate. These parameters are part of the severity scores of ASH.

## Imaging

Sonographically, ASH may not be distinguished from AFL. The demonstration of steatosis and the exclusion of extrahepatic cholestasis (if jaundice is present) is important. If the diagnosis can not be established (which is rare) liver biopsy may be performed. Liver biopsy may be important to determine the severity of the disease. It may also be helpful for the decision to use steroids as a treatment strategy, since those patients with a dense infiltration of polymorphic granulocytes respond best to corticosteroid therapy.

## Differential Diagnosis

Distinguishing ASH from NASH may be difficult. Laboratory markers including AST, ALT and GGT may

be helpful. Recently an ALD/NAFLD index (ANI) has been suggested for the differentiation between ALD and NAFLD. ANI, derived from easily available objective variables, accurately differentiates ALD from NAFLD in hospitalized, ambulatory, and pretransplantation patients. ANI can be determined with the use of the following formula:

$$ANI = -58.5 + 0.637\ (MCV) + 3.9\ (AST/ALT) - 0.406\ (BMI) + 6.35 \text{ for male gender.}$$

$$P = e^{ANI} / (1 + e^{ANI})$$

With $p > 0$ a diagnosis of ALD is favored, and with $p < 0$ a higher likelihood of NAFLD exists [21].

Hepatic steatosis with inflammation can also occur in Wilsons' disease and with certain drugs (Table 88.12).

It should be pointed out that chronic alcohol consumption may also be a cofactor which is present in other types of liver disease. The combination between ALD and hereditary hemochromatosis, hepatitis B and C and NAFLD is frequent and worsens the outcome of the disease. Therefore these types of liver disease should be excluded.

In general, differential diagnosis of ASH is not very difficult, since medical history and clinical findings suggest an alcoholic etiology of the disease. Primarily, the following diseases have to be considered: NASH, Wilsons's disease and certain drugs which may produce phospholipidosis. For the detection or exclusion of alcohol abuse in cases of unclear medical history, the use of markers for chronic alcohol consumption may be helpful (Table 88.11).

The possibility that ALD coexists with another form of liver disease, e.g. virus hepatitis, hemochromatosis and NASH should always be considered.

## Complications

Patients with ASH often have consumed alcohol until admission to the hospital and may therefore show symptoms of alcohol withdrawal during the course of their hospitalization. Mild to moderate ASH may resolve with only alcohol abstinence. Severe ASH may lead to complications of liver decompensation including portal hypertension, hepatorenal syndrome and hepatic encephalopathy. Mortality is high, in some series up to 25%.

## Natural Course and Prognosis

The natural course of ASH is illustrated in Fig. 88.21. Even patients with mild disease have a significant mortality. Patients who need to be hospitalized even without jaundice have a mortality risk of approximately 20%, while patients with severe disease (Discriminant Function [DF] > 32) have a short term mortality of 50% or more. The 4-year survival rate is estimated as follows:

- AFL 80%
- ASH 58%
- Cirrhosis 49%
- Cirrhosis + ASH 35% [11, 91]

Various scores predicting severity and prognosis of ASH do exist including the Maddrey score (DF), the Orrego index, a combined clinical and laboratory index (CCLI), the model for end stage liver disease (MELD), and the Glasgow Alcoholic Hepatitis Index (GAHI) [20, 24, 46, 64]. The oldest, but still extremely helpful score to identify patients with poor prognosis is DF. For calculation of DF see Chapter 30. *A DF greater than 32 identifies patients with a one-month mortality of 50%.*

More recent studies have shown that the MELD score allows valuable evaluation of prognosis, too. MELD integrates kidney function and can be easier standardized than the DF. For calculation of MELD score see Chapter 30. *A MELD score greater than 21 is associated with a three month mortality of 20%.*

In addition, the Orrego index correlates significantly with one year mortality, and the GAHI, similar to the MELD score, integrates kidney function as well

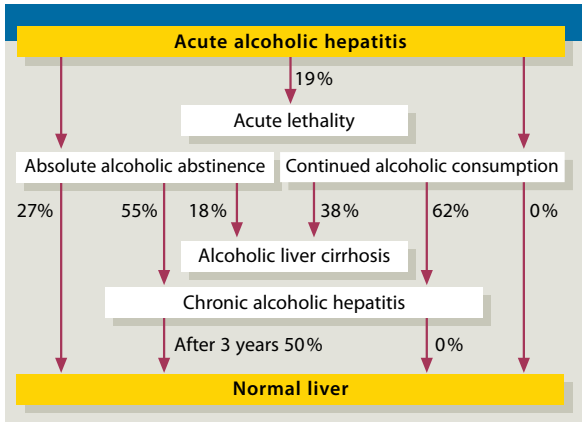
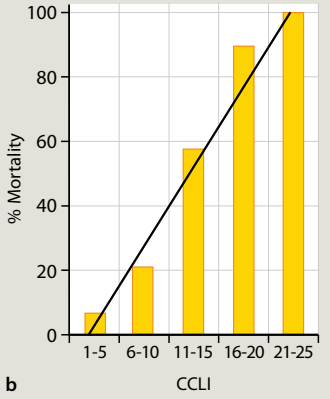



**Fig. 88.21** The natural course of alcoholic steatohepatitis [92]

**Fig. 88.22** Combined clinical and laboratory index (Orrego index) as a prognostic tool in alcoholic steatohepatitis. The index correlates well with one year survival (right side) [64]

a

| Clinics | Degree/Score | | Laboratory | Degree/Score | |
|---|---|---|---|---|---|
| Encephalopathy | 1–3 | 2 | Prothrombin time (sec > normal) | 4–5<br>>5 | 1<br>2 |
| Collaterals | 1–2 | 1 | Hemoglobin (% of normal) | 75–89.9<br><75 | 1<br>3 |
| Edema | 1<br>2–3 | 1<br>2 | Albumin (g/dl) | 2.5–2.9<br><2.5 | 2<br>3 |
| Ascites | 1–3 | 2 | Bilirubin (mg/dl) | 2.1–8<br>>8 | 2<br>3 |
| Spider naevi | >10 | 1 | Alkaline phosphate (U/dl) | >330 | 2 |
| Weakness | – | 1 | | | |
| Anorexia | – | 1 | | | |




b

as the age of the patient (Fig. 88.22; Table 88.16). A recent comparison between DF and GAHI showed a significant better prediction of three month outcome with 75% for the GAHI as compared top 53% for DF. In general, ASH with ascites and encephalopathy has a ninety day mortality of approximately 75%. If either ascites or encephalopathy is present, mortality is approximately 25% [20].

Most recently, a new score, the so-called Lille score has been reported to be able to identify patients with a poor prognosis and a poor response to steroid treatment [44].

Further factors that worsen the prognosis are the presence of MDBs, of less than 20% fat in the liver biopsy, and substantial alterations of the portal blood flow measured via Doppler sonography.

## *Therapy*

On the basis of the pathophysiology of ASH, various therapeutic strategies have been developed:

- Abstinence
- Nutritional therapy
- Drugs (corticosteroids, pentoxifylline, antioxidants, TNFα antibodies)
- Extracorporeal liver support and
- Liver transplantation.

**Table 88.16** Glasgow alcoholic hepatitis index [24]

| | Score | | |
|---|---|---|---|
| | 1 | 2 | 3 |
| **Age** | < 50 | > 50 | – |
| **White cell count** ($10^9$/L) | < 15 | > 15 | – |
| **Urea** (mmol/L) | < 5 | > 5 | – |
| **Prothrombin time** | < 1.5 | 1.5–2.0 | > 2 |
| **Bilirubin** (mg%) | < 7.25 | 7.25–14.5 | > 14.5 |

It has already been pointed out that ASH may vary from mild to severe and that in most cases, alcohol abstinence is mandatory to cure ASH. In more severe cases, prognosis worsens and therefore prognostic scores have been developed to identify those patients with poor prognosis and intensified therapeutic strategies.

**Abstinence.** In all types of ALD abstinence is mandatory, since abstinence has a significant effect on prognosis (Fig. 88.23). In the case of alcohol

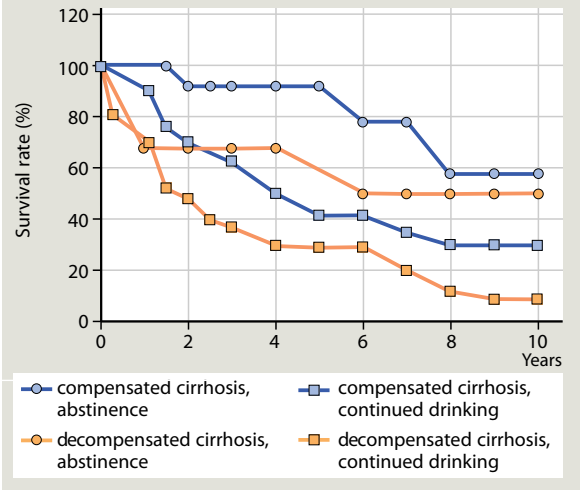



**Fig. 88.23** The role of abstinence in the prognosis of alcoholic cirrhosis. Abstinent patients have a better survival regardless of the severity of cirrhosis [56]

dependency, alcohol detoxification therapy needs to be performed. In most cases treatment of alcohol detoxification includes the administration of central acting psychoactive drugs such as benzodiazepines or chlormethiazole (mostly used in Europe) and ß-blockers to avoid severe withdrawal syndromes. Long-term therapy is based on psychological talking therapy. Relapse has been positively influenced by the use of anti-craving substances such as acamprosate or naltrexone [28].

**Nutritional therapy** (see also Chapter 91)

***Oral and/or enteral nutrition.*** As assessed in a large United States trial from Veterans Affairs Hospitals, the prevalence of malnutrition reaches 100% in patients with severe ASH, and a significant correlation of malnutrition with short- and long-term survival was established [32, 78, 81, 89]. So far, five studies have been published that investigated the efficacy of oral or enteral nutritional support in patients with ASH. In most studies, nitrogen balance and/or serum albumin levels were improved along with nutritional support, while mortality was unaffected. However, in a randomized multicentre trial, 71 patients with severe alcoholic hepatitis were enrolled to receive either prednisolone (40 mg p.o. qd) and a diet containing 2,000 kcal/day, or total enteral nutrition via a nasoduodenal tube also providing 2,000 kcal/day, for 4 weeks. The severity of ASH was confirmed using the DF. Although the differences in both the short-term mortality and the 1 year probability of survival between the two groups were not statistically different, several important issues became apparent [10].

1. There was a significantly better outcome during the 1 year follow-up after 4 weeks of treatment for the patients treated with total enteral nutrition.
2. Furthermore, there was a high number of drop-outs in the total enteral nutrition group, due to the nasogastric tube rather than from serious adverse events. From these data, it may be possible that a combination of total enteral nutrition and steroids may yield even better clinical results than either therapy alone.

In two multicenter trials of patients from United States Veterans Administration Hospitals, Mendenhall et al [52, 53] attempted to enhance the efficacy of enteral nutrition by adding oxandrolone, a synthetic anabolic steroid [52, 53]. In the first study, oxandrolone therapy was compared against prednisolone. In the second study, 273 patients with alcoholic hepatitis on active treatment received a standard hospital diet in addition to a hypercaloric diet (1,600 kcal/day; 60 g protein/day) for 30 days together with oxandrolone at 80 mg/day. After discharge, patients received 1,200 kcal/day containing 45 g/day of proteins in addition to 40 mg/day oxandrolone, for the following 60 days. Control patients received placebo and a low calorie, low protein diet. It was demonstrated that patients with moderate malnutrition experienced a significant improvement in DF and malnutrition through active treatment. The most important finding, however, was the decrease in mortality with active treatment. Another important result was that the treatment benefit could only be observed with successful nutritional therapy and not with oxandrolone medication. Patients from both studies were recently subjected to a meta-analysis, in which the authors concluded that the efficacy of oxandrolone treatment resulted from sufficient nutritional therapy rather than from the administration of oxandrolone.

***Parenteral nutrition.*** The data available do not allow one to distinguish the effect of parenteral nutrition on ASH from that on cirrhosis separately. Parenteral nutrition in patients with ASH and cirrhosis was performed in 244 patients with different study protocols [89]. Only randomized controlled trials will be discussed here. The treatment duration ranged from 21 to 30 days. Unfortunately, none of the studies could demonstrate an improved survival. All trials showed an improvement of visceral protein as assessed by serum albumin levels, and three studies reported a positive nitrogen balance in the treatment groups. Surprisingly, no long-term studies exist which address intermediate or long-term effects on survival in patients with alcoholic hepatitis or cirrhosis.

In patients with ASH, only one trial investigated whether beneficial effects on nutritional status may be achieved by the combination if intravenous nutritional therapy and oral oxandrolone. Oxandrolone administration again failed to result in further improvement of measured parameters with the exception of serum prealbumin and transferrin, which were highest in the patients treated with parenteral nutrition plus oxandrolone. Tolerability of nutritional therapy and oxandrolone was apparently good, since all patients completed the trial. However, long-term survival was not reported. Due to the lack of clear-cut evidence, oxandrolone cannot be advised for patients with ASH.

Guidelines for the nutritional therapy of ALD of various stages, and their complications are summarized

in Table 88.17. These are proposed recommendations from the American College of Gastroenterology as well as the European Society of Parenteral and Enteral Nutrition [43].

## Drugs

***Antioxidants.*** Since ROS have been implicated in the pathogenesis of ASH, various antioxidants have been used in the treatment of ASH with negative results. More recently, two studies have examined the effects of vitamin E and a cocktail of various antioxidants (vitamin C, vitamin E, selenium, methionine, allopurinol, desferrioxamine, N-acetylcystein, b-carotin) administered to patients with various severities of ASH. Both studies could not show a beneficial effect on hepatic function or mortality [81].

***Glucocorticosteroids.*** Corticosteroids inhibit Kupffer cell and neutrophil granulocyte function, decrease proinflammatory cytokines, such as TNFα, TGFβ, interleukin-1 and interleukin-8, and also decrease the immune response towards acetaldehyde protein adducts. Many studies assessing the use of steroids in ASH have been published. Various metaanalyses have been performed, coming to different results. While the metaanalysis from the 1990s show a therapeutic benefit, the metaanalysis from 1995 did not show such a benefit. A final metaanalysis from 2002 having integrated the new studies again concluded a beneficial effect of steroids in

**Table 88.17** Guidelines for the nutritional therapy of alcoholic liver disease of various stages, and their complications. This table shows a summary of recommendations issued by various international societies [43, 50]

| Disease/ complication | Aims | Calories (kcal/KG) | Protein (g/KG) | Carbohydrates (g/KG) | Lipids (g/KG) | Fluid/ Electrolytes | Vitamins/ trace elements |
|---|---|---|---|---|---|---|---|
| **Fatty liver** | Abstinence<br>Reduction of alcohol intake | n.r. | n.r. | n.r. | n.r. | n.r. | n.r. |
| **Alcoholic hepatitis** | Prevention of PEM<br>Prevention of encephalopathy, hypoglycaemia, inflammation | 40 | 1.5–2.0 | 4.0–5.0 | 1.0–2.0 | n.r. | Supplementation of deficiencies |
| **Liver cirrhosis without malnutrition** | Prevention of PEM | 35 | 1.3–1.5 | 4.0–5.0 | 1.0–1.5 | | |
| **Liver cirrhosis with malnutrition** | Prevention of malnutrition and decompensation | 35–40 | 1.5–2.0 | 3.0–4.0 | 2.0–2.5 | Fluid restriction<br>2.0–2.5 L/day | B-vitamins folate,<br>Thiamine, vitamin C and K |
| **Protein intolerance** | Recompensation, Prevention of PEM | ≥ 25 | 0.3–0.5 BCAA | 2.0–3.5 | 1.0–1.5 | Fluid restriction<br>2.0 L/day | B-vitamins folate,<br>Thiamine, vitamin B12 |
| **Intrahepatic cholestasis** | Improvement of cholestasis<br>Prevention of malnutrition | | | | 50% MCFA | | Fat-soluble vitamins (A, D, E, K) |
| **Ascites** and edema | Recompensation<br>Prevention of infection | | | | | 1.0–1.5<br>Sodium restriction | |

*BCCA* branched-chain amino acids; *MCFA* medium chain fatty acids; *PEM* protein energy malnutrition; n.r. no recommendation

a certain subgroup of patients with ASH [48, 67]. Analyzing the more recent placebo controlled double blind prospective carefully designed studies, it can be concluded, that under certain conditions steroids may be of benefit to a subgroup of patients with severe ASH. These are patients with a DF greater than 32, with encephalopathy and jaundice and who do not have gastrointestinal hemorrhages or infections. Early changes in bilirubin following 7 days of steroid treatment is a simple and powerful criterion for helping to identify patients who are responders to steroid treatment. Approximately 75% of patients with a DF greater than 32 are responders to steroids. At 6 months survival of patients with an early change in bilirubin levels is around 80%. With prednisolone (30 mg p.o. daily for 4 weeks) these patients may have a significantly better 1 month and 1 year survival. This benefit is lost after 2 years. Important side-effects of steroid treatment include sepsis and infections, which need to be taken carefully into consideration (for review see [81]).

***Pentoxifylline.*** One study in patients with ASH has been performed with pentoxifylline, which is a non-selective phosphordiesterase inhibitor [1]. Pentoxifylline is currently clinically approved for treatment of peripheral arterial occlusive disease. Pentoxifylline inhibits synthesis and secretion of TNFα, decreases the activation of neutrophil granulocytes and lymphocyte proliferation, and seems to have anti-fibrotic effects in animal experiments. The study has been performed in 101 patients with severe ASH (DF ≥ 32). Patients received pentoxifylline 400 mg p.o. tid for 4 weeks in a placebo controlled double-blind fashion. Short term mortality was significantly reduced in the pentoxifylline group, primarily due a decrease in hepatorenal syndrome. In addition, TNFα levels were found to be significantly reduced. The effect was greater compared to the effect expected by the use of glucocorticosteroids. No significant side-effects have been noted.

***TNFα antibodies.*** Another approach to counteract TNFα has been the use of a chimeric monoclonal TNFα antibody. Two studies with 20 and 12 patients (DF > 32 and biopsy-proven ASH) have been performed. In the first study by Spahr et al the antibody (infliximab) was given to 20 patients with ASH in a dose of 5 mg per kg body weight in addition to 40 mg prednisolone [86]. No survival difference was observed. However, there was a decrease in serum concentrations of interleukin 6 and 8, and of the DF. In the second study by Tilg et al, infliximab was given at the same dose in addition to nofloxacin to ASH patients with ascites [96]. Fifteen months survival was 83%, but two patients died due to septicemia.

More recently, Naveau et al performed a study with 36 ASH patients with a DF greater than 32. Infliximab in a dose of 10 mg per kg body weight was given three times on week 0, 2, and 4 [58]. In addition, 40 mg prednisolone was administered over 28 days. This regimen was compared to 40 mg prednisolone with placebo. They recorded a mortality of 39% in the infliximab-prednisolone group compared to 18% in the placebo group, which was not statistically significant. The mortality due to infections was 22% versus 11%, and due to severe infection 83% versus 28% which was highly significant ($p = 0.002$). No differences in DF was found at any time.

The dose of infliximab used in this study seems far too high since with this dose significant immune suppression occurs. In addition, a combination of steroids plus TNFα antibodies should not be administered concomitantly.

Preliminary data have been published with etanercerpt which is a soluble TNFα receptor FC fusion protein. A study was performed in 13 patients with a DF of more than 15 and/or hepatic encephalopathy for 2 weeks. Thirty-two milligram per kg body weight of etanercerpt was given as a loading dose, followed by 25 mg subcutaneously on day 4, 8 and 12. Thirty day survival was 92% and 90 day survival was 33%. Side effects were reported in 23% of patients [81].

**Extracorporeal liver support**

Extracorporeal liver support by the use of a molecular absorbent recirculating system (MARS) has been reported in severe ASH. In a pilot study with 8 patients (hepatic encephalopathy and hepatorenal syndrome I and II), the overall 90 day mortality was 50%, whereas the predicted 90 day mortality with the MELD score was 27%. In a second study of 11 severely ill patients from the same group, MARS reduced portal pressure significantly and the authors concluded that MARS may be useful in the management of portal hypertension in severe ASH with organ failure. However, it seems too early to recommend MARS as an established therapy in ASH (for review see [81)].

**Liver transplantation**

Orthoptic liver transplantation in ASH has been performed and the outcome has been compared to patients with AC alone [81, 97]. This Spanish study showed that there was no difference in 5 year survival between 36 patients with alcoholic cirrhosis plus ASH and 32 patients with alcoholic cirrhosis alone following liver

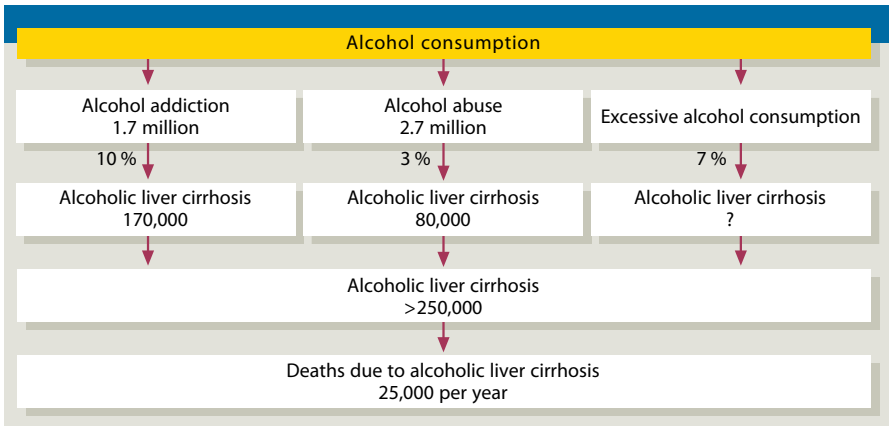



**Fig. 88.24** Alcohol consumption, liver cirrhosis and associated deaths in Germany [92]

transplantation. There was also no difference in the five year survival between those patients who had DF over 32 and those below 32. Ten percent of the patients returned to alcohol consumption after transplantation. Despite these data, 6 months abstinence from alcohol is generally required in most liver transplantation centers before liver transplantation will be performed.

## Alcoholic Cirrhosis

### *Definition*

Alcoholic cirrhosis (AC) represents end stage ALD and is irreversible. AC is defined by a continuous deposition of extracellular matrix, fibrosis, the generation of regenerative nodules and a simultaneous destruction of the normal architecture of the hepatic lobule. This morphological change leads to reduction in liver function and changes in hepatic blood supply. As a consequence, clinically important complications such as jaundice, coagulopathy, hepatorenal syndrome, portal hypertension with esophageal varices and ascites as well as hepatic encephalopathy may occur.

### *Epidemiology*

The exact prevalence of AC is difficult to obtain. Autopsy studies have shown a prevalence of 18% of AC in alcoholics and on the basis of liver biopsies this was estimated by 17–31%. Estimates of the frequency of AC in Germany are illustrated in Fig. 88.24. In the US, 13.8 million people still meet diagnostic criteria for alcoholism. Among these, more than two million are assumed to have ALD, and 14,000 people die of AC each year [47]. In the European countries there is a strong correlation between the prevalence of AC, the average annual consumption of pure alcohol and the death rate from cirrhosis (Fig. 88.25). This strong relationship between alcohol consumption and mortality due to AC has also been shown in other studies. During wine-rationing in France from 1941 to 1947 alcohol consumption declined significantly and this was associated with a decline in mortality due to AC. After World War II both alcohol consumption and AC mortality increased simultaneously. Also there was a decrease in the mortality due to AC during alcohol prohibition between 1916 and 1932 in the US. All these data clearly show a correlation between alcohol consumption and the risk for AC [70].

### *Pathogenesis*

AC can develop from ASH since inflammatory lesions in ASH are repaired by fibrosis. Therefore it is not surprising that the clinical feature of ASH may also occur in AC associated with increased serum transaminase activities, especially an increase in AST. However, AC may also develop over the years directly from AFL without any clinical symptoms. The major mechanisms of alcohol mediated fibrogenesis are illustrated in Fig. 88.26. Extracellular matrix consists of collagens, of non collagen glycoproteins, of glycosaminoglycans and elastic fibers. Furthermore, certain growth factors, matrix metalloproteinases as well as their inhibitors and cell receptors for components of the extracellular matrix belong to that

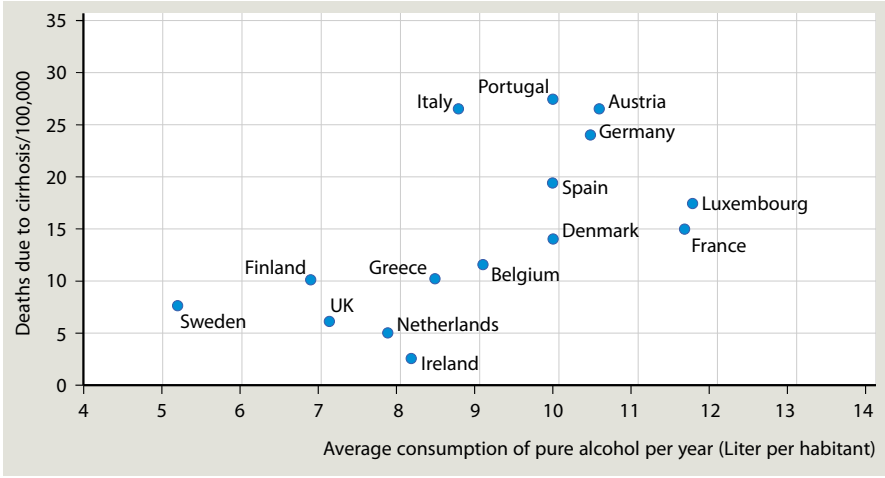



**Fig. 88.25** Correlation between average alcohol consumption and cirrhosis-associated death rates in European countries [92]

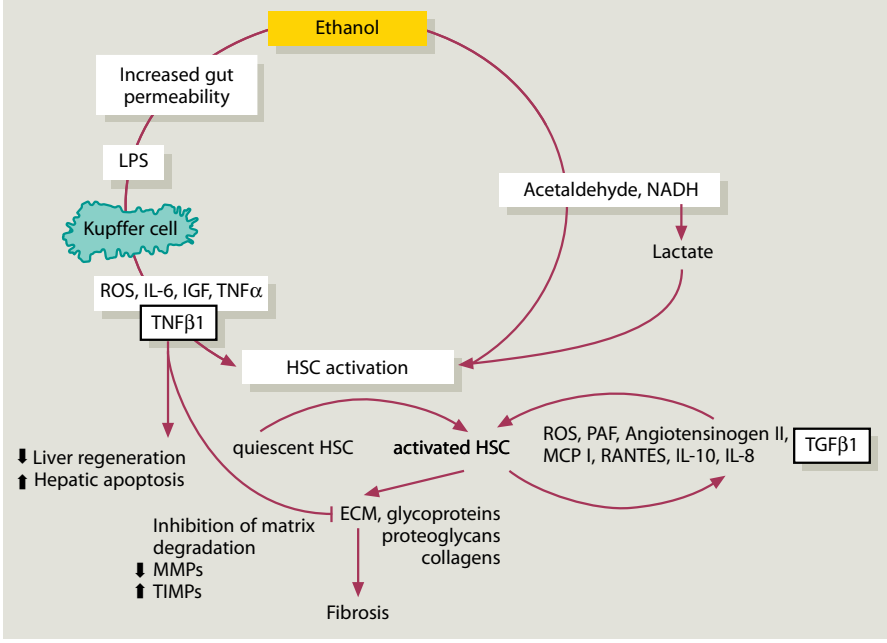



**Fig. 88.26** Pathogenesis of hepatic fibrosis leading to hepatic cirrhosis in alcoholic liver disease. Ethanol stimulates fibrogenesis by two mechanisms. (1) acetaldehyde and lactate (generated from pyruvate due to an excess of NADH in the hepatocyte from ethanol oxidation) both activate HSCs to transform into myofibroblasts with the capacity to produce extracellular matrix. Once activated HSCs also secrete cytokines that perpetuate their activated state. If liver injury persists, HSCs and portal myofibroblasts accumulate and synthesize large amounts of ECM. (2) Due to a leaky gut, LPS enter the liver and are responsible for the release of interleukins and especially TGFβ-1 from Kupffer cells. TGFß1 also activates HSCs through Smad signalling. TGFβ-1 decreases cell regeneration and stimulates apoptosis. It also inhibits matrix degradation by inhibiting MMPs and stimulating TIMPs [3]. *ECM* extracellular matrix, *HSCs* hepatic stellate cells, *IGF* insulin like growth factor, *IL* interleukin, *LPS* lipopolysaccharides, *MCP1* monocyte chemotactic protein type 1, *MMPs* matrix metalloproteinases, *PAF* platelets activating factor, *RANTES* Regulated on Activation, Normal T Expressed and Secreted, *ROS* reactive oxygen species, *TGFβ* transforming growth factor β, *TIMPs* tissue inhibitors for matrix metalloproteinases, *TNFα* tumour necrosis factor α

complex system. With respect to fibrogenesis and fibrolysis the reader is referred to Chapter 28. With respect to the effect of alcohol on this complex system, various mechanism have been identified [3, 66].

Acetaldehyde upregulates transcription of collagen type I directly as well as indirectly by upregulating the

synthesis of TGFβ-1. Thus, the increase in cirrhosis risk in patients with superactive ADH2 and inactive ALDH2 both leading to an increase in acetaldehyde following ethanol metabolism may be explained by the action of acetaldehyde on fibrogenesis. Acetaldehyde leads to the transformation of HSCs to active myofibroblasts which secrete collagen.

ROS which are produced in hepatocytes may also lead to collagen production in HCSs. Thus an induction of CYP2E1 leading to an increase in ROS may be associated with an increase in fibrogenesis. A correlation between CYP2E1 staining and fibrosis score has been recently shown in liver biopsies of patients with ALD.

Alcohol-induced hepatocyte apoptotic bodies can be scavenged by HSCs and Kupffer cells. This may lead to an increased expression of TGFβ-1 and an activation of HCSs.

Non-specific immunity consisting of Kupffer cells, natural killer cells and interferons may regulate the progression of hepatic fibrosis. For example macrophages inhibit hepatic fibrogenesis by killing HSCs and interferons by blocking TGFβ-1 signaling. Activation of this non-specific immune system may help to control the progression of hepatic fibrosis and this may be especially relevant in hepatitis C infection, where alcohol suppresses this immunity, one mechanism whereby alcohol accelerates hepatic fibrosis. Alcohol and hepatitis C virus infection promote hepatic fibrogenesis via increased oxidative stress and an increase in fibrotic cytokines.

Loss of vitamin A observed after chronic alcohol consumption may contribute to the transformation of HSCs into collagens producing myofibroblasts.

Risk factors of fibrosis in ALD include female gender, older age, increased body mass index in the past years before hospitalization when the patient was asymptomatic, high daily alcohol intake over more than 5 years, total time of alcohol abuse, an increased iron content of the liver and an increase blood glucose level which shows an unstable glucose homeostasis [68].

## Histopathology

Hepatic fibrosis usually starts around the hepatic central vein. In this area, ADH activity as well as CYP2E1 activity are both increased compared to other regions of the hepatic lobule. This leads to an increase in the hepatic redox potential, an increase in acetaldehyde accumulation and in the production of ROS, all factors associated with increased fibrogenesis. Thus, perivenular fibrosis is a histopathological feature with poor prognosis. Furthermore, in ASH perivenular and pericellular or "chicken wire" fibrosis is seen. AC is usually of the micronodular type but the cirrhotic process may become macronodular particularly if the patient becomes abstinent. The presence of fatty change and ASH are often seen in alcoholic cirrhosis, which is then associated with an increase in serum transaminase activity such as AST. In addition, MDBs may occur and an excess of stainable iron is frequently seen in the hepatocytes of patients with ALD. Sometimes a differential diagnosis is difficult between AC and hereditary hemochromatosis. Oncocytic hepatocytes are frequently seen in AC. The oncocytic appearance is due to the presence of large numbers of mitochondria which result in a deeply uniformly eosinophilic cytoplasm. As pointed out, macronodular cirrhosis with nodules ranging from 3 mm to 3 cm in size is not uncommon in the alcoholic [8, 31].

A typical histological feature of alcoholic liver damage is the occurrence of MBs, and interestingly, MB formation can be observed in cirrhotic livers with HCC, and the risk of developing HCC is significantly higher in cirrhosis with MBs than without. Recent data confirms the assumption that MBs represent a preneoplastic phenotype in the malignant transformation of hepatocytes as evidenced by a marked expression of markers of hepatocellular neoplasms such as $\alpha_1$-fetoprotein, ubiquitin B, fatty acid synthase and $\alpha_2$- macroglobulin by the MDB forming cells both on protein and mRNA level.

## Clinical Findings

At the early and compensated stage of AC most of the patients do not have any symptoms or complains [47, 56]. Serum aminotransferase activities are more or less normal. Frequently the diagnosis of AC is made during a routine check-up or when the patient decompensates by developing jaundice or ascites. The patients may present a small liver frequently associated with splenomegaly and variable expression of ascites, edema, jaundice and skin bleeding. Skin stigmata are similar in other causes of cirrhosis. Patients with alcoholic cirrhosis present with similar symptoms as patients with

other causes of cirrhosis, and the reader is referred to Chapter 79 and 80 for further detail. With respect to laboratory values in pure cirrhotic patients, serum transaminase levels are frequently normal. If they are elevated, there is also likely to be ASH in the cirrhotic liver; in this case, AST is always higher than ALT. ASH in AC has a particularly poor prognosis. In addition, it has been pointed out that the serum AST activity/platelet ratio index (APRI) may be a good marker to identify AC. However, this finding is not generally accepted.

Most recently, transient elastography (Fibroscan) may be an excellent method to quantify hepatic fibrosis and diagnose AC without biopsy. In this context it should be mentioned that serum hyaluronate levels seem to be a good intraindividual marker to monitor fibrosis [87]. Other serum fibrosis markers seem to be less sensitive to predict ongoing fibrosis.

## Differential Diagnosis

It is clear that all other types of cirrhosis have to be considered in the differential diagnosis. Therefore it is important to verify alcohol as a causal agent for the disease. As pointed out, this can be done by clinical experience, questionnaire and laboratory analysis. It should, however, be mentioned that various liver diseases may occur in one and the same liver. It is not rare that patients with hepatitis C or hepatitis B also drink alcohol, as do diabetics presenting with NAFLD. Due to the increased iron content of the liver, hereditary hemochromatosis also needs to be excluded.

## Natural Course and Prognosis

The natural course of decompensated alcoholic cirrhosis can be described by its complications [73]. Complications include portal hypertension with ascites, spontaneous bacterial peritonitis, bleeding from esophageal or fundal varices or from erosions of from hypertensive gastropathy, hepatic encephalopathy, and finally liver failure. Due to improvement in the therapeutic strategies of complications of cirrhosis, the main cause of alcoholic cirrhotic related death is HCC. The 5-year survival in patients with AC varies between 0–80% depending on the severity of the disease, diagnosed by various scores, and whether the patient continues to drink or not (Figs. 88.23 and 88.27) [21]. In this context, drinking behavior is probably the most important prognostic factor with respect to mortality [47, 50, 56].

## Therapy

Abstinence is the number one therapy in all types of ALD and this is also true for AC regardless of the severity of the disease. Patients with AC must adhere to complete and lifelong alcohol abstinence. Since alcohol abstinence determines the fate of the patients with AC, the effect of all therapeutic strategies have to be compared with the effect of abstinence.

A variety of therapies has been proposed in AC and many of them have been found ineffective. This is true for corticosteroids, propylthiouracil, D-penicillamine, anabolic steroids, silymarine, colchicine, and ursodeoxycholic acid.

However, patients with AC need adequate nutrition, with a minimal caloric intake of 30 kcal per kg of ideal body weight (Table 88.17) [43, 89]. In addition, repletion of water-soluble and fat-soluble vitamins should be performed and maintained. However, one should be cautious with the substitution of vitamin A or β carotene, which are toxic in the presence of alcohol consumption. The intervals of food intake are also important. Patients with alcoholic cirrhosis receive after an overnight fasting period more than 70% of the protein

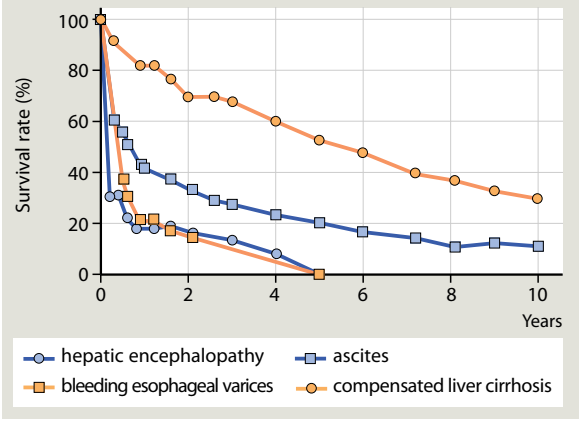



**Fig. 88.27** Survival rates of patients with various complications of alcoholic cirrhosis [56]

independent calories from adipose tissue in comparison to 40% in healthy controls. Therefore, patients with AC frequently need small amounts of food, including a snack during the night to improve their nitrogen balance.

S-adenosyl-methionine (SAMe), which is the active component for methyl transfer and for the generation of phospholipids, important molecules necessary for intact membrane structures, seems to be the only medication with a beneficial effect in AC. However, only one study has shown a significant positive effect with SAMe in patients with Child A and B AC. In a 2-year placebo controlled double-blind trial, SAMe was superior compared to placebo with respect to mortality (12% vs. 29%) [49]. These results, however, need confirmation.

Since the methyl group is transferred from SAMe to polyenylphosphatidylethanolamine, a further enzyme namely polyenylethanolaminemethyltransferase (PEMT) is needed to generate polyenylphosphatidylcholine (PPC) or lecithin. PEMT, however, is inhibited by acetaldehyde. Therefore the idea was to treat patients with alcoholic fibrosis with PPC to prevent A C. However, a multicenter double blind placebo controlled study in the US did not show a significant effect of PPC in alcoholic patients. This was due to the fact that short term counseling prevented the continuation of drinking in these patients which by itself prevented cirrhosis. In a subset of 51 patients, however, the application of PPC improved clinical chemistries [42].

With respect to the treatment of the complications of cirrhosis the reader is referred to Chapter 80.

Finally, orthoptic liver transplantation (OLT) is a therapeutic option for certain patients [97]. The results of OLT in alcoholic end stage cirrhosis are excellent with a 1-year survival of more than 90% and a 5-year survival of over 80%. To reduce the risk for relapse into alcoholism following OLT, most transplantation centers require a 6 month abstinence period prior to transplantation. Extrahepatic alcohol-associated diseases such as brain damage, severe chronic pancreatitis, or cardiomyopathy are contraindications for OLT. In addition, psychological evaluation is necessary to guarantee certain social prerequisites so as not to jeopardize the outcome of OLT. For example, a stable social network including work and family structure is necessary. Additionally, the patient must understand that he/she will need life-long medication with immunosuppressants which may impose an additional financial burden. Twenty to 50% of patients who survived transplantation surgery relapse into alcoholism depending on the definition of alcohol relapse. Severe alcohol relapse may occur in 10–15% of these patients. Therefore the patient with AC needs to be carefully evaluated for OLT. If the patient is a potential candidate for OLT, he or she should be referred to a liver transplantation center when minimal listing criteria are met [66].

## Hepatocellular Carcinoma

### *Epidemiology*

Case-control studies in countries with a high prevalence of alcohol use and a moderate prevalence of viral hepatitis, as well as studies from countries with a high prevalence of chronic viral hepatitis and a lower prevalence of alcohol use, report that chronic ethanol consumption is associated with an approximately twofold increased risk for HCC. The Odds Ratios increase further to five- to sevenfold when ethanol use exceeds 80 g per day for more than 10 years. In general, patients with AC show an HCC incidence of 1–2% per year. In industrialized countries, nearly all HCC develop in cirrhotic livers, although some studies demonstrated that HCC may evolve with non-cirrhotic alcoholic liver disease in 14–19% of subjects. A cross-sectional study from Italy indicates that the risk of development of HCC in individuals with alcoholic cirrhosis equals the risk of HCC in patients with cirrhosis as a result of chronic hepatitis C, while a more recent study in 200 patients with cirrhosis due to chronic hepatitis C (HCV) and 177 subjects with alcoholic cirrhosis found a higher risk for developing HCC in HCV-related rather than in alcoholic cirrhosis (16.5 vs. 5.1%) during a mean follow-up of 39 months (for review see [80)].

Although alcohol itself leads to liver cirrhosis and promotes HCC, it is also a co-factor for the development of HCC in other chronic liver diseases. Thus, chronic alcohol misuse may enhance and/or accelerate hepatocarcinogenesis in patients with HBV and HCV infection, with hereditary hemochromatosis, and with NAFLD. With respect to viral hepatitis, alcohol may stimulate oxidative stress and may, therefore, contribute to inflammation (see below). It has been shown that chronic alcohol consumption of more than 25 g per day leads to a 10-year earlier occurrence of HCC in a Japanese population, indicating an accelerating effect of alcohol in HBV-driven hepatocarcinogenesis. Chronic

alcohol misuse also increases the risk for HCV infection. Whether this is due to an impaired function of the immune system following alcohol ingestion or relates to a risky lifestyle of alcoholics is still unknown. In addition, alcohol may increase viral replication possibly by immunosuppression. Finally, alcohol may stimulate inflammation and, thus, oxidative stress.

In hereditary hemochromatosis, hepatic iron overload is a major factor in hepatocarcinogenesis and alcohol enhances iron deposition in the liver resulting in increased oxidative stress.

With respect to NAFLD, it became clear that type 2 diabetics have an increased risk for HCC. The pathogenesis of NAFLD includes the accumulation of fat in the liver which may be predominantly induced by hyperinsulinemia due to peripheral insulin resistance. Free fatty acids induce cytochrome P4502E1 (CYP2E1) and lead to the generation of ROS. Alcohol also increases CYP2E1 and enhances this pathophysiological pathway. In addition, TNFα is elevated in NAFLD and ALD resulting in further aggravation of peripheral insulin resistance and also in oxidative stress. It has been shown that the relative risk for HCC in type 2 diabetics is approximately 4, and it increases to almost 10 when more than 80 g per day are consumed [80].

## *Pathogenesis*

The pathogenesis of HCC in AC is complex. One major prerequisite is the presence of cirrhosis. The fact the chronic alcohol consumption results in HCC via AC can be either explained by (1) cirrhosis associated mechanisms (2) a long time process leading parallel to cirrhosis and HCC or (3) an early initiation of HCC followed by tumour promotion due to cirrhosis.

The mechanisms by which cirrhosis allows HCC to emerge are still incompletely defined but seemingly relate to profound alterations of the non-parenchymal matrix, the vascular supply and the surrounding microenvironment within the liver which all favor tumorigenesis. Along these changes, genomic changes arise which cause hepatocyte dedifferentiation, adenomatous, premalignant (dysplastic) and malignant lesions.

Various mechanisms independent of cirrhosis may stimulate hepatocarcinogenesis [82]. This includes (a) oxidative stress enhanced through a chronic inflammatory process, through the induction of CYP2E1 and by the increased iron content of the alcoholic liver, (b) disturbed methyl transfer resulting in DNA hypomethylation (c) reduced hepatic retinoic acid concentrations associated with the activation of tumour oncogenes such as AP-1 and hyperproliferation. A synopsis of the interaction of these factors is given in Fig. 88.28.

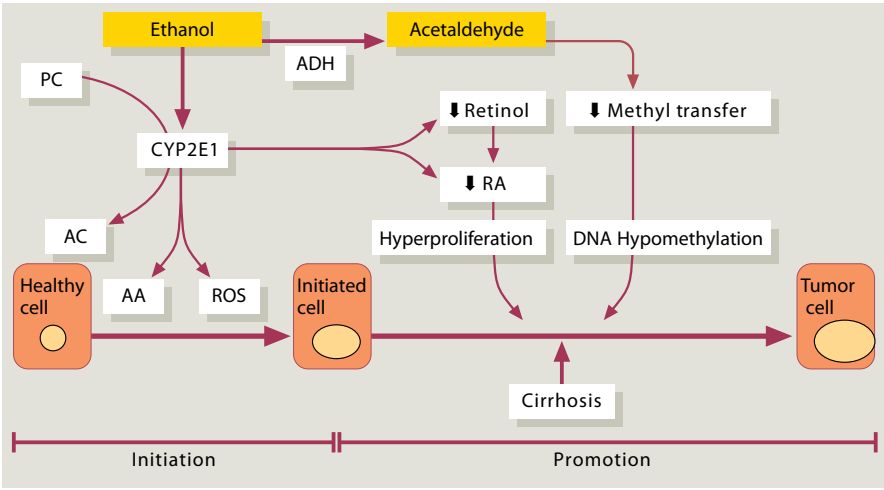



**Fig. 88.28** Simplified scheme of the pathogenesis of alcohol mediated hepatocarcinogenesis. Ethanol metabolism leads to the formation of acetaldehyde and ROS. Both acetaldehyde and ROS bind to DNA and affect initiation of carcinogenesis. Various PC (dietary, tobacco smoke, environmental) are activated through CYP2E1 to their AC. The reduction of hepatic RA through enhanced CYP2E1 metabolism leads to AP-1 gene activation and hyperproliferation. Acetaldehyde causes significant alterations in methyl transfer, resulting for examplein DNA hypomethylation, a situation associated with increased cancer risk. Modified according to [88]. AC activated carcinogen, PC procarcinogens, RA retinoic acid, ROS reactive oxygen species

Acetaldehyde, which is probably the most important carcinogen for the upper alimentary tract in the alcoholic may be of minor importance in the liver, since the liver possesses a highly effective enzyme system to detoxify acetaldehyde. However, acetaldehyde may indeed damage the antioxidative defense system, in particular the nuclear DNA repair system, is responsible at least in part for the reduced availability of S-adenosylmethinonine, the active methyl donor and may also be responsible for the increase in NFκB.

In various animal models attempts have been made to correlate the stages of initiation, promotion, and progression in hepatocarcinogenesis with specific precancerous histological features. Thus centers of focal growth have been observed which show a number of metabolic alterations, for example enzyme altered foci and preneoplastic nodules. Recently, such areas of preneoplastic tissue were also produced in rats by alternate treatment with N-nitrosodimethylamine as a cancer inducer and alcohol, strongly suggesting that ethanol may indeed act as a tumour promoter in hepatocarcinogenesis. Interestingly, MDB formation is high in HCC and the incidence of HCC is significantly higher in cirrhosis with MDBs than without. It was therefore hypothesised that MDBs may represent an initial phenotypical alteration in the carcinogenic transformation of hepatocytes. Another histological abnormality observed in experimental hepatocarcinogenesis is the occurrence of oval cells which originate from the portal triads after long term alcohol exposure. These cells do also appear after administration of a choline deficient ethionine supplemented diet which is known to stimulate hepatocarcinogenesis. Recently, the occurrence of oval cells has also been observed in patients with chronic alcoholic liver disease. Oval cell proliferation is enhanced by TNFα, by TGFß-1 and by various cytokines, all of them increased in ALD.

For more detailed information it is referred to a recent review article on molecular mechanisms of alcohol mediated carcinogenesis [82].

## *Clinical Findings, Diagnosis and Therapy*

A sudden, unexplained decompensation of AC may be due to HCC. Symptoms, diagnostics including imaging and clinical chemistry ($\alpha_1$-fetoprotein) and therapy of HCC in AC are the same as for HCC in other cirrhotic liver diseases. The reader is referred to Chapter 102 for a full discussion.

**Acknowledgement** This article is dedicated to my clinical teacher Professor Dr. Peter Czygan, Neuss, in gratitude for having guided me into Clinical Hepatology with broad knowledge and expertise based on scientific evidence, with curiosity and enthusiasm, but always with great empathy for the patient.

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# Nonalcoholic Fatty Liver Disease

# 89

Henryk Dancygier

## Chapter Outline



## Definition

Nonalcoholic fatty liver disease (NFLD) denotes liver lesions resembling alcohol induced liver injury in patients consuming less than 20–25 g ethanol daily. NAFLD should be considered part of a multiorgan system derangement and encompasses hepatic steatosis, steatohepatitis, cirrhosis and in some cases hepatocellular carcinoma. Nonalcoholic steatohepatitis (NASH) mimics alcoholic hepatitis and is defined histologically by the presence of hepatocellular injury (steatosis, hepatocyte ballooning, cell death), inflammation (intralobular neutrophils > mononuclear cells) and fibrosis [56, 95, 105, 120].

## Epidemiology

NAFLD is the most common liver disease in Western industrialized countries. While it was assumed initially that obese women are particularly at risk for developing NASH, it is well established nowadays that NASH also may occur in lean men and in children [8, 19]. As shown in a population based study from the USA, the incidence of NASH has increased in recent years from $4.2/10^5$/year in 1980–1985 to $38/10^5$/year in 1995–1999 [5]. The incidence of NAFLD in northern Italy was estimated at approximately 2% new cases per year [27]. The prevalence of NAFLD in the general population in industrialized western countries is estimated at 20–25%, and that of NASH at 2–3% [25, 120]. Seventy to 95% of obese patients with a long-standing BMI > 30 kg/m$^2$ have a fatty liver, 9–30% have NASH and 7–16% liver cirrhosis [54, 60, 150]. Approximately 70% of cases hitherto classified as cryptogenic cirrhosis are thought to represent the end stage of the NAFLD/NASH pathway with

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obesity (BMI > 30 kg/m$^2$) being an independent risk factor for the development of cryptogenic and alcoholic cirrhosis [39, 117, 135]. Thus, NAFLD is highly prevalent in the general population, is not associated with suspected liver disease as determined by elevated serum alanine aminotransferase levels, but is associated with many features of the metabolic syndrome (see below) [25]. Individuals with the metabolic syndrome have a significantly higher prevalence of unexplained elevations in alanine aminotransferase level and approximately two thirds of patients with elevated aminotransferases of unknown etiology histologically have NAFLD lesions [28, 57, 87, 102, 156].

The growing obesity epidemic in children has led to an increase in prevalence of NAFLD in this age group, and NAFLD is the most common disorder seen in pediatric hepatology practices. The entire spectrum of histologic features of NAFLD can be seen in children even with normal serum liver enzymes [1, 139].

Significant ethnic differences in the prevalence of NAFLD have been noted. In the USA Hispanics with NAFLD are overrepresented and whites are underrepresented compared with the base population, while the black population occupies a middle position. This racial variation may reflect differences in genetic susceptibility, but may also be influenced by socio-economic factors [31, 32, 170].

## Etiology

In the vast majority of patients in Western countries risk factors for the development of NAFLD include obesity (BMI ≥ 30 kg/m$^2$), hyperglycemia (≥ 110 mg/dL), hyperinsulinemia, hypertriglyceridemia (≥ 150 mg/dL), and a systolic arterial pressure of > 130 mmHg, i.e. factors that define the metabolic syndrome. [25, 53, 62]. *NAFLD is part of a multiorgan disorder and should be regarded as the hepatic component of the metabolic syndrome*, which is encountered in up to 90% of patients with NAFLD (Table 89.1) [108]. The prevalence and severity of NAFLD correlate with the number of components of the metabolic syndrome, particularly with the BMI, and with the degree of visceral adiposity [14, 164].

The relationship between type 2 diabetes mellitus and the liver is complex. 25–75% of patients with NASH suffer from type 2 diabetes mellitus or its precursors. Steatohepatitis may precede overt diabetes. Patients with NAFLD and type 2 diabetes mellitus are at increased risk for the development of cirrhosis. Among diabetics the risk of cirrhosis is quadrupled and among men with diabetes, the risk of hepatocellular carcinoma is doubled [58, 65, 178].

Most type 2 diabetics are also overweight and in the individual case it is not possible to assess the pathophysiologic significance of each risk factor separately. It appears, however, that the combination of both factors carries a higher NAFLD/NASH risk than each factor in isolation. However, also in nonobese patients and in those without type 2 diabetes mellitus NAFLD is closely associated with the metabolic derangements, such as insulin resistance, hypertriglyceridemia and hyperuricemia. Especially in normal weight patients, the development of NAFLD may precede the appearance of these metabolic changes [91].

In addition to the metabolic changes, *drugs* must be considered as potential causes of steatohepatitis. However, for many drugs incriminated with NAFLD, the causality is not proven. The hepatic side effects of the iodine containing antiarrhythmic drug amiodarone, however, are well documented. Due to its long half-life, serum levels of amiodarone may be detected months after treatment has been discontinued, and its accumulation in the liver may cause steatohepatitic lesions to progress despite withdrawal of the drug. Recent evidence suggests that severe steatohepatitis can be linked to chemotherapy and result from the administration of irinotecan and oxaliplatin [72].

*Chronic hepatitis C* infection is becoming increasingly important as a cause of metabolic syndrome and of NAFLD [154]. Hepatic steatosis is a common histologic feature found in more than 50% of individuals infected with HCV, with genotype 3a being more strongly associated with steatosis than other genotypes [78, 145].

*Gastrointestinal surgical procedures* for morbid obesity performed in the 1970s, such as jejunoileal bypass, extended small bowel resections resulting in short bowel syndrome, or gastroplasty have led to steatohepatitis in up to 25% of cases. Some of these patients even died of acute liver failure.

The occurrence of steatohepatitis has been documented in case reports in patients with Weber-Christian's disease, a-β-lipoproteinemia, lipodystrophy, dysmelia and small bowel diverticulosis. Occasionally Wilson's disease in children and adolescents may appear histologically as a steatohepatitis.

Fatty liver is identified in 40–55% of patients with *polycystic ovary syndrome*, with nearly 40% of the

**Table 89.1** Causes and risk factors of nonalcoholic fatty liver disease

| | |
|---|---|
| **Metabolic Syndrome**[a] | **Drugs**[b] |
| Abdominal obesity, identified as | - Amiodarone |
| Waist circumference | - Glucocorticoids |
| European men ≥ 94 cm (≥ 37 in.) | - Nifedipine |
| European women ≥ 80 cm (≥ 32 in.) | - Trimethoprim/Sulfamethoxazole |
| Ethnicity-specific values for other groups | - Tamoxifen |
| Plus any 2 of the following | - Synthetic estrogens |
| Triglycerides ≥ 150 mg/dL (or taking medication for the treatment of this risk factor) | - Chloroquine |
| | - Irinotecan |
| HDL-cholesterol | - Oxaliplatin |
| Men < 40 mg/dL | **Total Parenteral Nutrition** |
| Women < 50 mg/dL | **Various Diseases and Conditions**[c] |
| Blood pressure ≥ 130/85 mmHg | - Weber-Christian's Disease |
| Fasting blood glucose > 100 mg/dL | - A-β-Lipoproteinemia |
| **Type 2 Diabetes mellitus** | - Wilson's Disease |
| **Chronic Hepatitis C Virus Infection** | - Dysmelia |
| **Polycystic Ovary Syndrome** | - Lipodystrophy |
| **Severe Obstructive Sleep Apnea Syndrome** | - Small bowel diverticulosis with bacterial overgrowth |
| **Gastrointestinal Surgical Procedures** | - Adult onset type 2 citrullinemia |
| - Jejunoilealel bypass | - Chanarin-Dorfman syndrome[d] |
| - Extended small bowel resection | - Adult growth hormone deficiency |
| - Pancreato-duodenectomy | - Fatal burns |
| - Gastroplasty | - Citrin deficiency[94a] |
| | **Idiopathic** |

[a]Definition according to the IDF Epidemiology Task Force [12]. The clinical definitions of the metabolic syndrome provided by the World Health Organization and National Cholesterol Education Program (NCEP) Adult Treatment Panel III differ from the IDF definition [11, 160].

[b]Exact data on the prevalence of drug induced NASH are lacking. The causal link between the incriminated drugs and the histologic changes often is not confirmed

[c]Mostly case reports

[d]*Chanarin-Dorfman syndrome* (myopathy, hepatic steatosis, ichthyosis, catarct and hypoacusia) is a rare autosomal recessive inherited neutral lipid storage disorder probably due to ABDH5 mutation [144a].

women being lean. Insulin resistance is the underlying pathophysiological mechanism (see below) [44, 74].

Chronic intermittent hypoxia predisposes to liver injury. *Obstructive sleep apnea* is characterized by chronic intermittent hypoxia and is associated with NASH in obese subjects [152].

The causes of NAFLD are summarized in Table 89.1.

## Pathogenesis

The pathophysiology of NAFLD has been studied intensively in the past years and is becoming increasingly intricate. It involves

- Endocrine-metabolic
- Inflammatory
- Immunologic and
- Genetic factors

NAFLD/NASH results from the complex interplay of these factors with insulin resistance, proinflammatory cytokines, lipid peroxidation, mitochondrial dysfunction and oxidative stress currently viewed as the most important ones. The following discussion focuses on the pathophysiology of metabolic syndrome, obesity and HCV-related NAFLD/NASH.

### *NAFLD Associated with Obesity and Metabolic Syndrome*

NAFLD begins with the accumulation of fat in hepatocytes. This simple (bland) steatosis may be joined during the further course by inflammatory changes and fibrosis (steatohepatitis), and progress to liver cirrhosis and in some patients to hepatocellular carcinoma. It is unknown why the disease remains stationary, for

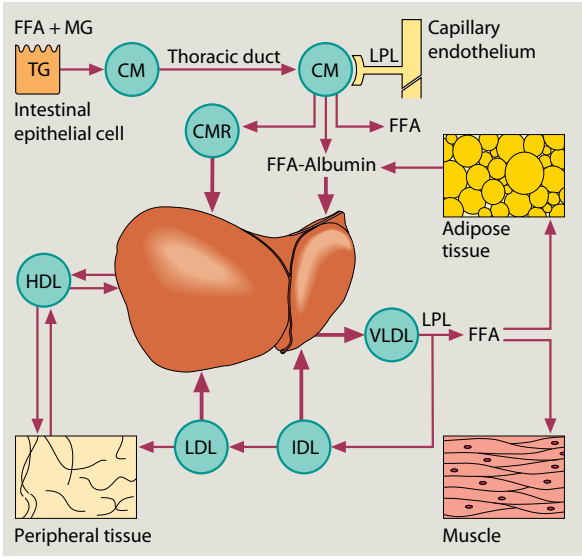


**Fig. 89.1** The liver plays a central role in lipid metabolism. CM chylomicrons, CMR chylomicron remnants, FFA free fatty acids, HDL high density lipoproteins, IDL intermediate density lipoproteins, LDL low density lipoproteins, LPL lipoprotein lipase, MG monoglyceride, TG triglycerides, VLDL very low density lipoproteins

example at the stage of simple steatosis in one patient, while it progresses to steatohepatitis and cirrhosis in others.

The liver plays a major role in lipid metabolism (Fig. 89.1) (see Chapter 6). *Steatosis of hepatocytes occurs whenever synthesis and/or supply of neutral fats (triglycerides) exceed their degradation or export from hepatocytes*. The "two-hit" hypothesis is still an attractive and simple pathophysiological model of NAFLD stating that steatosis with accumulation of free fatty acids (FFA) and triglycerides deals a first blow to the liver which renders the organ more susceptible to further hits [59].

## The Way to Steatosis

*Central to the pathogenesis of obesity and metabolic syndrome-related steatosis and NASH is the degree of visceral obesity and insulin resistance* [52, 77, 127, 149, 163, 164]. Insulin exerts its main physiologic functions predominantly in skeletal muscle, adipose tissue and the liver. Insulin resistance will therefore affect primarily these organs. In patients with insulin resistance, glucose uptake by skeletal muscle is decreased resulting in increased serum glucose levels after a glucose load. In a state of energy excess, glucose is taken up by the hepatocytes and transformed to fatty acids. Glucose enters the Krebs cycle via pyruvate and gives rise to citrate which crosses the mitochondrial membrane and enters into the cytoplasm. Here it is transformed to acetyl-CoA by ATP-citrate-lyase. Acetyl-CoA-carboxylase-1 (ACC-1) transforms acetyl-CoA to malonyl-CoA which is used by the enzyme complex fatty acid synthase for the synthesis of fatty acids. The emerging fatty acids are oxidized or used for synthesis of triglycerides (Fig. 89.2).

The de novo synthesis of fatty acids is regulated by insulin and glucose. The ability of insulin to activate hepatic lipogenesis is accomplished by its capacity to stimulate the transcription of the membrane bound transcription factor *sterol regulatory element-binding protein-1c* (SREBP-1c) that activates nuclear transcription of all genes required for lipogenesis. Surprisingly insulin stimulates hepatic SREBP-1c transcription even in the presence of marked insulin resistance, which results in an increased rate of de novo fatty acid synthesis. SREBP-1c also activates an isoform of acetyl-CoA carboxylase-2 (ACC 2) that generates malonyl-CoA at the mitochondrial membrane. Increased levels of malonyl-CoA result in increased fatty acid synthesis. Malonyl-CoA also inhibits the shuttle enzyme carnitine-palmitoyl transferase-1 (CPT-1), the protein responsible for fatty acid transport into the mitochondria thus reducing mitochondrial fatty acid oxidation. SREBP-1c also activates the transcription of PPAR-γ. In animals with insulin resistance and fatty liver, expression of PPAR-γ is increased [33].

Glucose-induced stimulation of lipogenesis also occurs through the transcription and activation of a second transcription factor, the *carbohydrate response element binding protein* (ChREBP). ChREBP stimulates the glycolytic generation of pyruvate that via citrate generated in the Krebs cycle gives rise to acetyl-CoA which is the parent compound for fatty acid synthesis (see above). ChREBP as SREBP-1c also stimulates the transcription of all lipogenic genes (Fig. 89.2).

In a healthy person insulin inhibits hormone-sensitive lipase in adipose tissue, thereby inhibiting hydrolysis of triglycerides and release of FFA. In insulin resistance states, despite high serum levels of insulin *lipolysis in adipose tissue is increased with consequent*

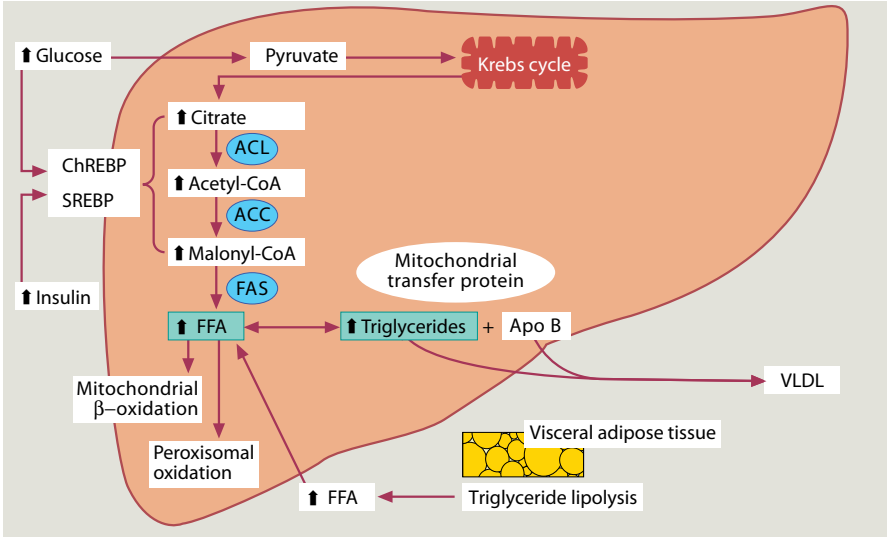


**Fig. 89.2** Mechanisms of insulin resistance-induced hepatic steatosis (see also text). Insulin resistance leads to hyperinsulinemia, hyperglycemia and increased levels of circulating FFA. In *adipocytes*, insulin resistance increases hormone-sensitive lipase activity, resulting in elevated rates of triglyceride lipolysis and enhanced FFA flux to the liver. FFAs can either be oxidized in the mitochondria to form ATP or esterified to produce triglycerides for storage or incorporation into VLDL particles. In *liver*, hyperinsulinemia induces SREBP-1c expression, leading to the transcriptional activation of all lipogenic genes. Simultaneously, hyperglycemia activates ChREBP, which transcriptionally also activates all lipogenic genes. The synergistic actions of SREBP-1c and ChREBP coordinately activate the enzymatic machinery necessary for the conversion of excess glucose to fatty acids. Thus, in the setting of insulin resistance, FFAs entering the liver from the periphery, as well as those derived from de novo lipogenesis, will be preferentially esterified to triglycerides

*ACL* ATP-citrate-lyase, *ACC* acetyl-CoA-carboxylase, *ChREBP* carbohydrate response element binding protein, *FAS* fatty acid synthase, *FFA* free fatty acids, *SREBP-1c* sterol regulatory element-binding protein-1c (Adapted from [33])

*rise in circulating FFA levels*. The visceral adipose tissue is the main source of portal venous FFA. The greater the visceral adipocyte mass the more pronounced is triglyceride hydrolysis and the higher are the levels of circulating FFA. Hepatic FFA uptake is directly proportional to the FFA serum levels. FFA taken up by the hepatocytes are either oxidized generating ATP or are used for triglyceride synthesis establishing a homeostatic balance between FFA and triglycerides. In patients with NAFLD, approximately two thirds of triglycerides stored in the liver arise from serum FFA, 25% from de novo lipogenesis and 10% from diet [63]. Triglycerides are either stored in hepatocytes or they are assembled with apolipoprotein B 100 and incorporated into very low density lipoprotein (VLDL) particles which may be secreted into the circulation (Fig. 89.2). *Microsomal transfer protein* (MTP) is a heterodimeric protein involved in transport of neutral lipids between membranes. It transports triglycerides into the lumen of the endoplasmic reticulum and plays a central role in the assembly of lipoproteins. Reduced MTP-transcription leads to diminished export of triglycerides from hepatocytes with consequent intracellular triglyceride accumulation resulting in steatosis. Genetic polymorphisms of the MTP-gene have been described and may be involved in the pathogenesis of NAFLD/NASH associated steatosis [118]. In addition, chronic hyperinsulinemia reduces the synthesis of apolipoprotein B 100 thereby impairing the VLDL-associated lipid transport from the hepatocyte [48].

In summary, insulin resistance causes several molecular and physiological alterations that result in the accumulation of triglycerides within the liver. In interpreting the pathophysiology of nonalcoholic steatosis it is important to understand that insulin resistance in patients with NAFLD is not expressed uniformly in liver, adipose tissue, and skeletal muscle. While the latter two are insulin resistant, hepatic insulin resistance is only partial with several metabolic pathways remaining relatively insulin sensitive. Insulin resistance of adipocytes and skeletal muscle cells increases the circulating

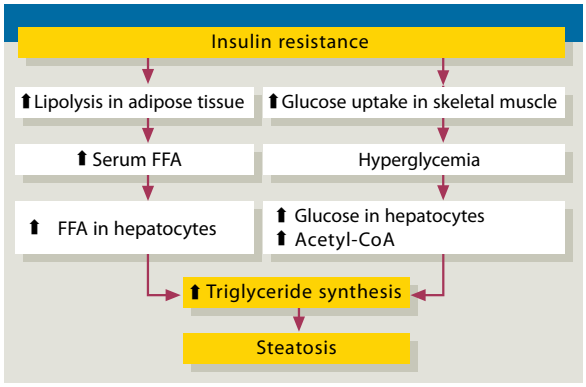


**Fig. 89.3** Simplified scheme of the pathophysiology of fatty liver in obesity and metabolic syndrome. Insulin resistance plays the pivotal role in the accumulation of triglycerides within hepatocytes

FFA levels and glucose load. Both have to be taken up by hepatocytes leading to a new intrahepatic balance between the expanding pool of FFA and hepatic triglycerides (Fig. 89.3). Although fatty acid oxidation and gluconeogenesis may be overactive in insulin resistance states, hepatic lipogenesis remains relatively sensitive to insulin and indeed is stimulated by insulin in patients with NAFLD/NASH [33, 82]. Thus, increased lipogenesis combined with impaired VLDL assembly and export lead to steatosis in patients with NAFLD.

## From Steatosis to Steatohepatitis

The transition from steatosis to steatohepatitis is characterized by increased *oxidative* and *endoplasmic reticulum stress* and by the increased expression of *proinflammatory cytokines*. Endoplasmic reticulum stress is a central feature of peripheral insulin resistance [126]. FFA enhance *lipid peroxidation* in the liver which generates highly reactive oxygen species (ROS), stimulates the expression of tumor necrosis factor α (TNF α), and damages mitochondria and DNA (Fig. 89.4). These mechanisms sensitise hepatocytes to TRAIL mediated cytotoxicity and induce hepatocyte apoptosis and mediate necroinflammation (see Chapter 14 and 23) [69, 71,

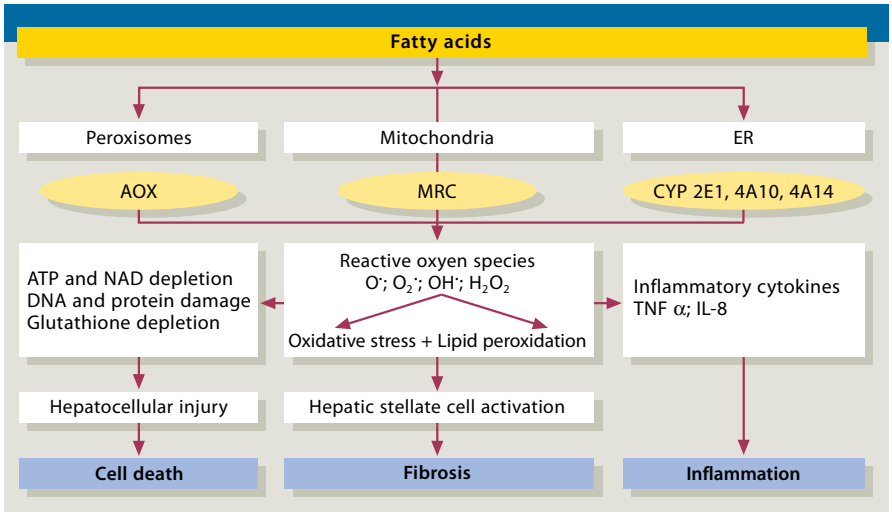


**Fig. 89.4** Overview of mechanisms leading to the histological hallmarks of steatohepatitis, i.e. hepatocellular injury/cell death, inflammation and fibrosis (see also text). The accumulation of fatty acids within the hepatocytes leads to their increased metabolism in peroxisomes, mitochondria and endoplasmic reticulum. Mytochondrial injury results in impaired activity of MRC. In peroxisomes, fatty acid β-oxidation is catalyzed by AOX that forms hydrogen peroxide. Microsomal ω-oxidation is catalyzed by cytochrome P450 enzymes 2E1, 4A10, and 4A14, which form ROS through flavoprotein-mediated donation of electrons to molecular oxygen. PUFA are extremely susceptible to lipid peroxidation by ROS. By-products of PUFA peroxidation are aldehydes that are themselves cytotoxic. ROS and aldehydes induce oxidative stress and cell death via ATP and NAD depletion, DNA and protein damage, and glutathione depletion. Additionally, they induce inflammation through the production of proinflammatory cytokines, leading to neutrophil chemotaxis. Finally, ROS and products of lipid peroxidation can lead to fibrosis by activating hepatic stellate cells.

AOX acyl-CoA oxidase, ER endoplasmic reticulum, MRC mitochondrial respiratory chain, PUFA polyunsaturated fatty acids, ROS reactive oxygen species (Adapted from [33]. With kind permission)

107, 138, 142]. In addition, FFA may also promote hepatic lipotoxicity by injuring mitochondria directly, and by inducing oxidative stress and TNF α expression via a lysosomal pathway [70, 101]. Enhanced hepatic lipid peroxidation is reflected by higher systemic lipid peroxidation products in patients with NASH [46]. Oxidative stress also induces a humoral immune response against lipid peroxidation products [10].

Structural and functional *mitochondrial alterations* affect ATP-synthesis and impair generation of energy. Impaired energy-homeostasis contributes to liver damage in NAFLD/NASH [130, 132, 149]. Dysfunction of mitochondrial respiratory chain leads to the generation of ROS which attack polyunsaturated fatty acids (PUFA) and initiate lipid peroxidation, further damaging mitochondrial membranes and impairing mitochondrial function. This vicious cycle further increases oxidative stress. With increasing impairment of mitochondrial fatty acid oxidation, alternative peroxisomal (β-oxidation; generation of $H_2O_2$) and microsomal (ω-oxidation; cytochrome P450 enzymes 2E1, 4A10, 4A14) pathways of fatty acid oxidation are being activated resulting in additional generation of ROS. The cumulative effect of extramitochondrial fatty acid oxidation results in continuing oxidative stress and mitochondrial dysfunction. The cytochromes most intensely associated with lipid peroxidation are CYP2E1 and CYP4A. In patients with NASH induction of CYP2E1 has been demonstrated [169].

Increased generation of *gut derived endotoxin* results in the activation of Kupffer cells and the secretion of proinflammatory cytokines, predominantly TNF α, interleukin-6 and interleukin-12 by Kupffer and inflammatory cells infiltrating the liver [172]. These mediators contribute through the above described mechanisms to hepatocyte injury.

Kupffer cells are responsible for clearing endotoxin and are activated via endotoxin interaction with Toll-like receptor 4 (TLR-4). TLR-4 signaling provides a direct link between Kupffer cells and the pathogenesis of NASH [143].

*Visceral fat* is directly associated with liver inflammation and fibrosis independent of insulin resistance and hepatic steatosis [163]. Obesity itself is characterized by an inflammatory state, and steatosis and inflammation actively influence each other. Lipids appear to be the primary stimulus to the activation of the innate immunity (leukocytes, receptors, and soluble mediators) [106]. Proinflammatory cytokines and inflammatory mediators produced in adipose tissue are central to the development of obesity-related insulin resistance by affecting signal transduction originating from the insulin receptor [83, 168]. Inflammatory cytokines also induce nitric oxide synthase whose overproduction in obesity appears to contribute to impairment of insulin action on skeletal muscle and pancreatic β-cell. Innate immunity is activated in obesity-related inflammation and innate immune processes not only cause insulin resistance but also end-organ damage in the form of NAFLD [106].

*Adipocyte hormones*, such as leptin and adiponectin, possibly also play a role in the pathophysiology of NAFLD/NASH.

*Leptin*, a product of the *ob*-gene primarily is involved in regulating body weight. It acts as an afferent signal in reducing appetite. Leptin is primarily produced by adipocytes, but also in the placenta, the gastric fundus, skeletal muscle and liver. It participates in insulin-induced signal transduction and in the regulation of glucose metabolism in skeletal muscle, liver and adipose tissue [43]. High leptin concentrations in serum in obese patients promote insulin resistance. The data regarding the pathophysiological significance of leptin in patients with NAFLD are conflicting. Experimental studies showing a correlation between leptin levels and fibrosis are opposed by investigations in human NAFLD where no relationship between leptin levels and fibrosis stage could be demonstrated [16, 45]. Animal experiments suggest that leptin-mediated neovascularization may play a role in the development of liver fibrosis and hepatocarcinogenesis in NASH [92].

*Adiponectin* is an antidiabetic and antiatherogenic polypeptide whose concentrations correlate with the systemic insulin sensitivity. Adiponectin gene polymorphisms modulate acute adiponectin response to dietary fat [116]. Adiponectin stimulates fatty acid oxidation in skeletal muscle, supports insulin action at the level of the hepatocyte and improves the postabsorptive insulin-induced inhibition of hepatic glucose release. In addition to its metabolic effects adiponectin also has antiinflammatory actions. Reduced adiponectin serum levels have been reported in human NAFLD and hepatic adiponectin expression was reduced in patients with NASH compared to patients with simple steatosis [90].

Hypoadiponectinemia and elevated TNF α levels are part of a metabolic disturbance characterized by visceral fat accumulation and are associated with the development of steatosis and NASH [173].

Reduced circulating adiponectin levels were reported to be related to hepatic insulin sensitivity, more extensive necroinflammation and higher serum aminotransferase activities [38, 86, 115, 177].

*Resistin* is mainly an adipocyte derived hormone associated with the production of proinflammatory cytokines. Elevated levels in NAFLD have been reported to be associated with insulin resistance and/or disease severity [128, 174]. Another study demonstrated that excessive ectopic fat accumulation in the liver and skeletal muscle of insulin-resistant subjects was associated with lower serum resistin concentration and not with hyperresistinemia [131]. Currently the pathophysiologic role of resistin in NAFLD remains undefined.

*Ghrelin* is a peptide of 28 amino acids that is predominantly synthesized and released from the stomach and duodenum. It is a potent stimulator of growth hormone release and also plays a role in appetite control. Ghrelin reduces insulin secretion in humans. Insulin resistance is a major factor controlling ghrelin levels in subjects with and without NAFLD [109]. The role of ghrelin in NAFLD, however, requires further research [162].

The role of *iron overload* in the pathogenesis of NASH is unknown, but an increased prevalence of the HFE gene mutations associated with hereditary hemochromatosis has been reported in North American patients with NASH, and the HFE C282Y heterozygous mutation was associated with advanced fibrosis among whites with NASH [30, 119].

*Genetic influences* in NASH are poorly understood, but available data suggest that genes may be involved in determining the susceptibility to NASH. A gene-pathway associated with NASH and polymorphisms of microsomal triglyceride transfer protein (MTP) gene, manganese superoxide dismutase (MnSOD) gene, and TNF gene has been described [75, 118, 146, 161]. Decreased MTP transcription reduces export of triglycerides from hepatocytes resulting in steatosis (see above). MnSOD gene dysfunction leads to reduced mitochondrial MnSOD activities with consequent impairment of mitochondrial function, and TNF polymorphisms, which influence TNF production, might be associated with the progression of NAFLD. Using microarray technology genes for maintaining mitochondrial function (copper/zinc superoxide dismutase, aldehyde oxidase and catalase) were found to be underexpressed, whereas genes for complement component C3 and hepatocyte-derived fibrinogen-related protein were overexpressed in NASH. Genes related to lipid metabolism and extracellular matrix remodeling were significantly dysregulated in NASH. In general, genes related to detoxifying enzymes are underexpressed whereas genes related to the activation of stellate cells and fibrogenesis are overexpressed [99].

## NAFLD Associated with Chronic Hepatitis C Virus Infection

There is a strong association between chronic HCV infection and fatty liver. More than 70% of patients infected by HCV genotype 3 present with steatosis, compared with 40% of patients infected by HCV genotype 1 [7]. NAFLD associated with chronic HCV infection is an important cofactor affecting progression of HCV-induced liver disease and response to therapy. The coexistence of steatosis (especially if greater than 33% of hepatocytes are fat-laden) and chronic HCV infection is associated with greater fibrosis and a low rate of sustained viral response to antiviral therapy [80, 129, 144].

*HCV genotype 3* exerts a direct cytopathic steatogenic effect and the degree of steatosis correlates directly with viral load (HCV RNA levels). Resolution of steatosis is often observed with the loss of viremia after antiviral treatment [42].

HCV genotype 3 affects hepatic triglyceride and lipoprotein metabolism in many ways. HCV entry into hepatocytes may be mediated by low-density lipoprotein receptor. Experimental studies in transgenic mice have shown that intracellular lipid accumulation occurs when the HCV core protein is strongly expressed and that the core protein can be found on the surface of lipid droplets within the cytoplasm in cell cultures transfected with HCV [22, 51, 114]. HCV core protein localizes in the periphery of triglyceride rich droplets and on the cytosolic surface of the endoplasmic reticulum membrane. HCV can affect de novo fatty acid biosynthesis, triglyceride assembly, secretion and lipid peroxidation. HCV derived proteins (core protein and NS4b) derived from HCV genotype 3a were found to be capable of inducing proteolytic cleavage of SREBP [167]. In addition, HCV core protein may interact with apoA2, which is a major component of high-density lipoproteins, and this interaction can lead to hepatocellular steatosis by inhibiting microsomal triglyceride transfer protein activity [148]. Another nonstructural protein, NS5A, has been found to interact with apoA1

and apoA2, and therefore can lead to altered cholesterol trafficking [9, 155]. Thus, steatosis in HCV genotype 3 infection is mainly due to blockade of lipoprotein assembly and secretion during viral replication. Apoptosis contributes to liver cell injury and the grade of steatosis in chronic HCV infection has been shown to correlate with the apoptotic index [166].

Although HCV virus, regardless of genotype, has been shown to cause insulin resistance through direct effects on viral proinflammatory cytokines and suppressors of cytokine signaling, steatosis in *HCV genotype 1* (non-genotype 3) is predominantly due to virus induced insulin resistance, in contrast to the direct cytopathic effect of HCV genotype 3. Of the HCV non-structural proteins, NS3 and NS5A act as key mediators in the induction of oxidative stress and inflammation. NS3 has been shown to be involved in ROS generation [154]. The degree of steatosis in genotype 1 is independent of HCV viral load.

## Pathology

The hallmark of NAFLD is *steatosis* which is present in all stages of the disease except for advanced cirrhosis where hepatic fat may disappear in some patients. Steatosis usually is *macrovesicular* with a large cytoplasmic fat vacuole pushing the nucleus aside to the cell periphery. Rupture of fat-laden hepatocytes may give rise to fat cysts and lead to the formation of lipogranulomas. The sole occurrence of pure *microvesicular* steatosis is rare (Table 89.2). In this form the hepatocyte cytoplasm is stuffed with tiny fat vacuoles imparting the cell a foamy appearance and leaving the nucleus in its central position. Commonly a mixed picture of macro- and microvesicular steatosis is present (Fig. 89.5). Steatosis usually begins in perivenular zone 3 and in severe cases may involve the entire acinus. Glycogenated vacuolated nuclei ("Lochkerne") occur predominantly in periportal (zone 1) hepatocytes and are thought to be an indication of hyperglycemia. Patients with severe steatosis are more likely to have steatohepatitis [47].

**Table 89.2** Causes of pure microvesicular steatosis

| |
|---|
| Reye's syndrome |
| Acute fatty liver of pregnancy |
| Drugs |
| • Tetracyclines |
| • Salicylic acid |
| • Valproic acid |
| • Tamoxifen |
| Alcoholic "foamy" degeneration |
| Urea cycle defects |
| Defects in mitochondrial fatty acid oxidation |

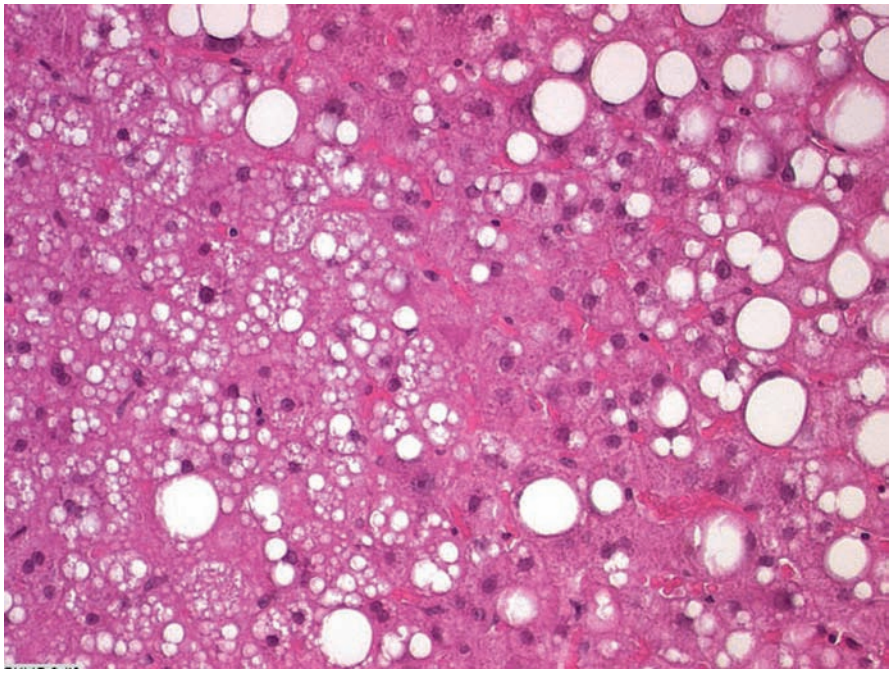

**Fig. 89.5** Macro- and microvesicular hepatic steatosis. Hematoxylin and eosin stain

Classical NASH is characterized by the combined occurrence of

- Hepatocyte injury (steatosis, ballooning [oxidative stress-induced keratin filament alterations with loss of keratin 8/18 immunostaining], necrosis, apoptosis)
- Inflammatory acinar cell infiltrates (neutrophil granulocytes > mononuclear cells) and
- Perisinusoidal ("chickenwire") fibrosis

that predominates in the perivenular zone (Figs. 89.6 and 89.7) [97, 105]. A significant subset of morbidly obese individuals may have portal fibrosis in the absence of NASH [3]. Mallory-Denk bodies, hepatocyte siderosis, and megamitochondria are facultative lesions and not mandatory for the diagnosis of NASH [36].

NASH by definition cannot be distinguished from alcoholic steatohepatitis (ASH). However, certain features, such as microvesicular steatosis, nuclear vacuoles

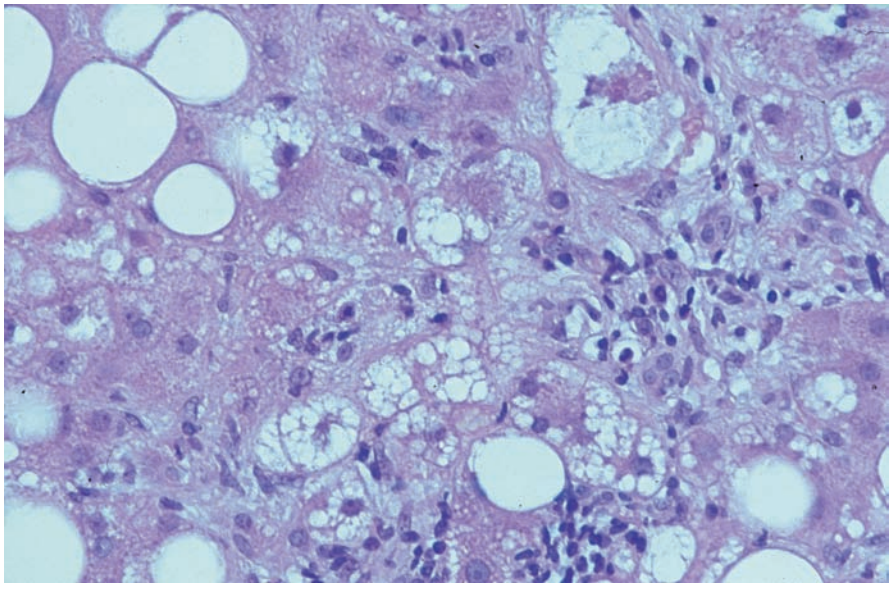

**Fig. 89.6** Nonalcoholic steatohepatitis. Hepatocyte injury with macro- and microvesicular steatosis and ballooning of hepatocytes is seen. Some hepatocytes contain hyaline material within their cytoplasm. The inflammatory cell infiltrate consists of mononuclear cells and neutrophil granulocytes. Hematoxylin and eosin stain

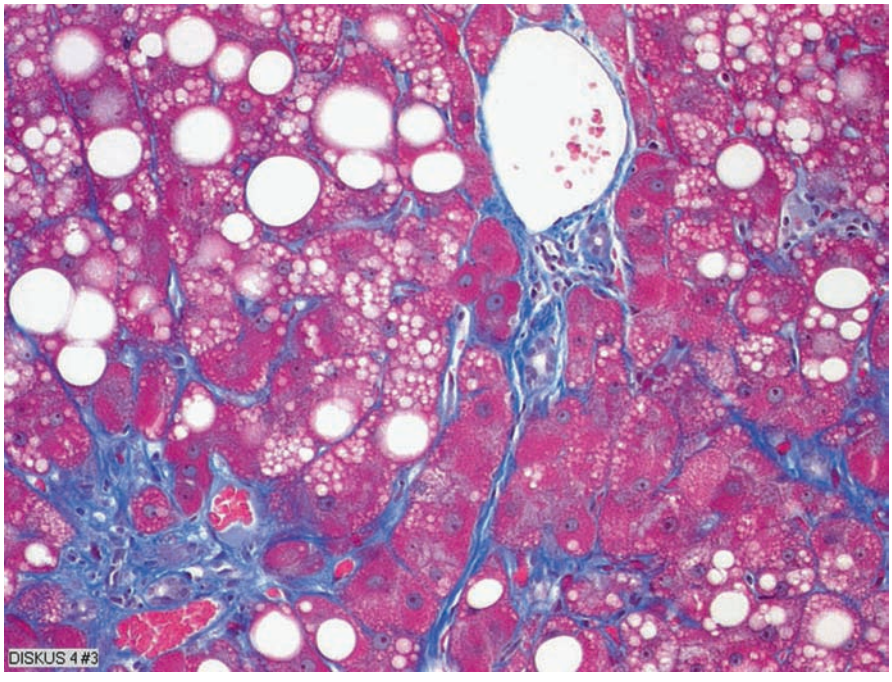


**Fig. 89.7** Perisinusoidal ("chickenwire") fibrosis in a steatotic liver. Masson trichrom stain

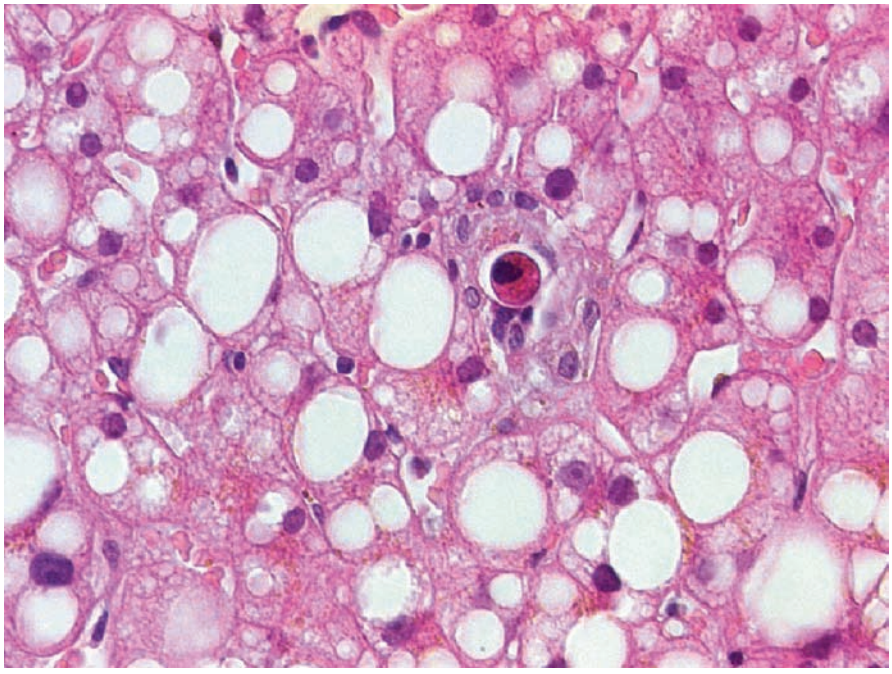

**Fig. 89.8** Apoptotic body in a steatotic liver. Hematoxylin eosin stain

and mononuclear inflammatory cells occur more often in NASH than in ASH [67]. The prevalence of various histopathological features of NASH and ASH is reported in Table 88.13. Despite the strict original histopathological definition of NASH, over the last years the term has been gradually "eroded and softened", and it is applied nowadays to virtually every inflammatory lesion in the setting of a nonalcoholic fatty liver. Since, however, even "innocent" appearing mild inflammatory reactions in a fatty liver may have an impact on long-term prognosis, it is important to also take notice of additional minor changes within a fatty liver, such as isolated ballooned or apoptotic hepatocytes (Fig. 89.8) [110].

Liver biopsy is still the gold standard in the definitive diagnosis of NASH and in grading and staging NAFLD lesions. One should be aware, however, that histologic lesions of NASH are unevenly distributed throughout the liver parenchyma; therefore, sampling error of liver biopsy can result in substantial misdiagnosis and staging inaccuracies, particularly for features of necroinflammation [98, 111, 141]. Histological scoring systems have been developed to allow a more objective classification of microscopic findings (see Chapter 29; Tables 29.4 and 29.5) [35, 94, 120].

The histopathologic features of a fatty liver do not allow one to make a specific diagnosis as to the cause of steatosis. It is therefore reasonable to diagnose steatosis or steatohepatitis microscopically, while the adjunct alcoholic or non-alcoholic descriptor should rest on clinical grounds. The term "nonalcoholic" should further be subclassified according to the clinical information. Thus a complete diagnosis should read, for example, NAFLD in metabolic syndrome, obesity, drug-induced NAFLD/NASH, etc. (Table 89.1). *A diagnosis of alcoholic liver disease should never be based on liver histology alone* [56].

## Diagnosis

### *Clinical Manifestations*

Most patients with steatosis or mild steatohepatitis are asymptomatic or present with nonspecific symptoms, such as a mild discomfort in the right upper quadrant or a slight general malaise. In some patients fatigue may be a significant problem similar in degree to that in primary biliary cirrhosis patients [121]. In the cirrhotic stage of NAFLD signs and symptoms correspond to those of cirrhosis of other etiologies (see Chapters 79 and 80). Usually patients with NAFLD attract one's attention during a general medical examination because of mildly elevated aminotransferase levels or a "bright liver" seen on ultrasound. After

careful exclusion of alcohol consumption of greater than 20–25 g per day and of other liver diseases, the presumptive diagnosis of NAFLD can be made, especially if the patient presents features of the metabolic syndrome or of one of its risk factors (Table 89.1). The amount of visceral fat may be estimated by determining the waist circumference [50]. The waist-to-hip ratio (men > 0.9; women > 0.85) correlates with the degree of hepatic steatosis. Fifty percent of patients with NAFLD have a hepatomegaly.

## Laboratory Findings

Serum levels of aminotransferases are only mildly elevated (2–4 times the upper limit of normal ALT > AST), but *an elevated ALT is not a marker for NAFLD*, and *normal aminotransferase levels do not exclude NAFLD*. The entire histologic spectrum of NAFLD may occur also in patients with normal ALT levels [113].

Alkaline phosphatase and γ-glutamyl transpeptidase levels are only mildly (up to 2 times the ULN) elevated in approximately one third of patients. Serum and liver iron and ferritin concentrations are elevated in 50–60% of patients. Hyperinsulinemia has been reported to be a major determinant of serum ferritin levels [179].

In up to 25% of patients with NAFLD non-organ-specific autoantibodies may be found (ANA > SMA > AMA). The titers of ANA are usually < 1: 320. High titer ANA is associated with insulin resistance [4, 104]. The demonstration of autoantibodies in patients with NAFLD should not be misinterpreted as evidence of autoimmune liver disease with consequent unnecessary antiinflammatory/immunosuppressive therapy.

In nondiabetic, nonobese patients with genotype 1 chronic hepatitis C, serum retinol-binding protein 4 (RBP4) levels might mirror a virus-linked pathway to steatosis. RBP4 has been suggested as a new marker for virus-induced steatosis in patients infected with hepatitis C virus genotype 1 [134].

More advanced NAFLD, as indicated by the presence of NASH with advanced fibrosis stage, has been reported to be strongly associated with low levels of circulating dehydroepiandrosterone (DHEA-S). The clinical significance of this finding is not known [49].

Attempts at distinguishing simple steatosis from steatohepatitis by laboratory methods are being reported but have not yet gained wide clinical acceptance. Plasma CRP levels are not predictive of the diagnosis of NASH in severely obese patients, whereas elevated high-sensitivity CRP levels have been reported to distinguish between NASH and simple steatosis [18, 142a, 177a]. The ratio of circulating TNF α/soluble TNF receptor 1 and IL-6/soluble IL-6 receptor levels has been reported to be significantly increased in NASH patients as compared with simple steatosis patients and healthy volunteers [2]. Hepatocyte apoptosis in fatty livers has formed the basis for using cytokeratin-18 as a serum marker capable of differentiating simple steatosis from steatohepatitis [171, 175]. Low serum levels of adiponectin have been exploited as markers of disease activity [86].

Recently a *clinical scoring system* for predicting NASH in morbidly obese patients based on demographic, clinical, and laboratory variables has been reported. The six predictive factors for NASH were arterial hypertension, type 2 diabetes mellitus, sleep apnea, AST > 27 IU/L, ALT > 27 IU/L, and non-black race. Each predictive factor equals one score point, except for non-black race with a score value of two points. The probability of NASH increases with the summed points (0–2: low, 3–4: intermediate, 5: high, 6–7: very high) [40]. A scoring system to separate NAFLD patients with and without advanced fibrosis has been constructed and validated based on routinely measured and readily available clinical and laboratory data. Age, hyperglycemia, body mass index, platelet count, albumin, and AST/ALT ratio were independent indicators of advanced liver fibrosis. By applying the low and high cutoff scores the absence or the presence of advanced fibrosis could be diagnosed with high accuracy [17].

Transient elastography is an accurate and reproducible method to identify patients without significant fibrosis, or with advanced fibrosis [124].

## Imaging Techniques

Ultrasound is the noninvasive imaging tool of choice in the clinical diagnosis of NAFLD. The liver displays a homogeneous hyperechogenicity ("bright liver") and if greater than 50% of hepatocytes contain fat its lower margin usually becomes rounded. If greater than 30% of hepatocytes are fat-laden, ultrasound diagnosis of hepatic steatosis has a sensitivity of 85% [147]. *Computed tomography and magnetic resonance imaging are not required in the diagnosis of uncomplicated NAFLD*.

Liver biopsy is the gold standard in the diagnosis of NAFLD (see above). In addition to grading inflammatory changes and staging fibrosis the results of initial liver histology have prognostic significance (see below) [110, 159]. Currently, however, a general recommendation to perform a liver biopsy in every patient suspected of having NAFLD/NASH cannot be given, since a specific treatment of NAFLD is not available and thus the results of liver biopsy have no therapeutic impact.

## Differential Diagnosis

The main disorder to be considered in the differential diagnosis of NAFLD is alcoholic liver disease. This is primarily based on a careful history seeking to quantitate the amount of ethanol consumed daily. The cutoff value between nonalcoholic and alcoholic liver disease has been set at 20 g alcohol per day, which roughly corresponds to one-two drinks per day. The CAGE-test (see Chapter 88) may help in evaluating a patient for alcohol abuse.

There are no specific laboratory findings for alcoholic liver disease. γ-GT is not a marker for alcohol abuse, although elevated γ-GT levels which decline during absolute alcohol abstinence strongly favor alcoholic liver disease. High red blood cell MCV values also suggest the diagnosis of alcohol abuse. Carbohydrate deficient transferrin (CDT) is only positive in patients consuming fairly large amounts of alcohol (approximately 60–80 g/day). Neither the total serum CDT concentration nor that of CDT-isoforms allow to accurately differentiate alcoholic from nonalcoholic liver injury [157].

In addition to alcohol, the diagnosis NAFLD further requires the exclusion of non hepatitis C viral infection, autoimmune, genetic (e.g. hereditary hemochromatosis, Wilson's disease), metabolic and drug-induced disease (except for the drugs reported in Table 89.1).

## Natural Course and Prognosis

Patients with a simple fatty liver, i.e. without further evidence of liver injury, such as necroinflammatory changes or fibrosis, have an excellent long-term prognosis. Nearly all these patients have stable nonprogressive disease and quality of life is not impaired. Fatty liver is reversible and regresses in approximately 50% of cases [26, 159].

Fibrosis in NAFLD progresses slowly over time with considerable variability in the rate of changes among patients. Changes of aminotransferases do not parallel changes in fibrosis stage. Diabetic and obese patients are at risk for higher rates of fibrosis progression [6, 68]. In obese patients the degree of steatosis correlates with the rate of fibrosis progression, but steatosis is reversible with weight loss [137]. The histological findings have a prognostic significance. In simple steatosis the risk of cirrhosis is approximately 1 (−2%) in 15–20 years, thus much better than alcoholic liver disease [55, 68]. If in addition to fat-laden hepatocytes, ballooned liver cells, Mallory-Denk bodies and/or fibrosis are present on initial biopsy, the probability of progression rises [79]. If NASH is present in the initial liver biopsy the risk of cirrhosis is approximately 14–28% in 10–20 years [110, 136].

NAFLD has long been an underrecognized cause of cryptogenic cirrhosis. 70% of cryptogenic cirrhoses are due to NAFLD, with obesity and type 2 diabetes mellitus being the most common risk factors [39, 135]. Clinical predictors of progression of NAFLD are a reversal of ALT: AST ratio, obesity, older age (> 45–50 years), type 2 diabetes mellitus, hypertriglyceridemia, arterial hypertension and the degree of insulin resistance. In other words, the more severe the metabolic syndrome, the higher the risk of progressive liver disease. The common occurrence of obesity and type 2 diabetes mellitus is the strongest predictor of fibrosis [15, 140]. There is no correlation between the degree of hepatic siderosis and progression of NASH.

Large epidemiological studies demonstrate obesity and type 2 diabetes mellitus as being also important risk factors for the development of hepatocellular carcinoma (HCC) [58, 65]. The risk of HCC is increased among patients with hepatitis C cirrhosis and type 2 diabetes mellitus compared to patients with sole chronic hepatitis C infection. The 5- year occurrence of HCC was 11.4% in patients with hepatitis C and type 2 diabetes mellitus and 5% in patients with hepatitis C only. Type 2 diabetes mellitus was independently associated with the development of HCC [165]. The oral glucose tolerance test appears to be useful for predicting prognosis of patients with liver cirrhosis. The cumulative survival rates of patients with liver cirrhosis and normal glucose tolerance (NGT) were 95%

at 5 years; liver cirrhosis and impaired glucose tolerance, 69% at 5 years; liver cirrhosis and type 2 diabetes mellitus, 57% at 5 years. Thus, the survival rates of patients with liver cirrhosis and type 2 diabetes mellitus significantly differ from those with NGT [122].

In a recent population-based cohort study the natural history of NAFLD in the community was investigated aiming to determine survival and liver-related morbidity as compared with the general Minnesota population of the same age and sex. The mean follow-up was 7.6 years. Survival was lower in the NAFLD group than the expected survival for the general population. Higher mortality was associated with age, impaired fasting glucose, and cirrhosis. Liver disease was the third leading cause of death (as compared with the 13th leading cause of death in the general Minnesota population), occurring in 1.7% of subjects [5]. Liver failure is the main cause of morbidity and mortality in NASH-associated cirrhosis. Compensated cirrhosis due to NAFLD is associated with a lower mortality rate compared with that due to HCV. It is also associated with a lower rate of development of ascites, hyperbilirubinemia, and HCC [37, 85]. However, fatty liver seems to be a strong independent predictor for the presence of significant coronary artery disease, and cardiovascular mortality is greater in patients with NASH emphasizing the fact that NAFLD should be considered part of a multiorgan system derangement [88, 112, 151].

## Therapy

The treatment of NAFLD is essentially the treatment of metabolic syndrome [29]. It is based on

- Lifestyle modifications (diet, increased physical activity)
- Pharmacological interventions and
- Bariatric surgery

### *Lifestyle Modifications*

Mild degrees of bland steatosis in non-overweight patients do not need treatment. Life style interventions aim at weight reduction and optimal glucose control. "Crash dieting" should be avoided, since it may trigger acute necroinflammatory flares and in rare cases may even lead to acute liver failure. Furthermore, crash diets do not provide sustained long-term weight loss. Diets should therefore aim at realistic goals of weight loss of approximately 10% of body weight over 6–12 months. This may be achieved by a diet reducing daily caloric intake by 500–1000 kcal ("start low, go slow") combined with a regular exercise program. Already a modest weight loss of approximately 10% of body weight leads to a disproportionately high reduction of visceral adipose tissue of up to 30% of baseline value, and a low-carbohydrate diet rapidly reduces intrahepatic triglyceride content [34]. Weight loss is associated with improvement of insulin sensitivity, reduction of oxidative stress, improvement of liver enzymes, histologic improvement, and improvement of quality of life [61, 66, 81, 84, 133]. NASH patients have lower levels of n-3 and n-6 polyunsaturated fatty acids (PUFA) in the liver. Addition of PUFA to the diet may improve serum liver chemistries and ultrasound features of steatosis in patients with NASH (see Table 89.3) [13, 41]. The addition of antioxidants, such as vitamin C or E to lifestyle changes does not improve results of lifestyle changes [96, 123].

### *Pharmacological Therapy*

The pharmacological approaches to NAFLD are listed in Table 89.3.

Peroxisomal proliferator activated receptors (PPARα, PPARδ and PPARγ) are a nuclear receptor family involved in nutrient sensing and the regulation of carbohydrate and lipid metabolism. PPARα and PPARδ appear primarily to stimulate oxidative lipid metabolism, while PPARγ is principally involved in the cellular assimilation of lipids via anabolic pathways [153]. The *thiazolidinediones* are agonists of the PPARγ transcription factor and have pleiotropic effects, including improved insulin sensitivity, reduced expression of SREBP-1c and ChREBP, pancreatic β-cell preservation, improved HDL-C levels, decreased triglyceride levels, and increased LDL particle size [176]. In addition, they may inhibit hepatic stellate cells. Considering the pathophysiology of NAFLD, this class of drugs therefore appears a rational therapeutic option. Several studies have shown that rosiglitazone (4–8 mg p.o. qd) and pioglitazone (45 mg p.o. qd) may improve liver enzymes, and decrease hepatic steatosis and necroinflammatory

**Table 89.3** Pharmacological therapy of nonalcoholic fatty liver disease

| Drug(s) | n | Effect on Histology | Duration of study | Results and Comments |
|---|---|---|---|---|
| **Thiazolidinediones** | | | | |
| Acosta RC et al. (2001) Gastroenterology 120 (Suppl): A546 | 8 | Improved | 2–12 months | Abstract. |
| Caldwell SH et al. (2001) Am J Gastroenterol 96: 519–25 | 10 | Liver biopsy in 7 patients at the end of treatment. No significant changes of histopathological lesions. | 3–6 months | Study with troglitazone. Withdrawn from the market because of hepatotoxicity. Only 5/10 patients completed 6 months of treatment. |
| Sanyal AJ et al. (2002) Hepatology 36 (Suppl): 382A | 21 | ↓ steatosis<br>↓ inflammation | 6 months | Abstract. **Randomized trial.** Pioglitazone + Vitamin E vs. Vitamin E alone. |
| Shadid S (2003) Clin Gastro Hepatol 1: 384–387 | 5 | No posttreatment liver biopsy. | 4–5 months | Pioglitazone 30 mg/day. |
| Neuschwander-Tetri BA et al. (2003) Hepatology 38: 1008–17 | 30 | Pretreatment (30/30) und posttreatment (26/30) liver biopsy. Necroinflammatory score und perisinusoidal zone 3 fibrosis improved in 22/30. | 48 weeks | **Single-arm interventional trial.** Rosiglitazone 4 mg bid + diet and exercise (based on recommendations rather than a specific program). 25/30 completed 48 weeks of treatment. 67% of patients gained weight.<br>(1) ↓ ALT. 6 months after end of treatment renewed ↑ALT and AST<br>(2) ↑ insulin sensitivity |
| Promrat K et al. (2004) Hepatology 39: 188–96 | 18 | ↓ NASH histological score (steatosis, hepatocellular injury, inflammation) and fibrosis in 2/3 of patients. | 48 weeks | **Single-arm interventional trial.** Pioglitazone 30 mg/day.<br>(1) ALT normalization in > 70%<br>(2) ↑ insulin sensitivity<br>(3) ↓ hepatic fat content (despite ↑ body weight) |
| Belfort et al. (2006) N Engl J Med 355: 2297–307 | 55 | ↓ steatosis, necroinflammatory activity and ballooning improved significantly.<br>No significant improvement in fibrosis. | 6 months | **Randomized controlled trial.**<br>Proof of concept study.<br>Pioglitazone 45 mg qd and diet vs. placebo and diet.<br>In the treatment group:<br>(1) ↓ ALT<br>(2) ↑ hepatic insulin sensitivity<br>(3) ↑ adiponectin<br>(4) ↓ TGF-β and TNF-α<br>(5) ↓ hepatic fat content<br>(6) ↑ body weight due to increase in whole body fat (Balas B, et al. (2007) J Hepatol 47: 565–70)<br>(Pioglitazone increases whole body fat. |

(*continued*)

**Table 89.3** (continued)

| Drug(s) | n | Effect on Histology | Duration of study | Results and Comments |
|---|---|---|---|---|
| Lutchman G, et al. (2006) Clin Gastroenterol Hepatol 4: 1048–52 | 18 | Serum obtained at day 0 and week 48 of therapy;<br>Paired liver biopsies | 48 weeks | **Open label study.**<br>Study assesses serum levels of adipokines and proinflammatory cytokines (adiponectin, leptin IL-1a, IL-6, TNFα) during pioglitazone treatment.<br>↑ adiponectin<br>↔ other cytokines<br>Improvement in steatosis, parenchymal inflammation and fibrosis |
| Ratziu V. et al. (2008) Gastroenterology 135: 100–10 | 63 | ↓ steatosis | 12 months | **Randomized controlled trial.**<br>Rosiglitazone (4 mg/day for the first month and 8 mg/day thereafter; n = 32) vs. placebo (n = 31). Rosiglitazone normalized aminotransferase levels (38% vs. 7%) and improved steatosis (47% vs. 16%). No improvement in other parameters of liver injury. Weight gain (mean 1.5 kg) main adverse effect of rosiglitazone. |
| Aithal GP et al. (2008) Gastroenterology 135: 1176–84 | 74 | Hepatocellular injury, number of Mallory-Denk bodies and fibrosis were reduced in patients treated with pioglitazone. | 12 months | **Randomized, placebo-controlled trial.**<br>All patients nondiabetics with histologically proven NASH.<br>Standard diet, exercise, and either placebo or pioglitazone 30 mg/day.<br>Pioglitazone therapy was associated with weight gain, reduction in glucose, HbA1c, insulin C peptide, ALT, γ-GT and ferritin levels. |
| **Thiazolidinedione + Vitamin E** | | | | |
| Sanyal AJ et al. (2004) Clin Gastroenterol Hepatol 2: 1107– 115 | 20 | Vit. E monotherapy:<br>↓ steatosis<br>Vit. E ± Pioglitazone:<br>↓ steatosis,<br>↓ hepatocellular ballooning<br>↓ number of Mallory-Denk bodies<br>↓ pericellular fibrosis | 24 weeks | **Open label, randomized, prospective study.**<br>Vitamin E 400 IU/day (n = 10) vs. Vitamin E 400 IU/day + Pioglitazone 30 mg/day (n = 10; 2 patients discontinued therapy because of pregnancy and hepatotoxicity, respectively).<br>In Vitamin E + Pioglitazone group:<br>↓ insulin resistance<br>↓ FFA<br>↓ blood insulin |

**Table 89.3** (continued)

| Drug(s) | n | Effect on Histology | Duration of study | Results and Comments |
|---|---|---|---|---|
| **Metformin** | | | | |
| Marchesini G et al. (2001) Lancet 358: 893–894 | 14 | No posttreatment liver biopsy. | 4 months | **Single-arm interventional trial.**<br>Metformin 500 mg tid, diet and exercise.<br>(1) ALT normalization in 50%<br>(2) ↑ glucose disposal<br>(3) ↓ liver volume |
| Nair S et al. (2002) Gastroenterology 122 (Suppl): A621 | 25 | No posttreatment liver biopsy. | 3–6 months | Abstract.<br>Metformin 20 mg/kg/day |
| Uygun A et al. (2004) Aliment Pharmacol Ther 19: 537–44 | 36 | No significant changes in necroinflammatory activity and stage of fibrosis between both study groups. | 6 months | **Randomized uncontrolled trial.**<br>Metformin 850 mg bis + diet vs. diet alone (low lipid and low calorie)<br>Metformin group:<br>(1) ↓ ALT (during first 3 months of therapy, thereafter increase to pretreatment levels)<br>(2) ↑ insulin sensitivity |
| Nair S et al. (2004) Aliment Pharmacol Ther 20: 23–8 | 15 | Biopsy proven NAFLD (15/15). In 10/15 patients posttreatment liver biopsy:<br>↓ steatosis (n = 3)<br>↓ inflammation (n = 2)<br>↓ fibrosis (n = 1) | 12 months | **Single-arm interventional trial.** Pilot study.<br>Metformin 20 mg/kg/day.<br>(1) ↓ ALT<br>(2) ↑ insulin sensitivity |
| Schwimmer JB et al. (2005) Aliment Pharmacol Ther 21: 871–9 | 10 | Histologically proven NASH. | 6 months | **Open label pilot study** in obese children (BMI 30.4 kg/m$^2$).<br>Metformin 500 mg bid.<br>(1) ↓ ALT and AST<br>(2) ↓ hepatic triglyceride content on magnetic-resonance-spectroscopy<br>(3) ↑ insulin sensitivity<br>(4) ↑ quality of life |
| Bugianesi E et al. (2005) Am J Gastroenterology 100: 1082–90 | 110 | Biopsy prior to therapy in 55/55 and after posttreatment in 17/55.<br>↓ steatosis<br>↓ inflammation | 12 months | **Open label, randomized study.**<br>Metformin 2 g/day (n = 55) vs. Vitamin E 800 IU/day (n = 28) vs. weight reduction (n = 27).<br>ALT normalized in 56% of patients in the Metformin-group. |
| Nobili V, et al. (2008) Clin Ther 30: 1168–76 | 57 | Biopsy proven NAFLD/NASH (28 study group, 29 control group) | 12–24 months | **Observational pilot study** in overweight/obese children 9–18 years old.<br>Metformin 1.5 g/day plus lifestyle modification vs. lifestyle intervention.<br>Metformin did not appear more effective than lifestyle intervention in ameliorating levels of ALT, steatosis, and liver histology. |

(*continued*)

**Table 89.3** (continued)

| Drug(s) | n | Effect on Histology | Duration of study | Results and Comments |
|---|---|---|---|---|
| **Gemfibrozil** | | | | |
| Basaranoglu M et al. (1999) J Hepatol 31: 384 | 46 | No posttreatment liver biopsy. | 1 month | Letter. |
| **Clofibrate** | | | | |
| Laurin J et al. (1996) Hepatology 23: 1464–1467 | 16 | ↔ | 12 months | Clofibrate 2 g/day. |
| **Atorvastatin** | | | | |
| Horlander JC et al. (2001) Gastroenterology 120 (Suppl): A544 | 7 | ↓ steatosis<br>↓ fibrosis<br>↓ inflammation | 12 months | Abstract. |
| **Probucol** | | | | |
| Merat S et al. (2003) J Hepatol 38: 414–418 | 30 | Biopsy proven NASH. | 6 months | **Double blind randomized study.** Treatment group (n = 20) Probucol 500 mg/day.<br>27/30 completed study. Significant decrease of ALT levels in the treatment group as compared to controls. |
| **Vitamin E** | | | | |
| Lavine JE (2000) J Pediatr 136: 734–738 | 11 | No posttreatment liver biopsy. | 4–10 months | Vitamin E 400–1200 IU/day. |
| Hasegawa T et al. (2001) Aliment Pharmacol Ther 15: 1667–1672 | 12 | 12/12 patients pretreatment liver biopsy. 9/12 patients posttreatment biopsy.<br>In 5/9 ↓ inflammation and ↓ fibrosis. | 12 months | Vitamin E 300 mg/day. Patients with weight reduction had an improvement of histopathological lesions. |
| **Vitamin E and C** | | | | |
| Harrison SA, et al. (2003) Am J Gastroenerol 98: 2485–90 | 49 | Posttreatment biopsy.<br>↓ fibrosis<br>↔ inflammatory activity | 6 months | **Prospective, randomized, placebo controlled study.**<br>45/49 completed study.<br>Vitamin E 1000 IU/day plus Vitamin C 1000 mg/d vs. placebo.<br>All patients received dietary counselling. Patients with diabetes mellitus "seemed" to benefit from treatment. |
| Nobili V, et al. (2006) Aliment Phramacol Ther 24: 1553–61 | 90 | No biopsy performed. | 12 months | **Double-blind placebo study** in children.<br>Balanced calorie diet, physical exercise + placebo vs. Vit. E 600 IU/d + Vit. C 500 mg/day<br>At 12 months no difference in ALT, insulin sensitivity, and weight loss between the two arms.<br>In subjects who lost more than 1 kg insulin sensitivity improved. |
| **Betain hydrochloride** | | | | |
| Abdelmalek MF et al. (2001) Am J Gastroenterol 96: 2711–2717 | 8 | 6/8 posttreatment biopsy.<br>↓ steatosis<br>↓ necroinflammatory activity<br>↓ fibrosis | 12 months | Betain 20 g/day. |

**Table 89.3** (continued)

| Drug(s) | n | Effect on Histology | Duration of study | Results and Comments |
|---|---|---|---|---|
| **N-acetylcysteine** | | | | |
| Gulbahar O et al. (2000) Gastroenterology 118 (Suppl): A6550 | 11 | No posttreatment liver biopsy. | 3 months | N-acetylcysteine 1 g/day. |
| **Ursodeoxycholic acid** | | | | |
| Laurin J et al. (1996) Hepatology 23: 1464–1467 | 24 | ↓ steatosis | 12 months | UDCA 13–15 mg/kg/day. Encouraging results. However, see UDCA–study Lindor KD (2004). |
| Guma G et al. (1997) Hepatology 26 (Suppl): 387A | 24 | No posttreatment liver biopsy. | 6 months | Abstract. |
| Ceriani R et al. (1998) Hepatology 28 (Suppl): 386° | 31 | No posttreatment liver biopsy. | 6 months | Abstract. |
| Lindor KD et al. (2004) Hepatology 39: 770–8 | 168 | In 95/168 posttreatment liver biopsy:<br>↔ steatosis<br>↔ necroinflammatory activity<br>↔ fibrosis | 2 years | **Prospective, randomized, placebo controlled study.**<br>UDCA 13–15 mg/kg/day vs. placebo.<br>UDCA not superior to placebo. |
| **Ursodeoxycholic acid and Vitamin E** | | | | |
| Dufour JF, et al. (2006) Clin Gastroenterol Hepatol 4: 1537–43 | 48 | Biopsy proven NASH.<br><br>Posttreatment liver biopsy in all patients: activity index significantly improved in treatment group A (UDCA + Vit. E) | 2 years | **Randomized placebo-controlled trial.**<br>(A) UDCA 12–15 mg/kg/day+ Vit. E 400 IU bid (n = 15) vs.<br>(B) UDCA + placebo (n = 18) vs.<br>(C) Placebo + placebo (n = 15)<br>Results: 8 patients dropped out.<br>(1) ↓ AST and ALT in group A<br>(2) ↓ ALT only in group B<br>(3) No change in AST and ALT in group C<br>(4) Histological improvement in group A |
| **Orlistat** | | | | |
| Harrison SA et al. (2002) Hepatology 36 (Suppl): 406A | 10 | ↓ steatosis<br>↓ fibrosis | 6 months | Abstract. Most patients with weight loss ≥ 10% of body weight had also a decrease in fibrosis. |
| Harrison SA et al. (2003) Am J Gastroenterol 98: 926–30 | 3 | ↓ steatosis<br>↓ fibrosis<br>↓ inflammation | 6–9 months | |
| Zelberg-Sagi S et al. (2006) Clin Gastroenterol Hepatol 4: 639–44 | 52 | Biopsy in 40 patients; posttreatment biopsy in 22 patients.<br>↓ steatosis and ↓ fibrosis in both groups | 6 months | **Double-blind randomized placebo-controlled trial.** 44 patients completed study.<br>Orlistat 120 mg tid vs. placebo for 6 months + all patients participated in identical behavioral weight loss program.<br>Significant ↓ BMI in both groups with nonsignificant difference between groups. |

(*continued*)

**Table 89.3** (continued)

| Drug(s) | n | Effect on Histology | Duration of study | Results and Comments |
|---|---|---|---|---|
| | | | | Reduction of steatosis as determined by ultrasound only in orlistat group.<br>Histologically no significant changes between both groups.<br>Main effects probably due to weight reduction and not to orlistat. |
| **Polyunsaturated Fatty Acids** | | | | |
| Capanni M, et al. (2006) Aliment Pharmacol Ther 23: 1143–51 | 56 | 56 patients with NAFLD.<br>42 PUFA group<br>14 control group No histology. | 12 months | **Pilot study**. n-3 long-chain PUFA 1 g/day.<br>PUFA significantly decreased serum AST, ALT, γ-GT, triglycerides and fasting glucose in comparison with controls.<br>Ultrasonographic features of steatosis improved in the PUFA group. |
| **Losartan** | | | | |
| Yokohama S et al. (2004) Hepatology 40: 1222–5 | 7 | ↓ necroinflammatory activity (5/7)<br>↓ fibrosis (4/7)<br>↓ siderosis (2/7) | 48 weeks | Losartan 50 mg/day.<br>Decrease in serum markers of fibrosis, of plasmatic TGF-β1 levels and of ferritin in serum. |
| **Leptin** | | | | |
| Javor et al. (2005) Hepatology 41: 753–60 | 10 | Paired liver biopsies at baseline and after treatment | Mean duration 6.6 months | Recombinant methionyl human leptin (r-metHuLeptin) in patients with lipodystrophy: significant improvement in steatosis and in hepatocyte ballooning.<br>Fibrosis unchanged.<br>Reductions in serum triglycerides, glucose, insulin and ALT levels. |
| **Folic acid** | | | | |
| Charatcharoenwitthaya P, et al. (2007) Liver Int 27: 220–6 | 10 | Biopsy proven NASH. No posttreatment biopsy. | 6 months | **Open-label pilot study**. Folic acid 1 mg/day.<br>Folic acid therapy safe and well tolerated but ↔ in AST and ALT. |
| **Pentoxifylline** | | | | |
| Satapathy SK, et al. (2004) Am J Gastroenterol 99: 1946–52 | 18 | Biopsy proven NASH. No posttreatment biopsy. | 6 months | **Open-label interventional study.**<br>Pentoxifylline 400 mg tid.<br>After therapy:<br>(1) ↓ fatigue<br>(2) ↓ AST and ALT (ALT normalized in 60%)<br>(3) ↑ insulin sensitivity<br>(4) ↓ TNF-α |
| Adams LA et al. (2004) Am J Gastroneterol 99: 2365–8 | 20 | Biopsy proven NASH. No posttreatment biopsy. | 12 | **Open-label interventional pilot study.**<br>Pentoxifylline 400 mg qid |

**Table 89.3** (continued)

| Drug(s) | n | Effect on Histology | Duration of study | Results and Comments |
|---|---|---|---|---|
| | | | | 9 patients dropped out, primarily because of nausea.<br>(1) ↓ AST and ALT |
| **Iron depletion** | | | | |
| Valenti L et al. (2007) Am J Gastroenterol 102: 1251–8 | 64 | | | **Case control study.** Phlebotomy vs. lifestyle modifications.<br>Phlebotomy reduced insulin resistance. |
| **Rimonabant**[a] | | | | |
| Banasch M et al. (2007) Liver Int 27: 1152–5 | 1 | Biopsy proven NASH. | 3 months | **Case report.** Rimonabant 20 mg p.o. qd.<br>10% weight loss, reduction of triglyceride levels, improvement of oral glucose tolerance and insulin resistance (HOMA-Index), improvement of ALT, AST and γGT. |

[a]Rimonabant has been withdrawn from the European market due to neuropsychiatric side effects (depression, suicide) in 2008.
↓ decrease, ↑ increase, improvement, ↔ no change. *ALT* alanine aminotransferase (GPT), *AST* aspartate aminotransferase (GOT), *FFA* free fatty acids, *IL* interleukin, *PUF* apolyunsaturated fatty acids, *TNF* tumor necrosis factor

activity in patients with NAFLD. Improvements in liver histology during thoazolidinedione therapy may be modulated by an adiponectin-mediated effect on insulin sensitivity and hepatic fatty acid metabolism. Since NAFLD, however, is a chronic disease running for decades, long-term drug use would be necessary. The duration of currently performed trials with thiazolidinediones does not exceed one year and, in addition, the safety profile of thiazolidinediones is not completely clear. Moreover, tioglitazone treatment is associated with weight gain that is attributable to an increase in adipose tissue mass [20]. Thus, despite encouraging results in short term studies the use of thiazolidinediones in non-diabetic patients with NAFLD currently cannot be recommended.

*Biguanide (metformin)* treatment may also improve insulin sensitivity, lower aminotransferases and decrease hepatic steatosis. The effects, however, are only transient and metformin treatment of NAFLD in the absence of diabetes cannot be recommended.

Lipid lowering agents, vitamin E and C, and ursodeoxycholic acid are of no clinical benefit in patients with NAFLD [103]. Statins, however, should not be withheld from patients with hyperlipidemia and NAFLD out of concern over an increased potential for statin-induced hepatotoxicity in patients with chronic liver disease [64, 100].

An interesting novel approach to treatment of NAFLD represents pharmacologic modulation of the endocannabinoid (EC) system. The EC system is a physiological network implicated in appetite control and energy homeostasis. Endogenous cannabinoids, such as anandamide acting at cannabinoid type 1 ($CB_1$) receptors stimulate appetite, and $CB_1$ antagonists show promise in the treatment of obesity [125]. *Rimonabant* is a selective $CB_1$ receptor blocker that reduces food consumption. In addition to its central nervous system effects rimonabant may lead to improvements in lipid profiles, insulin resistance and glucose homeostasis. $CB_1$ receptor is also present in the liver and antagonizing the EC system might prove beneficial in patients with NAFLD [21, 73, 76].

In patients with lipodystrophy *recombinant leptin* improves steatosis and hepatocyte ballooning [89]. In the rare patient with NASH due to adult growth hormone deficiency, *growth hormone* reverses steatohepatitic lesions [158].

*In summary*, currently there is no compelling evidence to recommend any drug in the treatment of patients with NAFLD.

### *Bariatric Surgery*

In patients with *morbid obesity* gastric bypass surgery leads to a marked weight loss and to improvement of metabolic and hepatic abnormalities associated with NAFLD [23, 93]. Roux-en Y gastric bypass induces considerable and persistent improvement in metabolic syndrome parameters. The reversibility of metabolic syndrome primarily depends on the amount of excess weight loss [24]. In a recent meta-analysis of 15 studies with 766 paired liver biopsies bariatic surgery was shown to improve steatosis, steatohepatitis and fibrosis in 92%, 81% and 65% of cases, respectively. NASH resolved completely in 69% of patients [114a].

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# Other Metabolic Diseases: Tabellary Overview

# 90

Henryk Dancygier

## Chapter Outline



Inborn errors of metabolism affecting the liver primarily manifest in newborns, infants and adolescents. In this chapter selected diseases are summarized in a table format. The interested reader is referred to textbooks on pediatric hepatology for further review.

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**Table 90.1** Diseases of carbohydrate metabolism [12]

| Type | Enzyme deficiency | Clinical manifestations |
|---|---|---|
| **Glycogen storage diseases** | | |
| **I (von Gierke's disease)** | Glucose-6-phosphatase | Glycogen accumulation in liver and renal tubular cells. Hepatomegaly, lactic acidosis, ketosis, hypoglycemia, hyperlipidemia |
| **II (Pompe's disease)** | Lysosomal α1→4 and α1→6 glucosidase (acid maltase) | Accumulation of glycogen in lysosomes. Cardiomegaly, death from heart failure by age 2 (juvenile onset variant). Macroglossia, mild hepatomegaly, no hypoglycemia, muscle dystrophy (adult onset variant) |
| **III (Limit dextrinosis, Forbe's or Cori's disease)** | 1,2 amylo-1,6-glucosidase (debranching enzyme) | Resembles type I glycogenosis. Fasting hypoglycemia. Hepatomegaly. Up to 25% of patients develop hepatocellular adenomas. Liver cirrhosis in adulthood. Muscle weakness |
| **IV (Amylopectinosis, Andersen's disease)** | Amylo-1,4–1,6-transglucosidase (branching enzyme) | Fetal hydrops. Hepatospenomegaly. Muscle weakness. Death from heart or liver failure in first years of life |
| **V (McArdle's syndrome)** | Muscle phosphorylase | No hepatic involvement |
| **VI (Hers' disease)** | Liver phosphorylase | Accumulation of glycogen in the liver. Hepatomegaly. Hypoglycemia and hyperlipidemia in first year of life. Relatively benign clinical course, generally good prognosis. Alterations tend to recede with age |
| **VII (Tarui's disease)** | Muscle and erythrocyte phosphofructokinase 1 | No hepatic involvement |
| **VIII** | Liver phosphorylase kinase | Accumulation of glycogen in liver. Hepatomegaly. Mild hypoglycemia |
| **IX** | Liver and muscle phosphorylase kinase | Accumulation of glycogen in liver and muscle. Hepatomegaly. Muscle spasms. Mild hypoglycemia |
| **X** | cAMP-dependent protein kinase A | Accumulation of glycogen in liver. Hepatomegaly. |
| **Galactosemia** | Galactokinase, uridyl transferase, 4-epimerase | Galactose is a substrate for aldose reductase, forming galactitol, which accumulates in the lens of the eye, causing cataract. With galactokinase deficiency only, solely eye symptoms are present<br>In uridyl transferase deficiency, galactose 1-phosphate accumulates and depletes the liver of inorganic phosphate. Vomiting, diarrhea, failure to thrive, hepatomegaly, jaundice, cerebral seizures occur. Galactose-free diet controls symptoms |
| **Fructose intolerance** | Fructose 1-phosphate-aldolase | Fructose-induced hypoglycemia despite the presence of high glycogen reserves, because fructose 1-phosphate allosterically inhibits liver phosphorylase. Dysfunction of liver, kidneys and central nervous system. Diets low in fructose, sorbitol and sucrose are beneficial |

**Table 90.2** Diseases of lipid metabolism [12]

| Disease | Enzyme/protein deficiency | Clinical manifestations[a] |
|---|---|---|
| **$GM_1$ gangliosidosis** | Lysosomal β-galactosidase-1 (A, B and C) | *Infantile form (type I)*:<br>Facial abnormalities (frontal bossing, depressed nasal bridge). Gum hypertrophy. Dermal melanosis. Failure to thrive. Blindness. Deafness. Decerbrate rigidity. Hepatocytes and Kupffer cells are finely vacuolated. On electron microscopy vacuoles containing fibrillar or granular material<br>*Juvenile form (type II)*:<br>Neurological symptoms at about 1 year of age (seizures, spasticity, ataxia, mental retardation). Hepatocytes are slightly vacuolated. Ganglioside-laden Kupffer cells are intensely PAS-positive. On electron microscopy Kupffer cells contain a granulofibrillar material<br>*Adult form (type III)*:<br>Less severe than type I and II |
| **Tay–Sachs disease** | Hexosaminidase A | Ethnic association with Ashkenazi Jews. Accumulation of ganglioside $GM_2$ in the brain. Seizures, mental retardation, paralysis. Cherry-red macular spot. Death by age 4–5 years<br>Hepatocytes appear normal on light microsopy. On electron microscopy they may contain concentrically laminated inclusions or membranous cytoplasmic bodies ($GM_2$ gangliosides) |
| **Sandhoff's disease** | Hexosaminidase A and B | No ethnic association. Similar to Tay Sachs. Cherry-red macular spot. Hepatomegaly. Kupffer cells contain PAS-positive granules. On electron microscopy laminated inclusions in hepatocytes and Kupffer cells ($GM_2$ gangliosides and tetrahexosyl ceramide) |
| **Fabry's disease (angiokeratoma corporis diffusum)** | α-Galactosidase A | Angiokeratomas, acroparesthesias, corneal opacities, recurrent febrile episodes, and renal or heart failure. Death is usually from renal or cardiovascular complications<br>Accumulation of globotriaosylceramide (GL-3) and cholesterol in Kupffer cells, portal tract macrophages and the endothelial cells of blood vessels. Stored material (concentrically laminated lysosomal inclusions) is intensely PAS-positive and resistant to diastase digestion<br>Amorphous material may accumulate in hepatocytes. Immunocytochemistry with anti-GL-3 antibody is diagnostic |
| **Gaucher's disease** | β-Glucosidase (glucocerebrosidase) | Lysosomal accumulation of glucosylceramide (glucocerebroside), mainly in macrophages ("Gaucher cells" in liver, spleen and bone marrow: striated wrinkled cytoplasm). Hepatosplenomegaly. CNS changes. Skin pigmentation. Involvement of bones and lungs<br>*Type I (non-neuronopathic)*:<br>Most common (90% of all cases). Ethnic association with Ashkenazi Jews<br>*Type II (acute neuronopathic)*:<br>Rarest form. Residual enzyme activity is lowest. Progressive neurologic deterioration. Death by age 2 years<br>*Type III (subacute neuronopathic)*:<br>Dementia, ataxia, bone and visceral involvement, supranuclear palsies, corneal opacities. Patients may survive for many years |
| **Niemann–Pick disease** | Sphingomyelinase | Ethnic association with Ashkenazi Jews. Seizures, blindness, paralysis, pulmonary infiltrates. Foamy Kupffer cells (contain sphingomyelins) and portal macrophages (in adults macrophages contain "sea blue" pigment). Hydropic hepatocytes |

(*continued*)

**Table 90.2** (continued)

| Disease | Enzyme/protein deficiency | Clinical manifestations[a] |
|---|---|---|
| **Sulphatide lipidosis (metachromatic leucodystrophy)** | Arylsulfatase A | Progressive paralysis. Dementia. Death by age 10 years<br>Sulphatide accumulation in CNS, liver, gallbladder (foamy macrophages in the tunica propria). Metachromatic granules are present in descending order of frequency in gallbladder epithelium, intrahepatic bile duct epithelium, portal macrophages, Kupffer cells and hepatocytes |
| **Wolman's disease and cholesterol ester storage disease** | Lysosomal acid lipase | Vomiting. Diarrhea. Malabsorption. Failure to thrive. Calcification of adrenal glands. Hyperlipidemia. Hepatomegaly. Lysosomal fat vacuoles in hepatocytes and Kupffer cells (foam cells, intensely PAS-positive). On polarizing microscopy cholesterol crystals display birefringence ("maltese crosses") |
| **Cerebrotendinous xanthomatosis** | Mitochondrial C27-steroid 26-hydroxylase | Accumulation of cholestanol in tissues. Neonatal hepatitis. Diarrhea. Juvenile cataracts. Tuberous xanthomas. Mental retardation, but also normal intelligence possible. Spasticity, ataxia, paralysis, polyneuropathy. Death usually in the fourth to sixth decade<br>Amorphous or crystalloid intracellular pigment inclusions in hepatocytes |
| **Abetalipoproteinemia (Bassen–Kornzweig syndrome)** | Microsomal triglyceride transfer protein (MTP; large subunit of the protein is absent) | MTP deficiency leads to marked impairment of secretion of mainly apolipoprotein B containing lipoproteins. Malabsorption of fat, steatorrhea, failure to thrive, acanthocytosis, retinitis pigmentosa, ataxia. Very low serum cholesterol, and extremely low triglyceride levels. Fat droplets in intestinal epithelial cells. Marked hepatic steatosis. Large fat droplets may rupture to form "fatty lakes". Mallory-Denk bodies. Untreated the disease progresses to micronodular cirrhosis |
| **Familial high density lipoprotein deficiency (Tangier disease)** | Cholesterol efflux regulatory protein (ATP-binding cassette-1 protein) | Cholesterol removal from cells is blocked. Tonsillar enlargement, orange discoloration of tonsils, lymphadenopathy, hepatosplenomegaly, peripheral neuropathy. Accumulation of cholesterol esters in reticuloendothelial cells.<br>*Liver*: clusters of foamy macropahges containing birefringent, needle-shaped cholesterol crystals |

[a]Most of these diseases are characterized clinically mainly by CNS symptoms. Although the abnormal material also is often stored in the liver, hepatic involvement rarely is clinically significant

**Table 90.3** Diseases of amino acid metabolism and urea cycle disorders [1, 3, 7, 11]

| Disease | Enzyme deficiency | Clinical manifestations |
|---|---|---|
| **Tyrosinemia type I** | Fumarylacetoacetate hydroxylase, often accompanied by other enzyme deficiencies, such as δ-aminolevulinic acid hydratase, methionine adenosyl transferase | *Acute form*:<br>Fulminant liver failure in the neonatal period possible. Failure to thrive, anemia, vomiting, diarrhea, hepatosplenomegaly, hemorrhages, death usually by age 1 year<br>*Chronic form*:<br>Liver and renal insufficiency. Failure to thrive, hypophosphatemia, vitamin D-resistant rickets, acute intermittent porphyria. Death usually by age 10 years<br>Liver changes occur in both forms: steatosis, cholestasis, siderosis, portal fibrosis, cirrhosis. Increased risk of hepatocellular carcinoma |
| **Cystinosis** | Defective carrier-mediated lysosomal transport system for cystine | Accumulation of L-cystine crystals in lysososmes. Fanconi's syndrome in first year of life. Impaired synthesis of melanin. Failure to thrive, hypothyroidism, retinopathy. Death usually due to renal insufficiency by age of 5 years<br>*Liver*: birefringent cystine crystals in hypertrophied Kupffer cells with little or no inflammatory reaction<br>Nodular regenerative hyperplasia may represent a late complication of cystinosis and cause portal hypertension |
| **Homocystinuria** | Cystathionine β-synthase | Accumulation of homocysteine and its metabolites. Lens ectopy, osteoporosis, kyphoscoliosis, cerebral defects. Hypercoagulable state with arterial and venous thromboembolic phenomena. Hepatomegaly. Steatosis, more prominent in the perivenular regions |
| **Urea cycle disorders** | Carbamoyl phosphate synthase, ornithin transcarbamylase, argininosuccinate lyase, argininosuccinate synthase, N-acetylglutamate synthase | Hyperammonemia under catabolic or protein-loading conditions. Early recognition is important since early treatment may prevent CNS injury. Diagnosis of urea cycle disorder should be considered in any patient with unexplained neurologic and psychiatric disorders with selective anorexia, even in adulthood. Vomiting, apathy, seizures, respiratory dysfunction<br>*Hepatic changes*: micro- and macrovesicular steatosis, periportal glycogenated nuclei, focal glycogenosis (clusters of hepatocytes with clear cytoplasm), portal inflammation and fibrosis. Depending on which enzyme is affected megamitochondria or small mitochondria occur |

**Table 90.4** Peroxisomal disorders [2, 12]

| Disease[a] | Deficiency | Clinical manifestations |
|---|---|---|
| **Zellweger's syndrome** | Absence of peroxisomes due to mutation of peroxisome-assembly-factor-1 mRNA | Impaired β-oxidation of long chained fatty acids, impaired bile acid synthesis<br>Cerebral, ocular, genital and cardiovascular malformations. Failure to thrive, mental retardation, arterial hypotonia, renal cysts. Death usually occurs by the age of 6 months<br>*Liver*: hepatomegaly, siderosis, cholestasis, portal inflammation and fibrosis, occasionally cirrhosis.<br>On electron microscopy: lack of peroxisomes |
| **Di- and trihydroxycholestanoic acidemia ("Alligator Bile syndrome")** | Di- and trihydroxy-coprostanic acid coenzyme oxidase | Obstructive jaundice, severe cholestasis |
| **Mevalonate kinase deficiency** | Mevalonate kinase | Mevalonate aciduria<br>Hyper IgD syndrome (periodic fever) |

[a]In the remaining peroxisomal biogenesis disorders (*neonatal adrenoleucodystrophy, infantile Refsum's syndrome* and *rhizomelic chondrodysplasia punctata*) the liver is not involved

**Table 90.5** Primary mitochondrial hepatopathies [4–6, 8–10, 12–14]

| Disease | Deficiency | Clinical manifestations[a] |
|---|---|---|
| **Defects in fatty acid oxidation** | Short and long-chain 3-hydroxyacyl-CoA dehydrogenase; many additional enzymes may be affected | Vomiting, apathy, cerebral coma in the newborn. Cardiomyopathy with sudden cardiac death. Muscle weakness, rhabdomyolysis, myoglobinuria. Micro- and macrovesicular steatosis of hepatocytes |
| **Hepatocerebral syndrome** | Deoxyguanosine kinase; mitochondrial DNA depletion syndrome | Hepatomegaly and progressive liver failure in the first weeks of life. Psychomotor delay, nystagmus. Severe hyperlactacidemia<br>*Liver*: multifocal injury of hepatocytes, irregular foamy steatosis, cholestasis, fibrosis, siderosis of hepatocytes and glycogen depletion |
| **Alpers–Huttenlocher's syndrome** | Mitochondrial DNA polymerase; mitochondrial DNA depletion syndrome | Fatal brain and liver disease in children and young adults<br>Mutations in the gene for the mitochondrial DNA polymerase (POLG) cause this disorder<br>Screening for A467T and W748S substitutions in POLG constitutes the most rapid and sensitive test available for confirming the clinical diagnosis of Alpers-Huttenlocher's syndrome |
| **Navajo neurohepatopathy** | Defect unknown; probably a mitochondrial depletion syndrome | Jaundice, ascites, Reye's syndrome-like episodes, cirrhosis, liver failure. Death in early childhood<br>*Liver histology*: multinucleated giant cells, micro- and macrovesicular steatosis, rosette formation, cholestasis, inflammation, bridging fibrosis and cirrhosis in some cases |
| **Pearson's syndrome** | Deletions of mitochondrial DNA segments | Three clinical presentations: infantile, childhood and classic type<br>Neonatal-onset of severe macrocytic anemia, variable neutropenia and thrombocytopenia. Vacuolization of marrow precursors, ringed sideroblasts in the bone marrow<br>Pancreatic fibrosis and acinar atrophy lead to exocrine pancreatic insufficiency with steatorrhea and malabsorption<br>*Liver*: hepatomegaly, steatosis, cholestasis, cirrhosis, progressive liver failure |

[a]Primary mitochondrial hepatopathies are characterized histologically by steatosis (mostly microvesicular), fibrosis, canalicular cholestasis, different degrees of siderosis and variable signs of hepatocellular injury (ballooning, hepatocyte loss)

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# Malnutrition and Nutrition in Liver Disease

# 91

Srinivasan Dasarathy and Arthur J. McCullough

## Chapter Outline



The liver is primarily a metabolic organ that regulates a complex array of physiological and biochemical processes, including protein and energy metabolism. Patients with advanced liver disease commonly have malnutrition or undernutrition but its assessment is confounded by many of the usual indicators of nutritional status that are altered directly by the hepatic pathophysiology rather than, or in addition to, preexisting or subsequent secondary malnutrition [23]. A central issue is the lack of a consensus on the definition of malnutrition in cirrhosis [23, 41]. It has been used to include hypoalbuminemia, cachexia, loss of skeletal muscle mass, loss of fat mass and loss of whole body weight. A number of clinical, anthropometric, biochemical, immunological, and body composition measures have been used by various authors to quantify malnutrition in cirrhosis [27]. It is important to identify the criteria used in each of these studies before the data can be interpreted. The consensus definition of malnutrition in cirrhosis is the decreased lean body mass and diminished skeletal muscle weight. It is important to underscore the fact that poor nutritional status is the single reversible prognostic marker in cirrhosis [39, 112]. Poor nutritional status accelerates deteriorating liver function and adversely affects clinical outcome both before and after liver transplantation.

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Nutritional management of patients with liver disease remains a much discussed but controversial area of clinical hepatology. At a strategic level, it is clear that adequate nutrition is a fundamental component to the survival and quality of life in these patients [87, 112]. The objective of nutritional support in patients with liver failure is to provide adequate nutrients to ensure the availability of specific substrates for energy and protein synthesis and for normal hepatocyte survival and function, without inducing or accentuating encephalopathy or otherwise compounding hepatic insufficiency. However, the logistics of identifying patients in need of nutritional therapy as well as determining the type, duration, and quantity of nutritional supplements remain unclear. In addition, questions persist regarding the cost-effectiveness of nutritional therapy, its effect on quality of life, and the definition of the appropriate goals and outcomes to be achieved from such therapy. These controversies stem from a number of factors: the definition of impaired nutrition is unclear, high quality human studies are limited, most studies on animal models have focused on a specific component, i.e., skeletal muscle mass (these are difficult to extrapolate to human studies), lack of significant benefit using currently available treatment options, and finally the absence of a rational therapeutic strategy based on an understanding of the pathophysiology of low whole body protein content as well as diminished skeletal muscle mass and fat mass in cirrhosis [3, 61, 96].

The importance of treating malnutrition has resulted in the development of guidelines for the nutritional management of patients with liver disease [96, 131]. The rationale for aggressive nutritional therapy in these patients is based on promising, but nascent clinical information and a number of novel mechanisms identified in animal models with liver disease and low muscle mass. First, malnutrition is common, but often under diagnosed. Second, these patients have alterations in energy metabolism and nutritional status that mimic starvation [75, 79]. Third, many of the complications associated with chronic liver disease (such as hepatic encephalopathy, ascites and sepsis) occur *pari passu* with a negative nitrogen state, such as occurs before and immediately after liver transplantation [10, 76, 108]. Finally, there is the expectation that correction of malnutrition will improve the clinical outcome of these patients.

Most clinicians employ a common sense approach to feeding patients with liver disease, whereas some investigators advocate the aggressive use of nutritional supplements and specialized formulations [96]. The aim of this more aggressive approach is to correct preexisting malnutrition and to stimulate hepatic regeneration. Aggressive nutritional therapy may, in fact, benefit certain types of patients with liver disease and emphasize the need for a greater understanding of the etiologic factors responsible for malnutrition so that the specific role of nutrition in the diagnosis, prognosis, and therapy of chronic liver disease can be improved and better defined.

## Definition

Malnutrition can be defined as the loss of protein stores (both visceral and muscle/somatic) and fat mass [2]. Determination of body cell mass (BCM) seem to provide the most accurate measure of malnutrition in chronic liver disease [54, 131]. Quantifying BCM, which comprises the central energy-expending mass of working tissue is labor intensive and expensive but provides the most accurate measure of body composition and consequently, nutritional status in patients with liver cirrhosis [95]. Most publications on nutritional assessment and intervention in cirrhosis have utilized clinical, biochemical, anthropometric measures or estimates of body composition that quantify loss of skeletal muscle mass as a surrogate of whole body protein loss with or without loss of fat mass [26, 101, 127, 135]. Each of these methods of measuring whole body protein mass has its own methodological and interpretational limitations. In contrast to children, where malnutrition is considered to be either *Kwashiorkor* (protein loss) or *marasmus* (proportionate loss of both protein and energy), a similar clear definition in adults is not available [2]. Given that skeletal muscle is the largest protein pool in the body, and loss of skeletal muscle mass is included in most measures of malnutrition in cirrhosis, loss of skeletal muscle mass is the primary indicator of malnutrition in patients with cirrhosis. This does not take into account loss of fat mass in cirrhosis that contributes to lower energy source as well as to adverse outcome in these patients [99]. Another potential difficulty in interpretation of published data is related to the differences in assessment of severity of malnutrition. Use of standardized terms permit the use of precise terminology and is essential when analyzing and interpreting data as well as in the design of future studies on nutritional consequences of cirrhosis.

In addition to these more classical forms of malnutrition it is also important to realize that over nutrition in the form of obesity also occurs in patients with liver disease [62]. Obesity, defined as a body mass index (BMI) ≥ 30 kg/m$^2$ in these patients is not only a potential etiologic factor for the development of advanced liver disease per se, it also contributes to the development of cirrhosis in patients with liver injury from alcohol and/or hepatitis C (see Chapter 88 and 89) [29, 51].

## Prevalence

### *Malnutrition*

Clinically obvious loss of protein content, lean body mass or skeletal muscle mass is uncommon in the pre-cirrhotic and early stages of cirrhosis, except in the setting of extrahepatic biliary obstruction [40]. The prevalence of malnutrition in cirrhosis ranges between 10% and 100% depending on the diagnostic criteria employed and correlates with the severity of disease defined by the Child-Pugh-Turcotte score [41, 80, 127]. Malnutrition has been reported in about 20% of patients with compensated cirrhosis (Child's A) and in 50–60% of patients with decompensated cirrhosis (Child's C) [41, 135]. The large range in reported prevalence rate may depend on how nutritional assessment is performed [6].

Although most of the earlier studies concentrated on patients with alcoholic liver disease the prevalence of malnutrition was high in all forms of cirrhosis [63]. In Fig. 91.1 the nutritional status assessed in both alcoholic and non-alcoholic cirrhosis shows that the prevalence of malnutrition is high and almost identical in these 2 different types of cirrhosis [6]. Based on anthropometric measurements of the lowest fifth percentile of a control population, the prevalence of malnutrition varied between 34–62% and 27–67% in these studies for alcoholic and nonalcoholic cirrhosis, respectively. There is also a high prevalence of malnutrition in patients awaiting liver transplantation with estimates ranging between 18% and 100% with the two most detailed studies showing that moderate or severe malnutrition occurred in 70–80% of these patients [6, 34]. As listed in Table 91.1, disease-specific patterns of malnutrition have also been shown in patients with cirrhosis awaiting liver transplantation. Therefore, clinicians need to be aware that malnutrition is very common in these patients, especially because it has prognostic significance.

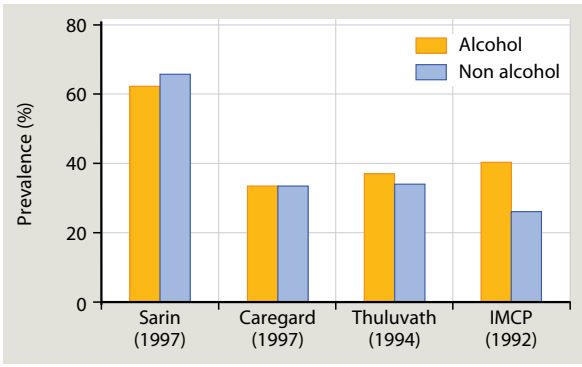


**Fig. 91.1** The prevalence of protein-energy malnutrition in alcoholic and non- alcoholic cirrhosis is shown for four available studies

**Table 91.1** Patterns in of malnutrition in chronic liver disease

| | Muscle wasting | Loss of fat stores | Synthetic function |
|---|---|---|---|
| **Alcohol** | +++ | + | ++ |
| **Viral** | ++ | ++ | + |
| **Primary biliary cirrhosis** | +++ | +++ | + |
| **Primary sclerosing cholangitis** | ++ | + | + |
| **Non alcoholic steatosis** | ++ | ± | + |

+, ++, +++: mild, moderate, or severe abnormalities, respectively

### *Obesity*

The prevalence of obesity is approximately 30% of the adult population in the US and its prevalence and is increasing at an alarming rate worldwide [59]. Obesity is associated with non-alcoholic fatty liver disease (NAFLD), which is a spectrum of histologic liver disease ranging from steatosis to non-alcoholic steatohepatitis (NASH) to cirrhosis and in some patients to hepatocellular carcinoma [62]. Obesity has also been reported as a risk factor for the progression of disease and development of cirrhosis in alcohol and viral related liver disease [29, 51].

Although the prevalence of obesity is unknown in patients with liver disease, obesity has been evaluated in patients awaiting transplantation [22]. Although the definition of obesity differed among the studies, it appears that moderate or severe obesity is present in 7–32% of patients at the time of transplantation [13, 92].

## Pathophysiology of Malnutrition

There are multiple factors (as listed in Table 91.2) involved in the etiology of malnutrition which accelerates significantly after the development of cirrhosis and portosystemic shunting [71]. Many of the factors can be treated successfully when nutritional status is recognized and treated as a priority.

### Nutrient Intake

Inadequate dietary intake (27–87%) occurs in patients with both alcoholic and non-alcoholic liver disease and has been attributed to anorexia, nausea, early satiety (especially in the presence of ascites) dysgeusia (related to zinc and/or vitamin A deficiency), unpalatable sodium, fluid and protein restricted diets and an elevated serum leptin concentration [64, 107]. In hospitalized patients, additional factors are often iatrogenic and related to diagnostic and therapeutic procedures. The importance of these observations is emphasized by observations that demonstrate that a regular oral diet can improve nitrogen retention and protein mass in cirrhotic patients.

**Table 91.2** Factors contributing to malnutrition in cirrhosis

*Non-hospitalized patients*
1. Inadequate diet[a]
   - Quantitative (poor intake due to anorexia, ascites)
   - Iatrogenic
   - (Protein, fluid/salt restriction)
2. Malabsorption[a]
   - Pancreatic and bile salt deficiency
   - Enteropathy
   - Gastrointestinal infections, bacterial overgrowth
   - Portal hypertension
3. Anorexia, nausea and vomiting
4. Alcohol toxicity on energy and protein
5. Altered protein and energy metabolism
   - Amino acid oxidation
   - Accelerated lipolysis and reduced lipogenesis
   - Insulin resistance and impaired gluconeogenesis
   - Increased proteolysis
   - Reduced protein synthesis, impaired meal related increase in protein accretion

*Hospitalized patients*
1. Fasting status[a]
   - Diagnostic testing
   - Gastrointestinal bleeding
   - Altered neurologic status
2. Purgation and neomycin toxicity
3. Unpalatable diets[a]
4. Stressful complications[a] (sepsis, renal failure)

[a]Indicates factors that can be currently altered or treated

### Absorption

Some studies also have demonstrated malnutrition in cirrhotics despite normal dietary intake. Therefore, other factors must be involved.

Although malabsorption is not usually considered a major cause of malnutrition in cirrhosis, moderate steatorrhea (<12 g daily) occurs in both cholestatic and non-cholestatic liver disease [100]. It has been reported to occur in as many as 40% of cirrhotic patients and may exceed 30 g daily in 10% of cirrhotic patients. This has been attributed to decreased intestinal bile acids, pancreatic insufficiency and a diminished capacity for long chain fatty acid absorption [97]. These abnormalities are more severe in cholestatic liver disease. Celiac sprue occurs more frequently in primary biliary cirrhosis; while inflammatory bowel disease and exocrine pancreatic abnormalities are more common in primary sclerosing cholangitis [58]. The use of oral lactulose or neomycin for hepatic encephalopathy may also exacerbate nutrient malabsorption in all forms of liver disease.

### Alcohol

Although alcohol is recognized as a direct hepatotoxin, it also has profound effects on protein and energy metabolism as recently reviewed elsewhere [18]. Additionally, alcohol has a direct effect on small bowel mucosal function and absorption [132]. From a practical approach, these adverse effects can be avoided by abstinence from alcohol use.

### Protein Metabolism

After an overnight fast, cirrhotics have an increased rate of protein breakdown that is not suppressed

normally in response to feeding [33]. Additionally, because of alterations in energy metabolism, suggested to be due to glucagon resistance and diminished glycogenolysis, amino acids are oxidized for energy, especially the ketogenic amino acid leucine (Fig. 91.2) [74]. This irreversible loss of nitrogen is also associated with an increased rate of gluconeogenesis.

Protein turnover studies in cirrhosis using tracer isotopes have yielded conflicting results [73, 74, 77, 78, 120–122]. These may be related to confounding variables such as differences in severity (Child's score) of liver disease and the magnitude of muscle loss at the time of evaluation. There are no direct studies in human cirrhotics measuring the rate of protein synthesis using incorporation of tracer amino acid in the skeletal muscle protein. Studies in human cirrhotics have quantified whole body protein kinetics that is primarily a measure of protein breakdown [120].

Estimation of rates of whole body protein breakdown using [$^{13}$C] leucine, in humans with stable cirrhosis (defined as Child's class A or B) in the fasted state, were not different from those in healthy controls [73, 74, 78]. However, when adjusted for body cell mass the measured rate of protein breakdown was increased. Studies using phenylalanine tracer showed a decreased whole body protein breakdown in patients with cirrhosis of Child's class B and C (decompensated) and no difference between compensated cirrhotics (Child's class A and B) and healthy controls [74]. In contrast, Tessari et al., using labeled leucine, observed that whole body protein breakdown was higher in cirrhotics compared to controls [122]. Contradictory results have been reported using different isotopic tracers such as [$^{15}$N] glycine and [$^{14}$C] tyrosine [72]. In summary, published data on estimated rates of protein synthesis from the whole body amino acid kinetic studies showed that the rates of protein synthesis in cirrhotics were either not different or lower than controls.

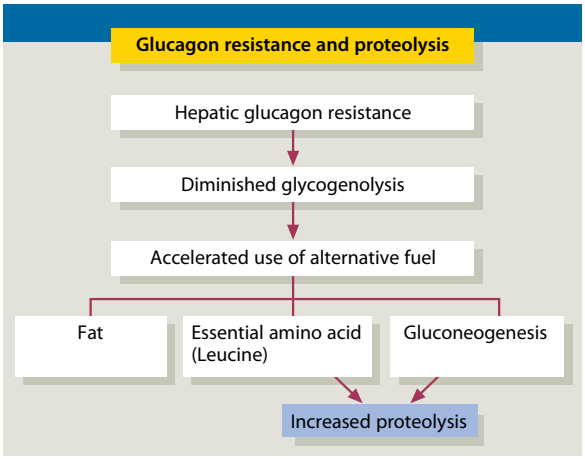


**Fig. 91.2** An hypothesis that decreased hepatic glucose production caused by hepatic glucagon resistance leads to the use of alternative fuels and increased proteolysis in cirrhosis

Despite the heterogeneity in the disease and methodologies used, the preponderance of evidence from studies in humans and animals suggest an unchanged or increased rate of protein breakdown and a decrease in the rate of protein synthesis in cirrhosis.

Regardless of the cause of the altered protein status of cirrhotic patients, their protein requirements are increased [85, 117]. According to recommended dietary allowance (RDA), healthy individuals have an average requirement of 0.6 g/kg/day [5, 91]. Allowing for two standard deviations and suboptimal composition of dietary protein in a mixed diet, the RDA recommendation is for 0.8 g/kg on a mixed diet. When adding the two standard deviations, this recommendation is increased to 1.2–1.3 g/kg/day.

## *Energy Metabolism*

Although highly variable, when corrected for lean body mass, resting energy expenditure has been shown to be increased in cirrhotics [93]. When measured energy expenditure is compared with predicted energy expenditure, 30%, 20% and 50% of cirrhotics have high, low, or normal metabolic rates. Patients with hypermetabolism have increased rates of morbidity and mortality after liver transplantation [68, 81]. Therefore, a clinician can tailor specific energy requirements for individual patients for using simple calculations (Fig. 91.3).

Regardless of the rate of energy expenditure, the preferred fuel substrate is clearly altered in cirrhosis [33]. Cirrhotics obtain 75% of their calories from fat after an overnight fast compared with 35% for controls, who would take 48–72 h of starvation to obtain the low respiratory quotient levels that cirrhotics obtain after only 12–18 h [111]. In addition, amino acids are oxidized for energy and converted to glucose via gluconeogenesis. Therefore, cirrhosis should be considered a disease of accelerated starvation with early recruitment of alternative fuels [17]. Consequently,

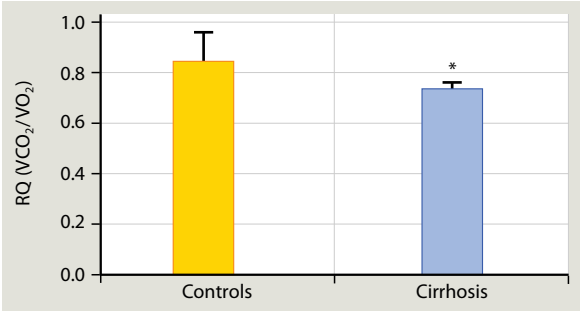


**Fig. 91.3** The respiratory exchange ratio (referred to as the respiratory quotient) is shown as the aggregated data from seven published trials for controls and patients with cirrhosis


**Fig. 91.4** The effect of a night-time snack with frequent feedings on nitrogen balance

food should not be withheld from cirrhotic patients for any extended period, and frequent enteral feedings need to be encouraged [96, 128]. Cirrhotic patients given an evening snack to supply energy during the sleeping hours have been shown to maintain a greater positive nitrogen (Fig. 91.4) [9, 88, 89, 104, 126].

## *Molecular Mechanisms*

A number of studies have examined the mechanisms of malnutrition specified by low lean body mass and diminished skeletal muscle mass in animal studies. These have used both isotopic tracer methodology and molecular studies on the cellular signaling pathways that regulate protein synthesis, protein breakdown and satellite cell function in vivo [32, 50, 73, 74, 78]. The primary pathway for regulation of skeletal muscle mass is shown in Fig. 91.5 [43, 44]. It can be seen that a number of interactions occur at each step between the signaling components at each level. A number of cytokines and hormones also regulate components of this critical signaling pathway and consequently affecting the lean body mass and skeletal muscle weight (Fig. 91.6) [16, 32, 43, 44]. Recent evidence from studies on the portacaval shunted rats also suggest that increased myostatin and decreased insulin like growth

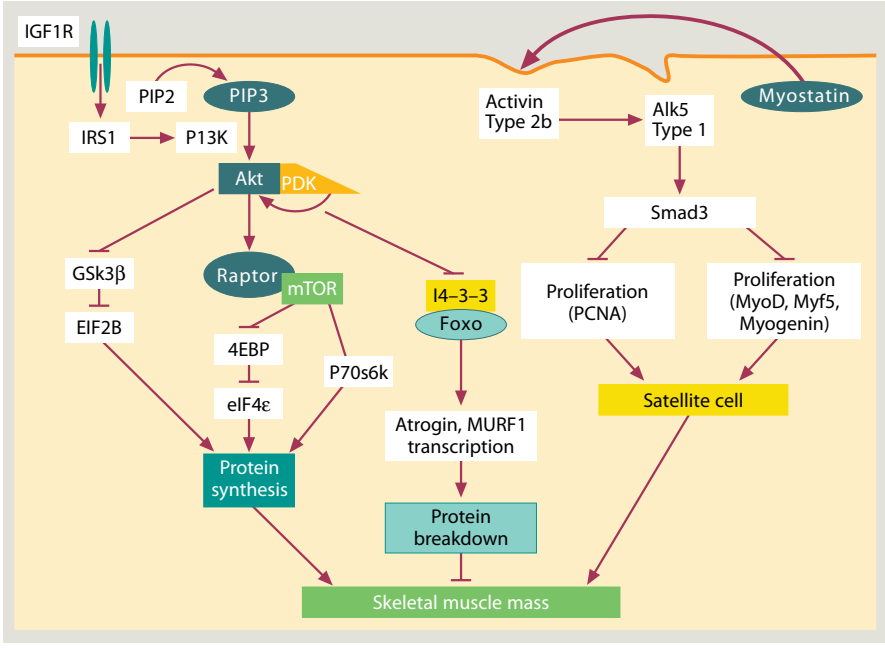


**Fig. 91.5** The primary pathway regulating protein synthesis consists of a series of hierarchical signaling proteins with multiple interactions at each level

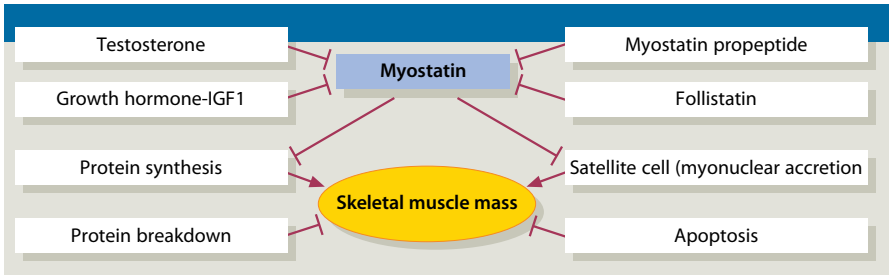


**Fig. 91.6** Myostatin is member of the TGFβ superfamily that is likely to be upregulated in cirrhosis (based on animal studies). Myostatin results in diminished skeletal muscle mass by multiple mechanisms

factor (which stimulates muscle protein synthesis may result in skeletal muscle atrophy in patients with cirrhosis and portacaval shunting [31]. A number of studies aimed at understanding the underlying mechanisms responsible for the diminished muscle mass with cirrhosis and portasytemic shunting are in progress. An understanding of these pathways and mechanisms will permit the development of targeted intervention strategies towards preventing and treating malnutrition and low muscle mass that occurs with cirrhosis of liver.

## Prognosis

Table 91.3 lists results from a number of studies, which suggest a causal relationship between malnutrition and survival in cirrhosis [7, 53, 112, 118]. Malnutrition has prognostic value in patients undergoing liver transplantation and other surgical operations [48]. Malnutrition predicts impaired immunity and increased susceptibility to infection in patients hospitalized for cirrhosis and ascites [76, 94]. The disappearance of ascites following placement of a peritoneovenous shunt improves nutritional status and immunity. Malnutrition has also been shown to correlate with poor hepatic function and severity of disease in cirrhotic patients [27]. These collective data suggest that the recognition and treatment of malnutrition are important considerations in the management of patients with cirrhosis, especially in those situations listed in Table 91.3.

**Table 91.3** Protein energy malnutrition as an adverse prognostic factor in cirrhosis

| Condition or procedure | Clinical outcome measured |
|---|---|
| 1. Cirrhosis (any etiology) | 1. Survival<br>Immunodeficiency |
| 2. Cirrhosis patients undergoing abdominal surgery | 2. Survival<br>Post-operative complications |
| 3. Hospitalized patients with ascites/cirrhosis | 3. Survival |
| 4. Alcoholic hepatitis | 4. Survival<br>Liver function |
| 5. Liver transplantation | 5. Patient/graft survival<br>ICU stay, postoperative complications |
| 6. Hepatectomy | 6. Post-operative complications |
| 7. Obesity and cirrhosis | 7. Post-transplant<br>Multi-organ failure |
| 8. Obesity associated NASH | 8. Cirrhosis |

Recent evidence indicates that obesity associated NASH is also associated with a poor prognosis (Fig. 91.7) [70]. Cirrhosis develops in 15–25% of NASH patients. Once developed, 40% of these patients may succumb to liver-related death over a 10-year period, the mortality rate being similar to or worse than cirrhosis associated with hepatitis C [103]. NASH is also now considered the major cause of cryptogenic cirrhosis. NASH-associated cirrhosis can also decompensate with the development of liver failure, progress to hepatocellular carcinoma (HCC), and re-occur post-transplantation [70, 103]. In contrast steatosis

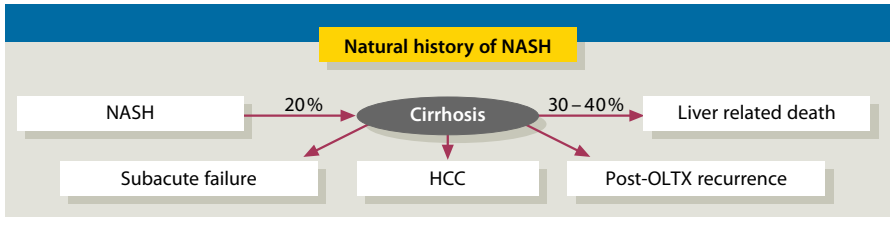


**Fig. 91.7** Natural history of NASH: *HCC* hepatocellular carcinoma; *OLTX* orthotopic liver transplantation

alone is reported to have a more benign clinical course, although progress of fibrosis in cirrhosis has occurred in 3% of these patients with steatosis alone.

Most, but not all studies show the importance of nutritional status on graft and/or patient morbidity and mortality in the transplant patient [12, 22, 112]. Early data showed malnutrition adversely affected survival. In patients awaiting liver transplantation, low skeletal muscle mass, body cell mass, lean body mass as well as hypermetabolism predicted decreased survival after transplantation, whereas the Child-Pugh score did not [112]. These collective data suggest that recognition and treatment of malnutrition should be an important consideration before liver transplantation.

There is much variability among transplant centers regarding the importance of obesity as a risk factor for patients undergoing liver transplantation [57]. Graft and patient survival are similar in patients post-transplantation. Nonetheless, many transplant physicians believe that obesity is a significant enough risk factor for liver transplantation to place certain restrictions on transplantation in obese patients [84, 123]. These concerns appear most relevant in obese patients with a body mass index of 35 kg/m$^2$ or greater. However, based on published data, obese and non-obese patients have similar long-term outcomes after liver transplantation [57, 123].

## Nutritional Assessment

Because malnutrition has prognostic value and influences clinical decisions regarding nutritional therapy, accurate methods of assessing nutritional status are vital. As stated earlier, the accurate definition of malnutrition is an important consideration when determining the method of assessment.

Nutritional assessment can be difficult in patients with chronic liver disease. Parameters of common use in clinical practice should diagnose protein (kwashiorkor or hypoalbuminemic) or combined protein calories (marasmic) forms of malnutrition (Table 91.4) [1, 2]. Fluid excess is universal in end-stage liver disease and may be present even in early stages of disease. This fluid excess causes weight-to-height parameters (such as percentage of ideal body weight or body mass index) to underestimate both the prevalence of chronic liver disease, which itself causes alterations in visceral protein synthesis, cellular immunity, and total lymphocyte count independent of malnutrition [95]. In contrast, markers of muscle mass (creatinine height index and midarm muscle circumference) and fat mass (triceps skin fold thickness) are less influenced by either alcohol or chronic liver disease [6]. When performing creatinine height index estimation, creatinine clearance should be measured to confirm the precision of the creatinine height index. Furthermore, it must be underscored that the creatine height index must be interpreted with the knowledge that plasma creatinine in cirrhosis is influenced by skeletal muscle mass, hepatic synthesis of creatine and renal function [113].

In addition to these anthropometric and laboratory parameters, a number of other methods have been used to estimate total lean body mass. These include the isotope dilution method, whole body potassium measurement and in vivo neutron activation analysis [52]. These have been considered some of the most accurate methods to estimate body composition but they are expensive and labor intensive and hence not

**Table 91.4** Measurements used to assess nutritional status

| | Malnutrition | | Alcohol Toxicity | Liver Disease | Renal Function |
|---|---|---|---|---|---|
| | Protein | Protein/calorie | | | |
| Visceral proteins | X | | X | X | |
| Lymphocyte count | X | | X | X | X |
| Cellular immunity | X | | X | X | X |
| % Ideal body weight | | X | | X | X |
| Anthropometry | | X | | (?) | (?) |
| Creatinine height index | | X | | (?) | X |
| SGA | | X | | X | ? |
| BIA | | X | | X | |
| DEXA | | X | | X | |
| Handgrip | | X | | X | |

*SGA* Subjective global assessment; *BIA* bioelectrical impedance analysis; *DEXA* dual emission X-ray absorptiometry

of great value in routine clinical practice. Other more practical methods include bioelectrical impedance (BIA), BodPod and dual emission X-ray absorptiometry (DEXA) [14]. Bioelectrical impedance has been considered by some authors to be inaccurate in cirrhotic patients with ascites or edema and has some imprecision even in those without ascites or edema [46, 133]. It may therefore be best used to serially monitor the response to nutritional feeding. DEXA is a non-invasive method with minimal radiation that provides accurate measurements of total body bone mineral, fat, and fat free soft tissue mass. The accuracy of this method is also influenced by fluid retention as with BIA.

Subjective global assessment is an evaluation of malnutrition based predominantly on a combination of multiple elements that include weight loss in the previous 6 months, changes in dietary intake, gastrointestinal symptoms, functional capacity, metabolic demands, signs of muscle wasting, and the presence of presacral or pedal edema [41, 127]. As a practical method for estimating malnutrition, it compares favorably with standard measures in patients without liver disease and also predicts outcome after OLT. Estimation of handgrip strength and respiratory muscle strength have been used as measures of muscle function. In patients with cirrhosis, handgrip strength has been shown to overestimate the prevalence of malnutrition but is a good predictor of complications before and after liver transplantation [127].

Therefore, nutritional assessment is useful in all types of cirrhosis, especially when a composite score emphasizing anthropometry, handgrip strength and creatinine height index is performed and combined with overall clinical judgment including subjective global assessment.

## Clinical Features

### Medical History

The duration, severity, and etiology of liver disease are pertinent to malnutrition in patients with liver disease (Table 91.5). Other disease processes, such as renal failure, cardiac and pancreatic disease in alcoholism, and diabetes and cardiac disease in hemochromatosis, should also be evaluated. Factors that may limit nutrient ingestion and absorption, such as anorexia, early satiety, vomiting, and diarrhea should be identified [23, 48]. If possible, dietary recall should estimate macronutrient and micronutrient intake and the consumption of alcoholic beverages. Because cirrhotic patients often self-select a higher proportion of their dietary intake at breakfast, a full day's calorie count is required to obtain an accurate assessment [128].

### Physical Examination

During physical examination signs of decompensation, such as mental status changes, asterixis, jaundice, ascites, leg edema, and bruising need to be evaluated. Weight should be recorded with special attention to fluid overload. Signs of specific nutritional deficiency, such as acrodermatitis from zinc deficiency or pellagra from niacin deficiency, should be sought. Patients with cholestatic liver disease and steatorrhea may have skin excoriations related to pruritus, xanthomas due to hyperlipidemia, or dermatitis from essential fatty acid deficiency [22, 23].

Evaluation of the oral mucosa, skin and hair are particularly important. In the oral mucosa, glossitis (raw

**Table 91.5** Nutritional assessment in advanced liver disease

| History | Physical examination | Laboratory tests |
|---|---|---|
| Weight loss > 10% of usual weight | Loss of skeletal muscle mass | Visceral proteins, albumin, pre-albumin and retinol-binding protein |
| Loss of appetite | Skin/hairs | Vitamin and mineral levels; assess blood vitamin levels |
| Decrease energy level | Acrodermatitis (zinc deficiency)<br>Dry rash (vitamin A deficiency)<br>Oral mucosa (vitamin B deficiency)<br>Peripheral neuropathy (thiamine deficiency)<br>Hyper-reflexia (magnesium deficiency) | Total lymphocyte count<br>Delayed hypersensitivity reaction |

tongue), atrophic tongue (slick tongue) and angular stomatitis are common findings and result from deficiencies in the B vitamins, iron, and folate. Less common are gingivitis (vitamin C deficiency) and altered taste/smell (zinc deficiency).

On the skin, petechiae (vitamin C deficiency) and purpura (vitamin A and zinc deficiency) may be present. Hair may demonstrate sparseness (protein, zinc and biotin deficiencies), follicular hyperkeratosis (vitamins A and C deficiency) or be corkscrewed and coiled (vitamin C deficiency).

## Nutritional Requirements

### Nitrogen

In stable cirrhotics, the average protein requirement to maintain positive nitrogen balance is 0.8 g/kg/day, which is similar to that required in the normal population. Because the range of protein required is larger in cirrhotics than in controls, compensated cirrhotics should receive at least 1.2 g/kg/day [85, 96]. Although nitrogen balance is achieved at this amount, nitrogen retention continues to improve up 1.8 g/kg/day. Even stable cirrhotics are protein deficient and this is an important management consideration. Cirrhotic patients undergoing surgery should receive protein doses between 1.2 and 1.5 g/kg/day (Table 91.6) [136].

### Energy

Abnormalities in extracellular fluid levels in fat and non-fat body compartments, portosystemic shunting, and variability in energy expenditure between different body requirements make the prediction of energy requirements quantitatively unreliable in cirrhosis [82, 85, 93].

**Table 91.6** Suggested guidelines for nutritional therapy in liver disease

| | Protein (g/kg/day) | Energy (kcal/kg/day) | Energy substrate | | Nutritional goal |
|---|---|---|---|---|---|
| | | | %CHO | %fat | |
| 1. Hepatitis (acute or chronic)[a] | 1.0–1.5 | 30–40 | 67–80% | 20–33% | Prevent malnutrition<br>Enhance regeneration |
| 2. Cirrhosis (uncomplicated) | 1.0–1.2 | 30–40 | 67–80% | 20–33% | Same as number 1 |
| 3. Cirrhosis (complicated)[b] | 1.2–1.8 | 40–50 | 72% | 28% | Restore normal nutritional status |
| (a) Malnutrition | | | | | |
| (b) Cholestasis | 1.0–1.5 | 30–40 | 73–80% | 20–27% | Prevent malnutrition<br>Treat fat malabsorption[c] |
| (c) Encephalopathy[d] | | | | | Provide nutritional needs without precipitating encephalopathy |
| Grade 1 or 2 | 0.5–1.2 | 25–40 | 75% | 25% | |
| Grade 3 or 4 | 0.5 | 25–40 | 75% | 25% | |
| 4. Liver transplant | | | | | |
| (a) Peri-transplant | 1.2–1.8 | 30–50 | 70–80% | 20–30% | Restore normal nutritional status |
| (b) Post-transplant | 1.0 | 30–35 | >70% | ≤30% | Retain and maintain ideal body weight |

[a]There are no convincing data indicating any extraordinary vitamin or trace metal requirements in liver disease. A typical substitution solution for parenteral feeding is provided. Vitamins: (10 cc/L) – ascorbic acid (20 mg/mL), vitamin A (660 IU/mL), vitamin D (40 IU/mL), thiamine HCL (0.6 mg/mL), riboflavin phosphate (0.72 mg/mL), pyridoxine HCI (0.8 mg/mL), niacinamide (8 mg/mL), D-pantothenic acid (3 mg/mL), vitamin E (2 IU/mL), biotin (12 mcq/m), folic acid (0.08 mg/5 mL), vitamin $B_{12}$ (1 mcq/5 mL). Trace metals: (1 mL/L) – zinc chloride (10.42 mq/5 mL, copper chloride (2.68 mq/5 mL), manganese (1.44 mq/5 mL), chromium chloride (0.012 mq/5 mL)

[b]Ascites and sepsis are two complications which are not listed but which should be treated aggressively as part of nutritional therapy (see text)

[c]Medium chain triglycerides may be necessary if fat malabsorption is present. Pancreatic enzymes are necessary if pancreatic insufficiency is present; especially in alcoholic cirrhosis and primary sclerosing cholangitis

[d]Branched chain amino acid (BCAA) formulations may be necessary. Available hepatic enteral formulations are 45% BCAA enriched and parenteral formulations are 36% BCAA enriched. This compares to standard for preparations which contain 26% BCAA. For outpatients, diets high in vegetable protein or casein hydrolysates may be better tolerated than standard dietary protein in the protein intolerant patient with cirrhosis

**Table 91.7** Vitamins in liver disease

- Many complications associated with liver disease are manifestations of vitamin deficiencies. These include: macrocytic anemia (folate and vitamin $B_{12}$ deficiency); neuropathy (pyridoxine, thiamine or vitamin $B_{12}$); confusion, ataxia and ocular disturbances (thiamine) and impaired adaptation to dark (vitamin A)
- Pyridoxine phosphate (the major active form of pyridoxine) rather than pyridoxine itself may be required to correct pyridoxine deficiency; especially in alcoholics
- The clinical response to thiamine is usually rapid and effective. However, the neuropathy may be irreversible and abnormalities in red cell transketolase may not improve
- Deficiencies in vitamin $B_{12}$ and folic acid may develop faster in cirrhotic patients due to diminished hepatic storage
- In the presence of active hepatic inflammation, fatty liver or hepatocellular cancer, normal or elevated levels of vitamin $B_{12}$ levels do not exclude a deficiency of this vitamin
- Decreased serum levels of vitamin A do not necessarily reflect vitamin A deficiency. Zinc deficiency may be causing decreased transport out of the liver
- In non-cholestatic liver disease, abnormal serum levels of fat-soluble vitamins should not be sought or treated in the absence of clinical or laboratory abnormalities, which indicate a functional vitamin deficiency. In cholestatic liver diseases, abnormal serum levels of the fat soluble vitamins may be treated even in the absence of clinical symptoms or laboratory abnormalities
- Suggestions for use of vitamin supplements: (a) water-soluble vitamins may be supplied with a multivitamin preparation; (b) fat-soluble vitamins should be used in a water-soluble form whenever possible. Vitamin A, Aquasol 1 capsule daily (50,000 IU), vitamin K, Synkayvite, one tablet daily (15 mg). Vitamin D treatment should be individualized by monitoring plasma 25-hydroxy vitamin D levels and serum and urinary calcium levels. Vitamin $D_2$ (ergocalciferol), 1.25 mg (50,000) U, one tablet 3–4 times a day, may be adequate. Vitamin E (D-α-tocopheryl polyethelene glycol 1,000 succinate (TPGS) 23 IU/kg/day)

Therefore, with knowledge that hypermetabolism may have adverse clinical sequelae energy expenditure should be measured with indirect calorimetry, especially in hospitalized patients undergoing liver transplantation [93]. If indirect calorimetry is not available, the daily resting energy expenditure for cirrhosis should be assumed as 25–30 kcal/kg body weight (based on ideal body weight if ascites and/or edema is present) for decisions regarding restorative or maintenance needs.

Energy is provided by both carbohydrates and fat (Table 91.6). It is essential to reiterate that (1) insulin resistance is universal in cirrhosis irrespective of the type of severity of the disease. Glucose intolerance is not typically an important management issue, except hypoglycemia, which develops in up to 50% of cirrhotic patients during sepsis and (2) lipid formulations are very useful in cirrhosis because of their low water content and high caloric density. Initial concerns that lipids might precipitate encephalopathy have not been confirmed [69, 83, 86].

### *Vitamins*

Approximately 40% of patients with non-alcoholic liver disease have fat soluble vitamin deficiencies (Vitamin A and E); 8–10% have deficiencies in the B complex vitamins (nicotinic acid, thiamine, Vitamin $B_{12}$, riboflavin and pyridoxine) and 17% have folate deficiency. These abnormalities are related more to disordered hepatic function and diminished reserve rather than deficient dietary intake or malabsorption. However, in severe advanced cirrhosis, malabsorption may play a role in both fat soluble vitamins and the B complex vitamins. These vitamin deficiencies are more prevalent and severe in alcoholic than non-alcoholic liver disease [55]. Specific issues regarding vitamins in liver disease are provided in Table 91.7.

## Specific Alterations in Liver Disease

### *Accelerated Starvation*

Cirrhosis is a disease of accelerated starvation with early recruitment of alternative fuels [38] (Table 91.8). Cirrhotic patients should avoid any extended period of time without feeding [9, 89, 134]. The advantage of a frequent feeding approach has been confirmed by nitrogen balance and indirect calorimetry measurements in cirrhotic patients [88, 126].

### *Hepatic Encephalopathy*

Advanced acute or chronic liver failure often presents with a constellation of neuropsychiatric abnormalities

**Table 91.8** Nutritional interventions for specific alterations in liver disease

| Specific alterations | Implications for nutritional management |
|---|---|
| **Accelerated starvation** | Frequent feedings with nighttime snack |
| **Hepatic encephalopathy** | Routine or prophylactic protein restriction should be discouraged |
| **Hepatic metabolism of non-essential amino acids** | Cysteine and tyrosine conditionally essential amino acids in cirrhosis |
| **Sodium restriction** | Salt restriction should be balanced against palatability of the diet. Risk of hyperkalemia to be considered when using salt substitutes rich in potassium |
| **Alcohol abuse** | Abstinence needs to be continually re-enforced |
| **Obesity** | Gradual weight loss should be instituted. Combination of dietary alteration and increased physical activity |
| **Micronutrient deficiency** | Zinc, thiamine, folate, vitamin B12, vitamin K |
| **Osteoporosis** | Calcium, vitamin D |

known as hepatic encephalopathy. These mental status alterations may range from mild behavioral changes to deep coma. Subclinical hepatic encephalopathy is present in up to 70% of patients with cirrhosis and currently does not require any specific nutritional therapy. Overt encephalopathy is almost always associated with some precipitating event such as gastrointestinal bleeding, infection/sepsis, fluid and electrolyte imbalances, constipation, acid–base abnormalities or the use of sedating drugs [60, 67]. In a minority of cirrhotic patients, hepatic encephalopathy may be precipitated by protein intake (especially animal protein) without any other precipitating factor [60]. These patients are considered protein intolerant. More than 95% of cirrhotic patients can tolerate diets containing as much as 1.5 g/kg/day of mixed proteins. Therefore, true protein intolerance is rare except for fulminant hepatic failure, or the occasional patient with 'endogenous' chronic encephalopathy.

In the few cirrhotic patients with protein intolerance, branched chain amino acid formulations are better tolerated than standard amino acid supplements and can achieve positive nitrogen balance [28, 116]. It should be emphasized, however, that there is no clearly proven benefit of branched chain amino acids over standard amino acids for the majority of patients with chronic liver disease [11, 66, 110, 125].

## Protein Restriction

There is no justification for the routine use of protein restriction as prophylaxis against precipitating hepatic encephalopathy [49, 124]. Available data indicate that the vast majority of cirrhotic patients and even patients with severe alcoholic hepatitis can tolerate large quantities of protein feeding (up to 1.75 g/kg/day). Despite these, it has been shown that the majority of patients with cirrhosis are on an inappropriately low protein diet [49].

## Non-essential Amino Acids

Administration of standard or specialized total parenteral nutrition solutions devoid of the non-essential amino acids tyrosine and cysteine may not achieve positive nitrogen balance in cirrhosis despite provisions of adequate amounts of essential amino acids [102]. This observation emphasizes that certain intrinsic liver functions may be rate limiting in cirrhosis such as the ability to synthesize cysteine from methionine and tyrosine from phenylalanine. Similarly, the non-essential amino acid, glutamine becomes conditionally essential in cirrhosis [119].

## Salt Restriction

Although frequently necessary, sodium and consequently salt restriction significantly decreases the palatability of the diet and consequently may diminish food intake [98]. In the hospitalized patient, very low 250–500 mg sodium (0.63–1.3 g salt) diets may be appropriate. In the non-hospitalized patient, every effort should be made to avoid salt restriction even though reduction in sodium intake has been suggested to improve outcome in some patients [42, 98]. In those patients with fluid overload who otherwise cannot be managed effectively, the least sodium restriction that is effective should be employed. A 2.5-g sodium (6.3 g salt) intake approximates a no added salt diet. This restriction can be tolerated by the motivated patient and is usually effective for fluid management in most patients and does not significantly limit calorie or protein intake.

### Alcohol Related Alterations

Alcohol has a direct inhibitory effect on muscle protein synthesis independent of associated liver injury. Alcohol is known to inhibit meal stimulated hepatic protein production which is an important contributor to skeletal muscle and whole body protein synthesis in humans [90]. Alcohol increases intestinal permeability which in turn initiates endotoxin/cytokine induced muscle proteolysis. Furthermore, despite having a high caloric density, alcohol produces less efficient energy per gram of nutrient than both carbohydrates and lipids [47].

### Obesity

Obesity is associated with hepatic steatosis which not only causes cirrhosis per se, is a risk factor in the development of cirrhosis in patients with alcohol and hepatitis C associated liver disease and may have an adverse impact after liver transplantation [29, 51, 57, 123].

## Nutritional Management

### Goals

Although early studies used survival as the primary outcome measure for determining the efficacy of nutritional therapy, more recently improvement in nutritional status, infection rates, immune function, nitrogen balance, and perioperative morbidity have been shown with nutritional therapy in cirrhotic patients. Nutritional therapy is most effective when used for longer periods or in certain subgroups such as in those with severe malnutrition, chronic hepatic encephalopathy, and decompensated liver disease [19, 20, 23, 89]. No reliable information is available regarding the cost-effectiveness of nutritional therapy or its effect on the quality of life of cirrhotic patients even though in enteral nutrition has been shown to be beneficial after liver transplantation [96].

Regarding goals specific for liver disease, nutritional therapy needs to correct pre-existing malnutrition while simultaneously providing sufficient amino acids to encourage hepatic regeneration and normalization of function without precipitating encephalopathy. The clinical emphasis should be placed on supplying the basic requirements of nitrogen and calories. Overall recommendations are provided in Table 91.9.

### Specific Patient Populations

#### Cirrhosis

Enteral feeding improves liver function, encephalopathy and perhaps survival in severely malnourished cirrhotics and in patients with decompensated alcoholic liver disease [96]. Nutrient intake is increased by enteral nutrition in all published studies to date and may in part be responsible for these benefits. Providing nutritional supplements (1,000 cal and 34 g of protein-casein based) for 1 year to patients with complicated alcoholic cirrhosis resulted in higher protein and caloric intake to levels, which simply met their required needs [65, 66].

#### Alcoholic Hepatitis

There have been eight published trials on the use of standard intravenous amino acid formulations as primary therapy for alcoholic hepatitis [35, 45]. The results are conflicting, but six of the eight studies showed improvement in either liver histology or function. One of these studies additionally showed a strong trend toward an improvement in mortality. Two of the studies concluded that supplemental amino acids were of no benefit. In one of these negative studies, the mortality was 3.3% in the 30 patients in whom positive nitrogen balance was achieved, but 58% in those patients who remained in negative nitrogen balance despite nutritional therapy. Controlled studies also reported enteral feedings to be equally effective as corticosteroids and act synergistically with corticosteroids [8, 25].

#### Fulminant Hepatic Failure

Fulminant hepatic failure defined as the development of hepatic encephalopathy within 8 weeks of the onset of liver disease is a life threatening illness associated with

**Table 91.9** Overall guidelines for the nutritional management of patients with liver disease

| |
|---|
| Nutritional assessment is useful in all types of cirrhotic patients |
| A composite score (emphasizing anthropometry and creatinine height index) combined with overall clinical judgment should be employed |
| The clinician should remember that all the methods for nutritional assessment in cirrhosis are influenced or potentially influenced by the presence of liver disease alone as well as abnormalities associated with liver disease such as renal failure, alcohol ingestion and expansion of the extracellular water compartment |
| Determine energy expenditure requirements with indirect calorimetry (if possible) in hospitalized patients or patients listed for liver transplantation. If energy expenditure requirements are estimated from prediction equations, calculate energy need based on ideal weight rather than actual weight if extracellular water (ascites/edema) is present |
| Assume protein calorie malnutrition is present in all patients |
| Assume an inadequate dietary intake; even in hospitalized patients |
| Qualitative stool fat should be done intermittently; especially in patients with alcoholic or cholestatic cirrhosis. If malabsorption is present; determine the cause and treat |
| Treatment with either neomycin or lactulose for hepatic encephalopathy may exacerbate malabsorption, which should be considered in nutritional management |
| Balance the need for sodium restriction with nutritional considerations and diet palatability |
| Multiple (5–6) small feedings with a carbohydrate-rich evening snack, which consists of approximately 10–15% of caloric needs, should be given. The need for breakfast feeding must also be stressed to the patient |
| For calories, complex rather than simple carbohydrates should be used. Lipids should supply 20–40% of caloric needs |
| Nutritional requirements may vary according to the specific type of patient and/or clinical situation as listed in Table 91.4 |
| Severely malnourished or decompensated cirrhotics should be given oral or enteral supplements as recommended in Table 91.4 |
| Long-term nutritional supplements may be necessary to provide recommended protein and caloric supplements |
| Patients with severe alcoholic hepatitis should be given supplemental standard protein 1.0 g/kg via an enteral or peripheral parenteral route |
| Peri-operative nutritional therapy should be given to those cirrhotic patients with significant malnutrition as defined by weight loss of more than 10% or for anthropometry or creatinine height index less than 5% of predicted values |
| Post-liver transplant, patients need higher amounts of protein and energy (see Table 91.6) |
| Pediatric patients undergoing liver transplant may benefit from branched-chain amino acids |
| Cirrhotics should never be treated prophylactically with protein restriction to prevent hepatic encephalopathy |
| Standard protein or amino acid mixtures should be supplied to meet the measured estimated nitrogen needs (as provided in Table 91.6) |
| Protein restriction should be implemented only if protein intolerance as manifested by encephalopathy occurs in the absence of precipitating factors is found |
| Protein restriction below the required amounts should not be continued for more than 3–4 days |
| Branched-chain amino acids should be given only if the required amount of standard feedings cannot be tolerated without precipitating hepatic encephalopathy |
| Monitor for hypoglycemia and treated aggressively with concentrated glucose solutions which may also decrease serum ammonia levels |
| Enteral feeding is the preferred route of feeding patients with insufficient oral intake. Enteral feeding tubes may be used even if non-bleeding varices are present |
| Do not use any nutritional product devoid of cysteine or tyrosine as the only nitrogen source for any prolonged period of time. |
| Be aware of clinically important issues related to vitamins listed in Table 91.7 |

a rapid development of protein calorie malnutrition; even when what is calculated to be adequate amounts of calorie and nitrogen are supplied. Up to four times the normal rate of protein breakdown accompanied by decreased hepatic amino acid oxidation leads to the accumulation of potentially toxic levels of certain amino acids (tyrosine, phenylalanine, and methionine).

Hypoglycemia occurs commonly and serum glucose levels must be maintained with concentrated glucose infused (20–40%) to avoid the risks of exacerbating cerebral edema. Lipid emulsions may be particularly useful in this setting. There is scant data on the nutritional management in fulminant hepatitis [130]. If necessary, the use of branched chain amino acids may be necessary to achieve this goal in patients with more advanced stages of encephalopathy. However, the efficacy of branched chain amino acids in this situation remains unproven [96].

### Weight Loss

Serum transaminases almost always improve with weight loss in the obese patient (with as little at 5–10% decrease in body weight), but they are poor predictors of histology which does not always improve and may, in fact, worsen if weight loss occurs too rapidly [115]. Gradual weight loss (1–2 lb/week) with an overall goal of 10% weight loss over 6 months is recommended as a safe and effective clinical strategy especially in patients who are 30% overweight.

With success, further weight loss can be attempted, if indicated. Multiple interventions and strategies, including diet modifications, physical activity, behavioral therapy, and pharmacotherapy with Orlistat, or a combination of these treatment modalities is recommended [30, 103]. The particular treatment modality should be individualized taking into consideration the BMI index and presence of concomitant risk factors and other diseases. Given the lack of clinical trials in this area, these overall recommendations are a useful and safe first step for obese patients with NAFLD. Bariatric surgery is an option in patients with morbid obesity and has been shown to be very effective in diminishing hepatic injury [37].

### Liver Transplant

There is no uniform approach among transplant centers regarding the management of nutrition in transplant patients [21, 39, 96, 109]. There is general agreement that malnutrition reflects the severity of chronic liver disease and should not be considered, in general, as an exclusion for patients receiving liver transplant. Most centers administer post-operative nutrition in a fashion similar to that given other patients after gastrointestinal major surgery. Therefore the recognition that malnutrition is a significant problem has been gaining momentum.

In the pre-transplant patient general principles of nutritional management as outlined in Tables 91.6 and 91.9 should be followed [48, 96]. In the post transplant patient, a number of benefits to nutritional therapy have been shown as demonstrated in Table 91.10 [96, 105]. As compared to conventional therapy, early enteral feeding was shown to decrease the number of viral infections and obtain better nitrogen retention post-operatively. Post-operatively, both nasogastric and jejunostomy tubes have been used successfully in these patients. The available information also indicates that both enteral and parenteral nutrition are equivalent in their ability to deliver nutrients and improve nutritional status at the tenth post-operative day.

**Table 91.10** Benefits of nutritional therapy post-transplant

- Improved nitrogen balance
- Less time in the ICU (trend)
- Lower hospital cost (trend)
- Fewer viral infections with early enteral feeding which is equivalent to parenteral feeding
- Both nasogastric and jejunostomy tubes have been used successfully

An increasing important nutritional problem which develops in the first 1–2 years after liver transplant is obesity which has been reported to occur in 30–70% of patients [4, 24, 56, 106, 114]. Therefore, it is important to follow the nutritional status of these patients post-operatively and treat obesity aggressively if it develops.

## Principles and Practical Implementation

The nutritional support of patients with liver disease follows the general principles applicable to any other type of patient. However, there are a number of principles particularly relevant to liver disease patients with guidelines provided in Tables 91.6 and 91.9.

### Glucose Requirement

Although insulin resistance and hypoglycemia are commonly observed in cirrhosis, hypoglycemia (<50 mg/dL) occurs in up to 50% of cirrhotic patients during episodes of stress. Therefore, serum glucose must be closely monitored in patients with fulminant or decompensated chronic liver disease.

### Route of Nutrient Administration

The least invasive route for nutritional supplements is oral which should be tried first. If attempts of oral supplementation fail, enteral feedings can be administered via a small caliber nasogastric or nasojejunal tube. The

presence of non-bleeding esophageal varices is not a contraindication to the use of enteral feeding tubes in these patients [96]. However, feeding tubes should not be used if there is active esophageal variceal bleeding.

The placement of a gastrostomy or a jejunostomy tube in cirrhotics with ascites is not recommended due to the possible complications of peritonitis or ascitic fluid leakage [36]. Unfortunately, this limits the potential of long term enteral feeding in many of these patients.

### Parenteral Nutrition

Parenteral nutrition should be initiated only if nutritional requirements cannot be supplied orally or enterally in situations such as gastrointestinal bleeding, ileus, or after abdominal surgery. Parenteral feeding has been suggested to be superior to enteral feeding in patients with portosystemic shunting because enteral feeding may worsen hyperammonemia in this specific patient population. However, entereal feeding has been shown to be as effective as parenteral feeding for maintaining nutritional status after liver transplantation and reduces the complications and cost [15]. Because of its relatively low caloric density, peripheral parenteral nutrition cannot supply total nutritional requirements and is usually not a good choice in cirrhotic patients with sodium and water retention. Peripheral parenteral nutrition may be useful in supplementing enteral or oral feeding; especially for providing amino acids in severe alcoholic hepatitis. Central parenteral nutrition is preferred in most cirrhotic patients despite the risk of central vein catheter replacement in patients with coagulopathy and thrombocytopenia.

## Branched Chain Amino Acids

The amount of protein recommended for different types of liver disease patients in various situations is provided in Table 91.6. If these amounts of protein cannot be provided without precipitating hepatic encephalopathy, formulations enriched with branched-chain amino acids should be substituted for standard formulations. There are oral or enteral feedings (Nutrihep – 50% BCAA enriched and Hepatic Aid II – 46% BCAA enriched) as well as intravenous formulations (Hepatamine and Hepatasol both 36% BCAA enriched) available. In addition to these formulations which have been tried in liver patients, there are also stress formulations (Freamine HBC and Aminosyn-HBC) both of which are approximately 45% BCAA enriched. However, these stress formulations have not been evaluated in patients with liver disease.

## Dietary Supplements

Many patients see advice regarding the efficacy and safety of vitamins, herbs, or other nutritional supplements[129]. Unfortunately, there is insufficient information in this area to make sound recommendations. General recommendations are provided in Table 91.11.

**Table 91.11** Use of nutritional supplements and medications in NAFLD

| Possibly harmful | | Possibly helpful | |
|---|---|---|---|
| Supplements | Medications | Supplements | Medications |
| St. John's wart | Acetominophen[d] | Vitamin E[h] | Betaine[k] |
| Ephedrine containing compounds | Tamoxifen[e] | MVI[i] | Ursodeoxycholic acid[k] |
| | Amiodarone[e] | SAMe[j,k] | Metformin[k,l] |
| Excessive vitamin A[a] | Iron[f] | Milk thistle[k] | Statins[k,m] |
| Glucosamine[b] | Estrogen[g] | | Thiazolidinediones[k,l] |
| Others[c] | | | |

[a]Vitamin A should not be used in excess of that contained in a daily multivitamin (MVI) which is 5,000 IU

[b]Since hexosamines in general cause insulin resistance, glucosamine should be used with some caution

[c]All other herbs should be considered as possible causes of injury and should be avoided

[d]Acetaminophen should be restricted to less than 2–3 g daily. Repeated or ongoing use of acetaminophen for longer than 3 days with daily does above 1.5 g should be discouraged. Many over the counter (OTC) medications contain acetaminophen. Therefore the amount of acetaminophen in the OTC medications should be carefully sought

[e]This drug may cause hepatic injury that histologically looks similar to NAFLD/NASH. Therefore, the benefit risk of using these drugs in NAFLD/NASH should be carefully considered

[f]Since iron may cause oxidative stress in the liver, iron supplements should only be used as per standard management for anemia. Transferrin saturation should not exceed 50%

[g]Estrogens used as oral contraception pills (OCP) or as hormonal replacement therapy (HRT) do not have to be discontinued

[h]Vitamin E should not be used at doses greater than 800 IU daily

[i]A daily multivitamin (MVI) with iron content <20 mg should be used

[j]SAMe = S-Adenosylmethionine

[k]The use of this supplement or medication should not be encouraged. However, there are uncontrolled studies suggesting their benefit in NAFLD

[l]This agent is approved for use in patients with type 2 diabetes

[m]The use of the statins used as cholesterol lowering agents is not contraindicated (and in fact, may be beneficial) in NAFLD. However, baseline and interval measurements of liver function tests should be performed

### *Obesity*

A number of different diets have been suggested including: the American Heart Association healthy heart diet, the Diabetic Diet as recommended by the American Diabetes Association, a low glycemic diet, and diets enriched with omega 3 polyunsaturated fatty acids [115]. However, the effect of these diets in NAFLD is unproven. Diets used to produce weight loss must always be individualized and related to the overall health status of the patient.

In general, patients should follow a well-balanced diet. One such diet is recommended by the National Cholesterol Education Program (www.nhlbi.nih.gov/about/ncep). This diet makes specific recommendations regarding total caloric intake, as well as the amount and type of fat and carbohydrate for patients who do not have to lose weight. If the patient has diabetes, specific recommendations have been made by the American Diabetes Association.

Overweight patients (BMI > 25 kg/m$^2$ based on dry weight) should be given a diet with a goal of losing and sustaining an initial weight loss of 10% of body weight. The weight loss should be gradual and should not exceed 2 lb/week. The National Heart, Lung and Blood Institute (NHLBI) guidelines for weight loss are proved in Table 91.12 as one typical diet that might be employed.

**Table 91.12** Weight loss diet[a]

| Nutrient | Recommended intake |
|---|---|
| Calories[b] | Approximately 500–1,000 kcal/day reduction from usual state |
| Total fat[c] | 30% or less of total calories |
| Saturated fatty acids[d] | 8–10% of total calories |
| Monounsaturated fatty acids | Up to 15% of total calories |
| Polyunsaturated fatty acids | Up to 10% of total calories |
| Cholesterol[e] | <300 mg/day |
| Protein[f] | Approximately 15% of total calories |
| Carbohydrate[g] | 55% or more of total calories |
| Sodium chloride | No more than 100 mmol/day (approximately 2.4 g of sodium or approximately 6 g of sodium chloride) |
| Calcium[h] | 1,000–1,500 mg/day |
| Fiber[g] | 20–30 g/day |

[a]This table provides guidelines for Step 1 weight loss as suggested in a monograph provided by the NHL BI, 2000 entitled "The Practice Guide. Identification evaluation and Treatment of Obesity in Adults"

[b]A reduction in calories of 500–1,000 kcal/day will help achieve a weight loss of 1–2 lb/week. Alcohol provides unneeded calories and displaces more nutritious foods. Alcohol consumption not only increases the number of calories in a diet but has been associated with obesity in epidemiologic studies as well as in experimental studies. The impact of alcohol calories on a person's overall caloric intake needs to be assessed and approximately controlled

[c]Fat-modified foods may provide a helpful strategy for lowering total fat intake but will only be effective if they are also low in calories and if there is no compensation by calories from other foods

[d]Patients with high blood cholesterol levels may need to use the Step II diet to achieve further reductions in LDL-cholesterol levels; in the Step II diet, saturated fats are reduced to less than 7% of total calories, and cholesterol levels to less than 200 mg/day. All of the other nutrients are the same as in Step 1

[e]Protein should be derived from plant sources and lean sources of animal protein

[f]Complex carbohydrates from different vegetables, fruits, and whole grains are good sources of vitamins, minerals, and fiber. A diet rich in soluble fiber, including oat bran, legumes, barley, and most fruits and vegetables, may be effective in reducing blood cholesterol levels. A diet high in all types of fiber may also aid in weight management by promoting satiety at lower levels of calories and fat intake. Some authorities recommend 20–30 g of fiber daily, with an upper limit of 35 g

[g]During weight loss, attention should be given to maintaining an adequate intake of vitamins and minerals. Maintenance of the recommended calcium intake of 1,000–1,500 mg/day is especially important for women who may be at risk of osteoporosis.

## Summary

Although more investigation is needed to study the effect of nutritional intervention in liver disease, clinicians need to recognize that malnutrition is virtually ubiquitous in all type of cirrhosis. It has prognostic significance, and its treatment has been shown to provide beneficial clinical outcomes. Therefore, it is important to identify malnutrition and treat it with increased amounts of conventional feedings while maintaining specialized formulations in only those patients who cannot tolerate standard dietary needs without precipitating encephalopathy.

Specific recommendations for the logistics of managing malnutrition in patients with liver disease are provided in Tables 91.6, 91.9, 91.11 and 91.12. The primary directive for nutritional therapy in cirrhosis is to use standard dietary methods to assure that nutritional requirements are provided and nutritional status is well maintained. The attainment of this strategy means that long-term outpatient management is even more important to these patients than when they are hospitalized for acute illness.

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# Hepatic Drug Metabolism and Drug Toxicity

# 92

Henryk Dancygier and Christian P. Strassburg

## Chapter Outline



Hepatic biotransformation and metabolic zonation are discussed in Chapters 8 and 9, respectively.

The liver plays a central role in the metabolism of endogenous substances and in the degradation and elimination of exogenous compounds. It is placed "strategically" between the intestinal and the systemic circulation. Thus, it is the first organ that comes into contact with enterally absorbed foreign compounds. The liver receives 1,500 ± 300 mL blood/min, corresponding to approximately 25% of the entire blood volume of an adult. Approximately 70% is supplied by the portal vein, the remaining 30% via the hepatic artery.

The hepatic microcirculation, the functional heterogeneity of hepatocytes, and the polarized structure of each hepatocyte mirror the division of work of liver cells. The liver plays a major role in biotransformation, i.e. the biochemical degradation of xenobiotics. The processes of biotransformation may be divided into *phase I* and *phase II reactions*. The former are non-synthetic reactions that include oxidation, reduction and hydrolysis of drugs, the latter are usually effected by specific transferases and include conjugation of the foreign compound or of its metabolites with activated glucuronic acid, activated acetic acid, active sulfate, aminoacids, methyl residues, glutathione as well as the formation of derivatives of mercapturic acid.

## Factors Affecting Hepatic Drug Metabolism and Drug Toxicity

Hepatic biotransformation and drug toxicity are influenced by many factors, such as age, gender, genetic factors, foreign compounds, endocrine and dietary factors, biorhythm, and liver disease.

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## Age

Age dependent factors affecting hepatic drug metabolism have been studied extensively. In humans the microsomal enzymes are already active at birth. However, phase II conjugation reactions are nearly completely absent in newborns and develop only during the first postnatal weeks [13, 34].

More than half of all drugs are prescribed to older patients with the percentage of older people in the general population steadily increasing. Thus, it is not surprising that most adverse drug reactions occur in adults. Exceptions include valproic acid induced liver damage in children younger than 3 years and Reye's syndrome in adolescents with salicylic acid playing an important role in its pathogenesis.

The question regarding age dependent changes of drug metabolism is difficult to answer. The results of many studies are conflicting and the data are difficult to interpret [37, 43]. Age dependent changes do not affect the liver in isolation. Rather, with advancing age a progressive decline in renal function, morphological and functional alterations of the gastrointestinal tract and changes in tissue composition are the rule. These factors combined determine the handling of xenobiotics. Thus, the question regarding liver-induced pharmacodynamic and pharmacokinetic changes must be viewed in connection with functional alterations of other organs. Naturally it is very difficult to assess these individual components separately. Furthermore, interindividual variability of metabolic rates usually by far exceeds age induced variations and makes interpretation of data difficult. Thus, for example, hepatic aminopyrine clearance already may vary sixfold between healthy individuals. Finally, most experiments have been conducted in animals and the results cannot be simply extrapolated to the human situation. Despite these reservations most studies indicate that *hepatic drug clearance and biotransformation decline with advancing age*. This may be due to a reduced hepatic blood flow of up to 40% in advanced age, a reduction of liver size and of absolute as well as relative (in relation to body weight) liver weight. Age dependent changes in lipoprotein composition of cell membranes alter membrane fluidity and contribute to altered drug clearance.

Generally, it may be asserted that with advancing age *phase I reactions are more strongly affected, while hepatic conjugation of drugs does not seem to be restricted significantly*. The inducibility of microsomal enzymes remains preserved in old age, while the extraction ("first-pass" effect) of drugs, such as labetalol, propranolol, lidocaine and clomethiazole is diminished. The biotransformation of chlordiazepoxide, chinidine and theophylline is reduced in old age. The age dependent increase in the plasma half lives of selected drugs is listed in Table 92.1.

**Table 92.1** Age dependent changes of plasma half lives of selected drugs

| Drug | Age (years) | Plasma ($t_{1/2}$) (h) |
|---|---|---|
| Penicillin | 30–65 | 0.35–0.65 |
| Streptomycin | 27–75 | 5.2–8.4 |
| Digoxin | 17–77 | 51–73 |
| Antipyrine | 26–78 | 12–17 |
| Phenobarbital | 30–70 | 20–107 |
| Diazepam | 20–70 | 20–80 |
| Practolol | 27–80 | 7.1–8.6 |

Source: From [5]

## Gender

Women seem to be more prone to develop drug-induced hepatitis after exposure to halothane, nitrofurantoin, methyldopa and sulfonamides [15]. On the other hand, men seem to be more prone to develop cholestatic reactions, for example to flucloxacillin. The causes for these observations are unclear.

## Genetic Factors

Drug biotransformation is also subject to genetic influences. In monozygotic twins the interindividual variations in drug metabolism are less pronounced than in dizygotic twins. The acetylation of sulfonamides and isoniazide has been studied extensively and is determined genetically. The bimodal distribution in the rate of acetylation is due to genetic polymorphisms within the N-acetyltransferase 2 gene. Ninety percent of Japanese, Chinese and Eskimos acetylate at a rapid rate ("rapid acetylators"), while the fraction of rapid and slow acetylators in the European population is roughly equal. Thus, when administering sulfonamides or isoniazide ideally the acetylation phenotype should be known. Isoniazide-induced hepatitis and the Lupus erythematosus syndrome

during therapy with procainamide or hydralazine primarily occur in rapid acetylators.

Genetic factors, however, not only affect conjugation, but also oxidation of drugs. Oxidative drug metabolism is characterized by a marked interindividual variability. The cytochrome P450 system represents a group of isoenzymes with selective, but overlapping substrate specificities (Table 92.2). The individual isoenzymes also differ with respect to their molecular weight and their immunologic features. If a certain enzyme variant

**Table 92.2** Pharmacogenetics of drug metabolism

| Drug-metabolizing enzyme | Frequency of variant poor-metabolism phenotype[a] | Selected substrates | Inducers | Inhibitors |
|---|---|---|---|---|
| **Phase I drug metabolism** | | | | |
| CYP2D6 | 6.8% in Sweden | Debrisoquin | None known | Fluoxetine, paroxetine, quinidine |
| | 1% in China | TCAs, SSRIs, antipsychotics, nicotine, β-blockers, codeine | | |
| CYP2C9 | Approximately 3% in England | Warfarin | Rifampicin, barbiturates | Isoniazide |
| | | Phenytoin | | |
| CYP2C19 | 2.7% among white Americans, 3.3% in Sweden, 14.6% in China, 18% in Japan | PPIs, proguanil, S-mephenytoin | Phenytoin | Cimetidine, ketoconazole |
| CYP3A4 | | Cyclosporine, atorvastatin, lovastatin, nifedipidine, erythromycin, midazolam, tamoxifen, cisapride, coffeine, cocaine | Rifampicin, dexamethasone, phenobarbital, phenytoin, carbamazepine, efavirenz, nevirapine | Azole antifungals, cimetidine, clarithromycin, erythromycin, grapefruit, indinavir, nelfinavir, ritonavir |
| Dihydropyrimidine dehydrogenase | Approximately 1% of population is heterozygous | Fluorouracil | | |
| Butyrylcholinesterase (pseudocholinesterase) | Approximately 1 in 3,500 Europeans | Succinylcholine | | |
| **Phase II drug metabolism** | | | | |
| *N*-Acetyltransferase 2 | 52% among white Americans | Isoniazid<br>Hydralazine | | |
| | 17% of Japanese | Procainamide | | |
| Uridine diphosphate-glucuronosyltransferase | 10.9% among whites | Irinotecan<br>Bilirubin | | |
| 1A1 (TATA-box polymorphism) | 4% of Chinese<br>1% of Japanese | | | |
| Thiopurine *S*-methyltransferase | Approximately 1 in 300 whites | 6-Mercaptopurine | 6-Mercaptopurine<br>Azathioprine | Salicylates, furosemide |
| | Approximately 1 in 2,500 Asians | Azathioprine | | |
| Catechol *O*-methyltransferase | Approximately 25% of whites | Levodopa | | |

*CYP* Cytochrome P450, *PPI* proton pump inhibitors, *SSRI* selective serotonin reuptake inhibitors, *TCAs* tricyclic antidepressants
[a]Polymorphism (poor metabolism phenotype) results in enhanced drug effect
Source: Adapted from [14, 42]

is present in ≥1% of the population, then *genetic polymorphism* is said to be present. Genetically determined enzyme deficiencies have pharmacokinetic and clinical implications. After administering the same dose, "weak metabolizers" will show higher drug plasma levels than persons who metabolize the drug normally. If nearly the total dose administered is excreted unchanged in the urine, then the person is said to be a deficient metabolizer. The ratio between the amount of unchanged drug in urine and its urinary metabolites is called the metabolic ratio. Four to 10% of the European population are phenotypically deficient metabolizers. If the dose is not reduced, a diminished "first pass" effect and accumulation will lead to an increased bioavailability of the drug.

Familial occurrence of hypersensitivity to phenytoin is rare, but has been reported [11].

Patients with Gilbert's syndrome are an interesting example of how genotype may influence drug toxicity.

## Gilbert's Syndrome and Pharmacogenomic Risk

The standard description of Gilbert's disease in hepatology text books appreciates this condition as an uncomplicated variant of the norm leading to transient unconjugated hyperbilirubinemia (see Chapter 52) [38]. This view is founded on the observation that Gilbert's syndrome does not lead to liver inflammation, histological changes or progressive fibrosis, and is therefore unlikely to lead to hepatic morbidity, or to be life limiting. Indeed, a greater risk appears to be associated with unwarranted invasive diagnostic procedures such as biopsies or endoscopic retrograde cholangiography, which are unnecessary and should be avoided when the clinical presentation is correctly interpreted and fluctuating non-hemolytic hyperbilirubinemia observed (Fig. 92.1). In addition, genotyping is likely to show the most common polymorphism associated with Gilbert's syndrome in Whites, a TA insertion into the promoter of the human *UDP-glucuronosyl transferase (UGT) 1A1* gene designated UGT1A1*28 [2]. However, this view may be too simple [25].

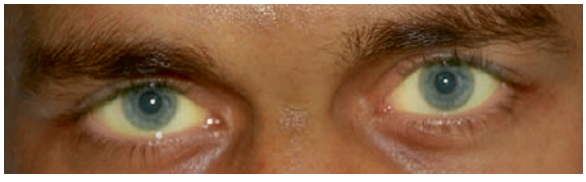

**Fig. 92.1** Typical phenotype of a young patient with Gilbert's syndrome. This patient experienced exacerbation of jaundice by ribavirin therapy that leads to hemolysis and an abundance of bilirubin in the presence of reduced bilirubin conjugation

UGT1A1 is the only physiological enzyme capable of forming water-soluble bilirubin glucuronides, which then undergo biliary or renal elimination. Bilirubin itself is not water-soluble and cannot be excreted; therefore, an absence of bilirubin excretion is a life threatening metabolic condition. Apart from bilirubin glucuronidation, UGT1A1 has several other endogenous and exogenous substrates [25, 26]. These include 2-hydroxy-estrone and estradiol, and a number of therapeutic drugs such as ethinylestradiol, gemfibrozil, metabolites of irinotecan, simvastatin and buprenorphine (Table 92.3). Furthermore, mutagenic xenobiotics such as N-hydroxy-PhIP undergo conjugation and detoxification by UGT1A1. Based on these functional considerations alterations in UGT1A1 activity are capable of modulating drug metabolism. In addition, there is a host of genetic variants in the *UGT1A1* gene: to date 113 variants (UGT1A1*1–*113) have been reported, which lead to differing degrees of functional variation.

**Genetic organization of glucuronidation enzymes (*UGT1A* genes).** UGT1A1 ("bilirubin UGT") is part of the *UGT1A* gene locus on chromosome 2, which is characterized by a unique organization [40]. At the 5′ (upstream) end of the gene locus 9 functional exon 1 domains are located, which are believed to be responsible

**Table 92.3** Drug toxicity and Gilbert's disease

| | | References |
|---|---|---|
| **Gilbert's disease** | UGT1A1*28 (main variant, "bilirubin UGT") | [2, 32] |
| **Established toxicity reactions** | Irinotecan | [1, 17, 18, 26] |
| | Atazanavir | [25, 36] |
| **UGT1A1 substrates** (potential risk) | Gemfibrozil[a] | [33] |
| | Ezetimibe | [12] |
| | Simvastatin, atorvastatin, cerivastatin[a] | [35] |
| | Ethinylestradiol | [7] |
| | Buprenorphine | [21] |
| | Fulvestrant | [3] |
| | Ibuprofen, ketoprofen | [23] |

[a]A severe drug reaction due to the inhibition of glucuronidation (UGT1A1) and cytochrome P450 (CYP) 2C8 and CYP2C9 when both drugs were combined led to the withdrawal of cerivastatin [33]

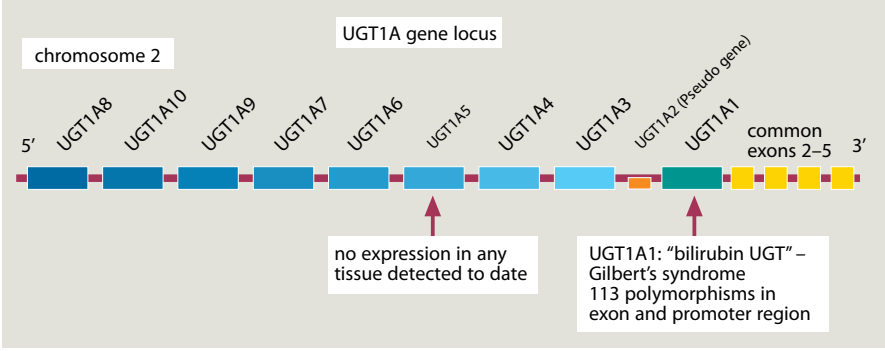


**Fig. 92.2** Graphic representation of the human UGT1A gene locus encoding UGT1A glucuronidation enzymes (not drawn to scale). Note individual first exons at the 5′ end of the chromosome and common exons 2–5 at the 3′ end. Refer to text for further explanations

for substrate specificity (Fig. 92.2). At the 3′ end (downstream) one copy of exons 2–5 is encoded. For the transcription of an individual UGT1A isoform, first exon sequence is combined with exon 2–5 sequence, which is shared by all transcribed UGT1A gene products to form individual UGT1As.

Regarding genetic variants, an alteration of an individual exon 1 would affect only this specific isoform, whereas an alteration of any of the exons 2–5 would affect the entire group of enzymes because this portion is present in all UGT1A gene transcripts. More than 40 of the UGT1A1 variants have been identified in the exon 2–5 region, which implicates their potential effect on glucuronidation by UGT1A gene products in general. Based on the complexity of this organization and its variants it is important to realize that Gilbert's syndrome represents one end of a spectrum that continuously extends to the most severe phenotype of Crigler–Najjar's disease type 1, which is fatal and completely lacks bilirubin glucuronidation and UGT1A1 activity [38].

**Genetic variation at the *UGT1A* gene locus.** The entire UGT1A locus (Fig. 92.2) spans approximately 200 kbp and has evolved by gene duplication events, which are suggested by the high homology of clustered UGTs such as UGT1A3, UGT1A4 and UGT1A5, as well as UGT1A7, UGT1A8, UGT1A9 and UGT1A10 [39]. The promoters regulating individual UGT1As and the corresponding first exons are in close proximity to each other. Genotyping data of the *UGT1A* genes has led to the discovery of hundreds of single nucleotide polymorphisms (SNPs) within the promoter regions and the *UGT1A* coding sequence, many of which are found to be in linkage dysequilibrium with each other. Studies have shown that haplotypes of numerous of these UGT1A variants occur simultaneously and influence glucuronidation by expressing proteins with altered function, or by modulating transcriptional regulation, or both [8, 22, 24, 31]. This is likely to influence drug metabolism and the disposition to unwanted drug side effects. In a patient with Gilbert's syndrome, who presents with an easily distinguishable phenotype of hyperbilirubinemia, the actual genotype is likely to be far more complex than the modulation of bilirubin glucuronidation alone. On the other hand, for the clinician this phenotype permits a unique opportunity to suspect genetically altered drug metabolism. There are currently two well documented examples of this pharmacogenetic risk disposition associated with Gilbert's syndrome.

**Irinotecan toxicity.** Irinotecan (camptothecin) represents a standard treatment option for metastatic colorectal cancer and other solid tumors [6, 9]. The prodrug irinotecan is converted to 7-ethyl-10-hydroxycamptothecin (SN-38) by ubiquitously expressed carboxyl esterases. SN-38 has a 100-fold higher anti-tumor activity (Fig. 92.3) [20]. SN-38 is mainly inactivated by

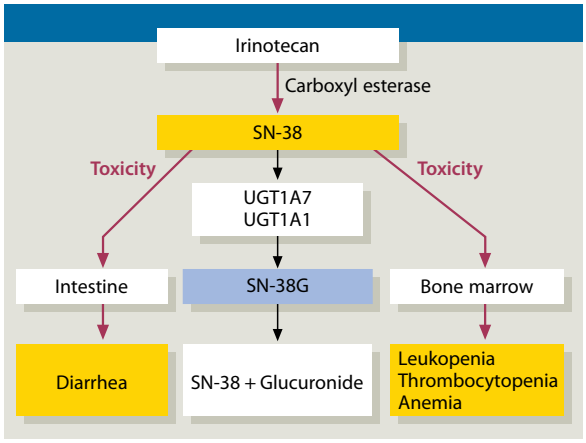


**Fig. 92.3** Metabolism and toxicity risk in irinotecan therapy. The active metabolite SN-38 is detoxified and eliminated after conjugation by UGT1A1 and UGT1A7. Reductions of UGT1A1 and UGT1A7 activity lead to increased toxicity

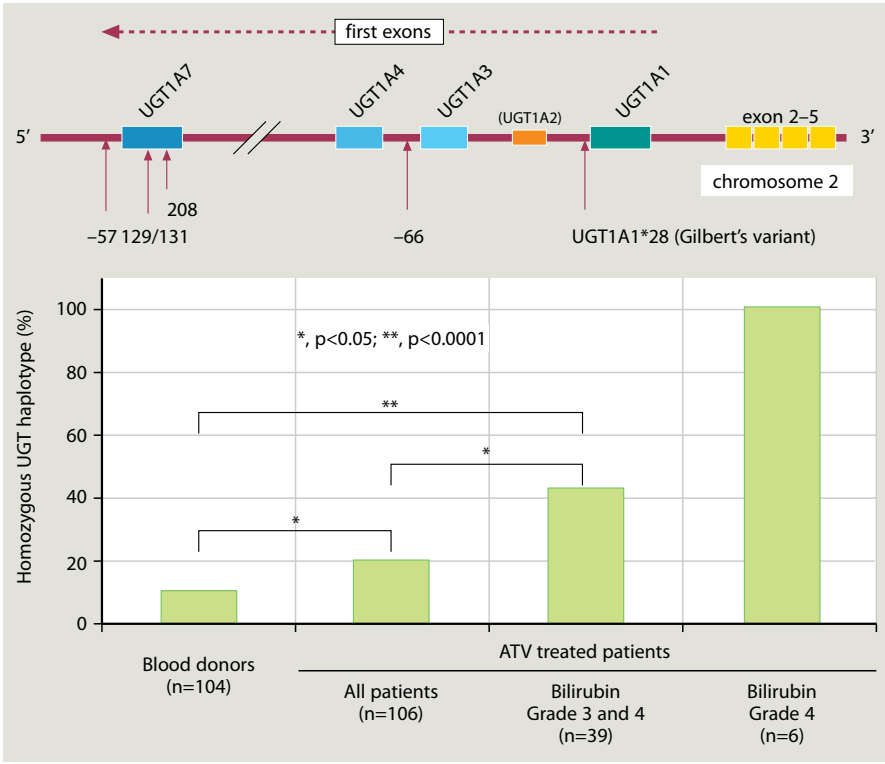


**Fig. 92.4** Jaundice is observed in approximately one third of patients treated with atazanavir (ATV). The homozygous presence of 4 single nucleotide polymorphisms in the UGT1A1 (Gilbert's), UGT1A3 and UGT1A7 genes (top graphic representation) occur in 9.6% of the normal population (*bottom* panel, *left* column). When atazanavir-treated patients are stratified according to grade of hyperbilirubinemia, the presence of the haplotype (homozygous) increases. All of the WHO grade 4 patients showed the homozygous haplotype (*lower* panel, *right* column), indicating that this is a risk allele for atazanavir-associated jaundice (Data adapted from [25])

UGT1A, which generate non-toxic SN-38 glucuronides that are eliminated via bile and urine [41]. Irinotecan has a narrow therapeutic range and leads to side effects such as myelosuppression (mainly leukopenia and thrombocytopenia), and diarrhea observed in 29–44% of patients frequently resulting in the necessity to discontinue or lower the dose of the drug [6, 16]. Based on irinotecan detoxification by UGT1A1, these side effects have been linked to genetic variations of UGT activity present in Gilbert's syndrome (UGT1A1*28) [1, 10, 17, 18]. However, the UGT1A7 protein was subsequently identified to exhibit a high specific activity with the active irinotecan metabolite SN-38 exceeding that of UGT1A1 [4, 31]. SNP association studies in Gilbert's patients have demonstrated the presence of homozygous promoter variants that reduce UGT1A7 transcription in up to 75% of patients with Gilbert's syndrome [31]. A recent study demonstrates, that the combination of variants of UGT1A1 and UGT1A7 can be used to more accurately predict irinotecan toxicity [26]. This example shows that a more complex genotype behind Gilbert's syndrome influences drug toxicity and can be exploited for the prediction of unwanted side effects.

**Jaundice in proteinase inhibitor therapy.** Hyperbilirubinemia is frequently observed in patients during treatment with anti-retroviral protease inhibitors, e.g. atazanavir or indinavir (Fig. 92.4) [36]. In many instances drug reactions occur when a single enzyme/pathway is the primary route of elimination of a drug such as in irinotecan toxicity. In the case of atazanavir the drug is not a significant substrate for glucuronidation but is capable of inhibiting UGT proteins, which include UGT1A1, UGT1A3, and UGT1A4 [44]. In the presence of the UGT1A1*28 variant atazanavir-treated patients were shown to develop variable degrees of hyperbilirubinemia linking this toxic reaction to Gilbert's syndrome [44]. In a recent study it was clarified that the probability of jaundice is related to a variant haplotype of three variants of the *UGT1A3* and *UGT1A7* genes in addition to the UGT1A1*28 Gilbert's variant. When these four variants were present as a homozygous trait, hyperbilirubinemia exceeded 85μmol/L (WHO grade 4) in atazanavir-treated patients. Homozygous carriers of all four variants amount to 9.6% of the white population, which is just below the prevalence of Gilbert's syndrome (16%) and represents a significant proportion of the population who carry this at-risk haplotype. These data demonstrate that the side effects of atazanavir treatment are associated with the presence of a haplotype of four functional UGT variants spanning three *UGT1A* genes, which are found among the group of Gilbert's syndrome patients.

Thus, drug metabolism by glucuronidation is influenced by complex genotypes and is linked to the clinical phenotype of Gilbert's syndrome. The improvement of drug safety and the establishment of individualized pharmacotherapy depend on identifying such risk constellations. Suggestions to incorporate pharmacogenomic data such as the above mentioned examples and tests into the drug approval process are a first step, which will in future contribute to individualized therapy and risk assessment. Pharmacogenetics will complement traditional methods for choosing drugs and for selecting dosing regimens for narrow therapeutic-index drugs [14, 42].

## Simultaneous Exposure to Several Drugs

Pharmacotherapy in clinical practice usually means polychemotherapy. The simultaneous intake of several drugs increases the risk of adverse drug reactions. Therefore the physician must be familiar with the effect of drugs and other xenobiotics on hepatic drug metabolism. Possible interactions have to be kept in mind while choosing an individual dose, in order to reduce the risk of undesired side effects.

The microsomal mixed function oxygenases of the liver may be induced or inhibited by foreign substances (Table 92.1). Such effects must be considered while applying drugs that are metabolized by the monooxygenase system since they may profoundly influence the interaction of drugs. Barbiturates, gluthetimide, dichlorphenazone, haloperidol, griseofulvin and ethanol are only a few examples of drugs that induce microsomal enzymes. If enzyme inducers are administered over a longer period of time, the dose of drugs that are degraded by microsomes must be increased in order to achieve the same pharmacological effect. After withdrawing the enzyme inducer the dose has to be adapted again in order to avoid overdosing.

The dose of drugs that are oxidized primarily by cytochrome P450 and whose metabolites are more active pharmacologically than the parent substance must be reduced during simultaneous treatment with an enzyme inducer.

Methylphenidate, chloramphenicol, disulfiram, allopurinol, dextropropoxyphene and some $H_2$-receptor antagonists inhibit microsomal degradation of many drugs. If they are administered over longer periods of time, the dose of drugs that are predominantly metabolized microsomally (for example, lidocaine, phenprocoumon, imipramine, metronidazole, propranolol, metoprolol, theophylline, chlordiazepoxide) must be reduced, in order to avoid side effects. The strongest microsomal inhibitor among the $H_2$-receptor blockers is cimitedine which, however, nowadays is only rarely prescribed. $H_2$-receptor blockers such as ranitidine that have a furan ring instead of an imidazole, however, may inhibit microsomal metabolism, albeit to a lesser degree.

Spironolactone increases the activity of NADPH-cytochrome-c-reductase without altering substantially cytochrome P450.

Proton pump inhibitors are extensively metabolized by the cytochrome P450 system, especially CYP2C19 and CYP3A4. Omeprazole inhibits the hepatic degradation of phenytoin, diazepam and phenprocoumon.

Induction or inhibition of microsomal enzymes not only has implications for the clinical efficacy of drugs. The degree of hepatotoxicity is also modified. Enzyme inducers, such as phenobarbital and ethanol increase the toxicity of substances (for example, bromobenzole, $CCl_4$, acetaminophen, isoniazid, valproic acid and certain antineoplastic drugs) which yield toxic metabolites during their degradation, while on the other hand these enzyme inducers delimit the extent of aflatoxin-induced parenchymal necrosis. Phenobarbital not only induces mixed function oxidases, but also glucuronyl transferase, a phase II reaction enzyme.

Microsomal enzyme induction is expressed morphologically by hyperplasia and hypertrophy of the smooth endoplasmic reticulum. This change may also be recognized by light microscopy ("induced hepatocytes"; see Chapter 24).

The use of "natural herbs" can also result in undesired interactions with other drugs. The concomitant intake of cyclosporin A and Hypericum perforatum (St. John's wort), for example, can lead to a significant fall of the immunosuppressant level in serum and elicit an acute, life threatening rejection reaction [19].

## Biliary Excretion of Drugs

The significance of biliary drug excretion in humans can only be appraised with difficulty, since it cannot be examined under physiologic conditions. Furthermore, due to methodological difficulties current data must be regarded as fairly inaccurate. Most clinical investigations dealing with biliary excretion of drugs were performed on bile which was obtained postoperatively

**Table 92.4** Biliary drug excretion in humans

| Drug (selection) | Dose (mg) | Biliary excretion (%/time) |
|---|---|---|
| Ampicillin | 1,000 p.o. | 0.03/12 h |
| Piperacillin | 4,000 i.v. | 13.4/12 h |
| Mezlocillin | 1,000 i.m. | 2.60/12 h |
| Cefamandol | 1,000 i.m. | 0.40/2 h |
| Cefazolin | 1,000 i.v. | 0.12/2 h |
| Chloramphenicol | 3,000 p.o. | 2.70/24 h |
| Doxycyclin | 200 i.v. | 4.00/24 h |
| Erythromycin | 500 p.o. | 0.04/12 h |
| Penicillin G | 600 i.v. | 0.12/24 h |
| β-Methyldigoxin | 0.2 i.v. | 12.4/48 h |
| Digitoxin | 0.6 i.v. | 1.50/24 h |
| Vincristin | 0.5 i.v. | 21.7/24 h |
| Acebutolol | 300 p.o. | 5,60/24 h |
| Practolol | 400 p.o. | 23–40/48 h |
| Spironolactone | 300 p.o. | 5–33/4 d |
| Phenylbutazone | 600 p.o. | 9.5/4 d |

Source: From [5]

through a T-drain, intraoperatively from the gallbladder, endoscopically during retrograde cholangiography or via a nasobiliary tube. All patients studied suffered from hepatobiliary disorders which make evaluation of physiologic biliary excretion of drugs impossible. Furthermore, the diversion of bile leads to a disruption of the physiological enterohepatic circulation thereby distorting pharmacokinetic data. Thus, a remarkable information gap that cannot be closed by extrapolating data from animal experiments to humans exists in this field. Nevertheless, biliary excretion in man is an important pathway for some drugs. The biliary secretion of a substance primarily depends on its chemical structure, polarity and molecular weight. Drugs excreted in bile usually are highly polar and possess ionizable residues. The canalicular hepatocyte membrane contains carrier dependent transport systems for biliary excretion of drugs (see Chapters 5 and 7). The biliary excretion of some drugs is listed in Table 92.4. Drugs or their metabolites excreted in bile may further be metabolized in the gut lumen or within the intestinal mucosa or be absorbed in the terminal ileum and enter the enterohepatic circulation.

## *Impact of Liver Disease*

Patients with liver disease more often than healthy persons develop drug-induced side effects. Hepatobiliary diseases may lead to clinical-pharmacological consequences that vary according to the severity of the underlying disease and the drugs administered.

Drugs with a hepatic extraction rate of >60% during a single pass through the liver are counted as *"high clearance" substances* (>800 mL/min; bioavailability <30–40%). Their systemic clearance in the first instance depends on hepatic circulation. Diseases associated with a compromised hepatic blood flow, such right heart insufficiency, portal vein or liver vein thrombosis, will primarily lead to a reduced systemic clearance of "high clearance" substances and thereby result in a higher bioavailability and/or toxicity of these drugs. After oral administration of "first pass" drugs to patients with portosystemic shunts, the concentration of the drug in the systemic circulation will be increased. Even an initial oral dose may lead to toxic blood levels and to a delayed decline of the serum concentration of these drugs (Table 92.5).

Drugs with a hepatic extraction rate of < 30% during a single liver passage belong to the *"low clearance" substances* (<300 mL/min; bioavailability >70–80%). Their systemic clearance essentially depends on the metabolic capacity of the liver. A diminished liver cell mass, for example in acute liver failure or in advanced cirrhosis, will result in a reduction of the systemic clearance of these drugs. Repeated administration of substances with a low hepatic extraction rate may lead to accumulation of the drugs. Toxic serum levels after one-time administration are not to be expected in these cases (Table 92.6).

Antipyrine is regarded as a model substance for drugs with low hepatic clearance, and indocyanine

**Table 92.5** High hepatic clearance drugs (hepatic extraction rate >60%)

| | |
|---|---|
| **Analgesics** | Pentazocine, meperidine, propoxyphene, salicylamide |
| **Sedatives** | Clomethiazole |
| **β-Adrenergic blockers** | Propranolol, labetalol |
| **Antiarrhythmics** | Lidocaine, verapamil |

Source: From [5]

**Table 92.6** Low hepatic clearance drugs (hepatic extraction rate <30%)

| | |
|---|---|
| **Analgesics** | Paracetamol, aminopyrine |
| **Barbiturates** | Pentobarbital, hexobarbital, phenobarbital |
| **Benzodiazepins** | Diazepam, chlordiazepoxide |
| **Xanthins** | Caffeine, theophylline |
| **Antibiotics** | Rifampicin, clindamycin |

Source: From [5]

green as one for drugs with a high hepatic clearance (see Chapter 35).

Diminished protein binding of drugs in decompensated cirrhosis promotes entry of drugs into the hepatocytes. Chronic liver disease may also have an impact on drug effects on other organs; for example, a diminished renal response to diuretics or an increased sensitivity to oral anticoagulants in patients with liver cirrhosis. Furthermore, it is known that analgesics and tranquilizers may elicit a hepatic encephalopathy in cirrhotic patients. This is believed to be due not only to an increased concentration of these substances in the central nervous system (CNS), but also to an increased drug sensitivity of the CNS. The excessive sedation of patients with liver cirrhosis by benzodiazepines is also due to this mechanism.

Obstructive cholestasis reduces the cytochrome P450 levels by damaging perivenular (zone 3) hepatocytes and thereby compromises the metabolic capacity of the liver. This may also be aggravated by retained bile acids which by their detergent action lead to a separation of enzymes from microsomal membranes.

As a consequence from what has been said, drug dosing in chronic liver disease ideally should be adjusted to liver function. The dose of drugs with a high hepatic clearance is not only determined by the state of the liver but also by the route of administration. While an intravenous dose has to be reduced by approximately 50%, oral dosing must be reduced to 10–20% of the normal dose, in order to avoid toxic plasma levels (Table 92.7).

Drugs with a low hepatic clearance should be dosed approximately two to four times lower than normal, largely independent from the route of administration (Table 92.8). Their bioavailability after a single dose (irrespective whether oral or intravenous) is not significantly increased. Another factor that has to be taken into consideration is the therapeutic-index of a drug. Drugs with a wide therapeutic-index do not need a significant adjustment of their dose even in severe liver disease.

Chronic liver diseases usually affect phase I reactions, while phase II reactions proceed unaltered for relatively long periods of time. A dose reduction is necessary only for those drugs which are metabolized primarily by phase I reactions, for example, the proton pump inhibitor lansoprazole. The capacity of hepatocytes to conjugate drugs is exceedingly high and remains preserved even in advanced liver disease. Therefore the dose of drugs that are primarily metabolized by phase II conjugation reactions, such as lorazepam, temazepam, clofibrate, ketoprofen does not need to be adjusted in chronic liver disease.

Finally the question arises as to how our detailed knowledge of hepatic metabolism and excretion of drugs (in analogy to renal clearance) can be translated into clinical practice in patients with chronic liver disease. While creatinine clearance is a reliable indicator of excretory kidney function allowing to predict renal elimination of drugs, there are no comparable data for the liver. *There is no global hepatic test capable of predicting reliably the pharmacokinetics of a drug in chronic liver disease*. Also the so-called liver function tests with markers whose elimination kinetics are well known, such as indocyanine, aminopyrine and galactose

**Table 92.7** Effect of liver cirrhosis on clearance, bioavailability and accumulation of some drugs with a high hepatic extraction rate

| Drug | Clearance (%)[a] | Bioavailability (%)[a] | Accumulation after oral application (%) |
|---|---|---|---|
| Pethidine | 60 | 160 | 270 |
| Pentazocine | 50 | 350 | 700 |
| Clomethiazole | 70 | 1,150 | 1,700 |
| Metoprolol | 76 | 170 | 220 |
| Propranolol | 60 | 160 | 280 |
| Labetalol | 70 | 190 | 270 |
| Verapamil | 50 | 240 | 490 |

[a]Referred to normal value
Source: From [5]

**Table 92.8** Effect of liver cirrhosis on the clearance of some drugs with a low hepatic extraction rate

| Drug | Clearance (%)[a] |
|---|---|
| Chlordiazepoxide | 40 |
| Diazepam | 25 |
| Nitrazepam | 70 |
| Hexobarbital | 50 |
| Chloramphenicol | 35 |
| Cefoperazone | 50 |
| Clindamycin | 50 |
| Metronidazole | 40 |
| Naproxen | 40 |
| Sulindac | 30 |
| Paracetamol | 80 |
| Triamterene | 10 |
| Theophylline | 30 |

[a]Referred to normal value
Source: From [5]

do not allow to reliably conclude on the capacity of the liver to metabolize drugs. Furthermore, this situation is complicated by the fact that liver diseases may be associated with changes that impact pharmacokinetic parameters in opposite ways in one and the same patient. Thus, in acute hepatitis liver cell mass diminishes while hepatic blood flow increases. Moreover, each liver disease is a dynamic process with changing clinical-pharmacologic parameters during its course. These complex processes result in the impossibility to make accurate predictions regarding the pharmacokinetics of drugs in liver disease. *Thus, the administration of drugs to patients with chronic liver disease cannot be calculated, and in the end it relies on the clinical experience of the physician*. The assessment of the metabolic situation will be based on simple laboratory measurements, such as serum albumin concentration, coagulation parameters, choline esterase and on the clinical appearance of the patient. Despite all theoretical considerations in clinical practice, drug dosing in liver disease follows the dictum "give, wait and see".

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# Drug- and Toxin-Induced Liver Injury

# 93

Henryk Dancygier

## Chapter Outline



The development of a plethora of new agents in the last decades was associated with an increased prevalence of adverse drug-induced hepatic reactions. Although most patients tolerate prescribed drugs well, approximately 2% of all cases of jaundice occurring in the hospital and up to 25% of cases with fulminant hepatitis are ascribed to drugs. A meta-analysis performed in the nineties reported an overall incidence of serious adverse drug reactions (ADRs) in hospitalized patients of 6.7% and of fatal ADRs of 0.32% [21]. Nutritional supplements are frequently considered to be harmless, but indiscriminate use of unlabeled ingredients or supplements containing anabolic steroids may lead to significant ADRs [9, 14, 27]. Also herbal agents may interact with hepatic drug metabolism and potentially are hepatotoxic [7, 16, 19, 20]. The broad clinical and pathologic spectrum of ADRs requires one to always consider drug-induced and toxic lesions in the differential diagnosis of hepatic diseases [11, 15, 21, 22, 30].

## Definition

Foreign compounds usually may damage the liver in two different ways (Fig. 93.1).
The *hepatotoxic pathway* is

- Dose dependent
- Predictable, and
- Reproducible in other individuals

Hepatotoxins may act directly or indirectly. *Direct hepatotoxins* damage the hepatocytes directly, i.e. without the need of toxic intermediates. *Indirect hepatotoxicity* involves reactive molecules generated in the metabolism of the parent substance which bind covalently to essential hepatocyte molecules and thus

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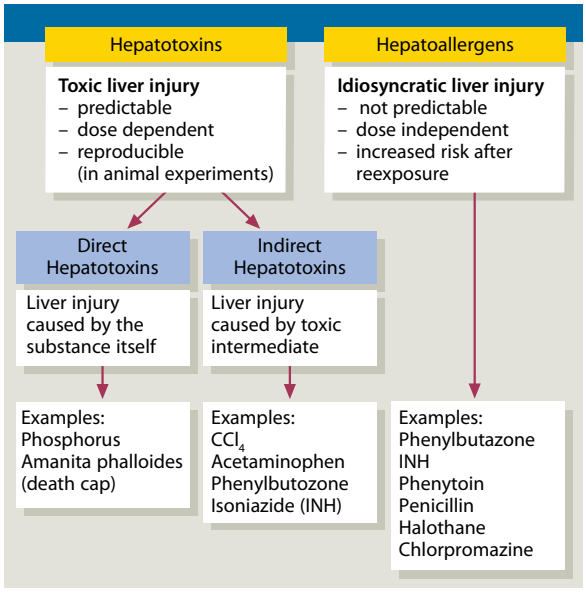


**Fig. 93.1** Main forms of drug- and toxin-induced liver injury. Some substances damage the liver by both toxic and idiosyncratic mechanisms

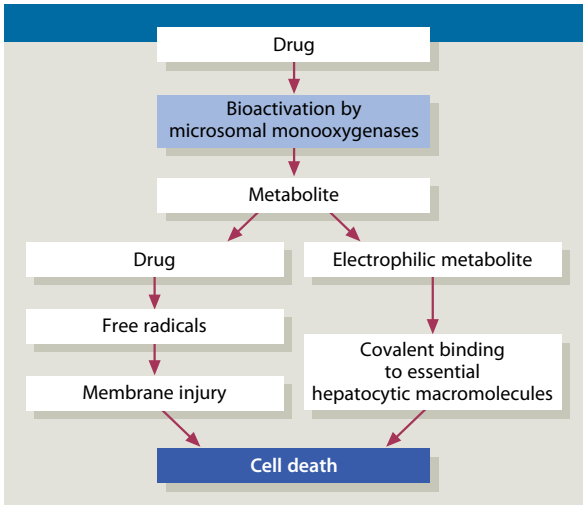


**Fig. 93.2** Pathophysiology of liver cell injury by indirect hepatotoxins

impair viability of liver cells (Fig. 93.2).

The vast majority of drugs are neither direct nor indirect hepatotoxins, but may damage the liver by *hypersensitivity (idiosyncratic) mechanisms*. This genetically based and immunologically mediated *idiosyncratic pathway* is

- Dose independent
- Unpredictable, and
- Not reproducible in other individuals

These hepatoallergens or their metabolic products alter the antigenicity of hepatocyte membranes and elicit immunologic processes, in the course of which hepatocytes are injured. Renewed exposure is associated with a markedly increased risk of liver damage. *Idiosyncratic and toxic mechanisms are not mutually exclusive*. With some substances, for example isoniazide and phenylbutazone, both mechanisms are operative.

## Diagnosis

### *Clinical Manifestations*

The cornerstone of the diagnosis of drug-induced liver disease is an exact *history*. Exposure to all xenobiotics (including "natural herbs", vitamin pills, dietary supplements, etc.) must be investigated, which often requires repeated anamnestic investigation. Establishing a temporal link between the onset of liver injury and the intake of the presumed injurious substance is of paramount importance. Follow-up after discontinuing the incriminated drug(s) or after inadvertent reexposure is equally important. One should also bear in mind that with some substances liver damage may become apparent only after the drug has been withdrawn. Ascribing unequivocally a causal relationship between a xenobiotic and liver injury often is hampered in clinical practice by the fact that most patients take several drugs concomitantly and the underlying liver disease itself may cause hepatic changes. In selected cases with mild injury the feasibility of reexposure may be examined which, however, in most clinical situations is not warranted.

Although in clinical practice a clear-cut distinction between a hepatotoxic and an idiosyncratic liver injury cannot be made based on clinical manifestations, for didactic reasons both entities are described separately in the following paragraphs.

#### Hepatotoxic Liver Injury

The clinical picture of hepatotoxic liver damage is characterized by the signs of *hepatic failure*. The course often is *bimodal*. Initial nonspecific symptoms, such as

nausea, vomiting and anorexia are followed by a short period of spontaneous improvement. After approximately 48 h, renewed clinical deterioration occurs with ensuing jaundice and signs of increasing liver failure. If the hepatic damage exceeds the regenerative capacity of the liver, without liver transplantation the patient will die in hepatic coma.

### Idiosyncratic Liver Injury

Idiosyncratic liver injury usually occurs 1–5 weeks after intake of the substance, but occasionally liver injury evolves for unknown reasons after a drug has been tolerated for months without any untoward side effects (for example: warfarin, phenprocoumon). The clinical picture ranges from asymptomatic (liver injury is detected incidentally by elevated liver enzymes in serum) to a general sense of malaise with a mild feeling of pressure in the right upper quadrant to (rare) liver failure. Idiosyncratic liver injury may be associated with fever, skin rash, arthralgias, eosinophilia and hemolytic anemia which are clinical indicators for an immune pathogenesis.

After discontinuing the causative agent, liver injury usually resolves within several days to weeks. Reexposure is followed by rapid clinical deterioration.

## Laboratory Findings

*No laboratory test is specific for drug-induced liver injury*. The correct interpretation of laboratory findings requires a close correlation of laboratory results with anamnestic and clinical data. Immunologic tests evaluating the proliferative response of the patient's lymphocytes to the incriminated drug are unreliable in predicting or diagnosing an idiosyncratic liver injury. Simple tests, such as the aminotransferases (AST, ALT), alkaline phosphatase (AP) and serum bilirubin are still the best parameters to detect a drug-induced or toxic liver injury. The zonal distribution of enzymes and subcellular structures (*metabolic zonation*; see Chapter 9) is the key to understanding the liver enzyme profiles in serum and the morphological patterns of drug-induced and toxic liver injury.

The AST is primarily localized in the mitochondria, the ALT in the cytoplasm. The glutamate dehydrogenase (GLDH) is concentrated in the mitochondria of perivenular (zone 3) hepatocytes. Elevations of AST serum levels indicate a liver cell injury that also includes mitochondrial damage. Injury of perivenular hepatocytes, due, for example, to ethanol, $CCl_4$, or hypoxia will result in elevated GLDH levels in addition to increases of serum aminotransferase levels. Cytochrome P450 activity is most concentrated in acinar zone 3. Thus, metabolic zonation, i.e. different endowment with enzymes in different acinar zones accounts for zonal toxicity.

**Table 93.1** Laboratory parameters in different drug-induced injury patterns

| Parameter | Hepatocellular injury | Cholestatic injury | Mixed liver injury |
|---|---|---|---|
| **ALT** | >2 ULN | – | >2 ULN |
| **AP** | – | >2 ULN | >2 ULN |
| **ALT: AP**[a] | ≥5 | ≤2 | 2–5 |

*ULN* upper limit of normal
[a]Expressed as the ratio of ULN

Isolated increases of AST, ALT, AP or total bilirubin to less than two times the upper limit of normal (ULN) should be interpreted as nonspecific biochemical alterations at first, not necessarily indicating liver damage. Only concomitant increases of ALT and conjugated bilirubin, each to more than two times the ULN, or the simultaneous elevation of AST, AP and total bilirubin, with at least one of these parameters being >2 times the ULN make the presence of liver injury very likely [2]. Depending on the level of ALT and AP elevation and on the ratio of ALT/AP (expressed as a multiple of normal) *hepatocellular*, *cholestatic* and *mixed patterns of injury* may be distinguished (Table 93.1).

If laboratory parameters are abnormal for less than 3 months, liver injury is said to be *acute*. If changes persist for more than 3 months, liver damage is *chronic*, which, however, should not be confused with chronic inflammatory liver disease, for example in hepatitis B and C.

## Histological Patterns of Injury

A generally accepted morphological classification system for drug-induced and toxic liver injury does not exist and *there is no histological alteration specific for toxic liver damage*. What has been said about laboratory findings in drug-induced liver injury applies also to liver histology.

**Table 93.2** Major histological patterns in drug-induced and toxic liver damage. Considering the millions of people taking (multiple) drugs for long periods of time, drug-induced liver injury is a relatively rare event and some of the examples listed here represent single cases reported in the literature

| Prevalent histological finding | Examples |
|---|---|
| **Zonal necrosis** | Acetaminophen [24, 29][a]<br>Allopurinol<br>Amanita phalloides (death cap)<br>$CCl_4$<br>Diclofenac [1]<br>Fe-sulfate<br>Yellow phosphorous<br>Halothane<br>Cocaine<br>Piroxicam<br>Salicylates |
| **Acute hepatitis ± bridging necrosis ± cholestasis** | ACE-inhibitors<br>β-Adrenergic blockers<br>Carbimazole<br>Chlorpromazine<br>Clopidogrel<br>Cyclophosphamide<br>Ecstasy<br>Ezetimibe<br>Flutamide<br>Halothane<br>Isoniazid<br>Ketoconazole<br>Methimazole<br>α-Methyldopa<br>Nonsteroidal anti-inflammatory drugs<br>Penicillin<br>Phenothiazine<br>Phenylbutazone<br>Phenprocoumon[b]<br>Phenytoin<br>Pioglitazone<br>Pravastatin[c]<br>Propylthiouracil<br>Rifampicin<br>Riluzole<br>Sulfonamide<br>Ticlopidine<br>Trazodone |
| **Fulminant hepatitis with liver failure** | Clozapine<br>Ecstasy<br>Fluconazole<br>Halothane<br>Ibuprofen<br>Isoniazid<br>Kava-Kava<br>Leflunamide<br>α-Methyldopa<br>Nefazodon<br>Phenprocoumon[b]<br>Phenytoin<br>Pyrazinamide |
| | Rifampicin<br>Rosiglitazon<br>Tolcapone |
| Chron**ic (autoimmune type) hepatitis** | Ezetimibe<br>Fluoxetine<br>Halothane<br>Isoniazid<br>α-Methyldopa<br>Nitrofurantoin |
| **Fibrosis** | Methotrexate<br>Vinylchloride<br>Vitamin A and Retinoids |
| **Granulomatous hepatitis** | Allopurinol<br>Amoxicillin-clavulanate<br>Carbamazepine[d]<br>Clofibrate<br>Diazepam<br>Diclofenac<br>Diltiazem<br>Halothane<br>Hydralazine<br>Hydrochlorothiazide<br>Interferon-α<br>Mesalamine<br>Methimazole<br>α-Methyldopa<br>Nitrofurantoin<br>Oral contraceptives<br>Oxyphenbutazone<br>P-aminosalicylic acid<br>Phenylbutazone<br>Phenytoin<br>Procainamids<br>Sulfonamides<br>Sulfonylureas |
| **Steatosis** | Antiretrovirals (+ lactic acidosis)<br>Ethanol<br>Cocaine<br>Methotrexate<br>Tamoxifen<br>Tetracyclines<br>Valproic acid |
| **Nonalcoholic steatohepatitis** | Amiodarone<br>Nifedipine<br>Estrogens<br>Glucocorticoids (long-term use) |
| **Cholestasis** | Anabolic steroids<br>Androgens<br>Carbimazole |

(*continued*)

**Table 93.2** (continued)

| Prevalent histological finding | Examples |
|---|---|
| | Ceftriaxone (gallbladder sludge)<br>Chlorpromazine<br>Erythromycin<br>Glimepiride<br>Gold<br>Ketoconazole<br>Methimazole<br>Paraquat |
| **Destructive cholangiopathy; ductopenia** [13] | Amoxycillin-clavulanate<br>Ampicillin<br>Arsenic<br>Azathioprine<br>Barbiturates<br>Carbamezepine<br>Chlorothiazide<br>Chlorpromazine<br>Cimetidine<br>Clindamycin<br>Cyproheptadine<br>Erythromycin<br>Estradiol<br>Flucloxacillin<br>Glycyrrhizin<br>Haloperidol<br>Imipramine<br>Methyltestosterone<br>Norandrostenolone<br>Phenytoin<br>Sulpiride<br>Tetracyclines<br>Thiabendazole<br>Tiopronin<br>Tolbutamide<br>Trimethoprim/sulfamethoxazole |
| **Acute cholangitis** [13] | Allopurinol<br>Chlorpromazine<br>Chlorpropramide<br>Trimethoprim/sulfamethoxazole |
| **Peliosis hepatis** | Anabolic steroids<br>Azathioprine<br>Oral contraceptives<br>Tamoxifen |
| **Veno-occlusive disease and/or Budd–Chiari syndrome** | Azathioprine<br>Busulfan<br>Cyclophosphamide<br>Dacarbazine<br>Mitomycin C<br>Oral contraceptives<br>Radiation treatment<br>Thioguanine |
| **Sclerosing cholangitis** [13, 25] | 5-Fluorouracil |
| **Nodular regenerative hyperplasia** | 6-Thioguanine |
| **Hepatocellular adenoma** | Anabolic steroids<br>$C_{17}$-substituted testosterone<br>Oral contraceptives |
| **Hepatocellular carcinoma** | Anabolic steroids<br>Androgenes<br>Thorotrast |
| **Angiosarcoma** | Diethylstilbestrole<br>Thorotrast<br>Vinylchloride |

[a]Blood lactate level is as an early predictor of outcome in paracetamol-induced acute liver failure. Lactate levels ≥3.5 mmol/L 4 h or ≥3.0 mmol/L 12 h after hospital admission correlate with a grave prognosis [3]

[b]Phenprocoumon-induced liver disease ranges from mild acute hepatitis to acute liver failure [26]

[c]Statins have an excellent safety profile. The most common clinical hepatic manifestation of statins is asymptomatic elevation in aminotransferases. Patients with elevated liver enzymes and preexistent steatosis are not at higher risk for statin hepatotoxicity [5, 6, 23]. Statin therapy is not associated with a higher risk of severe hepatotoxicity in patients with chronic hepatitis C [17]

[d]Carbamazepine is the drug most commonly involved in granulomatous hepatitis

The morphological spectrum ranges from drop out of single cells to necrosis of groups of hepatocytes with a variously intense inflammatory reaction, to micro- and macrovesicular steatosis, fibrosis, biliary lesions and vascular or neoplastic changes. *The same substance under different conditions may cause different histological patterns of injury*. The polymorphic histological picture does not allow one to reliably differentiate drug-induced liver injury from hepatic damage of a different etiology. The histological findings can only be interpreted correctly in conjunction with anamnestic, clinical and laboratory data. Thus, the correct histopathological interpretation requires a close cooperation between the clinician and the hepatopathologist. The most important histological patterns of drug-induced and toxic liver injury are listed in Table 93.2.

## Therapy

The most important therapeutic measure is discontinuing the offending drug. In most cases laboratory parameters will normalize within days to weeks. In Tables 93.3 and 93.4 therapy of acetaminophen and Amanita phalloides poisoning is outlined. L-carnitine treatment may be beneficial for patients with valproate-induced hepatotoxicity [4]. In acute liver failure liver transplantation may be life saving (see Chapter 78).

It is beyond the scope of this chapter to discuss all potentially hepatotoxic drugs and toxins. The reader is referred to three excellent textbooks that cover all aspects of this topic and from where he/she may retrieve the respective drug lists [11, 15, 30].

**Table 93.3** Examples of predictable hepatotoxicity

| Drug/toxin | Comments |
|---|---|
| **Acetaminophen** (Paracetamol) | Mechanism of liver damage:<br>**Paracetamol**[a] → CYP P450 → **Toxic Metabolite**[b] → if reduced glutathione consumed[c] → **Liver necrosis**<br>(NAPQI)<br>↓ ↓<br>Sulfation + Glucuronidation ↓ Excretion<br>Inactivation by binding to glutathione[c] ↓ Excretion in urine<br>Enzyme inducers (ethanol, barbiturates) increase acetaminophen toxicity<br>Depending on the activity of the microsomal monooxygenase system doses > 8–10 g/day lead to liver necrosis. Parenchymal necrosis initially is perivenular but later may become confluent and extend to the portal tracts. In chronic alcohol abusers (induction of microsomal enzymes) daily doses ≤6g may lead to liver damage |
| | **Paracetamol serum levels after ingestion of an overdose (8–10 g)**<br>After 4 h: >300 µg/mL → liver damage in 100% of cases; <120 µg/mL → no liver damage<br>After 12 h: >50 µg/mL → liver damage probable; <50 µg/mL → no liver damage |
| | **Treatment if liver damage is probable**<br>– Gastric lavage + gastrointestinal decontamination with activated charcoal<br>– N-acetylcysteine (glutathione precursor):<br>140 mg/kg b.w. p.o. followed by<br>150 mg/kg b.w. in 200 mL 5% dextrose infused over 15 min followed by<br>50 mg/kg b.w. in 500 mL 5% dextrose infused over 4 h followed by<br>100 mg/kg b.w. in 1,000 mL 5% dextrose infused over 16 h |
| **Tetracyclines** | Liver injury occurs with doses >1 g/day, especially if administered i.v.<br>Histologically a microvesicular steatosis without a noteworthy necrosis or inflammatory reaction is present. The histological appearance corresponds to that seen in acute fatty liver of pregnancy and in Reye's syndrome |
| | **Mechanism**<br>Impairment of lipoprotein synthesis with compromised excretion of triglycerides from hepatocytes. Increased uptake by hepatocytes of free fatty acids. Lipid peroxidation, oxidative stress, reactive oxygen species<br>Liver injury is reversible. After discontinuing tetracyclines complete histological recovery occurs usually within 10 days |

(*continued*)

**Table 93.3** (continued)

| Drug/toxin | Comments |
|---|---|
| **α-Amanitine, phalloidin** | Are the main toxic components of Amanita phalloides (death cap). The lethal dose is approximately 50 g<br>**Mechanism**<br>Impairment of protein synthesis by binding of toxins to RNA-polymerase II<br>**Clinical course**<br>Latency period 5–24 (48) h after ingestion<br>Gastrointestinal phase (vomiting, diarrhea) 24–48 h<br>Hepatorenal phase >48 h<br>Death in hepatic coma<br>**Therapy** [10, 12, 18]<br>*Basic therapy*<br>Gastric lavage (continuous)<br>Activated charcoal 40–60 g daily and lactulose 60–100 g daily<br>Enema (twice daily)<br>Hemoperfusion (alternatively forced diuresis)<br>*Antidotes*<br>Silibinin 20 mg/kg b.w. in 500 mL 5% dextrose qid<br>Penicillin G 1 million units/kg b.w. i.v.<br>Thioctic acid 200 mg i.v. five times daily<br>*Supportive therapy*<br>Glucose infusions + insulin + electrolytes<br>Red blood cells, fresh frozen plasma<br>Antithrombin III<br>Prophylaxis of brain edema<br>Vitamins (K and B)<br>*Liver transplantation*[e]<br>Liver transplantation should be strongly considered in patients with an interval between ingestion and diarrhea <8 h. From day 4 after ingestion, prothrombin index lower than 10% (approximately INR of 6) alone is a reliable tool for deciding emergency transplantation [10] |

[a]Eighty-five to 90% of acetaminophen is metabolized in the liver by direct sulfation (30%) and glucuronidation (55%). Approximately 5% of an ingested dose is excreted unchanged in the urine. Five to 10% is metabolized by cytochrome P450 (primarily isoforms CYP 2E1, CYP 1A2 and CYP 3A4) isoenzymes

[b]*N*-acetyl-*p*-quinone imine (NAPQI) is considered to be involved in paracetamol-induced hepatotoxicity. It is formed by oxidation by the CYP 2E1 and CYP 3A4 isoenzymes

[c]After therapeutic doses, NAPQI is normally detoxified by conjugation with glutathione and eventually excreted in the urine as a mercapturic acid metabolite. With increased doses, the formation of the NAPQI metabolite exceeds the conjugation capacity of the glutathione system, which leads to liver necrosis

[d]Initiation of recurrent daily intake of 4 g of acetaminophen in healthy adults is associated with ALT elevations. Therefore, history of acetaminophen ingestion should be considered in the differential diagnosis of serum aminotransferase elevations, even in the absence of measurable serum acetaminophen concentrations [28]

Source: According to [8]

**Table 93.4** Examples of idiosyncratic liver injury[a]

| Drug/toxin[a] | Comments |
|---|---|
| **Antidepressants** | Hepatitis like reactions occur in approximately 1% of patients taking mono-amino-oxidase (MAO)–inhibitors<br>Latency period 1–6 months. Mortality rate up to 15% !<br>Tricyclic antidepressants (imipramine, amitryptiline) cause liver injury less often (approximately 0.5%) than derivatives of hydrazine |
| **Antituberculous drugs** | |
| Isoniazide (INH) | **Mechanism**<br>1. INH → acetylhydrazine → reactive metabolite → liver injury<br>2. Hypersensitivity reaction<br>Increased toxicity in rapid acetylators (East Asians) and after microsomal enzyme induction<br>INH-induced liver injury is increased by concomitant administration of rifampicin<br>Jaundice occurs in 1% of cases. Approximately 10–20% of patients develop an asymptomatic elevation of serum AST levels within the first 2 months of treatment, which however normalize despite continuing administration of INH<br>A cholestatic hepatitis occurs in only approximately 0.1% of cases<br>In patients younger than 35 years INH-induced liver injury is rare. Persons between the ages 50–65 years are at greatest risk to develop INH-induced liver damage<br>The latency period to the appearance of overt liver injury may be 2–12 months |
| Paraaminosalicylic acid (PAS) | PAS-hepatitis occurs in approximately 1–2% of patients. It has a high mortality rate of approximately 20% |
| Rifampicin | Asymptomatic elevation of serum AST levels occur in approximately 20% of patients. Jaundice in 8%. Severe liver injury in 0.5%<br>Latency period: 1–2 months<br>**Mechanism**<br>Rifampicin inhibits the binding of bilirubin to the intracellular transport proteins Y (ligandin) and Z. It also impairs the canalicular excretion of bilirubin. Since rifampicin also induces microsomal enzymes bilirubin concentration may normalize after 2–3 weeks despite continued administration of the drug |
| Halothane | An asymptomatic elevation of serum aminotransferase levels occurs in 10–20% of patients<br>The incidence of severe halothane-induced liver injury is very rare (1: 10,000 anesthesias; mortality rate 1: 40,000 anesthesias). Repeated halothane exposures within 4–6 weeks lead to an increased incidence of halothane-induced liver injury<br>70% of affected patients are women |

[a]Newer drugs with reported idiosyncratic liver injury [22]

| | | | |
|---|---|---|---|
| Clozapine | Floxacillin | Lisinopril | Piroxicam |
| Diclofenac | Flutamide | Lovastatin | Terbutaline |
| Doxepin | Glyburide | Norfloxacin | Ticlopidine |
| Etoposide | Ketoconazole | Ofloxacin | Trazodone |
| Etretinate | Labetalol | Pentamidine | |

Source: According to [8]

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# Hepatic Granulomas

# 94

Henryk Dancygier

**Chapter Outline** (see Chapter 27)



Hepatic granulomas may occur in many liver diseases. The term granulomatous liver disease (synonyms: liver granulomas, granulomatous hepatitis) is not a final diagnosis but simply describes the presence of granulomas in liver tissue. *The challenge is not to diagnose a granuloma but to find its cause* [5].

## Definition

Granulomas are nodular accumulations of transformed (activated) macrophages, which often develop an epithelioid (epithelial-like) appearance. Fibroblasts, plasma cells, lymphocytes and multinucleated giant cells may be admixed to a variable degree.

## Epidemiology

Exact data regarding the incidence and prevalence of hepatic granulomas is lacking. Granulomas are found in 2.5–10% of all liver biopsies.

## Etiology

Hepatic granulomas have a broad range of underlying etiologies. The most important causes are listed in Tables 94.1 and 94.2. The different etiologic categories encompass

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- Systemic infections
- Malignant tumors
- Chemical compounds and drugs
- (Auto)immune diseases, and
- Idiopathic forms

**Table 94.1** Causes of hepatic granulomas (selection)

| |
|---|
| **Infections** |
| *Bacteria* |
| Mycobacteria (typical and atypical) |
| Brucellae |
| Yersiniae |
| Pseudomonas pseudomallei |
| Treponemas |
| Listeriae |
| Tropheryma whipplei |
| *Fungi* |
| Candida sp. |
| Blastomycetae |
| Coccidioides immitis |
| Histoplasma capsulatum |
| Cryptococcus neoformans |
| Nocardiae |
| *Protozoa* |
| Leishmania donovani |
| Toxoplasma gondii |
| Fasciola hepatica |
| Schistosomae |
| Toxocara canis |
| *Worms* |
| *Rickettsiae* |
| R. burneti (Q fever) |
| R. conorii (Fievre boutonneuese) |
| *Viruses* |
| Epstein-Barr-Virus |
| Cytomegalovirus |
| Hepatitis A, B, C |
| **Chemical Substances and Xenobiotics** |
| Beryllium |
| Copper |
| Talc |
| Mineral oil |
| Drugs (see Table 94.2) |
| **(Auto)Immune Diseases** |
| Sarcoidosis |
| Crohn's disease |
| Ulcerative colitis |
| Primary biliary cirrhosis |
| Lupus erythematosus |
| Wegener's granulomatosis |
| Polymyalgia rheumatica |
| **Neoplastic Diseases** |
| Hodgkin's lymphoma > Non Hodgkin's lymphoma |
| Renal carcinoma |

**Table 94.2** Selection of drugs that may cause hepatic granulomas

| |
|---|
| Allopurinol |
| Carbamazepine |
| Chlorpromazine |
| Clofibrate |
| Contraceptives |
| Diazepam |
| Diltiazem |
| Diphenylhydantoin |
| Gold |
| Halothane |
| Hydralazine |
| Hydrochlorothiazide |
| α-Interferon |
| Isoniazide |
| Mebendazole |
| Methyldopa |
| Nitrofurantoin |
| p-Aminosalicylic acid |
| Penicillin |
| Phenylbutazone |
| Phenytoin |
| Procainamide |
| Quinidine |
| Sulfonamides |
| Sulfonyl ureas |
| Tolbutamide |

Fifty to 75% of all hepatic granulomas in Western countries are due to sarcoidosis, tuberculosis, primary biliary cirrhosis or drugs [1–3 ]. More than 90% of patients with miliary tuberculosis have liver granulomas, as do up to 70% of patients with extrapulmonary tuberculosis. Hepatic granulomas can be found in approximately 25% of patients with isolated pulmonary tuberculosis. Despite all diagnostic efforts the cause of hepatic granulomas remains enigmatic in 10–35% of cases.

## Pathogenesis and Pathology

Granulomas are the expression of an immunologic and/or inflammatory reaction that results in a focal accumulation of macrophages surrounded by lymphocytes, plasma cells, fibroblasts and a variable amount of fibrous tissue. Under the influence of proinflammatory cytokines macrophages are activated and transformed into epithelioid cells. Neighbouring macrophages can fuse and create giant cells. Granulomas may exhibit a central necrosis or be non-necrotizing (*caseating* and

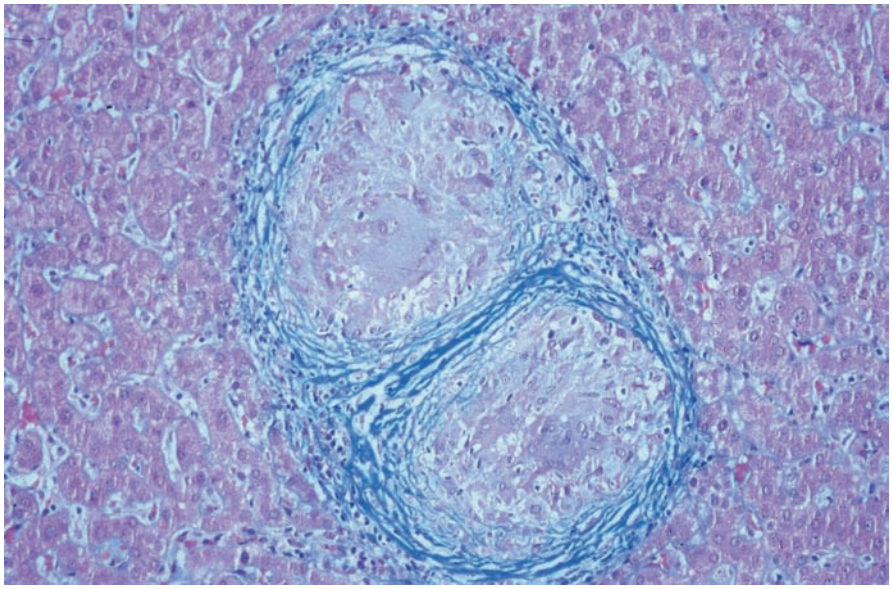

**Fig. 94.1** Epithelioid hepatic granulomas surrounded by a mild concentric fibrosis. Trichrome stain (× 200)

*non-caseating granulomas*) (Fig. 94.1; see also Figs. 27.1 and 27.2).

*Fibrin ring granulomas* ("doughnut granuloma") are granulomas with a central vacuole surrounded by a fibrin ring and epithelioid cells. *Lipogranulomas* contain a central fat vacuole. Granulomas may occur in all segments of the liver and in all acinar areas. Sarcoid granulomas, however, are preferentially localized in the portal/periportal area and the granulomas in primary biliary cirrhosis occur within the portal tracts.

## Diagnosis

### *Clinical Manifestations*

Most patients with hepatic granulomas are asymptomatic. Occasionally patients complain of nonspecific upper abdominal discomfort, fatigue, anorexia, weight loss which may be associated with fever of unknown origin [7]. These symptoms, however, are usually due to the underlying disease rather than to the granulomas themselves. In severe forms the liver may be enlarged.

### *Laboratory Findings*

There is no constellation of laboratory findings characteristic of hepatic granulomas. Commonly only a mild increase in the serum levels of alkaline phosphatase, γ-GT and aminotranferases is observed. Normal serum concentrations of aminotransferases, however, do not exclude even extensive granulomatous involvement of the liver. In sarcoid granulomas the serum levels of angiotensin-converting enzyme may be increased (see Chapter 95).

### *Imaging Techniques*

Sonography, computed tomography and magnetic resonance imaging are not able to visualize hepatic granulomas, except in the very rare cases in which several granulomas aggregate to form large space occupying lesions. Even in these cases findings are nonspecific. The diagnostic method of choice is percutaneous liver biopsy. A biopsy under laparoscopic guidance does not improve the rate of granuloma detection. A diagnostic laparoscopy, however, is useful if, for example peritoneal tuberculosis is suspected.

## Differential Diagnosis

The diagnostic challenge in granulomatous hepatitis is in attributing a specific cause to granulomas. With a combined histological, clinical, serological, and molecular approach the cause can be clarified in only approximately

two thirds of patients [4]. The diseases and conditions listed in Tables 94.1 and 94.2 must be considered in the differential diagnosis of granulomas.

Only in single cases the histopathological findings yield a specific etiology. The most common cause of non-caseating epithelioid granulomas in Western countries is sarcoidosis. Sarcoid granulomas contain fairly large amounts of connective tissue, and they heal leaving behind a concentric fibrosis. A central caseation suggests tuberculosis. However, acid fast rods are demonstrable in only approximately 10% to maximal 50% of tuberculous granulomas. So-called fibrin ring granulomas may be caused by allopurinol. They may also occur in Hodgkin's lymphoma, CMV- and EBV-infection, leishmaniasis, hepatitis A, toxoplasmosis and rickettsial diseases (Q-fever and boutonneuse fever). Birefringent material within a granuloma indicates foreign material, for example talc or starch, which may be a hint to intravenous drug abuse. Many eosinophils suggest an allergic pathogenesis, for example drugs or parasites. Occasionally parasite ova may be visualized within a granuloma, which then are evidentiary for its parasitic etiology.

## Course and Prognosis

Both depend on the underlying disease. The granulomas themselves usually have no relevant impact on liver structure and function. Depending on location and size, however, they may cause obstructive cholestasis and portal hypertension in rare cases. A portal hypertension due to granulomas occurs only in sarcoidosis, primary biliary cirrhosis and schistosomiasis [8]. Usually granulomas heal without sequelae.

## Therapy

Treatment of hepatic granulomas depends on the underlying disease. Infectious causes are treated by antimicrobial therapy; in primary biliary cirrhosis ursodeoxycholic acid and corticosteroids may be used. Drugs used in the treatment of hepatic sarcoidosis with cholestasis and portal hypertension or in severe idiopathic granulomatous hepatitis are listed in Table 95.2 [6].

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# Sarcoidosis of the Liver

# 95

Henryk Dancygier

## Chapter Outline



## Definition

Sarcoidosis is a multisystem disease of unknown etiology, characterized by the occurrence of epithelioid, non-caseating granulomas in one or several organs. Although the liver is affected in the majority of the cases, involvement of the liver rarely clinically predominates. The clinical spectrum of hepatic sarcoidosis encompasses asymptomatic patients with mild elevations of alkaline phosphatase and γGT serum levels, a chronic cholestatic liver disease, hepatic vein thrombosis and nodular transformation of the liver with portal hypertension.

## Epidemiology

The disease has a worldwide distribution. The prevalence rates are high in Scandinavia, Japan and the United States, and low in South America and in Central Asia. The yearly incidence of new cases is 55–64/$10^5$ inhabitants in Scandinavia, 11/$10^5$ in whites and 35/$10^5$ in Afro-Americans in the US. Men and women are equally affected and 99% of all cases of sarcoidosis occur between 20 and 60 years of age. Hepatic granulomas may be demonstrated in more than 70% of the cases of pulmonary sarcoidosis. Liver involvement without lung disease occurs in approximately 13% of cases [5, 8, 9].

## Pathogenesis

The exact pathogenesis of sarcoidosis is still unknown. Environmental, infectious, genetic and immunologic factors appear to be important. It is assumed that antigenic exposure in genetically susceptible individuals

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elicits an exaggerated cellular immune response that results in granuloma formation.

Familial clustering of sarcoidosis is seen in approximately 20% of affected Afro-American and 5% of white families. Monozygotic twins fall ill more often than dizygotic. Genetic associations with HLA-A1, -B8 and HLA-DR3 have been described in whites with sarcoidosis with a strong heterogeneity of sarcoidosis associated HLA polymorphisms.

The clustering of sarcoidosis in circumscribed geographical areas suggests exposure to organic or anorganic agents. Spread of disease by transmission from person to person seems probable.

Many infectious agents may cause granulomas. No definite agent has been identified yet in sarcoidosis. An abnormally enhanced immunologic reaction to mycobacteria or human herpesvirus 8 has been discussed [9].

## Pathology

Sarcoid granulomas are epithelioid and non-caseating. Occasionally some granulomas may show a central fibrinoid necrosis which must not be mistaken for caseation. Granulomas are spread diffusely throughout the liver and preferentially located in the portal/periportal areas. Usually all granulomas are in approximately the same developmental stage. Young acinar granulomas are composed of relatively few loosely assembled epithelioid cells, while mature lesions are sharply rounded and may also coalesce to form granulomatous aggregates. Giant cells and epithelioid cells express angiotensin converting enzyme (ACE), collagenase, lysozyme and $\alpha_1$-antitrypsin. Epithelioid cells, but not giant cells also express interleukin-1. The epithelioid cells are placed in a fine lattice of reticulin fibers. The inflammatory cellular response is scant with a few $CD4^+$ lymphocytes, plasma cells and some eosinophils.

Characteristic but not pathognomonic inclusions in sarcoid granulomas are

- *Schaumann bodies*: concentrically layered basophilic lamellas, measuring 25–100 µm in diameter consisting of Ca-phosphate, Ca-carbonate and iron often with central birefringent crystals. They occur more frequently in giant cells of sarcoid granulomas in the lymph nodes than in the liver.
- *Asteroid bodies*: star-shaped lipoproteins, 5–20 µm in diameter, usually situated in globular vesicles in giant cells. They consist of non-collagenous filaments and myelin-like membranes and may be also be visualized by immunocytochemistry with antibodies against ubiquitin.
- *Residual bodies*: single or multiple, homogenous cytoplasmic granules (centrospheres) measuring 1 µm in diameter that may push the nucleus to the periphery of the cell. They may represent end products of activated lysosomes.

The concentric accumulation of fibroblasts heralds the healing of sarcoid granulomas by fibrosis. Collagen increasingly becomes hyaline (paramyloid) and may disappear completely or (especially in large confluent granulomas) leave behind hyalinized fibrous scars. Occasionally single giant cells containing inclusions as the last remnants of previous sarcoid granulomas may be present in these scars.

Granulomas are the most salient but not the only histopathological changes of the liver in sarcoidosis. *Cholestatic alterations* occur in approximately 60% of the cases. Among them are ductular lesions that resemble those seen in primary biliary cirrhosis (cholangionecrotic) or an onion skin-like periductal fibrosis reminiscent of primary sclerosing cholangitis [1, 2, 10]. These changes may lead to ductopenia and be accompanied by a pseudoxanthomatous transformation (cholate stasis) of and copper accumulation in hepatocytes [7]. *Necroinflammatory changes* are scant and may appear as randomly distributed parenchymal necroses, apoptotic bodies and/or as a mild to moderate portal inflammation.

Large confluent granulomas may form *pseudotumors*. Involvement of central veins or portal venous branches may cause a *granulomatous phlebitis*. Nodular regenerative hyperplasia, probably due to disturbances of microcirculation, develops in up to 6% of cases. Healing of large granulomas by fibrosis may lead to nodular transformation of the liver with consequent *portal hypertension* [6, 7, 12].

## Diagnosis

### Clinical Manifestations

The majority of patients with hepatic granulomas are asymptomatic regarding the liver. A hepatomegaly is

present in 5–50%, and some of the patients complain of nonspecific right-sided abdominal discomfort. Up to 15% of patients have a splenomegaly. Occasionally fever is present.

Jaundice is very rare. A chronic intrahepatic cholestatic syndrome may occur in less than 5% of the patients. Even more rarely are signs of portal hypertension or a Budd-Chiari syndrome [11, 12].

### Laboratory Findings

Serum levels of alkaline phosphatase (AP) and γGT are mildly elevated in 5–35% of cases. Aminotransferase levels are usually normal or only slightly increased. In cholestatic variants, bilirubin concentration rises, though usually not greater than 5 mg%. AP, however, in these cases may reach levels of to 10–15 times the upper limit of normal.

A polyclonal hypergammaglobulinemia is usually present. ACE levels in serum are elevated and may correlate with the activity of the disease. Repeated measurements of ACE levels are warranted since they may hint towards a relapse.

### Imaging Techniques

Liver biopsy is the method of choice to demonstrate hepatic granulomas (see Chapter 94). If granulomas are present on the liver surface, they appear laparoscopically as small (≤ 2 mm) and well-delineated whitish nodules. Even if no granulomas are seen laparoscopically, liver biopsy usually will yield positive results. Increased serum levels of AP and γGT in a patient with known pulmonary sarcoidosis predicts with high probability the presence of hepatic sarcoidosis, and liver biopsy is not mandatory in these cases.

The sonographic appearance of the liver is normal in approximately half of the patients, and the remaining patients display nonspecific changes. Confluent granulomas forming tumor-like nodules ("sarcoidoma") appear as space occupying lesions and must be further differentiated by biopsy. Enlarged abdominal lymph nodes occur in up to 30% of cases with abdominal sarcoidosis. In uncomplicated hepatic sarcoidosis, CT and MRI do not add significant diagnostic information to ultrasound findings.

## Differential Diagnosis

*Cholestasis* in sarcoidosis may have different causes (Table 95.1). The chronic intrahepatic cholestatic syndrome in sarcoidosis has much in common with the cholestasis in primary biliary cirrhosis (PBC). In sarcoidosis, however, cholestasis is not the first sign of disease. Usually patients already have extrahepatic signs of sarcoidosis, and during the further course of the disease cholestasis appears as an additional feature. Furthermore, antimitochondrial antibodies (AMA) are absent in sarcoidosis, while more than 95% patients with PBC are AMA-positive. The common occurrence of sarcoidosis with PBC or primary sclerosing cholangitis is seen in less than 1% of cases.

*Portal hypertension* in hepatic sarcoidosis generally is due to secondary biliary cirrhosis. Other causes include mechanical compromise of sinusoidal blood flow by periportal granulomas or scars left behind by large confluent granulomas, nodular transformation of the liver, compression of portal vein by enlarged hilar lymph nodes, thrombotic occlusion of liver veins or portal vein, or a granulomatous venulitis.

*Ascites* in sarcoidosis is only rarely due to peritoneal sarcoidosis but is nearly always the result of portal hypertension.

## Course and Prognosis

In the vast majority of asymptomatic patients the course is benign and sarcoidosis of liver heals spontaneously [13]. Complications though may occur in a few patients, and sarcoidosis can cause end-stage chronic liver disease, which is often unrecognized [8]. A *chronic intrahepatic cholestasis* (similar to primary biliary cirrhosis) with progressive destruction of interlobular bile ducts by portal granulomas after 10–20 years may lead to secondary biliary cirrhosis with signs of portal

**Table 95.1** Causes of cholestasis in sarcoidosis [6]

- Hepatic granulomas
- Destruction of interlobular bile ducts
- Sarcoidosis of extrahepatic bile ducts
- Compression of extrahepatic bile ducts by enlarged hilar lymph nodes
- Associated primary biliary cirrhosis
- Associated primary sclerosing cholangitis

hypertension [2]. More rarely, granulomas may cause strictures of the intrahepatic bile ducts similar to primary sclerosing cholangitis.

Compression of liver veins by granulomas may lead to a *Budd-Chiari syndrome* [11].

Death due to *liver failure* is extremely rare. If patients with sarcoidosis succumb to their disease it is usually due to cardiac and pulmonary involvement, and not to hepatic dysfunction.

## Therapy

The treatment of hepatic sarcoidosis may represent a challenge. While most patients do not require therapy, some need life-long treatment. There are no established therapeutic guidelines. The decision whether drug treatment should be initiated depends on the severity of the disease in the individual patient.

Most patients with hepatic sarcoid involvement develop a spontaneous remission after several weeks to months. Only in patients with a complicated course should drug treatment be contemplated. Drugs that may be used in severe hepatic sarcoidosis or idiopathic granulomatous hepatitis are listed in Table 95.2. Patients with intrahepatic cholestasis should receive *ursodeoxycholic acid* 10–15 mg/kg p.o. daily [4]. Although their efficacy has not been proven in controlled trials, the drugs of choice in severe hepatic sarcoidosis are *corticosteroids* (prednisone 40 mg p.o. qd slowly tapering over a few weeks). Their use should be restricted to severe cases, as their use in hepatic sarcoidosis is not generally recommended. In patients with severe adverse reactions to steroids or in whom steroids prove ineffective, *immunosuppressants* (azathioprine 2–3 mg/kg p.o. daily or methotrexate 15 mg p.o. qw) may be tried [3]. Response to conventional immunosuppression, however, is variable and unpredictable. *Transplantation* is feasible and safe in this population but recurrence is possible [8].

**Table 95.2** Drugs used in the treatment of severe hepatic sarcoidosis and in idiopathic granulomatous hepatitis[a]

| | |
|---|---|
| **Prednisone** | 40 mg/day |
| **Methotrexate** | 15 mg qw |
| **Azathioprine** | 50–200 mg/day |
| **Chloroquine phosphate** | 200–400 mg/day |
| **Ursodesoxycholic acid** | 10–15 mg/kg body weight/day |

[a]Controlled trials proving the efficacy of these drugs are lacking. Therefore, their general use in hepatic sarcoidosis is discouraged and should be limited to patients with severe hepatic disease

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# Effects of Chronic Liver Disease on Other Organs: Tabellary Overview

# 96

Henryk Dancygier

The outstanding significance of the liver in metabolism and its central anatomical location in the splanchnic circulation explains why acute liver failure and end stage liver disease have far reaching implications for the function of other organs and systems. Table 96.1 summarizes the most important complications of liver cirrhosis [1]. For a detailed discussion the reader is referred to Chapters 32, 33, 78, 79, 80 and 91.

**Table 96.1** Complications of liver cirrhosis[a]

| | |
|---|---|
| **Gastrointestinal** | Ascites<br>Varices<br>Portal hypertensive gastro-enteropathy |
| **Infectious** | Spontaneous bacterial peritonitis<br>Systemic infections |
| **Neuro-psychiatric** | Hepatic encephalopathy |
| **Pulmonary** | Hepatopulmonary syndrome<br>Portopulmonary hypertension<br>Hepatic hydrothorax |
| **Endocrine** | Thyroid dysfunction<br>Gonadal dysfunction<br>Glucose intolerance, diabetes mellitus |
| **Cardiovascular** | Hyperdynamic circulation<br>Cirrhotic cardiomyopathy<br>Heart insufficiency |
| **Neoplastic** | Hepatocellular carcinoma<br>Malignant lymphoma (in chronic hepatitis C)<br>Breast cancer in men (?)[2] |
| **Nutritional** | Malnutrition<br>Cachexia |

[a]Skin changes in patients with liver cirrhosis are reported in Chapters 33 and 79

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# Hepatic Involvement in Extrahepatic Disease: Tabellary Overview

# 97

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The following table summarizes liver changes occurring in various extrahepatic disorders.

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**Table 97.1** Involvement of the liver in extrahepatic disease

| Organ/disease | Hepatobiliary manifestations |
|---|---|
| **Cardiovascular system** | |
| Heart failure [37] | Acute right heart failure leads to dilatation and congestion of central veins and sinusoids<br>Chronic circulatory congestion causes atrophy of perivenular hepatocytes, steatosis, and necrosis with perivenular fibrosis up to "cirrhose cardiaque"<br>Distension of the liver capsule (especially if acute) causes a sense of pressure and pain in the right upper quadrant. Ascites and jaundice are possible. On ultrasound hepatic veins are dilated<br>*Laboratory findings*: Bilirubin mildly ↑. aminotransferases are ↑ (usually < 400 IU/L) in 30% of the cases. LDH and GLDH ↑<br>In acute hepatic congestion aminotransferase concentrations in serum increase to up to several 1,000 IU/L and normalize again within a few days |
| Shock liver [18] | Hypoxic injury to perivenular (zone 3) hepatocytes with development of perivenular necrosis<br>*Laboratory findings*: ↑ aminotransferases and LDH levels to several 1,000 IU/L within a few hours after the initiating event (ischemic "hepatitis"; better "hepatosis") followed by rapid normalization within a few days after successful shock treatment<br>If reticulin fiber network remains preserved. necrotic tissue is cleared within 2–6 weeks and complete healing (restitutio ad integrum) ensues |
| Hereditary hemorrhagic telangiectasia (Osler–Rendu–Weber disease) [8, 9, 19, 20] | Liver involvement in 30–70% of the cases reported. More prevalent in females<br>Intrahepatic vascular malformations associated with blood shunting (arteriovenous, arterioportal and/or portovenous)<br>*Clinical manifestations*: Hepatic symptoms only occur in a minority of patients with vascular malformations in the liver<br>Hyperdynamic circulation. Hepatomegaly. Portal hypertension. High output heart failure<br>*Histology*: irregular fibrosis with interspersed telangiectatic vessels. A significant parenchymal reaction or an inflammatory activity is lacking |
| **Lungs** | Lung diseases associated with pulmonary hypertension may damage the liver via chronic right heart failure<br>Pneumococcal pneumonia often is accompanied by elevated aminotransferase levels in serum (mild liver injury) |
| Obstructive sleep apnea [46] | Obstructive sleep apnea is a risk factor for elevated liver enzymes and steatohepatitis independent of body weight |
| **Gastrointestinal tract** | |
| Chronic inflammatory bowel disease (Crohn's Disease; ulcerative colitis) [7, 34, 44] | Abnormal hepatic biochemistries are present in approximately one-third of the patients with chronic inflammatory bowel disease (IBD)<br>Primary sclerosing cholangitis (PSC) is the most common hepatobiliary disease in patients with IBD. PSC occurs in approximately 7% of patients with ulcerative colitis, less often in those with Crohn's disease<br>*Laboratory findings*: ↑↑AP and γ-GT, ↑ ALT and AST, p-ANCA positive<br>Diagnosis is made by ERCP or MRCP<br>The so-called pericholangitis is not a discrete disease entity, but a variant of PSC with involvement of the smallest bile ducts. ERCP findings are often normal and diagnosis is made by liver biopsy<br>Secondary biliary cirrhosis and cholangiocarcinoma are complications of long-standing PSC<br>Steatosis, granulomas, and chronic inflammation are frequent nonspecific findings<br>After operative resection of the terminal ileum in patients with Crohn's disease, an increased prevalence of gallstone formation is observed<br>Hepatic amyloidosis may develop after long-standing (decades) IBD. However, this is an extremely rare event |
| Celiac disease [2, 10, 14, 29, 32, 43] | Think of celiac disease in patients with elevations of aminotransferases of unknown origin even in the absence of diarrhea!<br>Clinical spectrum ranges from mild liver abnormalities to hepatic failure (rare)<br>*Histology*: steatosis, progressive hepatitis without apparent cause<br>A gluten-free diet improves liver abnormalities and may even reverse hepatic failure |

**Table 97.1** (continued)

| Organ/disease | Hepatobiliary manifestations |
|---|---|
| Short-bowel syndrome | Steatosis, steatohepatitis<br>Cholestasis<br>Increased incidence of gallstones |
| Autoimmune pancreatitis [47] | *Histology*: portal inflammation with or without interface hepatitis, large bile-duct obstructive features, portal sclerosis, lobular hepatitis, and canalicular cholestasis. Hepatic infiltration with IgG4-bearing plasma cells ("IgG4-hepatopathy")<br>Glucocorticoid therapy reduces IgG4-bearing plasma cell infiltration and ameliorates other histological findings |
| **Endocrine system** | |
| Diabetes mellitus [11, 15, 39] | Nonalcoholic Fatty Liver Disease<br>*Histology*: steatosis, glycogenated nuclei, periportal fibrosis, nonalcoholic steatohepatitis<br>Patients with type 2 diabetes mellitus have an increased incidence of gallstones. Biliary complications of cholelithiasis (cholangitis, perforation of gallbladder) are more frequent in diabetics than in non-diabetics<br>Emphysematous cholecystitis occurs predominantly in male diabetics<br>Hepatic abscesses are more frequent in diabetics<br>Risk of acute liver failure is increased in type 2 diabetes mellitus<br>Risk of hepatocellular carcinoma is increased in obese patients with type 2 diabetes mellitus<br>Insulin resistance is associated with a reduced response to therapy in patients infected with chronic hepatitis C virus |
| Hypothyroidism [21] | Significant liver changes are very rare. Occasionally mild ↑ of AST and LDH due to prolonged half live (slowed metabolism) of these enzymes<br>Very rarely a pericentral sclerosis with portal hypertension and ascites may occur |
| Hyperthyroidism [25] | Hypoxic liver injury (steatosis, necrosis) of perivenular hepatocytes<br>*Laboratory findings*: AP (also bone isoenzyme) and aminotransferases slightly ↑ |
| Oral contraceptives | Increased incidence of hepatocellular adenomas, gallstones, cholestatic liver disease and liver vein thrombosis |
| Anabolic androgenic steroids | Liver cell adenoma, focal nodular hyperplasia, hepatic peliosis, cholestasis |
| **Renal disease** | |
| Uremia | Mild reactive ↑ of aminotransferase levels are without clinical significance |
| Renal cell carcinoma | *Stauffer's syndrome*<br>Very rare paraneoplastic reaction of the liver in hypernephroma. Hepatomegaly with Kupffer cell hyperplasia and nonspecific reactive hepatitis. AP, γ-GT and bilirubin in serum are ↑, prothrombin level is ↓. These findings normalize after nephrectomy |
| **Hematopoietic system** | |
| Acute leukemia | *Histology*: Blast infiltration of the liver. In acute lymphatic leukemia blasts infiltrate predominantly the portal tracts; in acute myelocytic leukemia sinusoidal infiltration<br>Mild hepatomegaly; cholestasis (AP, γ-GT and bilirubin in serum ↑)<br>Rarely: acute liver failure<br>Hepatosplenic candidiasis |
| Chronic myelocytic leukemia | Marked hepatomegaly in approximately 50% of patients<br>*Laboratory finding*: ↑↑ AP<br>*Histology*: intrasinusoidal leukemic infiltrates. Extramedullary hematopoiesis in the liver<br>*Complication*: portal hypertension |
| Osteomyelofibrosis | Hepatomegaly in 70–85% of cases<br>Laboratory findings: AP, γ-GT and bilirubin in serum are ↑<br>*Histology*: extramedullary hematopoiesis in the liver in nearly all patients<br>*Complication*: portal hypertension |
| Polycythemia vera | Involvement of the liver is very rare. Portal vein and liver vein thrombosis occurs. Veno-occlusive disease |
| Essential thrombocytopenia | Involvement of the liver is very rare. Portal vein and liver vein thrombosis occurs. Veno-occlusive disease |

(*continued*)

**Table 97.1** (continued)

| Organ/disease | Hepatobiliary manifestations |
|---|---|
| Hodgkin's lymphoma [6, 13] | Hepatomegaly may also occur in the absence of lymphomatous infiltration of the liver. Only every other patient with histologically proven hepatic granulomas has a hepatomegaly<br>Cholestasis may occur as a paraneoplastic phenomenon or may be due to bile duct compression by enlarged lymph nodes<br>*Laboratory findings*: liver enzymes are normal or AP is ↑ |
| Non-Hodgkin's lymphoma | Hepatomegaly in 50% of the cases. Hepatic involvement occurs more often in low-grade lymphoma than in high-grade lymphoma<br>*Histology*: lymphomatous infiltrates<br>Cholestasis due to compression of extrahepatic bile ducts by lymphoma occurs more often than in Hodgkin's lymphoma<br>Primary malignant lymphomas of the liver occasionally can cause acute liver failure |
| Chronic lymphocytic leukemia | Marked lymphocytic infiltration in advanced stages<br>*Laboratory findings*: AP, AST, ALT, γ-GT and bilirubin i.S. are ↑ |
| Hairy cell leukemia | Sinusoidal and portal leukemic infiltrates are nearly always present. Liver tissue occasionally "melts down" forming cavities that on ultrasound appear as space occupying lesions |
| Multiple myeloma | Involvement of the liver is rare. Diffuse or focal accumulation of tumor cells possible. In *light chain disease* perisinusoidal deposition of immunoglobulin light chains |
| Sickle cell disease [1, 4, 27] | The clinical spectrum of liver involvement ranges from mild ↑ of liver enzymes (AP, AST, ALT) in asymptomatic patients to dramatic disease with marked hyperbilirubinemia and acute liver failure<br>*Histology*: Sickle-formed erythrocytes agglutinate within the sinusoids. Erythrophagocytosis by Kupffer cells<br>Liver pathology (sickle cell hepatopathy) is due to several factors, such as ischemia, post-transfusional viral hepatitis, iron overload and gallstones |
| Thalassemia major | Posttransfusional siderosis of liver<br>Pigment gallstones |
| Paroxysmal nocturnal hemoglobinuria | Thrombosis of portal vein, splenic vein and liver veins |
| Hemophagocytic syndrome [12] | Occurs primarily in leukemias and malignant lymphomas<br>Fever, jaundice, hepatomegaly or splenomegaly<br>*Laboratory findings*: ALT, AP and bilirubin in serum are ↑. Factor V is ↓<br>*Histology*: sinusoidal dilatation with hemophagocytic histiocytosis |
| Macrophage activating syndrome [5] | Macrophage activating syndrome is a rare hematological disorder associated with uncontrolled systemic T-cell activation. It may be associated with lobular hepatitis and severe bile duct injury with cholestasis |
| Antiphospholipid antibody syndrome [36] | May be associated with autoimmune intrahepatic cholangiopathy |
| **Collagen vascular diseases [33]** | AST, ALT and AP may be slightly ↑. In order to assess liver involvement biopsy is necessary |
| Polyarteritis nodosa [3, 22] | Liver cell necrosis. Hepatic infarcts<br>Nodular regenerative hyperplasia<br>Necrotizing angiitis of small hepatic arteries (see Figure 62.2)<br>Aneurysms of hepatic artery<br>Sclerosing cholangitic lesions of small intrahepatic bile ducts. Bile duct strictures |
| Giant cell arteritis [24] | Hepatic granulomas<br>Arteritis of hepatic artery |
| Rheumatoid arthritis | Steatosis, focal liver cell necrosis, sinusoidal dilatation, Kupffer cell hyperplasia, amyloidosis |
| Felty's syndrome | Lymphocytic infiltration<br>Nodular regenerative hyperplasia |
| Systemic lupus erythematosus (SLE) | Nodula regenerative hyperplasia<br>"Lupoid" hepatitis has nothing to do with SLE. It is a historical term for autoimmune hepatitis<br>Lupus anticoagulant (antiphospholipid antibodies) may lead to hepatic vein thrombosis<br>Jaundice in patients with SLE is usually due do hemolysis and not to liver disease<br>Hepatic idiosyncratic reactions to nonsteroidal anti-inflammatory drugs occur more often in SLE patients |

**Table 97.1** (continued)

| Organ/disease | Hepatobiliary manifestations |
|---|---|
| **Various diseases and conditions** | |
| Septicemia, septic shock | Jaundice<br>*Laboratory findings*: AP, AST, ALT, GLDH are ↑<br>*Histology*: nonspecific hepatitis, liver cell necrosis, ductular cholestasis<br>Sepsis is the most common cause of a ductular cholestasis. Endotoxin-induced damage of biliary cells plays a major pathophysiologic role |
| Henoch–Schönlein purpura [49] | Hepatobiliary involvement is extremely rare<br>Vasculitis of peribiliary arteries with ischemic necrosis of bile ducts. Bile duct strictures may lead to secondary biliary cirrhosis |
| Langerhans cell histiocytosis [28] | Sclerosing cholangitis with infiltration of bile ducts with Langerhans cells. Focal tumor-like infiltrates or cystic liver lesions may be present |
| Erdheim–Chester disease [23] | Erdheim–Chester disease is a rare non-Langerhans form of histiocytosis. It may mimic Klatskin's carcinoma |
| Systemic mastocytosis [30, 31, 35] | The liver is involved in approximately 60% of cases of systemic mastocytosis<br>Hepatomegaly. Portal hypertension and ascites are rare. May present as cholestatic liver disease<br>*Laboratory findings*: ↑ AP<br>*Histology*: mast cell infiltration (special stains: toluidine-blue; chloro-acetate esterase, immunohistology with mast cell tryptase and CD117 antibodies), fibrosis, nodular regenerative hyperplasia. Portal veopathy. Veno-occlusive disease |
| Whipple's disease | Very rarely hepatic granulomas |
| Graft-versus-host disease [16] | Marked injury of intrahepatic bile ducts. Lymphocytic infiltration is scant<br>Cholestasis. Focal liver cell necrosis |
| Sarcoidosis | See Chapter 95 |
| Amyloidosis | See Chapter 87 |
| HELLP syndrome | See Chapter 100 |
| Neurofibromatosis (von Recklinghausen's disease) | Obstructive jaundice due to occlusion of the ampulla of Vater by neurofibromas (very rare) |
| Heat stroke [42, 45] | "Hepatitis" of unknown cause. Often cholestatic with signs of liver failure<br>*Laboratory findings*: AST, ALT are ↑ up to several 1,000 IU/L. Prothrombin activity is ↓<br>*Histology*: microvesicular steatosis and congestion in the early stages. Perivenular (zone 3) necrosis in severe cases |
| Turner's syndrome [41] | NAFLD<br>Nodular regenerative hyperplasia<br>Multiple focal nodular hyperplasias<br>Cirrhosis |
| Generalized pustular psoriasis [48] | Jaundice<br>*Laboratory findings*: γ-GT, AP, aminotransferases are ↑<br>*Histology*: neutrophilic cholangitis<br>*MRCP*: bile duct features similar to primary sclerosing cholangitis |
| Cystinosis [38] | Nodular regenerative hyperplasia with severe portal hypertension |
| Chronic granulomatous disease [17, 26] | AP and ALT ↑<br>High incidence of liver abscesses and drug hepatotoxicity. Noncirrhotic portal hypertension<br>*Histology*: granuloma, lobular hepatitis, venopathy of the central vein, nodular regenerative hyperplasia |
| Anorexia nervosa [40] | Acute liver insufficiency is a rare complication of anorexia nervosa with extremely poor nutritional status. Starvation-induced autophagy and endoplasmic stress may be involved in liver cell death |

↑ Elevated, ↓ decreased. *AP* alkaline phosphatase, *AST* aspartate aminotransferase, *ERCP* endoscopic retrograde cholangiopancreatography, *γ-GT* γ-glutamyl transpeptidase, *GLDH* glutamate dehydrogenase, *LDH* lactic dehydrogenase, *MRCP* magnetic resonance cholangio-pancreatography, *NAFLD* nonalcoholic fatty liver disease, *SLE* systemic lupus erythematosus

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# Intrahepatic Cholestasis of Pregnancy

# 98

Henryk Dancygier

## Chapter Outline



## Definition

Intrahepatic cholestasis of pregnancy (ICP) is an intrahepatic cholestasis of unknown origin occurring in the second or third trimester of pregnancy. It is characterized by general pruritus and an increase in total bile acid levels in serum. The outcome for the mother is excellent, though the sequelae for the fetus may be grave [5, 14].

## Epidemiology

ICP is the most common pregnancy specific liver disease. Its prevalence exhibits geographical, ethnic and seasonal variations. The highest prevalence rates with 9–14% are reported from Bolivia and Chile with certain indigenous populations displaying prevalence rates of up to 27% [17]. For unknown reasons, prevalence rates have been falling in South America in the last 2 decades to current rates of 4–6.5% [5]. The prevalence of ICP in the United States, Canada, Australia and Central Europe is only 0.2–0.5% [2]. The highest prevalence rates are in Europe, where in Sweden and Finland they are 1–2%, with the highest rates observed during the winter months.

A positive family history is present in nearly half of women. ICP recurs in 40–70% of cases in each subsequent pregnancy. Women with multiple pregnancies are at a higher risk of recurrence.

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## Etiology and Pathogenesis

The exact etiology and pathogenesis of ICP are unknown. Genetic, hormonal and not precisely defined environmental factors are assumed to be important. The high prevalence of the disease in certain populations and the frequently positive family history indicate a genetic background. Genetically determined variants of canalicular transporters have been reported to represent risk factors for the development of ICP [11, 22, 23]. At least ten different MDR3 mutations have been identified in ICP, and MDR3 mutations may account for 15% of cases of ICP [10, 21].

Estrogens definitely are involved in the development of cholestasis. An exaggerated hepatic reaction to estrogens is being discussed. Genetically susceptible patients often develop cholestasis after the intake of estrogens, for example oral contraceptives. Withdrawal of oral progesterone during pregnancy leads to a regression of ICP in some women. Therefore it is supposed that progesterone may also promote the clinical manifestation of ICP in some women. In addition to hormonal factors, liver injury by reactive oxygen species due to a reduced activity of glutathione peroxidase and selenium deficiency (selenium is a cofactor for this enzyme) is discussed.

The common final pathophysiological pathway appears to be a change in the lipid composition and fluidity of hepatocyte membranes with consequent dysfunction of canalicular and ductular transport systems.

The pathophysiological significance of markedly increased bile acid concentrations in serum with shifting of molar ratios from primary to secondary and from trihydroxylated to dihydroxylated bile acids is unclear. Bile acids probably are involved in the origin of pruritus. However, the observation that an excruciating pruritus may be present or even worsen with low and decreasing bile acid levels indicates that additional factors must be operative. Enhanced stimulation of centrally expressed opiate receptors by enkephalins is discussed in this regard.

Recently an increased intestinal permeability was detected in ICP patients during and after pregnancy. It was hypothesized that a "leaky gut" may participate in the pathogenesis of ICP by enhancing the absorption of bacterial endotoxin and the enterohepatic circulation of cholestatic metabolites of sex hormones and bile salts [18].

## Pathology

Histologically, a bland cholestasis is present with bile deposits in the hepatocytes and canalicular bile plugs (bilirubinostasis) predominantly in perivenular hepatocytes. Inflammatory changes are lacking or only very scant.

## Diagnosis

### *Clinical Manifestations*

The cardinal symptom of ICP is pruritus. It begins on the palms and the soles, and becomes generalized within a short period of time. It may be so severe as to cause chronic insomnia and daytime fatigue. Pruritus sets in after the 30th week of pregnancy in 80% of the women. Approximately 10–20% of patients develop dark urine and a mild jaundice 1–4 weeks after the onset of pruritus that may be accompanied by mild nausea and a sense of pressure in the right upper quadrant. Fewer than 2% of patients complain of only a mild jaundice without pruritus. Often cholestasis is preceded by a urinary tract infection.

A thorough history often will reveal a mild steatorrhea that may result in weight loss and vitamin K deficiency.

### *Laboratory Findings*

The aminotransferases are elevated up to twofold to tenfold the upper limit of normal in 80% of affected women. ALT levels are slightly higher than AST concentrations, and ALT is more sensitive than AST. Bilirubin concentration in serum is normal or only mildly increased, up to 6 mg/dL. The γ-GT can be normal or elevated up to twofold to fourfold the upper limit of normal in approximately half of the patients. Elevations of serum AP levels up to fourfold the upper limit of normal are difficult to interpret, since the enzyme may be placental in origin. A *rise of total bile acid concentration in serum up to 10–100-fold the upper limit of normal* with a *shift of the ratio of cholic acid to chenodeoxycholic acid (CA:CDCA > 1)* is characteristic of ICP. This marked

increase in serum bile acids may be the only laboratory abnormality present in some women. Therefore, *in patients with pruritus in pregnancy and normal routine liver chemistries measurement of bile acid levels in serum may be reasonable*.

### Imaging Techniques

On ultrasound the liver appears normal. Ultrasound is primarily performed in order to exclude the presence of dilated bile ducts which would suggest obstructive cholestasis rather than ICP. Magnetic resonance imaging is not indicated and computed tomography is contraindicated in pregnancy. A liver biopsy is not needed to diagnose ICP, but may be required to exclude more serious liver disease.

## Differential Diagnosis

ICP is a diagnosis of exclusion and one should be aware that in Europe and in North America ICP is a very rare disease. The diagnosis is based on the signs and symptoms described above and ICP must be differentiated from other pruritogenic diseases (with or without jaundice) in pregnancy. Some differential diagnostic features of pregnancy-specific diseases associated with pruritus and cholestatic jaundice are listed in Table 98.1. However, one should keep in mind that the most common cause of jaundice during pregnancy is acute viral hepatitis.

Fever, abdominal pain and encephalopathy are not part of an uncomplicated ICP. Hepatomegaly, marked right upper quadrant pain and fever usually indicate a mechanical obstruction to bile flow and/or inflammation of bile ducts. Bile duct obstruction by stones, chronic inflammation of the pancreatic head, primary bile duct disorders, such as primary biliary cirrhosis and primary sclerosing cholangitis or drug induced cholangiopathies should be excluded. An acute viral hepatitis should be considered in endemic areas in patients with an appropriate history. Atypical presentation of ICP includes pruritus without elevation of aminotransferases, onset of jaundice prior to pruritus, onset of symptoms before the 21st week of pregnancy or persisting pruritus and cholestasis after delivery. In all these instances the differential diagnosis must include the entire spectrum of non-pregnancy specific pruritogenic and cholestatic dermatological and internal diseases.

## Course and Prognosis

ICP is a benign disease which never leads to chronic progressive liver disease. Hepatic dysfunction normalizes within 2–4 weeks after delivery. Only in isolated cases may cholestasis persist for longer periods of time. In these cases the risk of post-partum bleeding is increased. Usually pruritus lessens within a few hours after delivery and may even disappear before delivery, especially after withdrawing oral progesterone. ICP, however, tends to recur with each pregnancy, and especially in multiple pregnancies relapse rates of 40–70% occur.

**Table 98.1** Differential diagnosis of pruritus typical of pregnancy and cholestatic jaundice

| | Pruritus | Jaundice | ↑ ALT | Pain |
|---|---|---|---|---|
| **1st Trimester** | | | | |
| Hyperemesis gravidarum | Rare | 10% | 25% | No |
| **2nd and 3rd Trimester** | | | | |
| ICP | >90% | 10–20% | ~80% | No |
| AFLP | (Yes) | 90–100% | Yes | Diffuse |
| HELLP | Yes | Yes | Yes | Diffuse |
| Toxemia of pregnancy | Yes | Yes | Yes | In impending liver rupture |

*ICP* Intrahepatic cholestasis of pregnancy, *AFLP* acute fatty liver of pregnancy, *HELLP* hemolysis, elevated liver enzymes, low platelets (HELLP syndrome)
Source: According to [5]

The main risk in ICP is to the fetus. Fetal complications are placental insufficiency, premature labor, and sudden fetal death. Premature labor occurs in 44% of cases, meconium stained amniotic fluid is seen in approximately 27%, and signs of immaturity of the newborn are observed in 60% of cases. The prevalence of still births is increased and the perinatal mortality rate reaches approximately 10% [2, 5, 6].

The pathophysiology of fetal injury is unclear and there are no reliable markers that indicate fetal threat. Bile acids are believed to play a role in the pathophysiology of ICP. Fetal complications correlate with maternal bile acid levels, with premature delivery, asphyxial events, and meconium staining occurring only in the 19% of cases with maternal bile acid levels greater than 40 µmol/L [7]. Pregnancies of women with very high serum bile acid levels must therefore be monitored very attentively. Preterm delivery between the 36th and 38th week of gestation is the goal of management, provided fetal lungs are mature. It is probably due to this increased vigilance that perinatal mortality has been lowered from approximately 10% to 4% in the last few years [16].

A recent, large Finnish population study with more than 10,000 patients showed that patients who have had ICP subsequently have more gallstones and cholecystitis, more nonalcoholic pancreatitis, more hepatitis C, and more nonalcoholic cirrhosis. Therefore, in some patients, ICP may be an indicator of more serious, subsequent liver disease [10, 20].

## Therapy

The most important aims of treatment are amelioration of pruritus and, above all, decreasing the incidence of fetal complications. Diagnosis of ICP necessitates the referral to a center specialized in high-risk pregnancies.

*Ursodeoxycholic acid* (UDCA) is the drug of choice in ICP [8, 9]. UDCA 14–16 mg /kg p.o. qd, divided in two to three daily doses, over 3 weeks leads to improvement of symptoms and laboratory chemistries [4, 15]. High-dose UDCA (1.5–2.0 g p.o./day) reduces abnormal maternal and fetal bile acid levels and is completely safe for the fetus [13]. UDCA improves pruritus and biochemical cholestasis, and facilitates deliveries at term in ICP patients, with a higher birthweight compared with historical controls [24].

*S-adenosyl L-methionine* (SAMe) improves estrogen-induced impairment of bile flow and membrane alterations in animal experiments. Results in humans, however, are conflicting. Although SAMe is less effective than UDCA it may have an additive effect to UDCA [3, 19]. *Antihistamines* are not effective. Moreover, they impair the reaction capacity and increase fatigue.

Oral *cholestyramine* 4–6 g p.o. bid or 3–4 g p.o. tid may aleviate pruritus but usually is of limited efficacy. With prolonged regular cholestyramine intake malabsorption of fat soluble vitamins ensues. Vitamin K should be administered 10 mg i.m. qw to avoid fetal bleeding. Bleeding due to hypoprothrombinemia occasionally develops but is readily reversed by vitamin K 5 to 10 mg i.m. qd for 2–3 days.

*Dexamethasone* 12 mg p.o. qd for 7 days with stepwise tapering thereafter exhibits a beneficial effect in isolated cases and may promote fetal lung maturity.

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# Acute Fatty Liver of Pregnancy

# 99

Henryk Dancygier

## Chapter Outline



## Definition

Acute fatty liver of pregnancy (AFLP) is a rare, poorly understood life threatening disorder occurring almost exclusively in the third trimester of pregnancy. It resolves after delivery and usually does no recur. Histologically, the disease is characterized by microvesicular steatosis. Clinical signs and symptoms are those of acute liver failure [1, 3, 5].

## Epidemiology

The disease is distributed worldwide without preference of geographical regions or ethnic groups. The incidence in hospital-based studies is 1:1,500 to 1:7,000 deliveries. Unlike the HELLP syndrome (see Chapter 100), 40–50% of patients with AFLP are nulliparous, with an increased incidence in twin pregnancies. The incidence estimated from a recent population-based cohort of women was lower than reported in earlier hospital-based studies. The estimated incidence of AFLP was 5.0 cases per 100 000 maternities, 18% of women had twin pregnancies and 20% were underweight (BMI < 20 $kg/m^2$) [4]. The risk of recurrence in a new pregnancy is not increased.

## Etiology and Pathogenesis

Both are unknown. Impairment of mitochondrial fatty acid oxidation in genetically susceptible women is thought to cause microvesicular steatosis. Homozygous mutations of fetal long-chain 3-hydroxyacyl-CoA

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dehydrogenase (LCHAD) associated with maternal AFLP are well documented [2]. LCHAD is part of a trifunctional mitochondrial enzyme that catalyzes several steps of fatty acid β-oxidation. However, it is unclear how dysfunction of fetal fatty acid metabolism leads to liver disease in the mother.

## Pathology

The lobular architecture and the trabecular arrangement of liver cells are preserved. The salient histopathological feature is microvesicular steatosis predominantly of perivenular (zone 3) hepatocytes. Affected liver cells appear foamy. Unlike in macrovesicular steatosis the cell nucleus is not pushed to the periphery but remains in its central position. Occasionally ballooned hepatocytes, necrotic cells and focal inflammatory cell infiltrates are present. In rare cases, findings may be indistinguishable from viral hepatitis. In contrast to acute viral hepatitis or drug-induced liver injury, however, hepatocyte necrosis and inflammatory infiltrates are not prominent in the vast majority of patients with AFLP. Accumulation of small fat droplets also occurs in pancreatic acinar and in renal tubular epithelial cells.

## Diagnosis

### *Clinical Manifestations*

The disease begins between the 30th and 38th gestational week, not uncommonly preceded by an infection of the upper respiratory tract. Initial symptoms include fatigue, anorexia, headache, and are soon joined by nausea and vomiting, abdominal discomfort, and jaundice, followed by a rapidly progressive hepatocellular failure in severe cases. Polydipsia, with or without polyuria, that is not explained by the amount of fluid lost by vomiting is a common early symptom. One to 2 weeks after the onset of symptoms 90–100% of patients develop a moderate jaundice that only exceptionally is accompanied by pruritus. The patients become apathetic and in severe cases hepatic coma ensues.

Symptoms persist until delivery followed by a slow recovery and complete resolution after 1–4 weeks. Only in individual cases a worsening of clinical manifestations occurs after delivery.

On physical examination signs of chronic liver disease are absent. The liver is small, and the liver margin is smooth and only rarely palpable.

### *Laboratory Findings*

Laboratory findings resemble those of fulminant viral hepatitis except that aminotransferase levels usually are less than 500 U/L, and hyperuricemia (10–20 mg/dL) may be present. γ-GT levels are normal, and AP is slightly elevated. A conjugated hyperbilirubinemia with total bilirubin levels between 5–15 mg/dL occurs. With progressive hepatocellular failure ammonia levels in serum and glutamine concentrations in cerebrospinal fluid increase, and hypoglycemia and lactic acidosis occur.

### *Imaging Techniques*

Sonography and magnetic resonance imaging are only of minor importance -in the diagnosis of AFLP. They help primarily to exclude other hepatobiliary disorders.

Liver histology is diagnostic, provided the coagulation parameters allow for a biopsy to be performed. If biopsy is performed after delivery – in the resolution phase of the disease – the hepatocellular fat may already have disappeared and the liver may appear quite normal. It is this normal histological appearance of the liver that retrospectively is in good agreement with the initial presumptive diagnosis of AFLP.

## Differential Diagnosis

The differential diagnosis includes diseases with right upper quadrant pain, such as acute cholecystitis and the many causes of acute liver failure (see Chapter 78), primarily viral and drug-induced hepatitis. A mild increase in serum aminotransferases, marked thirst and a mild rise in blood pressure suggest AFLP rather than viral hepatitis. If feasible, a liver biopsy yields a quick answer.

Hepatitis E, which often runs a severe course in pregnancy should be considered in the respective endemic regions (see Section 63.3).

Herpes simplex hepatitis is rare but has a mortality rate of up to 40%. On microscopy typical viral inclusions in the liver are seen. A vesicular eruption of the genital and oral mucosa in a patient with elevated aminotransferases should arouse the suspicion of acute herpetic hepatitis. Immediate intravenous antiherpetic (e.g., acyclovir) therapy improves the outcome.

If the clinical picture does not improve after delivery, non-pregnancy specific liver diseases have to be considered.

In Table 99.1 the distinguishing features between AFLP and the liver in toxemia of pregnancy are listed.

A microvesicular steatosis may also occur in Reye's syndrome, be drug-induced (valproic acid or high doses of intravenous tetracyclines) or toxic, for example in Jamaica vomiting sickness (herbs). Inborn urea cycle and fatty acid oxidation enzyme defects lead to microvesicular steatosis in children (Table 99.2).

**Table 99.1** Differential diagnosis between acute fatty liver of pregnancy and liver in toxemia

| | Acute fatty liver of pregnancy | Toxemia of pregnancy |
|---|---|---|
| **Abdominal pain** | 50% | 100% |
| **Jaundice** | 90–100% | 40% (serum bilirubin ≤ 6 mg/dL) |
| **Aminotransferases in serum** | <10 × ULN | >10 × ULN |
| **Sonography** | Diffuse changes | Focal changes |
| **Liver histology** | Microvesicular steatosis | Fibrin thrombi, liver cell necrosis, hemorrhages |
| **Hepatocellular failure** | Yes | No |

*ULN* Upper limit of normal

**Table 99.2** Causes of microvesicular steatosis

| |
|---|
| **Acute fatty liver of pregnancy** |
| **Reye's syndrome** |
| **Drug-induced** |
| • Valproic acid |
| • Tetracyclines |
| **Toxic** |
| • Ethanol[a] |
| • Nonalcoholic steatohepatitis[a] |
| • Herbs (Jamaica vomiting sickness) |
| **Inborn errors of metabolism (enzyme defects)** |
| • Fatty acid oxidation |
| • Urea cycle |

[a]Usually a macrovesicular or mixed steatosis is present. The so-called foamy degeneration in alcoholic liver disease is extremely rare

## Course and Prognosis

*AFLP is an obstetrical and hepatological emergency.* The diagnosis has to be established without delay and therapy must be instituted immediately. If the diagnosis is missed the prognosis is dismal. *So far no patient with AFLP has recovered spontaneously before delivery!* Maternal and fetal mortality rates are high in severe cases (up to 70–90%). Maternal mortality is usually due to acute liver and renal failure (60%). Hypoglycemia and severe infections occur in 50% of patients, upper gastrointestinal bleeding in 33% and parenchymal hemorrhages in various organs due to hepatic coagulopathy in 30%. Acute pancreatitis occurs in fewer than 10% of cases [6].

Increased awareness of the severity of AFLP, early recognition and prompt delivery or termination of pregnancy have reduced maternal mortality to 0–20%. Most patients improve markedly after delivery, recover completely and have no recurrences. Hepatocellular failure progresses after delivery in only very few patients, necessitating liver transplantation.

Despite recent data suggesting that maternal and neonatal outcomes are better than previously reported, fetal mortality rates remain high, approximately 104 per 1,000 births [4]. Treatment in a neonatal intensive care unit in a specialized obstetrical-pediatric center improves child prognosis.

## Therapy

There is no specific therapy of AFLP. The presumptive diagnosis of AFLP is made on compatible clinical and laboratory features and requires immediate hospitalization in a specialized center and intensive supportive care. Therapeutic decisions (treatment of acute liver failure, immediate termination of pregnancy) are made without histological confirmation of the diagnosis. Orthotopic liver transplantation is the ultimate therapeutic option. Preterm delivery by caesarean section improves fetal survival.

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# The Liver in Toxemia of Pregnancy

# 100

Henryk Dancygier

## Chapter Outline



## Definition

*Preeclampsia* denotes the occurrence of hypertension, proteinuria and edema after the 20th week of gestation in a previously normotensive woman. If cerebral seizures or a coma supervene, *eclampsia* is said to be present.

## Epidemiology

Preeclampsia occurs at the end of the second and in the third trimester in 5–10% of all pregnancies, eclampsia in 1:150–350 pregnancies and in 3.6% of twin pregnancies. Liver involvement, although infrequent, always indicates severe preeclampsia with significant perinatal morbidity and mortality.

## Etiology and Pathogenesis

The etiology is unknown. Pathophysiologically deranged placental development including poorly developed uterine placental spiral arterioles with placental ischemia or infarction play an important role. Diffuse or multifocal vasospasm may result in ischemia and necrosis of multiple organs including the placenta. Placental ischemia possibly leads to the release of reactive oxygen species and lipid peroxides that damage the endothelium. Generalized endothelial dysfunction activates the coagulation system with formation of platelet and fibrin microthrombi which result in arteriolar occlusion with organ ischemia and organ failure. Thus, preeclampsia is a multisystem disease with predominant involvement of kidneys, brain, cardiovascular system and the liver [2, 3].

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## Pathology

The histopathological correlate of severe preeclampsia in the liver is hyaline fibrin thrombi within the sinusoids, periportal single cell and group necrosis and parenchymal hemorrhage. The development of subcapsular hematomas predisposes to liver rupture. Focal areas of microvesicular steatosis may also occur. Unlike acute fatty liver of pregnancy, however, steatosis is only mild and is predominantly localized in periportal (zone 1) hepatocytes.

## Diagnosis

### Clinical Manifestations

The clinical manifestations are characterized by the signs and symptoms of severe preeclampsia, such as visual disturbances, headaches, edema, hyperreflexia, changes of the ocular fundus (Table 100.1). The hepatic manifestations are not prominent initially. With continuing liver damage abdominal pain, nausea, vomiting, and mild jaundice may occur and with extensive hepatic parenchymal necrosis acute liver failure may supervene. Rapidly developing large subcapsular hematomas lead to capsular tension with right upper quadrant pain.

**Table 100.1** Criteria for severe preeclampsia (From [4])

| |
|---|
| **Blood Pressure** |
| > 160 mmHg systolic or > 110 mmHg diastolic |
| **Proteinuria** |
| ≥ 5 g in 24 h |
| **Acute Renal Failure** |
| **Oliguria** |
| < 400 mL/ 24 h |
| **Grand mal Seizures** (eclampsia) |
| **Pulmonary Edema** |
| **HELLP–Syndrome** |
| **Thrombocytopenia** |
| < 100000/mm$^3$ |
| Symptoms indicating significant organ involvement: headache, visual disturbances, epigastric or right upper quadrant pain |

### Laboratory Findings

Serum levels of aminotransferases are variable from mild to 10- to 20-fold the upper limit of normal (depending on the extent of parenchymal necrosis). Bilirubin levels are usually less than 5 mg/dL.

### Imaging Techniques

Ultrasound, CT and MRI show focal lesions corresponding to parenchymal necrosis and hemorrhages. Subcapsular hematomas are easily visualized by all three techniques.

## Complications

### HELLP Syndrome

HELLP syndrome is a complication of severe preeclampsia and is characterized by hemolysis (H), elevated liver enzymes (EL), and low platelet count (LP).

It occurs in 2–12% of cases of severe preeclampsia and in 0.2–0.6% of all pregnancies [2]. The incidence is higher in whites and the risk of recurrence is increased (3–27%) in a following pregnancy. Approximately two thirds of cases occur between the 27th and 36th week of gestation, and 30% post partum. Since the diagnostic criteria of HELLP syndrome are not defined exactly these epidemiological data should be interpreted with caution.

Clinically useful criteria of HELLP syndrome are

- Hemolysis
  - Abnormal blood smear
  - Total bilirubin in serum > 1.2 mg/dL (elevated indirect bilirubin)
  - LDH > 600 U/L
- Elevated liver enzymes
  - AST > 70 U/L
- Low platelets
  - Platelet count < 150,000/mm$^3$

HELLP syndrome may be subdivided, based on platelet count, into severe (platelets < 50,000/mm$^3$), moderate (50–99,000/mm$^3$), and mild (100–150,000/mm$^3$) [1].

The *pathophysiology* of HELLP syndrome is characterized by vascular endothelial injury, fibrin deposition in blood vessels, and platelet activation with platelet consumption. Variably extensive areas of hemorrhage and necrosis beginning from zone 1 and occasionally involving the entire lobule occur in the liver. Subcapsular hematomas may lead to capsular tears, and intraperitoneal bleeding.

*Clinically,* the disease begins with general malaise (90%) followed by epigastric and right upper quadrant pain (65–95%). Nearly 50% of patients complain of nausea and vomiting, and 30% of headaches. Jaundice occurs in only 5% of cases. A polyuria due to a transient nephrogenic diabetes insipidus is observed rarely [1].

Since initial symptoms are nonspecific complete blood count and liver enzymes should be determined already when general malaise occurs in a pregnant woman after the 27th week of gestation.

The hallmark of HELLP syndrome is *microangiopathic hemolytic anemia*. Serum LDH levels are elevated. Aminotransferase elevation (AST > ALT) is variable, from mild to 10- to 20-fold the upper limit of normal, and bilirubin is usually less than 5 mg/dL. Platelet counts usually decrease to less than 100,000/mm$^3$. Fibrinogen, prothrombin time and partial thromboplastin time are normal in the majority of cases.

Sonography and computed tomography of the liver may show subcapsular hematomas, intraparenchymal hemorrhage and infarction or hepatic rupture with intraperitoneal bleeding.

*Complications* of severe HELLP syndrome are disseminated intravascular coagulation (20–40%), abruption of placenta (16%), acute renal failure (7%) and pulmonary edema (6%) [1].

The *differential diagnosis* of HELLP syndrome includes the early stages of acute fatty liver of pregnancy (AFLP). Serum fibrinogen is usually decreased and prothrombin time and partial thromboplastin time are prolonged in AFLP. Elevation of aminotransferases is less pronounced in HELLP syndrome than in AFLP.

Further diseases that have to be differentiated from HELLP syndrome are the hemolytic uremic syndrome and thrombotic thrombocytopenic purpura (see textbooks of Internal Medicine).

## Prognosis and Therapy

Severe preeclampsia is a life threatening condition for mother and child. Depending on the severity of HELLP syndrome, perinatal fetal mortality rates may reach 35%. Newborns have an increased risk of thrombocytopenia.

No specific therapy is needed for the hepatic involvement of preeclampsia, and its only significance is as an indicator of severe disease with need for immediate delivery to avoid eclampsia, hepatic rupture, or necrosis [2]. Corticosteroids are of no proven benefit, but successful treatment has been reported in individual cases. Obviously, hepatic rupture requires immediate surgical intervention.

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# Benign Tumors

# 101

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## Chapter Outline



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**Table 101.1** Classification of primary liver tumors [1–6]

| | Benign tumors | Malignant tumors |
|---|---|---|
| **Epithelial tumors** | Hepatocellular adenoma<br>Biliary adenoma<br>Biliary cystadenoma<br>Biliary papillomatosis | Hepatocellular carcinoma (HCC)<br>Fibrolamellar carcinoma<br>Hepatoblastoma<br>Cholangiocellular carcinoma (CCC)<br>Combined HCC/CCC<br>Biliary cystadenocarcinoma<br>Squamous cell carcinoma |
| **Nonepithelial tumors** | Hemangioma<br>Infantile hemagioendothelioma<br>Angiomyolipoma<br>Myelolipoma<br>Lymphangioma/lymphangiomatosis<br>Leiomyoma | Angiosarcoma<br>Epithelioid hemagioendothelioma<br>Kaposi sarcoma<br>Lymphoma<br>Embryonal sarcoma<br>Rhabdomyosarcoma<br>Leiomyosarcoma<br>Malignant fibrous histiocytoma<br>Malignant schwannoma |
| **Tumor-like lesions** | Hamartomas<br>Mesenchymal hamartoma<br>Biliary hamartoma<br>Cysts<br>Focal nodular hyperplasia<br>Nodular regenerative hyperplasia<br>Hepatic peliosis<br>Inflammatory pseudotumor<br>Focal steatosis<br>Compensatory lobular hyperplasia<br>Postnecrotic regenerative nodules<br>Dysplastic nodules<br>Hereditary hemorrhagic telangiectasia | |

## Introduction

In Chapters 101 and 102 only primary liver tumors, i.e. tumors that originate in the liver are discussed. An overview of these tumors is given in Table 101.1 (see also Table 37.1).

For ultrasound, CT and MRI images of tumors see Chapters 37 and 38.

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# 101.1 Hepatocellular Adenoma

## Definition

Hepatocellular adenoma (HCA) is a well demarcated, benign monoclonal epithelial tumor composed of cells resembling normal hepatocytes. Arterial vessels and draining veins are distributed throughout the tumor, while portal tracts and central veins are absent. Large HCA are surrounded by a thin capsule-like fibrous sheet.

## Epidemiology

Before the era of oral contraceptives HCA was a rarity. Since the early 1970s an increase in incidence has been observed, which could be attributed to the use of oral contraceptives. The tumors are seen almost exclusively in women of childbearing age with a long-term history of oral contraceptive use, but rare cases occurring in children are also documented. Without the use of oral contraceptives the incidence of HCA is $1/10^6$. With long standing use the incidence increases to $3–4/10^5$ and is directly related to the duration of drug intake and hormone dose. Women taking oral contraceptives for 9 years have a 25-fold greater frequency of developing HCA than do women without hormone consumption [18]. Modern drug compounds with lower hormone concentrations seem to cause HCA less frequently.

## Etiology

Oral contraceptives are the most frequent cause of HCA with the estrogen component playing the main causative role [9]. In men the administration of anabolic androgens ($C_{17}$-alkylated steroids) may induce the formation of HCA.

In addition HCA are encountered in 28% of patients with glycogen storage disease Type I and in 10% of patients with glycogen storage disease Type III [19]. In HCA associated with glycogen storage disease, men are affected more often than women, the tumors are more often multiple, and they have a higher propensity to undergo malignant transformation. Isolated cases of HCA may be associated with virilizing and feminizing ovarian tumors, polycystic ovary syndrome, Klinefelter's syndrome, severe combined immunodeficiency, Hurler's disease, β-Thalassemia and the intake of clomiphene and carbamazepine.

## Pathogenesis

Hormonally and metabolically induced liver cell proliferation leads to the formation of HCA. However, the exact pathogenetic mechanism of how sex hormones initiate hepatocellular proliferation is unknown. Sex hormones are promoters of hepatocellular tumors and sex hormone receptors have been detected on some HCA [7]. HCA may regress after discontinuation of oral contraceptives. On the other hand, hormonal stimulation during pregnancy and in the postpartum period increases the risk of HCA development and the growth of preexisting adenomas, and is associated with a heightened risk of rupture.

HCA are consistently *monoclonal tumors* (in contrast to most focal nodular hyerplasias which are polyclonal). They have been divided up into three subtypes depending on the molecular alteration detected in the tumors: (1) hepatocyte nuclear factor-1α (HNF-1 α)-inactivated, (2) β-catenin-activated, and (3) inflammatory subtype. These molecular features are closely related to clinical and pathological characteristics. There is a higher risk of malignant transformation for β-catenin activated HCA cases [4, 17]. β-catenin is a multifunctional cytoplasmic protein that plays a role in hepatic physiology. It regulates hepatocyte proliferation and differentiation during development and regeneration. Mutants of β-catenin may translocate into the nucleus and activate gene transcription, thereby stimulating hepatocellular proliferation. In addition β-catenin plays an essential role in the E-cadherin/catenin complex in the maintenance of cell–cell adhesion, the loss of which may lead to tumor invasion.

Methylation of tumor suppressor proteins in HCA and the association of germline HNF-1α mutations (HNF-1α inactivation) with familial liver adenomatosis also highlight the importance of genetic factors in the development of HCA [1, 20].

## Pathology

HCA usually develops in a normal liver with the exception of livers affected by glycogen storage diseases. Most tumors are solitary, but multiple lesions (>10 adenomas = *liver cell adenomatosis*) may occur, especially in persons taking androgenic steroids or with glycogen storage diseases [5, 12]. Most HCA are found in a subcapsular location and measure 5–15 cm in diameter, although lesions up to 30 cm in diameter and HCA arising from the liver as a pendulous mass have been described. Due to lipid accumulation their cut surface is light brown to yellow. The tumors are well demarcated and compress the surrounding liver tissue. A true capsule is not present but large tumors are surrounded by a thin capsule-like fibrous layer containing large vessels. Prominent arterial vessels and draining veins are distributed throughout the tumor parenchyma. The extensive hypervascularity makes these tumors prone to hemorrhage and regressive changes, such as necrosis.

*Microscopically*, HCA consist of somewhat irregularly arranged sheets of normal looking or slightly atypical appearing hepatocytes; however, frank cellular atypia is not a feature of HCA. The normal acinar structure is lacking. Due to cytoplasmic glycogen and fat, the tumor cells are larger than normal hepatocytes and their cytoplasm is clearer. Large amounts of Dubin-Johnson pigment (lipomelanin) or Mallory-Denk bodies are rarely present. Immunohistochemical expression of $\alpha_1$-fetoprotein is lacking. The adenomatous cells may be arranged in a pseudoglandular, rosette-like pattern surrounding a lumen containing a bile plug. The nucleus-to-cytoplasm ratio is normal and mitoses are absent. Foci of malignant transformation are very rarely encountered and dysplastic areas ("atypical adenoma") may be misinterpreted for hepatocellular carcinoma (HCC). In contrast to intestinal adenomas, HCA are not prone to the adenoma-carcinoma sequence and only exceptionally may harbor HCC [3, 10]. Distinguishing HCA from a well differentiated HCC may be difficult. Nuclear irregularities, a high nucleus-to-cytoplasm ratio, prominent nucleoli, numerous mitoses, as well as multiple chromosomal aberrations suggest a carcinoma. The potential relevance of endothelial cell immunophenotyping for the differential diagnosis between HCA and well differentiated HCC is of questionable value [11]. Adenomas with β-catenin activation have a higher risk of malignant transformation, while hepatic adenomas displaying HNF-1α mutations have an overall benign course [23].

Usually HCA do not have a collagenous stroma. They do possess, however, a well developed reticulin fiber network. Kupffer cells, endothelial cells, and stellate cells are present. Portal tracts, bile ducts and central veins are typically absent. Some HCA may contain small groups of hematopoietic cells. Hemosiderin laden macrophages suggest previous hemorrhage. Thick-walled arteries run at the periphery of the tumor, while inside the HCA thin walled, dilated vessels and blood filled sinusoids resembling peliosis may be observed.

Using genetic molecular techniques, it has recently been shown that "telangiectatic focal nodular hyperplasia", hitherto considered to be a variant of focal nodular hyperplasia (FNH), displays a molecular pattern closer to that of HCA than to FNH. This suggests that these lesions may represent a telangiectatic subset of HCA [15]. Histological analysis of *telangiectatic adenoma* shows cellular atypias in 19%, and a significant inflammatory infiltrate in 91% of cases. In up to 3% of the telangiectatic variant of HCA foci of well-differentiated hepatocellular carcinoma have been reported [16].

## Diagnosis

### *Clinical Manifestations*

Most HCA are asymptomatic. Generally they are discovered incidentally during a sonographic examination. Telangiectatic HCA has been reported to occur in a characteristic background of overweight patients often associated with a biological inflammatory syndrome [16]. When symptoms occur they usually result from an increase in size of the adenoma, from intratumoral hemorrhage, or from intraperitoneal rupture.

Twenty to 25% of patients complain of recurrent mild to moderate right upper quadrant or epigastric pain, diminished appetite, and nausea. Fever is rare. Giant HCA may present with chronic iron deficiency anemia, requiring repeated blood transfusions [6]. Acute abdominal pain due to tumor bleeding or life threatening intraperitoneal rupture occurs in approximately 30% of the cases, oftentimes during monthly menstruation.

Rare complications of HCA are systemic AA amyloidosis and paraneoplastic syndromes caused by secretion of cortisol, ACTH or ACTH-like peptide by the tumor [8, 13, 22].

### Laboratory Findings

Serum chemistries are not helpful in the diagnosis of HCA. Liver enzymes are normal in most patients, though a few may have mild nonspecific elevations of aminotransferases, alkaline phosphatase and γ-GT. Serum $\alpha_1$-fetoprotein is not increased.

### Imaging Techniques

Imaging methods (US, CT, MRI) can detect even small (<1 cm) HCA as sharply demarcated masses, but their specificity is low (see Figs. 37.3 and 38.5).

On *ultrasonography* adenomas may be hypo-, iso- or hyperechoic. Irregularly delineated echopoor intratumoral areas represent hemorrhage and/or necrosis. On color or power Doppler, peritumoral blood flow in a "basket"-like pattern may be seen. Peripheral arterial vessels penetrate the tumor parenchyma, and diffuse contrast enhancement of the tumor may be observed during the arterial phase.

On *dynamic CT* hepatic adenomas are hypodense, becoming hyperdense in the arterial phase and isodense in the portal venous phase.

*MRI* images of HCA show variable signal intensity, depending on the amount of fat, glycogen, bleeding and necrosis present. A recent study has suggested that MRI imaging features may depend on the molecular pathology of HCA with HNF-1 α-mutated HCA and inflammatory HCA being associated with specific MRI patterns related to diffuse fat repartition and sinusoidal dilatation, respectively [14].

Contrary to previous views HCA do contain Kupffer cells. Therefore they cannot be differentiated reliably from other space occupying lesions by *scintigraphy* with ($^{99m}$-Tc) sulfur colloid (normally taken up by Kupffer cells) or by MRI using Kupffer cell specific contrast agents.

Since at the present time all imaging modalities, however dynamic, yield nonspecific results in the diagnosis of HCA, the "confirmation" of high quality sonographic findings by CT or MRI is neither warranted nor cost effective.

As long as malignancy cannot be ruled out, a space occupying liver lesion of unknown etiology should be biopsied even if the value of histologic examination of an adenoma may be limited by the lack of specific features in a small specimen. Small specimens will, however, allow for the differentiation of a benign from a malignant lesion, which will be of utmost importance to the patient.

Percutaneous needle biopsy of an HCA is associated with an increased risk of subcapsular and intraperitoneal bleeding. This risk can be minimized by using a correct biopsy technique. Approaching the tumor through a tract of normal liver tissue that will serve as a cover to the puncture site is preferred to choosing the shortest, most direct path.

## Differential Diagnosis

It is most important to distinguish HCA from a highly differentiated HCC. There are no reliable sonographic, CT or MRI criteria that enable this distinction. Even on liver biopsy this differentiation might be challenging. Features that favour a diagnosis of HCC include thickened liver cell plates with more than three cells, a "nodule-in-nodule" growth, the formation of pseudoglandular structures, and loss of the reticulin fiber network.

The loss of Kupffer cells in HCC has been the basis for attempts using scintigraphy and MRI to differentiate HCC from HCA, using substances that are stored in cells of the macrophage-phagocyte system. Unfortunately, these efforts too do not yield specific results.

In contrast to HCA, focal nodular hyperplasia is characterized by a central fibrous scar containing vessels that can be visualized by contrast sonography, dynamic CT and MRI-angiography. Clinically, most FNH are silent whereas HCA cause abdominal pain much more often. The detection of bleeding and/or necrosis in a tumoral mass favors the diagnosis of HCA.

The differentiation from metastases is by liver biopsy.

## Prognosis and Therapy

A woman on an oral contraceptive should be advised to discontinue the drug. Monitoring by ultrasound will determine if the tumor regresses, in which case a resection generally will not be needed. Due to the risk of rupture, intraabdominal bleeding and malignant transformation (rare, though HCA associated with glycogen storage disease have a higher risk of malignant transformation) are two indications for operative resection, especially for enlarging tumors or HCA greater than 5 cm in diameter. A ruptured adenoma should be stabilized by selective arterial embolization prior to resection [21]. In cases of non-resectable tumors, arterial chemoembolization may be attempted, or in very rare instances, orthotopic liver transplantation is an option.

Molecular predictors such as β-catenin activation and HNF-1α mutations might identify patients with a higher risk for malignancy or with a more benign course, respectively [2]. These cytogenetic techniques are becoming increasingly available and will, in the future, hopefully assist in therapeutic decision making (i.e. whether to pursue a conservative or an aggressive operative approach.)

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## 101.2 Nodular Regenerative Hyperplasia

Nodular regenerative hyperplasia (NRH) was first described as "miliary hepatocellular adenomatosis" by Ranstrom in 1953 [10]. The term NRH was subsequently coined by Steiner in 1959 [13].

### Definition

NRH is characterized by multiple regenerative hepatocellular nodules up to 3 mm in diameter that compress the surrounding parenchyma and are not surrounded by fibrous tissue. In general, the regenerative process is diffuse throughout the entire hepatic parenchyma, but in rare cases NRH may be circumscribed.

### Epidemiology

In a large autopsy study, 64 cases of NRH were discovered in 2,500 consecutive autopsies (2.6%) [15]. There is no gender predilection. The majority of patients are 50 years or older with an increasing incidence in patients over 70–80 years, likely reflecting the higher prevalence of systemic disease in an elderly population. Cases of NRH have also been described in children, however, and familial occurrence has been reported [3].

### Etiology

The conditions associated with NRH are listed in Table 101.2. NRH is observed in a variety of extrahepatic diseases, including hematologic, lymphoproliferative, and, particularly rheumatologic disorders, such as rheumatoid arthritis, Felty's syndrome, progressive systemic sclerosis, and its CREST variant (calcinosis cutis, Raynaud's phenomenon, esophageal dysmotility, sclerdodactyly, telangiectasias). Liver involvement in primary hypogammaglobulinemia mainly consists of NRH leading to chronic cholestasis and portal hypertension [7]. Recently the occurrence of NRH in a patient with cystinosis has been reported [9].

**Table 101.2** Conditions associated with nodular regenerative hyperplasia [8]

| | |
|---|---|
| **Rheumatological** | Rheumatoid arthritis<br>Felty's syndrome<br>Systemic lupus erythematosus<br>Polyarteritis nodosa<br>Progressive systemic sclerosis<br>Antiphospholipid syndrome |
| **Hematological** | Idiopathic thrombocytopenic purpura<br>Essential thrombocythemia<br>Polycythemia vera<br>Sickle cell anemia<br>Macroglobulinemia<br>Myeloid metaplasia<br>Chronic myelogenous leukemia<br>Chronic lymphocytic leukemia<br>Hodgkin's lymphoma<br>Non-Hodgkin's lymphoma<br>Primary hypogammaglobulinemia |
| **Drugs** | Azathioprine<br>Bleomycin<br>Busulfan<br>Carmustine<br>Chlorambucil<br>Cyclophosphamide<br>Cytosine arabinoside<br>Doxorubicin<br>5-Fluorouracil<br>Oxaliplatin<br>6-Thioguanine |
| **Hereditary/ congenital** | Agenesis of portal vein<br>Cardiac abnormalities<br>Cystinosis<br>Familial |
| **Other** | Toxic oil syndrome<br>Metastatic disease<br>Primary biliary cirrhosis<br>Celiac disease<br>Congestive heart failure<br>Bacterial endocarditis<br>Tuberculosis |

Some patients with NRH have received oral contraceptives, anabolic steroids, corticosteroids and immunosuppressive agents. However, a true causality between these drugs and the development of NRH has not been established. Physicians should be aware of the potential occurrence of NRH and its sequelae during chemotherapy, for example with 5-fluorouracil and oxaliplatin-based chemotherapy for metastatic colorectal cancer or 6-thioguanine for inflammatory bowel disease [4, 5]. A statistically

significant association between NRH and the conditions with which it has been described, however, has not been demonstrated.

## Pathogenesis

The pathogenesis of NRH is not well understood. Currently two theories have been advanced. The *vascular hypothesis* assumes that occlusive lesions of the portal venous system lead to an impairment of the microcirculation [6, 15]. The diminished blood flow causes atrophy and necrosis of central hepatocytes and acinar segments with a compensatory regenerative hyperplastic response of the still well perfused periportal hepatocytes. Obliterative lesions of portal vessels, mainly of portal vein branches, are often observed in NRH. Vasculitic and sclerotic alterations of intrahepatic arterial vessels associated with systemic disease may occasionally be seen too and may lead to secondary obliteration of adjacent portal veins. The second hypothesis views NRH as a *primary proliferative disorder* of the liver [14]. Recent experimental data in mice show that inducible inactivation of Notch1–receptor was associated with the development of NRH, supporting the idea that in special circumstances hepatocellular proliferation may occur, and that NRH may develop without associated vascular abnormalities [2].

NRH occurring in primary hypogammaglobulinemia is associated with intrasinusoidal T cell infiltration, portal vein endotheliitis, autoimmune diseases and peripheral lymphocytic abnormalities suggesting an autoimmune mechanism [7].

## Pathology

The main features of NRH are multiple hyperplastic nodules made up of hepatocytes without intervening fibrous septa. However, some degree of periportal and perisinusoidal fibrosis with very thin fibrous septations between the hepatic lobules may be seen [1]. The liver parenchyma between regenerating nodules is atrophic. The entire organ is diffusely transformed and on laparoscopic examination the numerous small nodules, usually 1–3 mm in diameter, impart its surface a micronodular cirrhotic appearance. Isolated larger nodules may occasionally develop.

*Partial nodular transformation* is a circumscribed variant of NRH that mainly affects the perihilar region and is composed of nodules that are larger than in diffuse NRH. Hemorrhage and necrosis may develop in larger nodules.

Due to the lack of significant fibrosis, the changes may be very subtle and difficult to identify in needle biopsies on routine hematoxylin–eosin stains. The diverse orientation and nodular growth of liver cell plates are best visualized using reticulin stains of large bore biopsies (Fig. 101.1). The nodules develop in periportal zone 1. Growing nodules collide with each other and compress central veins and portal tracts, which leads to pressure atrophy of the parenchyma between individual nodules.

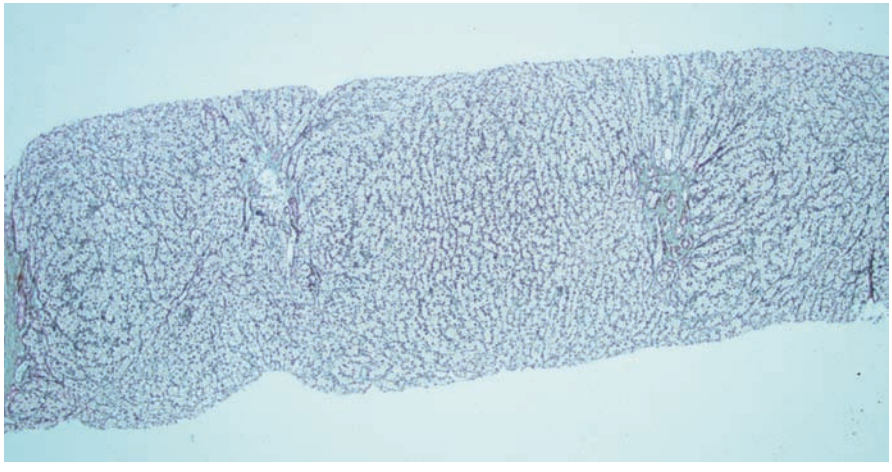

**Fig. 101.1** Nodular regenerative hyperplasia. Reticulin stain best visualizes the nodular growth of liver cells without intervening fibrous septa. Gomori (×50)

An *obliterative portal venopathy* is the characteristic vascular lesion but veno-occlusive changes of central veins or their slitlike compression may also be seen. In areas of hepatocellular atrophy the sinusoids may be dilated. The vascular lesions are best visualized by elastic fiber stains.

The regenerative nodules are composed of hepatocytes that resemble the cells of the surrounding parenchyma. The intranodular liver cells, however, are generally somewhat larger and clearer than their internodular counterparts. The orientation of intranodular liver cell plates differs from that of the extranodular regions, as the hepatocytes grow in irregularly arranged plates that are more than one cell thick. Immunohistochemical staining for $\alpha_1$-antitrypsin but not for $\alpha_1$-fetoprotein has been shown to be increased in periportal areas [8]. There is no inflammation or cholestasis, bile ducts are normal, and proliferation of bile ducts is absent.

## Diagnosis

### Clinical Manifestations

NRH develops insidiously and most patients remain asymptomatic for many years. It is not uncommon to encounter NRH as an incidental finding at autopsy. Symptoms are generally caused by the underlying disorder, e.g. rheumatological or hematological disease. When patients develop hepatic symptoms these are mostly due to portal hypertension and its complications, while synthetic liver function is preserved. Data on the frequency of occurrence of ascites, splenomegaly and esophageal varices in NRH varies between 5% and 50%. Bleeding of esophageal varices or the rupture of a nodule with acute abdominal symptoms are rare events, as is liver failure due to NRH. Partial nodular transformation may be complicated by portal hypertension relatively early in its course.

Obstructive jaundice may ensue if regenerative nodules compress or encase bile ducts.

### Laboratory Findings

Serum levels of alkaline phosphatase and γ-GT are slightly elevated in approximately 25% of patients. Serum aminotransferases, albumin, prothrombin time, and bilirubin levels are usually normal [11].

### Imaging Techniques

On ultrasound examination the nodules are either not or barely visible, and the liver displays a nonspecific irregular echopattern. Contrast-enhanced computed tomography and magnetic resonance imaging can visualize larger nodules without being able to characterize the lesions as NRH. Hypovascular areas inside nodules may be caused by hemorrhage or necrosis.

*Histological evaluation is the only way to make a definitive diagnosis of NRH*. Liver biopsies are obtained either by a percutaneous or a laparoscopic route using large bore needles. Regenerative nodules may be missed if the needle is too narrow, as is often the case with transjugular liver biopsy [11]. Laparoscopy is a rapid, simple and safe procedure that combines direct inspection of the diffuse surface nodularity with the relative ease of obtaining several large biopsies under direct visual control, thus minimizing the risk of bleeding. Open wedge biopsies are nowadays rarely required.

## Differential Diagnosis

The most important differential diagnosis is micronodular liver cirrhosis. The lack of fibrosis is the cardinal feature that differentiates NRH from cirrhotic remodeling. Larger nodules must be distinguished from liver cell adenoma, focal nodular hyperplasia and macroregenerative nodules. Partial nodular transformation must be differentiated from incomplete cirrhosis. It is important to note that different nodular lesions may coexist in the same liver, e.g. NRH and hepatocellular adenoma.

## Natural History and Prognosis

The natural history of NRH has not been well studied and the literature appears to emphasize symptomatic cases. In most cases, however, the course of NRH is likely much more indolent than is suggested by the literature. Of the 64 patients out of 2,500 autopsies

mentioned above, only one was diagnosed with NRH prior to death [14]. In most cases the disease seems stationary and does not progress. However, rare cases of hepatic failure necessitating orthotopic liver transplantation have been reported [3].

NRH is a hyperplastic-regenerative and not a neoplastic process. Nevertheless, in individual cases a relationship with adenomas might exist. Formation of large adenoma-like nodes and areas of large cell dysplasia may be the starting point for the development of hepatocellular carcinoma [12].

## Therapy

There is no treatment for diffuse NRH. In progressive disease, management is directed at treating the underlying disease and the sequelae of portal hypertension (see Chapters 53 and Section 80.1). It is not known whether NRH reverses once the underlying disorder has been treated successfully. Only in very rare cases will orthotopic liver transplantation be necessary. Few patients with severe portal hypertension due to partial nodular transformation will require surgical resection or a porto-caval shunt procedure.

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## 101.3 Focal Nodular Hyperplasia

### Definition

Focal nodular hyperplasia (FNH) is a tumor-like lesion composed of hyperplastic hepatocytes. It typically has a central stellate scar containing thick-walled arterial vessels. Fibrous septa radiate from the central core of connective tissue and separate the tumor parenchyma into multiple nodules.

### Epidemiology

The estimated prevalence of FNH is 0.4% to 3% in non-selected autopsy cases and 0.3% in clinical series. FNH is the second most common benign hepatic tumor (the most common is hemangioma). It occurs in all age groups, preferentially between 20 and 50 years. It is also seen in children where it comprises 2% of hepatic tumors [17, 22]. FNH is more prevalent in women, with the female to male ratio being 3–4:1. The incidence of FNH is about twice as high as that of hepatocellular adenoma (HCA). The coexistence of FNH and HCA has only rarely been documented.

FNH may be associated with hepatic hemangiomas in up to 23% of patients, vascular malformations in other regions (e.g. telangiectasias in the brain, arterial aneurysms, dysplastic arteries), brain tumors (meningioma, astrocytoma, glioblastoma multiforme) and portal vein atresia [8, 10, 19]. Whether this coexistence represents a syndromatic association ("multiple FNH syndrome") or whether it is purely coincidental is not known.

### Etiology and Pathogenesis

The cause of FNH is unknown. The association between the intake of anabolic steroids and the development of FNH is not clear. In contrast to HCA, oral contraceptives do not have an etiologic role and growth of FNH is generally not stimulated by estrogens or during pregnancy, although increases in the vascularity of the lesion during pregnancy have been described [11, 21]. A recent case-control study suggests that cigarette smoking may be associated with an increased risk of development of FNH [18].

Among the various pathogenetic concepts, the *vascular hypothesis* is currently favored. According to this hypothesis FNH is considered to be the result of a hyperplastic response of hepatic parenchyma to increased arterial blood flow secondary to vascular malformations [16]. It is possible that an as yet not well-defined portal tract injury subsequently leads to the enlargement, neoformation and proliferation of arteries, and to the development of venous shunts (portal and hepatic). A quantitative gene expression study suggested a role for angiopoietins in angiogenesis in FNH [15]. The blood flow pattern in FNH, i.e. extensive arterial inflow with virtual lack of portal venous flow, and the frequent association of FNH with hemangiomas appear to lend support to the arterial hypothesis. In addition, *genetic hypotheses* are discussed. In contrast to HCA, most FNH are polyclonal [7, 14]. Telangiectatic FNH is of monoclonal origin and most probably represents a variant of HCA rather than a true FNH (see Section 101.1) [1].

### Pathology

The right liver lobe is involved in about half of the patients. In 5–10% of cases both lobes are affected simultaneously. In 80% of patients the lesion is solitary, in 12% two and in 7% multiple nodes are present. Monolobar FNH occupying an entire liver lobe is extremely rare. In 85% of cases the diameter of FNH is ≤5 cm, 12% measure 5–10 cm and very few lesions are larger than 10 cm in diameter. Most FNH are located beneath the liver capsule and very rarely an FNH may be pedunculated. FNH in men tend to be smaller and more often atypical compared to those in women [9].

The *macroscopic appearance* is characteristic. FNH forms a well circumscribed, yellowish to light-brown colored, unencapsulated globular mass, relatively sharply demarcated from the surrounding liver parenchyma. A central or eccentrically located stellate fibrous region with connecting tissue septa radiating to the periphery is characteristic but not pathognomonic, and is not always present. On its cut surface these fibrous septa subdivide the tumor into multiple smaller nodules of liver parenchyma imparting the lesion the appearance of a "focal cirrhosis".

*Microscopically* FNH consists of cords of nearly normal appearing hepatocytes, proliferating bile ductules and fibrous septa containing blood vessels. The hyperplastic hepatocytes are usually somewhat clearer

than normal liver cells and may contain glycogen and fat droplets. Bile canaliculi are present, but they usually do not contain bile. The hepatocytes generally are arranged in plates of two to three cells and are supported by a well-developed reticulin fiber network. The liver cell plates are separated by endothelium lined sinusoids. Stellate and Kupffer cells are also present. However, the normal acinar architecture with portal tracts and terminal hepatic venules is missing. In nearly all FNH signs of cholestasis are present, as evidenced morphologically by cholate stasis (feathery change), copper accumulation in hepatocytes bordering fibrous septa, and by intracytoplasmic bile droplets [3]. An impressive ductular reaction may be present in between the central scar, the fibrous septa and the tumor parenchyma (Fig. 101.2). It is assumed that the proliferating ductular cells arise from metaplastic liver cells.

Abnormal capillary and thick-walled arterial vessels run within the scar and the fibrous septa [6]. They may display obliterative lesions, such as intimal hyperplasia, subintimal fibrosis, smooth muscle proliferation and occasionally thrombotic occlusion. Portal veins usually are not present. Often the fibrous septa are infiltrated by plasma cells, lymphocytes (occasionally forming lymphatic aggregates), mast cells and neutrophils. Necrosis and hemorrhages might be present but are not as frequent as in HCA.

The *telangiectatic type* of FNH (see above) lacks a central scar and is characterized by prominent dilatation of intralesional sinusoids. It probably is not a true FNH but a special type of HCA. *Variant forms* of FNH without a central fibrous region are difficult to distinguish from other types of large regenerative nodules or adenoma and may show cytologic atypia resembling dysplasia of large cell type [12].

## Diagnosis

### *Clinical Manifestations*

In the majority of patients FNH is asymptomatic and is discovered incidentally during a sonographic examination. Approximately 20% of patients complain of mild epigastric pain that might be attributed to FNH. Due to pressure on neighbouring structures large FNH may cause a sense of fullness and nonspecific epigastric discomfort. A rare intratumoral hemorrhage may cause more pronounced pain.

Occasionally a flow murmur in the epigastrium or the right upper quadrant may be heard on auscultation of a large and highly vascularized FNH. Very rarely multiple FNH-tumors may lead to signs and symptoms of portal hypertension.

Unlike in HCA bleeding and rupture of a FNH with subsequent hemoperitoneum is a rarity. Oral contraceptives are almost always involved in these

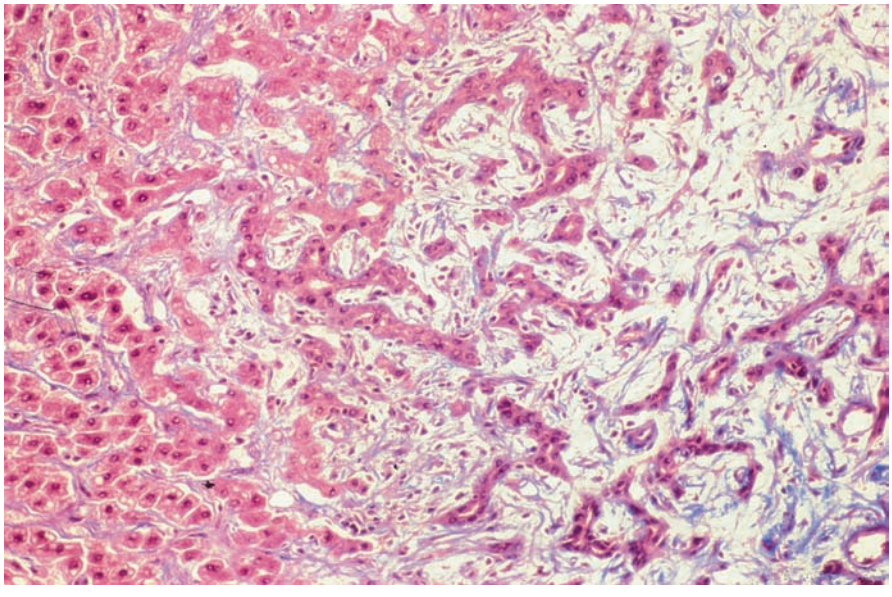

**Fig. 101.2** Marked ductular proliferation in a focal nodular hyperplasia. Trichrome stain (×200)

infrequent cases. The risk of bleeding is increased in the telangiectatic variant of FNH [1].

### Laboratory Findings

Serum chemistries are unremarkable with occasional slight elevations of alkaline phosphatase and γ-GT levels. Alpha-fetoprotein is normal.

### Imaging Techniques

In typical FNH (central scar containing feeding artery, radiating fibrous septa with vessels) ultrasonography, contrast enhanced computed tomography and magnetic resonance imaging yield characteristic, though nonspecific images. The diagnosis can be confirmed by histologic examination.

In typical cases *ultrasonography* shows a well-defined, mostly isoechoic focal lesion with a somewhat irregular echopattern, sharply demarcated from the surrounding parenchyma by an echopoor rim. Inside the lesion a hyperechoic band may be seen that represents the central scar. This typical finding, however, is present in only a minority of patients. Using color Doppler and contrast enhanced ultrasound the vascular pattern can be delineated, showing predominantly an arterial flow with vessels radiating peripherally from a central feeding artery in a spoke-wheel pattern (see Fig. 37.2). Contrast-enhanced ultrasound improves the specificity of sonographic findings.

*CT-scanning* shows a hypo- or isodense lesion with a rapid homogeneous centripetal enhancement in the arterial phase. In the portal venous phase the central fibrous region remains hyperdense compared to the remainder of the lesion that becomes isodense again.

*Magnetic resonance imaging* yields analogous findings, showing an isointense tumor on T1-weighted images, and a hyperintense central scar in T2-weighted images. With intravenous gadolinium contrast injection, the tumor mass shows an early homogeneous enhancement followed by a late central enhancement (see Fig. 38.4). When typical features of FNH are present the specificity of MRI for the diagnosis of FNH is >95%. However, in up to 80% of patients, especially those with FNH smaller than 3 cm in diameter, a central scar may not be detectable. Liver-specific MR-contrast agents enable the diagnosis in the majority of cases with atypical FNH.

The central artery, the tortuous intratumoral vessels, hypervascularization and delayed venous filling can also be visualized by *angiography. Hepatobiliary scanning* with trimethylbromoiminodiacetic acid (TBIDA) typically reveals a "hot spot" of radioactivity in the clearing phase usually persisting 60 min after application of the substance [2]. Because of the presence of Kupffer cells, the lesion takes up $^{99m}$Technetium ($^{99m}$Tc) sulfur colloid, though this is a nonspecific finding. Neither angiography nor scintigraphic imaging are nowadays required in the diagnosis of FNH.

If ultrasonographic, CT or MR imaging yield typical findings of FNH, a histological confirmation is not necessary and the lesion may be followed clinically and by sequential ultrasound examinations. The vast majority of FNH do not enlarge. If, however, US, CT or MRI fail to establish the diagnosis, which may easily occur if the typical central hypervascular scar is lacking, *histological examination* of the lesion is mandatory. The histological diagnosis depends largely on the size of the tissue sample obtained. Even if this is too small to allow for a definite diagnosis of FNH, histology will permit the exclusion of malignancy. Lesions deeply embedded in the hepatic parenchyma should be biopsied percutaneously. More superficially localized lesions should undergo open biopsy or tissue resection during laparoscopy. This procedure will yield enough material for a definite diagnosis and will reduce the associated bleeding risk.

## Differential Diagnosis

FNH must be distinguished from other hepatic mass lesions, especially metastases, HCA and hepatocellular carcinoma. The diagnosis is not difficult if a typical configuration with a central scar, fibrous septa and a spoke-wheel vascular pattern is seen. However, even a typical angiographic pattern does not allow for a definitive differentiation of a well-vascularized and hyperperfused FNH from hepatocellular carcinoma.

Calcifications within an FNH occur rarely. They are encountered more often in fibrolamellar carcinoma. When interpreting liver biopsies one should be aware that FNH-like changes may be observed in liver tissue adjoining a fibrolamellar carcinoma.

In small biopsy samples differentiation from liver cirrhosis might be difficult. A periseptal ductular proliferation, the complete lack of the normal acinar architecture, absence of lipofuscin, the presence of cholate stasis and multiple thick-walled vascular profiles support the diagnosis of a FNH [5].

## Natural History and Prognosis

FNH is not a premalignant condition and malignant transformation has not been reported. The idea that FNH might be a precursor of fibrolamellar carcinoma has not been substantiated. On long-term follow up changes in FNH size are rarely observed, with fewer than 5% of cases enlarging or decreasing in size [20]. Isolated cases with increasing FNH-size during pregnancy or in women taking oral contraceptives have been described, although this is not the rule.

The risk of bleeding and rupture is much less than in HCA with the exception of the telangiectatic variant of FNH (see above) that appears to have a risk of bleeding similar to that observed in patients with an HCA [1].

## Therapy

Small lesions or asymptomatic FNH deeply embedded in hepatic parenchyma need no treatment [4, 13, 20]. The patient should be reassured and the lesion followed clinically and by sequential imaging, mostly ultrasound. The discontinuation of oral contraceptives is not necessary. Large, symptomatic FNH or asymptomatic but rapidly growing tumors should be resected surgically. This is indicated in only a small minority of patients. Arterial embolization or ligation are alternative treatment options in selected patients.

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## 101.4 Bile Duct Adenoma

### Definition

Bile duct adenomas usually are solitary, small (mean size 1.3 mm), unencapsulated, firm, grey-white, subcapsular nodules consisting of proliferating bile ducts [2, 5].

### Histology

*Microscopically* they consist of a cluster of small, uniform, non-dilated biliary acini and tubules lined by a columnar epithelium, and embedded in a loose fibrohyaline stroma (Fig. 101.3). Mononuclear inflammatory cells are regularly found in the fibrous matrix, whereas lymphoid follicles are only occasionally encountered.

The adenomatous biliary cells express antigens that can also be demonstrated in the peribiliary glands of the normal liver. This led some authors to assume that biliary adenomas arise from peribiliary glandular elements and that they in fact represent peribiliary gland hamartomas instead of true neoplasms [3].

### Clinical Manifestations

Biliary adenomas are asymptomatic. Because of their small size they are missed on non-invasive imaging and are usually detected incidentally at laparoscopy or laparotomy performed for other reasons where they can be mistaken for metastatic carcinoma.

### Differential Diagnosis

The differential diagnosis includes *biliary microhamartomas* (*von Meyenburg complexes*). These acquired hamartomatous malformations of the finest branches of the biliary tree are found in the periportal region and are composed of ectatic bile ducts lined by low cuboidal epithelial cells. Their lumina communicate with bile ducts and often contain bile. Obliteration occurs frequently and causes cystic dilatation. When they are large and/or multiple they are often associated with polycystic kidney or liver disease (see Chapter 57).

Occasionally differentiation of a bile duct adenoma from a *well-differentiated cholangiocarcinoma* or from *metastatic adenocarcinoma* may be difficult. The absence of atypia, dysplasia and glandular variation helps to make the correct diagnosis.

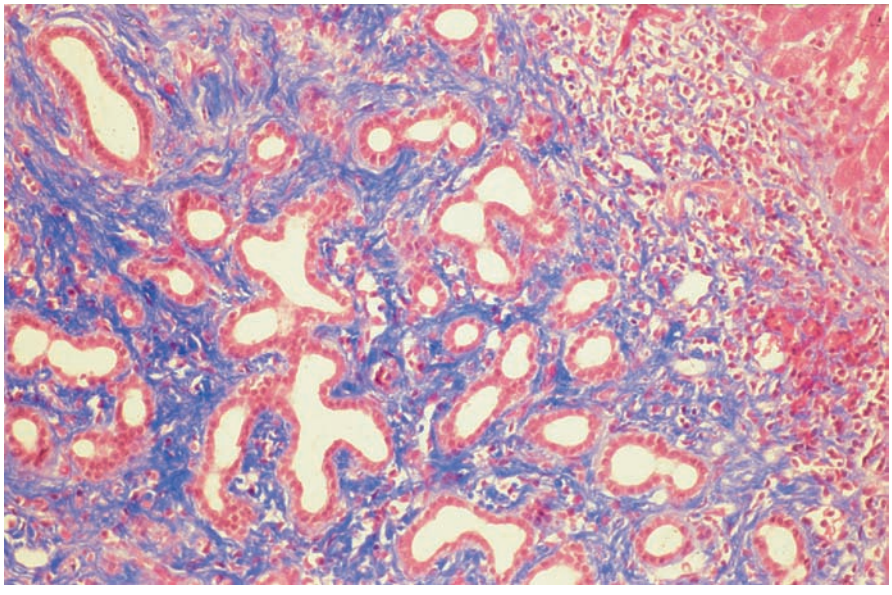

**Fig. 101.3** Biliary adenoma. Trichrome stain (×200)

A variant of bile duct adenoma with clear-cell morphology mimics *renal cell carcinoma* [1].

Diffuse macroscopically recognizable *hyperplasia of the peribiliary glands* of intrahepatic and extrahepatic bile ducts is an extremely rare condition of unknown pathogenesis. This condition has been described in a patient with hepatic necrosis [4].

## Therapy

Since bile duct adenomas do not cause adverse clinical effects no treatment is required.

Adenomas of the common bile duct and of the ampulla/papilla of Vater are discussed in Chapter 115.

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# 101.5 Biliary Cystadenoma

## Definition and Epidemiology

Biliary cystadenoma, also called hepatobiliary cystadenoma with mesenchymal stroma, is a very rare benign tumor that probably arises from ectopic embryonic tissue.

It occurs almost exclusively (95%) in women of all age groups. Patients between 30 and 50 years are most commonly affected.

## Pathology

The lesion has a slight predilection for the right liver lobe, is usually solitary and surrounded by a fibrous capsule. At the time of diagnosis most tumors are very large with a diameter between 25 and 30 cm. The tumor is always multilocular containing a mucinous-gelatinous or serous and clear fluid. Histologically two variants are distinguished, a *serous type* that is exceedingly rare and a more common *mucinous type*. The epithelium is tall and columnar, may display papillary foldings and cystic invaginations, but may also be atrophic and flattened with focal ulcerations. Due to the presence of spindle-shaped stromal cells the matrix may resemble ovarian stroma [1–3]. Inflammatory changes, scar formation and calcification may occur in the septa running between the cysts.

## Diagnosis

### *Clinical Manifestations*

The clinical picture is not characteristic. Small tumors are asymptomatic, large lesions may cause right upper quadrant abdominal pain. Depending on the size and location of the mass it also may be palpable. Rupture, hemorrhage and obstruction of the biliary tree by the tumor rarely occur [5].

### *Laboratory Findings*

Specific laboratory changes do not exist. Serum $\alpha_1$-fetoprotein and CEA are not elevated. Elevated Ca 19–9 levels have been described, but this finding is nonspecific [6].

### *Imaging Techniques*

A biliary cystadenoma appears as a solitary, well encapsulated, multilocular cystic mass on sonography, CT and MRI.

Because laboratory examinations and imaging techniques yield nonspecific results, the definitive diagnosis of a biliary cystadenoma is usually made by the histologic examination of the resected specimen. A preoperative biopsy is not advisable.

## Differential Diagnosis

The differential diagnosis includes first and foremost a cystadenocarcinoma [7]. Alveolar echinococcosis, the rare mesenchymal hamartoma and unilobar Caroli's disease are further conditions to be considered [4].

## Therapy

Complete operative resection is the treatment of choice. Because the mucinous type may undergo malignant transformation, complete surgical resection is mandatory and curative. Incomplete resection may lead to recurrence.

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## 101.6 Biliary Papillomatosis

### Definition

Biliary papillomatosis is a rare disease characterized by multiple papillary adenomas with thin fibrovascular stalks involving both the intrahepatic and extrahepatic biliary tree.

### Epidemiology, Etiology and Pathogenesis

There are no systematic epidemiological data on biliary papillomatosis. Women are slightly more often affected than men. The etiology and pathogenesis of the disease are unknown.

### Diagnosis

#### *Clinical Manifestations*

The clinical manifestations are characterized by repeated episodes of abdominal pain, recurrent suppurative cholangitis with jaundice, septicemia and hemobilia. Blood clots in the common bile duct and hemobilia may cause acute pancreatitis [4–7, 9].

#### *Laboratory Findings*

The laboratory findings are nonspecific. Cholestatic parameters (AP and γGT) and aminotransferase levels may be increased. If cholangitis is present inflammatory markers, such as the concentration of C-reactive protein and the erythrocyte sedimentation rate, rise. There are no specific tumor markers for biliary papillomatosis.

#### *Imaging Techniques*

The diagnosis is made by ERCP, MRCP and cholangioscopy. The intra- and extrahepatic bile ducts show multiple saccular and segmental dilatations with polyps, mucin plugs, sloughed tumor debris and blood clots resulting in multiple contrast medium filling defects. Whenever possible several intraductal forceps biopsies should be obtained. Thickening and irregularities of the bile duct wall may be visualized by intraductal endoscopic ultrasound. However, the findings are nonspecific and do not allow for the exclusion of malignancy.

### Differential Diagnosis

Cystically or aneurysmatically dilated bile ducts in mucin-hypersecreting variants (intraductal papillary mucinous tumors) should be differentiated from cystadenoma, cystadenocarcinoma and liver abscess.

### Prognosis

Biliary papillomatosis has a strong malignant potential. The majority of patients experience transformation to papillary cholangiocarcinoma. Gastroenteric metaplasia with aberrant expression of cytokeratin 20, mucin core proteins and mucin carbohydrate antigens is frequent in the neoplastic areas, and increasing grades of biliary epithelial dysplasia give rise to in situ and then invasive carcinoma [1, 8]. Histologically, phenotypically and clinically the biliary papillary neoplasms resemble intraductal papillary mucinous neoplasms of the pancreas.

Thus, the prognosis of biliary papillomatosis is grave and the disease runs a relentless course with most patients dying from septicemia, acute liver failure or cholangiocarcinoma. Survival rates between 20% and 50% at 3 years have been reported [6].

### Therapy

Management is difficult. Antibiotic treatment of recurrent cholangitis cannot prevent progression of the disease. The rare segmental forms of biliary papillomatosis may be amenable to surgical resection. Intraluminal radiation with $^{192}$iridium and orthotopic liver transplantation yield encouraging results [2, 3].

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# 101.7 Hemangioma

## Definition

Hemangioma is a benign tumor originating from hepatic blood vessels. Diffuse systemic hemangiomatosis denotes the occurrence of hemangiomas in multiple organs, including the liver [2, 10, 11].

## Epidemiology

Cavernous hemangioma is the most common benign liver tumor. It occurs in any age group and is more common in women than in men, with a ratio of 3–5:1 [4]. The incidence in autopsy studies varies between 0.4% and 20% depending on the accuracy of liver dissection. On ultrasound examination the prevalence is approximately 1%.

## Pathology

Most hemangiomas are well-defined solitary lesions. However, especially large hepatic hemangiomas may have irregular edges and a less well-defined interface with the surrounding liver parenchyma [8]. Their gross appearance is spongy-reddish or bluish and they vary in size from a few millimeters to >20 cm in diameter, with most measuring <5 cm. Hemangiomas with a diameter > 10 cm are defined as giant hemangiomas [1].

*Microscopically* dilated, endothelium lined, blood filled, anastomosing cavities are seen (Fig. 101.4). Depending on its developmental stage the hemangioma may be purely cavernous, partially or totally thrombosed. It may heal by increasing sclerosis leaving behind a scarred nodule.

## Diagnosis

### *Clinical Manifestations*

Most hemangiomas are asymptomatic and are found incidentally during a sonographic examination of the abdomen. Depending on their size and subcapsular

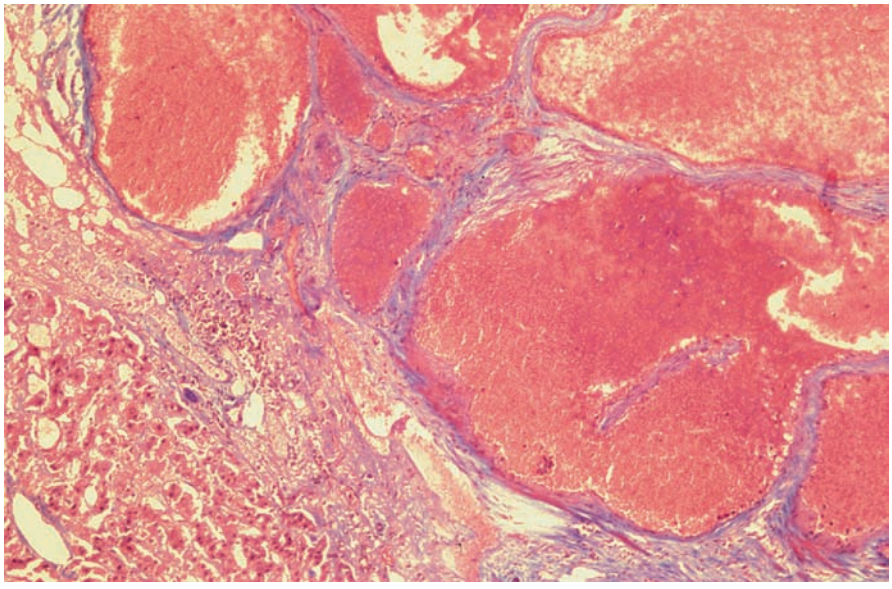

**Fig. 101.4** Hemangioma with large blood filled vascular cavities separated by delicate fibrous strands. Trichrome stain (×200)

location they may cause a vague discomfort in the right upper abdomen. Occasionally a large hemangioma may cause abdominal symptoms because of a mass effect leading to compression of bile ducts with consequent cholestasis. Giant hemangiomas may lead to disseminated intravascular coagulation (*Kasabach-Merrit syndrome*).

## Laboratory Findings

Small hemangiomas do not cause any laboratory abnormalities. Large hemangiomas may lead to slight elevations of γ-GT and alkaline phosphatase serum levels, and cause a thrombocytopenia. Giant hemangiomas have been associated with elevations of indirect reacting serum bilirubin, due to intravascular hemolysis of trapped red cells.

## Imaging Techniques

The diagnostic work-up primarily relies on imaging procedures, such as ultrasound, computed tomography and magnetic resonance imaging. Angiography and scintigraphic techniques have been largely replaced by these modalities in the diagnosis of hepatic hemangiomas. See also Chapters 37, 38 and 39.

*Sonography* is widely available and should be the imaging technique applied first. Even small hemangiomas (<1 cm) are accurately detected by ultrasound [5]. Approximately 80% of hemangiomas appear sonographically as round, well delimited, hyperechoic lesions with faint acoustic enhancement. The echogenicity may vary because the tumors may undergo regressive changes, such as cystic and fibrotic alterations and calcification. Larger hemangiomas may display a more irregular echopattern. The sensitivity and specificity of ultrasound is 60–75% and 60–80%, respectively. Color-Doppler ultrasound imaging may demonstrate feeding vessels in the periphery of the tumor, but due to intratumoral sclerosis and thrombosis no significant flow deep within the hemangioma itself. Contrast-enhanced ultrasound with bolus injections of contrast medium demonstrates the typical features of a hemangioma, such as peripheral nodular contrast enhancement and a homogenous centripetal filling (*iris-diaphragm sign*) in approximately 80% of patients (see Fig. 37.1) [3].

Hemangiomas are often missed on standard *CT scan*. The reliable diagnosis of a liver hemangioma requires triple phase (dynamic) CT. After administration of contrast material hemangiomas appear as hypodense lesions, typically showing foci of peripheral globular enhancement during early-phase imaging. On delayed images, the mass usually shows a centripetal filling in of the lesion, with contrast enhancement persisting for at least 20 min because of

the characteristic lack of intratumoral shunting. If a lesion demonstrates globular peripheral enhancement, isodense with the aorta during the early phase of contrast-enhanced helical scanning, a diagnosis of hemangioma can be confidently made (see Figs. 38.1, 38.2 and 38.3). Smaller lesions may enhance uniformly on early-phase imaging, whereas larger hemangiomas with fibrotic regions show corresponding persistent areas of nonenhancement. The pattern of globular enhancement has been reported to be 88% sensitive and 88–100% specific for distinguishing hemangiomas from hypervascular metastases [9].

*MRI* is a highly accurate method to diagnose hepatic hemangioma. Characteristic findings are a strong hyperintense signal on T2-weighted images and decreased signal intensity on T1-weighted images compared with normal liver tissue. In cases with intratumoral fibrosis, hemorrhage, or thrombosis an atypical, heterogenous appearance may be present. The use of heavily T2-weighted images has been shown to give a sensitivity of 100% and a specificity of 92% for the diagnosis of hemangioma [9].

Despite the high accuracy of ultrasound, CT and MRI in the diagnosis of hepatic hemangioma, there remain cases in which a specific diagnosis cannot be reached with these indirect imaging techniques. This applies especially to patients with underlying malignancies in whom metastasis must be excluded when a new liver mass occurs. In these patients a *fine needle biopsy* should be performed in order to exclude a malignancy. Despite the hypervascular nature of a hemangioma, fine needle biopsies can be safely performed if the hemangioma is embedded within the liver, surrounded by normal hepatic parenchyma. A biopsy with a 20–22-gauge needle will generally yield tissue suitable for histological examination. Even if the tissue sample is too small to allow for a definitive diagnosis of hemangioma, it will usually be sufficient to rule out malignancy. Subcapsular hemangiomas should not be biopsied percutaneously. Instead a laparoscopy will allow for a quick visual diagnosis (Fig. 101.5).

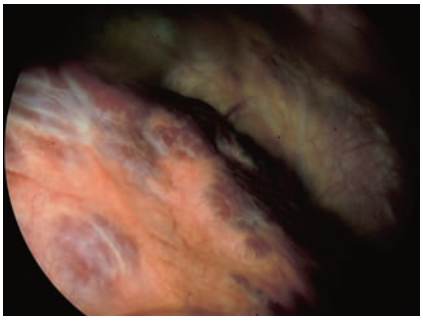

**Fig. 101.5** Laparoscopic appearance of several superficial hemangiomas of the left liver lobe

## Differential Diagnosis

The differential diagnosis of a hepatic hemangioma includes primary malignant liver tumors and hypervascular metastases. Small, incidentally detected hemangiomas in asymptomatic patients, with a characteristic morphological appearance on US, CT or MRI do not need an invasive work-up, but can be followed by serial ultrasound examinations, for example, in intervals of 3–6 months. If the appearance of the lesion remains unchanged after 6–12 months, then no further surveillance is necessary [5].

In contrast, large lesions with an irregular echopattern or an atypical appearance on CT or MRI should be biopsied without delay. If a safe biopsy of the tumor with the passage of the needle through normal liver tissue is not possible, a laparoscopy should be performed.

A rare differential diagnosis of liver hemangioma includes localized hepatic changes in patients with hereditary hemorrhagic telangiectasia (Rendu-Weber-Osler disease) [12]. In theses patients dilated arterial and venous vessels are found, and angiographically arterio-venous shunts may be visualized. Occasionally these lesions are accompanied by nodular regenerative hyperplasia.

An hepatic peliosis may also mimic a hemangioma (see Chapter 60).

## Natural History and Prognosis

The vast majority of liver hemangiomas remain stable on follow-up and do not enlarge [5]. However, due to thrombosis, fibrosis and calcifications the appearance of the lesion may change. During pregnancy or estrogen treatment hemangiomas may grow. Thus, regular sonographic follow-up examinations in women with known hepatic hemangiomas receiving hormone therapy appear warranted [6].

Even with large hemangiomas the risk of spontaneous rupture is extremely low. A blunt abdominal trauma, however, may lead to rupture of a large subcapsular hemangioma with a subsequent life threatening acute abdomen.

## Treatment

Most hemangiomas do not need treatment. Indications for treatment include rupture and hemorrhage, abdominal symptoms because of a mass effect, Kasabach-Merrit syndrome and a diagnostic inconsistency that can otherwise not be solved.

Treatment options include hepatic arterial embolization and resection. The resection of large hemangiomas, located centrally in the liver hilum may be technically very demanding and sometimes impossible. In cases of an unresectable giant hemangioma with disseminated intravascular coagulation the patient should be evaluated for orthotopic liver transplantation [7].

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# 101.8 Infantile Hemangioendothelioma

## Definition and Epidemiology

Infantile hemangioendothelioma is a benign vascular tumor of embryonic origin and, although very rare, it is the most common mesenchymal hepatic tumor of infancy. Other organs, especially the skin may also be affected. It is observed in infants within the first 6 months of age. Fewer than 5% of cases are detected beyond 1 year of age. Girls are affected twice as often as boys [1].

## Pathology

Most tumors are multinodular. The nodules consist of proliferated anastomosing blood filled spaces, are unencapsulated, with a soft consistency and a red-brown spongy appearance. The size varies from a few millimetres to >20 cm in diameter.

Two *microscopic types* have been described [3]. In *type 1* the vascular spaces resemble capillary channels lined by a simple endothelial layer with a scant collagenous matrix. The stroma of larger tumors contains proliferated bile ducts, areas of hemorrhagic necrosis, fibrosis, dystrophic calcifications and regularly foci of extramedullary hemopoiesis.

*Type 2* tumors exhibit poorly formed vascular spaces with papillary proliferations of a multilayered endothelium. This type has to be distinguished from angiosarcoma. Transitional forms between infantile hemangioendothelioma type 2 and angiosarcoma have been described [3].

## Diagnosis

### *Clinical Manifestations*

Approximately half of the patients present with an abdominal mass, anorexia, vomiting, failure to thrive and lethargy. Rarely, a systolic bruit may be auscultated over large tumors due to intratumoral arteriovenous shunts. These patients may develop congestive heart failure.

Spontaneous rupture with subsequent hemoperitoneum is a rare complication. Malignant transformation is a very rare event [3, 11, 12].

### *Laboratory Findings*

Mild nonspecific elevations of liver enzymes (aminotransferases, AP, γGT) are observed. Anemia, thrombocytopenia due to platelet sequestration, disseminated intravascular coagulation (Kasabach-Merrit syndrome) and liver failure have also been described. Infantile hemangioendotheliomas may express type 3 iodothyronine deiodinase and cause severe hypothyroidism in some patients [5, 8]. Alpha-fetoprotein is only seldom elevated [10].

### *Imaging Techniques*

On US, CT and MRI the tumor appears as a complex, inhomogeneous mass lesion with areas of cystic degeneration, hemorrhages and large draining veins. Color-Doppler sonography and angio-MRI have widely replaced conventional hepatic angiography and may establish the vascular nature of the tumor and document its multiple arteriovenous shunts [9]. Imaging studies, however, cannot differentiate between hemangioendothelioma and angiosarcoma.

Percutaneous liver biopsy is contraindicated because of the associated bleeding risk.

## Therapy

Uncomplicated infantile hemangioendothelioma does not require treatment since spontaneous involution of tumors over a time period of 5–8 years may occur [4].

In symptomatic patients treatment is mandatory. Therapeutic options, however, are limited and due to the rarity of the tumor studies with a large number of patients are not available.

Medical treatment options include corticosteroids, interferon alpha, and, in life-threatening cases, cyclophosphamide [4, 6]. In infants with hypothyroidism supranormal doses of L-thyroxine may be required for normalization of thyroid function until the tumor involutes or is resected [5, 8].

Radiation therapy, hepatic artery embolisation or ligation are alternative treatment options [7].

Surgical resection has the highest rate of success but is technically demanding and sometimes not feasible in patients with a giant hemangioendothelioma or with multiple lesions. Orthotopic liver transplantation should be considered if all other therapies fail or are not eligible [2, 14].

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## 101.9 Lymphangioma and Hepatic Lymphangiomatosis

The existence of a solitary lymphangioma of the liver is controversial, since the few published cases may well represent misdiagnosed mesenchymal hamartomas (K.G. Ishak, personal communication, 2000).

However, the occurrence of multiple small (1–5 cm in diameter) hepatic lymphangiomas, denominated lymphangiomatosis, is well documented. They consist of anastomosing, cystically dilated, endothelium-lined lymph vessels containing lymph-like chylous fluid. In most patients hepatic lymphangiomatosis is associated with lymphangiomas in other organs, including the spleen, skeleton, kidneys, gastrointestinal tract, soft tissues, brain, lungs and mediastinum [1, 5].

Symptoms depend on the size and location of tumors. Small solitary lymphangiomas are asymptomatic, whereas lymphangiomatosis may lead to liver failure.

On ultrasound and computed tomography lymphangiomas appear as cystic lesions, and hepatic lymphangiomatosis has been reported to mimic polycystic liver disease [4]. Fine needle biopsy may lead to peritoneal lymphorrhea [2].

Small, solitary lesions need no treatment. Large, symptomatic lesions and lymphangiomatosis may require surgical resection or orthotopic liver transplantation [3].

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## 101.10 Mesenchymal Hamartoma

Mesenchymal hamartoma of the liver is a rare developmental cystic tumor that occurs most commonly in the first 2 years of life with a male-to-female ratio of 2:1.

Grossly the tumor is unencapsulated and consists of solid and cystic components. Microscopically the lesion is composed of immature mesenchymal cells, bile ducts, and hepatocytes.

Due to accumulation of fluid in the cysts, it usually progresses in size. Rapid enlargement may occasionally cause respiratory distress and edema of the lower extremities, and mimic malignancy. Intratumoral arteriovenous shunts may cause heart failure, and even death as a result of respiratory complications [1].

On US and CT imaging studies the tumor appears as a cystic mass composed of multiple cystic areas separated by septa. In younger patients, mesenchymal hamartoma may appear more solid because the cysts are smaller [2]. The MR imaging features usually reflect the predominantly cystic nature of this tumor. Signal intensities can differ because of varying concentrations of protein in the cysts [3].

Surgical resection or orthotopic liver transplantation of symptomatic progressive tumors is the sole therapeutic option.

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## 101.11 Lipomatous Tumors

### Lipoma

True hepatic lipomas are exceedingly rare tumors. They consist of mature adipocytes with interspersed isolated blood vessels. One case of tuberous sclerosis associated with hepatic lipoma has been reported [10]. The tumors usually remain asymptomatic and are detected incidentally on imaging studies. On ultrasound examination they are hyperechogenic with a dorsal acoustic shadow of varying intensity. On CT scanning the well-delineated mass has a density comparable to subcutaneous fat and, on MR imaging hepatic lipomas have a high signal intensity on T1-weighted images with only a minimal signal decrease on T2-weighted sequences [2, 11 21]. Treatment is not required.

### Pseudolipoma

This pseudotumor is mostly found incidentally at autopsy or during an abdominal operation. It manifests as a small, yellowish lesion surrounded by a fibrous capsule immersed in a superficial depression of the liver surface at the level of Glisson's capsule.

Pseudolipomas result from the detachment of epiploic appendixes from neighbouring colon or omentum and subsequent adhesion to the liver surface.

Histologically, a localized accumulation of fat cells is seen with a varying admixture of connective tissue, calcifications and even ossifications.

These pseudotumors have no clinical significance [13].

### Focal Steatosis

See also Chapter 37. Circumscribed steatotic areas in the liver are a frequent finding. They do not represent tumors, and their only clinical significance lies in the fact that they must not be confused with a neoplastic liver lesion and cause a cascade of complex and costly diagnostic investigations.

The cause of focal steatosis is unknown. These focal changes in fat distribution are possibly caused by regional circulatory disturbances [7].

On ultrasound examination, focal steatosis usually occurs anterior to the central portal vein branches as a more or less well-delineated hypoechoic area compared to the remainder of the liver (see Fig. 37.4). Even if large, these "mass lesions" do not encroach upon the vascular architecture of the liver, which differentiates them from true tumors. The experienced sonographer has no difficulty in diagnosing focal fatty liver. CT and MRI studies are not required.

### Angiomyolipoma

#### *Definition and Epidemiology*

Angiomyolipomas are rare, benign mesenchymal tumors. Most angiomyolipomas affect the kidneys. The isolated occurrence in the liver is rare. Females are affected more often than males, and the mean age at the time of diagnosis is approximately 50 years. An association of angiomyolipoma with tuberous sclerosis has been described [6, 10]. The concomitant occurrence of angiomyolipoma with focal nodular hyperplasia, bile duct adenoma, hemangioma and hepatocellular carcinoma has been reported in single cases [3, 8, 15].

#### *Pathology*

On gross examination the tumors are red to yellow, reflecting their heterogeneous composition of fat, vessels and muscle cells. They are well demarcated from the surrounding liver parenchyma and vary in size from a few millimeters to >30 cm in diameter.

Angiomyolipomas derive from perivascular epithelioid cells and share a similar gene expression profile with hepatic stellate cells [12, 16, 18, 19].

Histologically the tumors are heterogeneous with varying proportions of adipocytes, blood vessels and smooth muscles. In two thirds of cases bone marrow elements are found (*angiomyomyelolipoma*). If only adipocytes and vascular structures are present the lesion is described as an *angiolipoma. Myelolipomas* contain adipocytes and hemopoietic cells. In rare cases only trace amounts of fat are found [24]. If the smooth muscle component (spindle shaped, epithelioid)

predominates, the differentiation from mesenchymal tumors may be difficult. Some angiomyolipomas resemble epithelial tumors and may mimic hepatocellular carcinoma or metastases from clear cell renal carcinoma [23]. Despite the benign nature of the tumor on histological examination, angiomyolipomas frequently display a growth pattern mimicking invasiveness, which might lead to an erroneous diagnosis of malignancy. Therefore, pathologists should not overdiagnose malignancy based solely on the histological growth pattern of angiomyolipomas [20].

Immunohistochemical studies have shown angiomyolipomas to express the melanocytic markers HMB-45, MART-1 (Melan A), microophthalmia transcription factor, tyrosinase, smooth muscle actin, vimentin, desmin and KIT (CD 117) (Figs. 101.6 and 101.7) [5, 16].

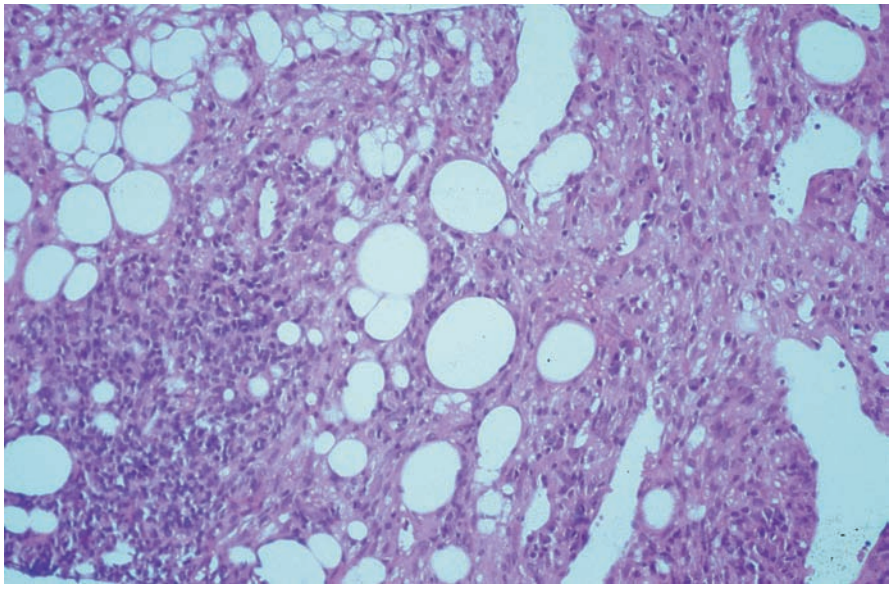

**Fig. 101.6** Angiomyolipoma. Hematoxylin & Eosin (×200). Sonographically the tumor appeared as a homogeneous hyperechoic lesion indistinguishable from a hemangioma

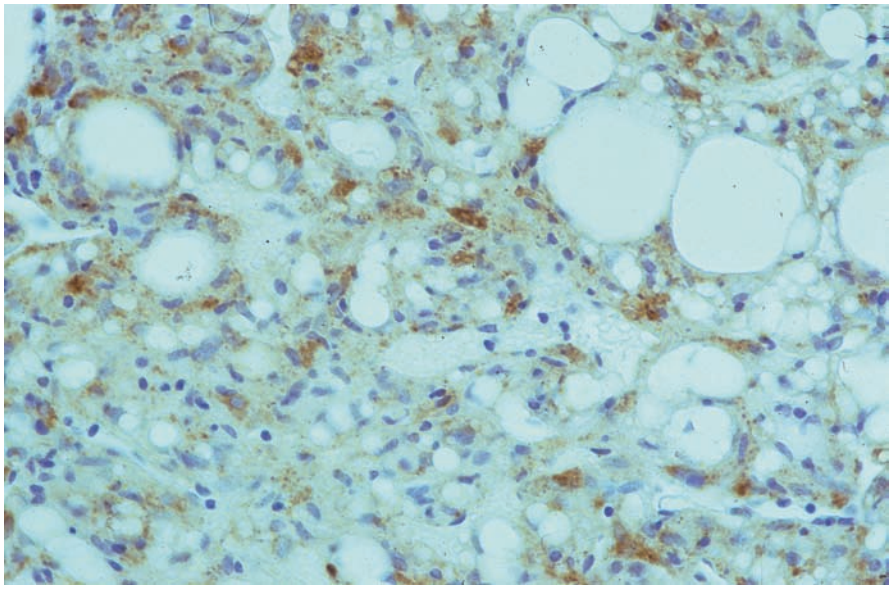

**Fig. 101.7** Angiomyolipoma (same case as in Figure 101.6). Immunocytochemical staining of the melanocytic marker HMB-45

## Diagnosis

### *Clinical Manifestations*

Clinically, most angiomyolipomas are asymptomatic and are found incidentally. However, the tumors may attain a large size, cause hepatomegaly, abdominal discomfort, nausea and in rare cases a Budd–Chiari syndrome [14].

### *Laboratory Findings*

There are no specific serologic diagnostic markers for angiomyolipoma.

### *Imaging Methods*

Imaging tools visualize the hypervascular nature and the fatty component of the lesion. However, because of the variability of the different tissue components the appearance on US, CT and MR imaging is heterogeneous. On ultrasound examination the tumors are well-delineated, hyperechoic with an irregular echopattern. They cannot be distinguished from a hemangioma. On CT scanning they are predominantly hypodense with an irregular contrast enhancement. On MRI tumors show hypointensity or hyperintensity on T1-weighted images and heterogeneous hyperintensity on T2-weighted images [22]. The combined application of dynamic spiral CT, MRI and contrast-enhanced ultrasound allow for the detection of a vascular pattern with a prominent efferent tumor vein [1, 27]. The specificity of this vascular pattern, however, has not been documented yet.

The main clinical challenge is the exclusion of primary hepatic or metastatic malignancy. If imaging procedures do not allow for the establishment of a definitive diagnosis and do not permit the exclusion of a malignancy, then a needle biopsy with special immunostains should be performed. HMB-45 is the best marker available for diagnosis [25].

## Therapy

Most patients with angiomyolipoma are asymptomatic and do not need treatment. They may be managed expectantly and monitored on a regular basis [26]. Spontaneous rupture and malignant transformation are exceedingly rare [4, 9].

Symptomatic and large tumors should be resected. The prognosis after surgical resection is excellent and recurrence is rare [17, 22].

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## 101.12 Fibrous Tumors

### Solitary Fibrous Tumor

This very rare tumor is also called *localized fibrous mesothelioma* or *submesothelial fibroma*. It can reach a size of up to 20 cm in diameter and may be pedunculated. Microscopically it is composed of parallel bundles of collagen and reticulin fibers. The cellular component is variable.

Diagnosis is by liver biopsy.

Symptomatic tumors are treated by surgical resection.

### Inflammatory Pseudotumor

#### *Definition and Epidemiology*

An inflammatory pseudotumor is a rare tumor with fewer than 200 cases reported in the literature. It has also been called *inflammatory myofibroblastic tumor* and *plasma cell granuloma* of the liver. It is found primarily in men, with a male to female ratio of 3.5:1. The mean age at diagnosis is approximately 33 years [8, 15, 16, 18, 23].

Inflammatory pseudotumors may occur alone or be associated with various conditions, such as autoimmune pancreatitis, $IgG_4$ associated primary sclerosing cholangitis, primary biliary cirrhosis, and malignancy (mainly gastrointestinal and biliary tract cancers) [5, 7, 9, 14, 19, 25]. An association of an inflammatory pseudotumor with rare hereditary diseases, such as the Papillon-Lefevre syndrome (palmar-plantar hyperkeratosis and severe periodontal destruction) has also been described [3].

#### *Etiology and Pathogenesis*

The etiopathogenesis is poorly understood. Viral (HIV, EBV), fungal and bacterial infectious have been hypothesized to play a role. Recently Actinomyces and Bacteroides caccae were demonstrated within an inflammatory pseudotumor of a patient and in hepatic

tissue of another patient with an inflammatory pseudotumor, respectively [1, 21]. The concomitant occurrence of inflammatory pseudotumor with $IgG_4$ associated diseases, such as primary sclerosing cholangitis and autoimmune pancreatitis suggest an immune background in its pathogenesis [25].

## Pathology

The majority of inflammatory pseudotumors are solitary lesions, with 90% occurring within the liver and 10% located at the hepatic hilum. The size of the tumor varies from 1 to 25 cm in diameter. The lesion is usually vaguely demarcated from the surrounding liver parenchyma and might infiltrate portal and hepatic vein branches as well as bile ducts.

Microscopically, inflammatory pseudotumor consists of proliferating fibrovascular tissue and infiltrating chronic inflammatory cells (Fig. 101.8). There is a polyclonal proliferation of plasma cells, lymphocytes, foamy histiocytes, fibroblasts and myofibroblasts. The cells may be arranged in whirls alongside collagen bundles and proliferated blood vessels. The latter occasionally display obliterative endophlebitic lesions [17]. Granulomas may be present.

## Diagnosis

### Clinical Manifestations

Depending on its size and location the tumor may cause symptoms due to mass effect on neighbouring structures. Clinical manifestations include fever, malaise, weight loss, and right upper quadrant pain. Cholestatic jaundice due to recurrent pyogenic cholangitis and portal hypertension may occur [22].

### Laboratory Findings

Laboratory data often reveal evidence of an inflammatory process indicated by leukocytosis, an elevated erythrocyte sedimentation rate, and a positive C reactive protein. Rarely serum CA19–9 levels may be elevated [10].

### Imaging Techniques

Imaging studies yield nonspecific results. On ultrasound most lesions appear hypoechoic. The most common CT findings are a single hypodense lesion with either no significant change or a delayed persistent enhancement after administration of contrast medium.

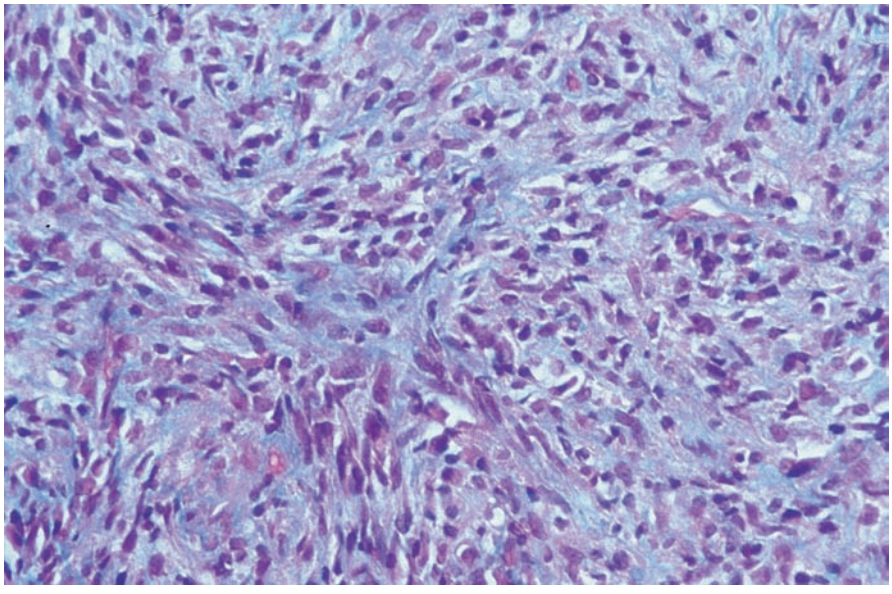

**Fig. 101.8** Inflammatory hepatic pseudotumor. Sonographically the tumor appeared as a hypoechoic mass. Trichrome stain (×400)

MRI findings also are nonspecific. Signal intensity characteristics on both unenhanced T1- and T2-weighted images vary from hypointense to hyperintense. Mostly, however, on unenhanced MR imaging there is hyperintensity on T2-weighted images.

Recently a case of hepatic inflammatory pseudotumor identified by FDG-PET has been reported [6].

Microscopic examination of tumor tissue is the only reliable method of establishing the diagnosis and liver biopsy therefore should be performed early in the work-up of a hepatic mass lesion of unknown cause [13].

## *Differential Diagnosis*

The differential diagnosis includes all solid tumors, primarily hepatocellular carcinoma. The ultrasound, CT and MRI appearance of a hepatic hilar pseudotumor is indistinguishable from that of cholangiocarcinoma [20]. The histological differential diagnosis includes an organized chronic hepatic abscess, a rhabdomyosarcoma, a malignant fibrosing histiocytoma and a sclerosing hepatocellular carcinoma.

## *Therapy and Prognosis*

Inflammatory pseudotumors have a small but definite malignant potential and treatment is therefore advised. Regression, either spontaneous or following therapy with steroids, antibiotics or nonsteroidal anti-inflammatory drugs, as well as malignant transformation are rare, but documented [2, 4, 7,12, 24]

The prognosis after complete surgical resection is excellent.

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## 101.13 Various Rare Tumors

In addition to the tumors described in the preceding sections, some uncommon benign tumors of the liver are mentioned here for completeness. They are, however, of limited clinical importance, and have been described primarily as case reports or in small series. These tumors or tumor-like lesions comprise leiomyomas, benign schwannomas, chondromas, benign teratomas, granular cell tumors, adrenal-remnant tumors, pancreatic and splenic heterotopias, endometrial cysts, focal extramedullary hematopoietic tumors, tumor-like reactive lymphoid hyperplasias, ectopic hepatic pregnancy and epidermoid cysts.

*Leiomyomas* are well-circumscribed smooth muscle tumors that occur extremely rarely in the liver. They have been described in renal transplant recipients, in patients infected with HIV and EBV, and in an asymptomatic hepatitis B carrier [3–5]. They have no specific radiologic or ultrasound characteristics. A case with a myxoid matrix appearing on CT scan as a cystic lesion has also been reported [6].

*Spongiotic pericytoma (Ito cell tumor)* appears to originate from hepatic stellate cells. So far, only one case has been reported [1]. The polycyclic tumor contained multiple nodular cell aggregates, and tumor cells expressed vimentin, CD34, CD105, CD99, CD56, and smooth muscle actin. It remains unclear whether this tumor is benign or malignant [2].

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# Malignant Tumors

# 102

Henryk Dancygier

## Chapter Outline



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The most common malignant liver tumors are hepatic metastases. The approach to liver metastases has changed in the last years. Liver metastases from colorectal cancer in particular are approached nowadays more aggressively. Depending on the clinical situation they may be resected or treated with locally ablative methods, such as ethanol injection, cryotherapy, laser or radiofrequency ablation or by transarterial chemoembolization.

In the following sections only primary malignant liver tumors are discussed. Cholangiocarcinoma is discussed in Chapter 116.

## 102.1 Hepatocellular Carcinoma

### Definition

Hepatocellular carcinoma (HCC) is a malignant epithelial tumor originating from hepatocytes and consisting of liver cell-like but abnormal cells. The basic histologic structure with a trabecular-like arrangement of tumor cells surrounding sinusoidal lumina may nearly always be detected in histologically different HCCs [107, 200].

### Epidemiology

HCC belongs to the most common malignant tumors worldwide with nearly 1 million new cases occurring yearly. In high incidence areas (>20 new cases/$10^5$ inhabitants/year) approximately 20–40% of all malignant tumors are HCCs. Men are affected more often than women (8:1 in high incidence areas, 2–3:1 in low incidence areas). The mean age at manifestation in Western Europe and in the US is between 50 and 60 years.

The incidence of HCC exhibits marked geographical variations (Fig 102.1). High incidence areas with 100–150 new cases per $10^5$ inhabitants per year are East, West and Central Africa, China, Korea, Thailand and Vietnam. Moderate incidence rates of 5–20 new cases per $10^5$ inhabitants per year are observed in Japan, the Middle East, in South and in Central America. Low incidence rates of up to five new cases per $10^5$ inhabitants per year occur in North, West and Central Europe, North and South America, Australia, Central Asia and in the white population of North and South Africa. The incidence in central Europe is 3–7/$10^5$ inhabitants, in the Mediterranean area 5–20/$10^5$ [61]. The geographical variation in the incidence of HCC probably is related to the different geographical distribution of risk factors (see below) [30, 193].

In high incidence areas younger, previously healthy people are affected more often, and HCC relatively frequently develops in a noncirrhotic liver and progresses rapidly. $\alpha_1$-Fetoprotein levels in serum nearly always are elevated. HCC patients in the USA and in Europe tend to be older, HCC develops on the background of cirrhosis in approximately 90% of cases,

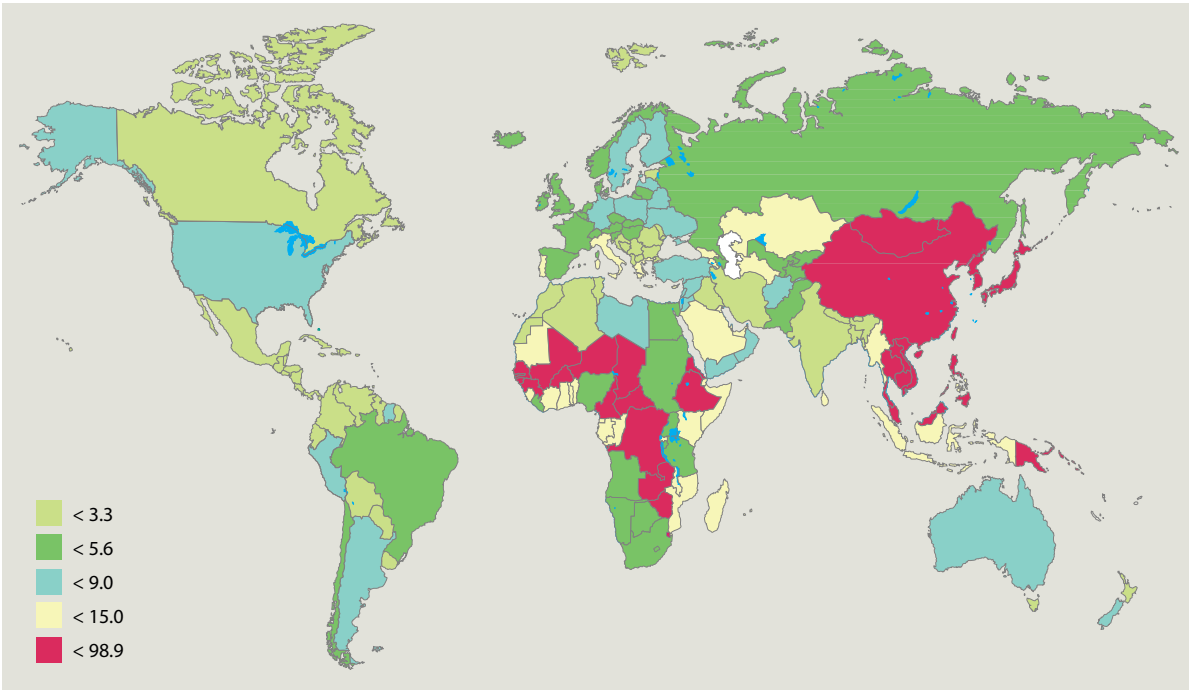


**Fig. 102.1** Worldwide incidence of hepatocellular carcinoma (Adapted from [17]. With permission)

$\alpha_1$-fetoprotein levels in serum are elevated more rarely, and the course is slower and less aggressive than in high incidence areas. Overall HCC incidence, especially in younger people, is rising in Western countries, possibly reflecting the increased incidence in chronic hepatitis C infection [17, 60, 186].

Ethnic factors affect the development of HCC, and among patients with chronic hepatitis C and cirrhosis, liver cancer risk was reported to be increased fourfold in Asians and doubled in African-American men, compared with Whites [169].

## Etiology

The risk factors for HCC are listed in Table 102.1.

*Liver cirrhosis* is a precancerous condition and each patient with liver cirrhosis, irrespective of its etiology carries an increased HCC risk. The risk of HCC correlates with the duration and etiology of liver cirrhosis (Fig. 102.2). Patients with chronic hepatitis B and C, with hereditary hemochromatosis and with alcoholic liver disease have the highest relative risk of HCC [12, 54, 216]. Increased age, anti-HCV-positivity, prolongation of partial thromboplastin time and low platelet counts define a subgroup of patients with liver cirrhosis at a particularly high risk for HCC [238]. *HCV genotype 1b* is associated with a statistically significant higher risk of developing HCC than other HCV genotypes [23]. Although interferon therapy reduces the risk of HCC, 3.5% of patients with chronic hepatitis C still develop HCC after interferon-induced sustained viral response. Elderly, male patients, with an advanced histologic stage seem to be at a high risk for the development of HCC even after achieving a sustained viral response [113].

On a worldwide scale, *chronic HBV infection* is the most important cause of HCC. More than 60% of HCC cases in hyperendemic areas are HBsAg positive. The lifetime risk of a HBsAg-positive person developing a HCC is approximately 40%. The presence of HBeAg and HBV-DNA further increases this risk [37, 249].

*Alcoholic liver disease* is the most common risk factor for HCC in Western countries. Liver iron overload and C282Y mutation are associated with a higher risk of HCC in patients with alcoholic cirrhosis 1[65].

*Insulin resistance states*, such as type 2 diabetes mellitus, obesity in men, and nonalcoholic fatty liver disease are becoming increasingly significant as risk factors

**Table 102.1** Risk factors for hepatocellular carcinoma[a]

| |
|---|
| Chronic hepatitis B (± D), C |
| Liver cirrhosis (especially of viral, hemochromatotic and alcoholic origin) |
| Insulin resistance states |
| Diabetes mellitus |
| Obesity (in men) |
| Nonalcoholic fatty liver disease |
| Toxins |
| Ethanol |
| Nictotin |
| Aflatoxin |
| Oral contraceptives [152] |
| Anabolic androgenic steroids |
| Membranous obstruction of inferior vena cava[b] [212] |
| $\alpha_1$-antitrypsin deficiency[b] [68] |
| Glycogen storage disease type I and III [46, 51] |
| Galactosemia |
| Progressive familial intrahepatic cholestasis (PFIC)[b] [112] |
| Ataxia telangiectasia[b] |
| Porphyria cutanea tarda [73] |
| Tyrosinemia[c] [153, 243] |

[a]The first three categories are by far the most important
[b]Single cases are documented. Evidence is inconclusive to establish a causal relation with HCC
[c]HCC occurs in approximately one third of patients who survive the first 2 years of life. HCC may also develop in a transplanted liver

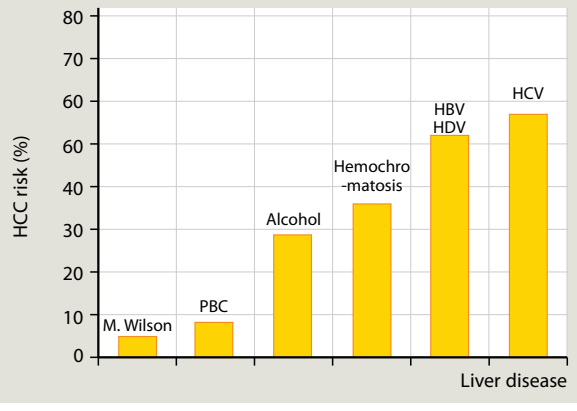


**Fig. 102.2** HCC-risk in patients with liver cirrhosis of different etiologies (From [12])

for HCC (particularly in affluent Western countries), and are responsible for the majority of HCC cases that hitherto could not be attributed to a "traditional" risk factor [26, 63, 64, 66, 123, 154]. Type 2 diabetes mellitus is associated with a two to three fold increase in the risk of HCC, regardless of the presence of other major HCC risk factors [48]. Recent evidence also suggests that individual risk factors, such as alcohol, chronic virus hepatitis B and C, type 2 diabetes mellitus, obesity and tobacco interact and are synergistic risk factors for HCC [36, 37, 39, 84, 94, 157, 171, 239, 254].

Up to 45% of patients with *hereditary hemochromatosis* die of HCC if treated inadequately (see Chapter 82). The risk of HCC in a cirrhotic hemochromatotic liver is increased up to 200-fold [53, 69, 170]. Rarely HCC may also occur in a non-cirrhotic hemochromatotic liver or in liver cirrhosis after successful iron depletion by phlebotomy. For practical purposes it may be assumed that HCC in hereditary hemochromatosis develops in a cirrhotic liver.

*Oral contraceptives* increase the risk of HCC approximately fourfold after an intake for 5 years. This association is proven in areas with low incidence rates of HCC. After discontinuing therapy, an increased HCC risk persists for approximately 10 years.

*Anabolic androgenic steroids* promote tumor formation. It is not entirely clear, however, whether the described tumors are highly differentiated HCCs or whether they instead represent hepatocellular adenomas. Peliotic areas frequently occur within these tumors. They are not associated with increased $\alpha_1$-fetoprotein serum levels and rarely metastasize.

Inborn or acquired *membranous obstruction of the inferior vena cava* above the junction with the liver veins may be associated with the development of HCC [212]. The cause is unknown. Relapsing parenchymal losses alternating with regenerative phases leading to a chronic increase in hepatocyte turnover are being incriminated.

*Glycogen storage disease* (type I and III) usually is associated with hepatocellular adenomas, but occasional HCCs also have been described [46, 51].

The inborn error of metabolism associated with the highest risk of HCC is *hereditary tyrosinemia type I*. In one study 16 out of 43 children developed a HCC [243]. All of the children had liver cirrhosis prior to the formation of HCC.

The risk of HCC is increased approximately 60-fold in patients with *hepatic porphyrias* [105].

HCC has been reported in patients with familial adenomatous polyposis, Alagille's syndrome, ataxia telangiectasia, Weber-Rendu-Osler's disease, biliary atresia and congenital hepatic fibrosis. But the evidence for a causal relationship is weak, and the concomitant occurrence of HCC and these disorders probably is incidental in most cases.

There is epidemiological evidence that increased consumption of coffee reduces the risk of liver cancer, though inference on causality remains open [19, 76, 88, 124].

## Pathogenesis

The exact pathogenesis of HCC is unknown. It is clear, however, that hepatocarcinogenesis is a multistage process involving an interplay of exogenous and endogenous factors, of direct carcinogens (for example, chemical and viral [HBV] carcinogenesis) and indirect carcinogenic mechanisms (for example, chronic necroinflammation) (Fig. 102.3).

The *chronic necroinflammatory process* is characterized by recurrent destruction of hepatic parenchyma associated with continuous stimulation of regeneration and hepatic remodeling. Cytokines and immunomodulatory substances, such as interleukins, interferon and tumor necrosis factor-α, proteases and growth factors are released and may result in the emergence of premalignant foci of dysplastic hepatocytes which ultimately may undergo malignant transformation [1, 93].

The *molecular pathogenesis* of HCC is not uniform. HCCs are genetically highly heterogenous tumors, with multiple chromosomal abnormalities having been described, but not all of them are operative in each HCC [190]. A high rate of premalignant changes in noncancerous tissue before the formation of a solitary tumor has been revealed by complementary DNA microarray analysis [111]. Mutations have been detected in genes involved in DNA-repair (p53, HBV x gene), cell cycle control (retinoblastoma gene 1, cyclin D, p16), in apoptosis and growth inhibition, in cell–cell interactions and in signal transduction (β-catenin, E-cadherin). Epigenetic modifications of tumor suppressor genes, such as gene-silencing by DNA-methylation have also been demonstrated [194]. Recently, etiology-dependent and etiology-independent oncogenes have been identified [201]. As in other malignant tumors, overexpression of protooncogenes and silencing of tumor suppressor genes ultimately results in the formation of HCC [33, 244].

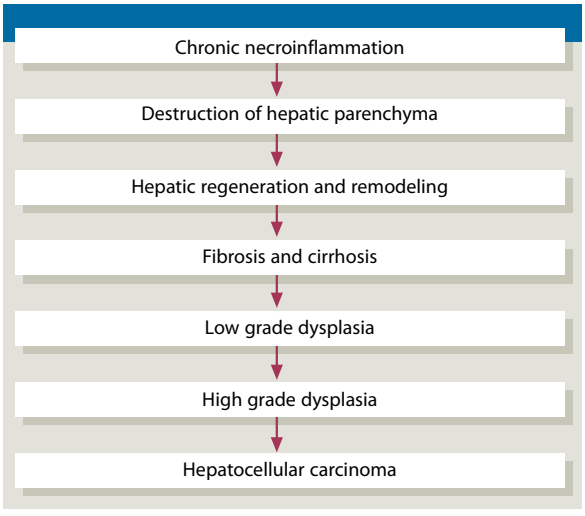


**Fig. 102.3** The pathogenesis of hepatocellular carcinoma is a multistage process

In addition to mutations and epigenetic alterations, *genetic susceptibility* plays a role in HCC development. Genetic polymorphisms in drug metabolizing enzymes, such as UDP-glucuronosyltransferases, N-acetyl transferases, glutathione-S-transferases and cytochrome P450 enzymes are associated with HCC risk and age of onset [236].

Several *growth factors*, such as insulin-like growth factors, epidermal growth factor (EGF), transforming growth factor-β (TGF- β) are involved in hepatocarcinogenesis, although their precise role still must be defined [5, 28, 189, 222]. Thus, EGF gene polymorphism has been shown to be associated with risk for development of HCC in liver cirrhosis through modulation of EGF levels [222]. Evidence from animal experiments suggests that embryonic liver fodrin (ELF), a TGF-β adaptor and signaling molecule, functions as a critical adaptor protein in TGF-β modulation of angiogenesis as well as cell cycle progression. Loss of ELF in the liver leads to cancer formation by deregulated hepatocyte proliferation and stimulation of angiogenesis in early cancers [5, 205]. Angiopoietin-1 and angiopoietin-2 have been shown to be involved in tumor angiogenesis, and increased expression of angiopoietin-1 and angiopoietin-2 has been show to occur in the development in HCC [230].

The *telomere hypothesis* of cancer initiation applies also to human HCC. Telomere shortening may initiate cancer by induction of chromosomal instability. Telomere shortening correlates with increasing aneuploidy of chromosome 8 in human HCC. The inactivation of cell cycle checkpoints coincides with further telomere shortening and an accumulation of DNA damage in HCC [93, 183–185, 198].

While definitive proof of a *stem cell* origin of HCC is still lacking, there is data suggesting that human liver tumors can be derived from hepatic progenitor cells rather than from mature cell types. Recent evidence suggests that most combined hepatocellular-cholangiocarcinomas arise from hepatic progenitor cells that retained their potential to differentiate into the hepatocytic and biliary lineages [135, 204]. Chemical carcinogenesis models in the liver demonstrate varying degrees of oval cell proliferation. There is also preliminary evidence that HCC may maintain a bipotential phenotype consistent with an oval cell origin [211].

## Chronic Hepatitis B Virus Infection

(See also Section 63.3).

Chronic HBV infection is of major significance in the development of HCC in the Far East and in the Sub-Sahara regions. In Asia HBV genotype C is associated with an increased risk of HCC [32]. The geographical distribution pattern of the HBV carrier state coincides with the incidence of HCC in these regions. Serologic markers of a past or present HBV infection occur in approximately 80% of HCC patients. The relative risk of HCC in HBsAg-carriers is approximately 220-fold compared with HBsAg-negative persons in these geographical zones. Approximately 25% of European HCC patients have evidence of a HBV infection. Considering the lower prevalence rates of HBV infection in Europe, the association of HBV with HCC is as strong as in high incidence areas [6].

Active HBV replication plays a pivotal role in HBV-induced carcinogenesis, and the *incidence of HCC depends on the viral load and on the duration of HBV infection*. The cumulative incidence of HCC is 1.3% in persons with serum HBV-DNA levels of less than 300 copies/mL and increases to an incidence of 14.9% in those with HBV-DNA levels of $10^6$ copies/mL or more. Elevated serum HBV-DNA level (≥10,000 copies/mL) is a strong risk predictor of HCC independent of HBeAg, serum alanine aminotransferase level, and liver cirrhosis [38]. However, once HBV-induced cirrhosis is established the risk of HCC further increases significantly.

The risk of HCC is particularly high in perinatally acquired HBV infection. Perinatal transmission and maternal viral load are important risk factors in hepatocarcinogenesis. In the Far East (except for Japan) HBV is transmitted predominantly perinatally (i.e. "vertically"), and HBV infection is active and replicative during pregnancy and delivery in most women. In Africa, spread of HBV is primarily horizontal. Following perinatal transmission, the probability of becoming a chronic HBV carrier is 95%. The risk of HCC is approximately 40% in horizontal transmission during childhood, and 10% in horizontally acquired adulthood HBV infection [35]. The latency period between HBV infection and the development of HCC is approximately 30 years and may reach even 60 years in individual cases.

HBV induces HCC formation in several ways. HBV replication promotes and maintains chronic necroinflammation, thus promoting hepatic carcinogenesis indirectly [81]. In addition, HBV integrates into the host's genome and may act as a direct carcinogen. In nearly 90% of HBV-associated HCCs, HBV-DNA was found to be integrated into cellular genes [161]. Integration of viral DNA into protooncogenes and tumor suppressor genes (p53) may initiate malignant transformation. HBV-DNA integration may induce the production of the transactivator protein hepatitis Bx antigen (HBx) that may stimulate tumor promoter signaling pathways [37, 106]. HBx deregulates DNA methyltransferase promoter activity, and promotes both specific regional hypermethylation and global hypomethylation [180]. Its integration into the host's genome results in alterations of tumor suppressor gene p53 which leads to inhibition of apoptotis and cell cycle arrest. In addition, it up-regulates vascular endothelial growth factor and stimulates angiogenesis [133, 205]. These epigenetic modulations by HBx suggest a mechanism for HBV-mediated hepatocarcinogenesis even in the absence of cirrhosis [233].

HBV maintains its pro-oncogenic properties also in the case of occult HBV infection and there is clear evidence that *occult HBV is a risk factor for development of HCC*. Occult HBV infection is characterized by the persistence of HBV-DNA within the liver tissue of HBsAg-negative individuals. Both integrated viral DNA and covalently closed circular HBV genomes may be detected in patients with occult HBV infection. Moreover, the presence of free HBV genomes is associated with persistence of viral transcription and replication [187, 188, 245].

## Chronic Hepatitis C Virus Infection

HCV is carcinogenic and chronic HCV infection is closely related to the development of HCC. In Japan and Southern Europe HCV may be demonstrated in up to 80% of patients with HCC. HCV genotype 1b is associated with a higher HCC risk. The latency period between HCV infection and the development of HCC is 20–30 years.

The precise mechanism of hepatocarcinogenesis in humans by HCV is currently unclear. The virus replicates within the tumor tissue, but (in contrast to HBV) HCV-RNA is not integrated into the host's genome.

Presumably chronic necroinflammation with the continuous stimulation of proliferation and regeneration, and the development of cirrhosis are the background for malignant transformation. In addition, HCV core protein promotes proliferation of human hepatoma cells by activation of the MAPK/ERK pathway through upregulation of transforming growth factor-α transcription via activation of nuclear factor-κB [199]. In contrast to HBV-induced HCC, *HCV-associated HCC almost exclusively develops in a cirrhotic liver* by a multistep and multicentric process [174].

Recent studies have found trace HCV viral material both among sustained responders and in patients with chronic liver disease who are HCV-RNA negative, suggesting the entity of *occult hepatitis C*. Little is known of the potential importance of occult type C hepatitis for the development of HCC. The finding of occult hepatitis B in noncirrhotic HCCs among patients with hepatitis C who achieved sustained virologic response after treatment raises interesting questions regarding coinfections and the natural history of both of these viral hepatitis agents [77].

## Liver Cirrhosis

*Liver cirrhosis is the most important precancerous condition*. Irrespective of its etiology, it is an independent risk factor for HCC, especially in countries with low to moderate HCC incidence rates. In Europe liver cirrhosis is usually due to chronic ethanol consumption and/or to chronic HBV or/and HCV infection. The yearly incidence of HCC in patients with virus-induced liver cirrhosis is approximately 1.5–3%. The risk of HCC is higher in HBV-HCV coinfection compared to each viral disease alone. Alcoholic liver cirrhosis is associated with malignant transformation in 3–10% of cases after a follow-up of 10–20 years. Alcohol is believed to act as a cocarcinogen.

The exact pathogenetic mechanisms of malignant transformation in a cirrhotic liver are unknown. Presumably the continuous necroinflammatory process associated with loss of liver parenchyma not only leads to the formation of regenerative nodules, but also to genomic injury. Subsequent impairment of cellular proliferation and differentiation results in the occurrence of liver cell dysplasia and finally in the emergence of malignant foci (see below "Pathology") [15].

## Alcoholic Liver Disease

See Chapter 88.

## Nonalcoholic Fatty Liver Disease

See Chapter 89.

## Aflatoxin $B_1$ and Other Toxins

*Aflatoxin* is a genotoxic and mutagenic mycotoxin produced by the ubiquitary fungus *Aspergillus flavus*. It is the most potent experimental liver carcinogen. Aspergillus flavus contaminates food that has been stored improperly. Epidemiologic studies in Africa have shown a clear correlation between aflatoxin contaminated food and risk of HCC. Aflatoxin $B_1$ is transformed by cytochrome P450 enzymes (CYP1A2 and CYP3A4) to its active moiety aflatoxin $B_1$–8, 9–epoxide, which is a potent carcinogen that can form DNA adducts. In hyperendemic areas with aflatoxin exposure and chronic HBV infection a particularly high prevalence of mutation in codon 249 of the p53 gene is found with an exchange of arginine for serine (Arg249Ser-p53) [87, 160].

The algal toxin *microcystine* may contaminate well water and is held responsible for the development of HCC in certain regions in China.

Chewing *betel nut* that is prevalent in Asia is also believed to be associated with an increased incidence of HCC.

Other chemical carcinogens, such as vinyl chloride, thorotrast or arsenic induce the development of hepatic angiosarcomas.

## Pathology

HCCs develop in a multistage process presumably from multicentric, clonally proliferating cells. Most small (<1 cm) HCCs are well-differentiated in their early stages. Growth and proliferation are associated with increasing dedifferentiation.

The aim of many studies in the past decades was to define precancerous changes of HCC, in order to lower morbidity and mortality of HCC by finding its precursor lesions. The early detection of such indicator lesions would enable the implementation of effective screening and therapeutic strategies, and ideally would prevent the development of overt carcinoma.

The terminology of precancerous lesions is rather confusing and mirrors our lack of understanding of the exact pathogenesis of HCC [95]. The following discussion focuses on purely descriptive terms which hopefully will be complemented and correlated with gene expression patterns in the near future. A combined morphologic and molecular approach will allow for better characterization of premalignant lesions. Currently, the biggest diagnostic *challenge in clinical practice is to distinguish macroregenerative and dysplastic nodules from highly differentiated HCC*. This may be especially difficult or sometimes even impossible based on the small tissue samples obtained by liver biopsy. Most pathologic studies of precancerous hepatic lesions have therefore been performed on explanted or autoptic livers.

### *Macroregenerative Nodules*

Macroregenerative nodules (MN) are circumscribed, regenerative nodules measuring 1–3 cm in diameter. They usually occur within macronodular cirrhosis, but can also develop in other chronic liver diseases or after extensive loss of liver parenchyma. Though these tumor-like nodules have been assigned many names in the past, such as adenomatous hyperplasia, adenomatoid hyperplasia, and cirrhotic pseudotumor, they should not be used anymore.

MN may grow and form daughter nodules ("nodule in nodule"). Histologically MN contain portal tracts, bile ducts, and arterial and venous vessels. Their parenchyma is made up of hyperplastic liver cell plates containing 2–3 cell layers. The hepatocytes are cytologically uniform and resemble or equal those of the surrounding parenchyma. Cellular atypias are rare, but occasionally dysplastic changes may occur. During follow-up of 33 months, up to 31% of MN may transform into HCC. In addition, HCC may develop outside the MN. Thus, MN may serve as indicator lesions that characterize a cirrhotic subpopulation with a high risk of HCC [16].

### *Liver Cell Dysplasia*

The term liver cell dysplasia (LCD) was introduced in 1973 when it was shown to be prevalent in livers harboring HCC [4]. LCD denotes the trabecular rearrangement of groups of hepatocytes of various size and shape with enlarged, pleomorphic, hyperchromatic nuclei, and prominent nucleoli. Usually LCD occurs in regenerative nodules on the background of liver cirrhosis [15, 223]. DNA changes associated with LCD are not uniform, but aneuploidy and numeric chromosomal aberrations are usually present. LCD may be a precursor of HCC and should therefore be regarded as a precancerous lesion. Japanese authors have subdivided LCD into a large cell and small cell variant [242].

#### Large Cell Dysplasia

This form is more common than small cell dysplasia and is characterized by enlarged hepatocytes with a broad eosinophilic cytoplasm and enlarged nuclei without a change in the nucleo-cytoplasmic ratio. The nuclei are polymorphous, hyperchromatic and may contain many nucleoli. Occasionally dysplastic hepatocytes may contain several nuclei. Large cell dysplasia usually is aneuploid.

Large cell dysplasias occur in up to 7% of all cirrhotic livers and in approximately 65% of cirrhotic livers containing a HCC. The discussion whether large cell dysplasia indeed represents a premalignant lesion is controversial. It can also occur in long-standing cholestasis. In this clinical setting it is reversible and has been interpreted as a regenerative reaction [168].

At present, large cell dysplasia is regarded rather as a degenerative, non-premalignant lesion of non-proliferating hepatocytes, while small cell dysplasia maintains its increased proliferative potential.

#### Small Cell Dysplasia

The cytoplasm of liver cells with small cell dysplasia is basophilic, the nuclei are hyperchromatic and the nuclear-cytoplasmic ratio is shifted in favor of the nuclei leading to a phenomenon of "nuclear crowding".

### *Dysplastic Foci*

This term denotes clusters of dysplastic hepatocytes measuring up to 1 mm in diameter. They consist of large or (more often) small dysplastic hepatocytes, are more or less well delineated, and show no invasive growth. They often are localized in the periportal area but do not contain portal tracts themselves. Dysplastic foci occur in almost every advanced cirrhosis.

### *Dysplastic Nodules*

Dysplastic nodules predominantly occur in long-standing cirrhosis of HBV and HCV origin. They measure several millimeters to centimeters in diameter. They bulge from the cut surface and appear clearer than the surrounding parenchyma. Dysplastic nodules are composed of large and small dysplastic hepatocytes with a clear, basophilic or steatotic cytoplasm. Their architecture may be normotrabecular or pseudoglandular. According to cytologic criteria they may be subdivided into low and high grade forms.

#### Low Grade Dysplastic Nodules

The hepatocyte display only mild cytologic variability. The nuclear-cytoplasmic ratio is usually normal or only slightly shifted in favor of the nucleus. Nuclear atypia is mild. Hepatocytes may contain fat droplets and Mallory-Denk bodies. The liver cells are arranged in plates not thicker than two cell layers. Mitoses usually are absent.

#### High Grade Dysplastic Nodules

The liver cell plates are thicker than two cell layers and often show a pseudoglandular arrangement. The cytoplasm often is basophilic. The nuclei are enlarged and hyperchromatic, the nuclear-cytoplasmic ratio is increased. Mitoses may occur. However, unequivocal signs of malignancy, such as stromal invasion or lymphatic spread of tumor cells are absent. Occasionally a "nodule in nodule" formation may be observed.

*Iron-free foci* or *nodules* in hereditary hemochromatosis represent a special form of dysplastic foci/nodules. They exhibit cytologic and architectural signs of dysplasia and are precancerous lesions [53].

### *Progression from Dysplasia to Hepatocellular Carcinoma*

The progression from a dysplatic nodule to HCC is characterized by molecular changes that are still not elucidated in all their details, but which become increasingly deciphered with microarray techniques. Gene expression profiles can discriminate not only between dysplastic nodules and overt carcinoma, but also between different histological grades of HCC, thus allowing for a clear molecular distinction between dysplastic nodules and overt HCC [149, 162, 167]. However, it will probably take some more years until these sophisticated techniques will be available in clinical practice.

The histologic transition from a high grade dysplastic nodule to a well-differentiated HCC may be difficult to diagnose even for experienced pathologists. A diameter of >1.5 cm, reduction or loss of reticulin fibers, high grade nuclear atypia, increased mitotic activity, multinucleated hepatocytes, pseudoglandular arrangement of hepatocytes, increased capillarization of sinusoids are all features that favor the diagnosis of HCC. The transition from a premalignant lesion to a small HCC is associated with a sharp increase in glypican 3 expression. Thus, immunohistochemical glypican 3 expression may help to distinguish a small HCC from a dysplastic nodule [136]. Despite all diagnostic efforts, however, assigning a lesion as high grade dysplastic or already as definitely malignant is often impossible, especially in small liver biopsy samples. This notwithstanding, the clinician should expect the

pathologist in addition to grading and staging hepatic lesions also to comment on the presence or absence of liver cell dysplasia, particularly when dealing with biopsies from nodular lesions.

## *Hepatocellular Carcinoma*

Excellent descriptions of the macroscopic and microscopic pathology of HCC dating back to the early years of the last century are available [58, 59, 176]. Relatively little has been added to these seminal works during the last decades. However, aside from a few exceptions the descriptive morphology of a HCC is clinically not very relevant, neither from the epidemiological nor from the biological point of view.

### Gross Anatomy

Eggel's macroscopic classification of 1901 has essentially maintained its value until today [59]. Accordingly, three macroscopic types of HCC may be distinguished:

- Multinodular type
- Massive, and
- Diffuse type

The multinodular type is characterized by many, quite well-delineated tumor nodes scattered throughout the liver. The massive or expanding type exhibits a main tumor mass, sometimes with a few satellite nodules, which may be separated from the surrounding liver tissue by a fibrous capsule. In the diffuse or spreading type many tumor masses with ill-defined borders coalesce and intermingle with surrounding liver tissue. Combinations between these main types are common. Often tumor nodes show regressive changes, such as hemorrhages, necrosis or biliary extravasates. HCC tends to invade vessels and to spread hematogenously.

Japanese authors have classified HCC into an infiltrative, expanding, mixed (infiltrative and expanding) and a diffuse type. This classification, however, does not yield important additional insights, and essentially corresponds to Eggel's work.

Japanese investigators again have drawn attention to the existence of a *small HCC* as a discrete clinical entity. It is said to behave a priori in a biologically different way than the usual HCC. Small HCC is defined as a tumor less than 2 cm in diameter that is usually well-differentiated, contains portal tracts, is surrounded by a fibrous capsule, and almost always develops in a cirrhotic liver. Uninodular small HCCs have an excellent postoperative prognosis with 5 year survival rates of >90% [100, 115, 218]. However, it is still a matter of debate if small HCC is indeed a separate clinical entity or just the T1 stage of a regular HCC.

*Pedunculated HCC* is rare, with fewer than 50 cases described. It probably originates from accessory lobes of the liver and usually arises from the lower surface of the right liver lobe. Pedunculated HCC is not substantially different from HCC in general. Despite an average weight of >1 kg the slow growth and good prognosis relate to its extrahepatic location. Due to its peduncle it may be resected quite easily [3].

### Microscopic Anatomy

Histologically most HCCs display cellular and architectural similarities with the normal liver. Tumor cells are typically arranged in trabeculae separated by vascular sinusoidal spaces (Fig. 102.4). Even in less well-differentiated HCCs these features may be found every now and then.

The histological spectrum of HCCs ranges from well-differentiated tumors that may be difficult to distinguish from the normal liver, to anaplastic, highly undifferentiated carcinomas [96].

Microscopically, different *growth patterns* may be distinguished:

**Trabecular (platelike)**. The trabecular variant is the basic pattern from which all other forms may be derived. Tumor cells grow in cords of variable thickness. The trabeculae are separated by sinusoid-like, occasionally cavernous, endothelium-lined spaces. Kupffer cells are diminished or completely absent. The connective tissue stroma is only scant or lacking. Reticulin fibers are usually absent.

**Pseudoglandular (acinar)**. The pseudoglandular variant may derive from the trabecular form by central degeneration of solid trabeculae or by dilatation of bile canaliculi into gland-like spaces. The tumor appears acinar, glandlike. The pseudoglandular spaces may be filled with bile, cellular debris, fibrinous exudate, or a homogenous, colloid-like material reminiscent of thyroid follicles.

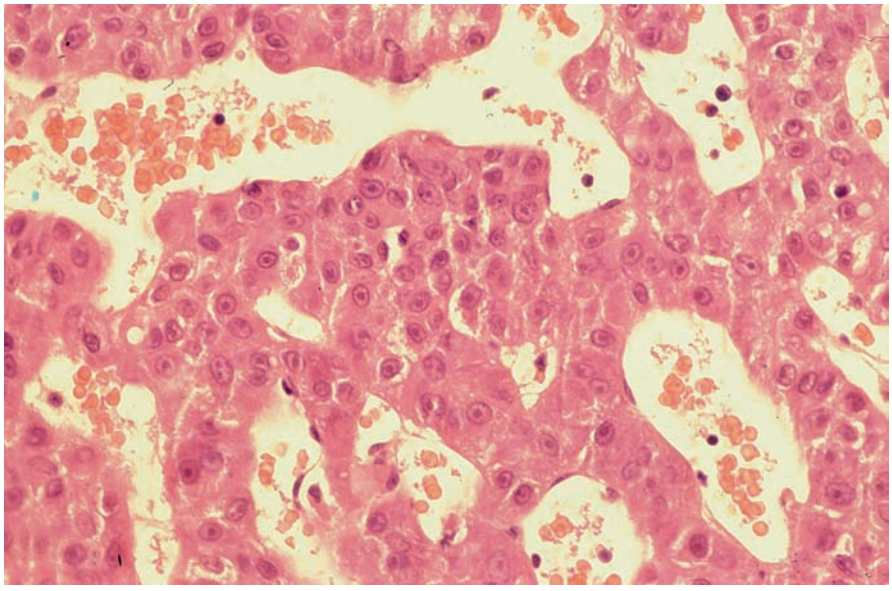

**Fig. 102.4** Differentiated hepatocellular carcinoma. Trabecular arrangement of tumor cells separated by vascular sinusoidal spaces. Hematoxylin and Eosin stain (×400)

**Solid (compact)**. The solid variant is basically a trabecular HCC in which the sinusoids are compressed by growing cancer cells imparting the tumor a solid appearance.

**Scirrhous**. Scirhhous HCC (SHCC) accounts for approximately 5% of all HCCs. The majority of SHCC (88%) are located close to the liver capsule [121]. Cords of tumor cells are embedded in an abundant fibrous stroma, and the diagnosis of SHCC is made on the basis of a scirrhous histological pattern exceeding 50% of the tumor area. Common stromal components of SHCC are collagen types I and III, and almost all stromal cells are α-smooth muscle actin-positive [175]. This variant is most often seen following radiation, chemotherapy or infarction. Cholangiocarcinomas and metastases may have a similar appearance, and indeed some pathologists believe that scirrhous HCC actually is a cholangiocarcinoma in many cases.

The so-called *sclerosing hepatic carcinoma* belongs to this group. It usually arises in a noncirrhotic liver and is characterized clinically by (paraneoplastic) hypercalcemia. Sclerosing carcinomas do not constitute a separate histopathological entity.

**Fibrolamellar**. See Section 102.2

The *cytological features* of HCCs may vary:

**Liver cell-like**. Most often tumor cells resemble normal hepatocytes. The extent of nuclear hyperchromasia and nuclear polymorphism as well as the nuclear to cytoplasmic ratio depend on the degree of tumor differentiation. The cytoplasm is finely granular and often more basophilic than that of normal hepatocytes. Canaliculi are often demonstrable between tumor cells.

**Pleomorphic**. Pleomorphic variants are characterized by a loss of the trabecular pattern with marked variation in cellular and nuclear size and shape with the occasional occurrence of bizarre giant cells.

**Clear cell**. The clear cytoplasm of tumor cells in this variant is primarily due to the presence of glycogen, but also of water and fat. These tumors must be distinguished from metastatic renal carcinoma or from adrenal tumors infiltrating the liver. Groups of clear cells also may occur in well-differentiated HCCs.

**Oncocytic**. Oncocyte-like cells with an eosinophilic, granular cytoplasm are typical of fibrolamellar carcinoma. Their granularity is due to a large number of mitochondria.

**Spindle cell (pseudosarcomatous** or **sarcomatoid)**. Spindle cell tumors have a sarcomatoid appearance and should be characterized further immunohistochemically for the expression of desmin, actin, myosin and myoglobin. If epithelial and spidle cell components occur concomitantly the term *carcinosarcoma* is applied by some pathologists.

**Lymphocytic infiltration**. This is a rare subtype of HCC with a lymphoid stroma. It has a better prognosis than regular HCC [67, 241].

**Intracellular inclusions**. Intracellular inclusions occur in many HCCs. Globular *hyaline inclusion bodies* are found in up to 15% of cases. They commonly consist

of $\alpha_1$-antitrypsin, fibrinogen or other export proteins. Albumin and fibrinogen may also appear as *pale bodies*. *Mallory-Denk bodies* are the expression of impaired metabolism of intermediary filaments. They are readily immunostained by anti-ubiquitin antibodies. *Ground glass inclusions* may be due to HBsAg expression or to fibrin accumulation. Some HCCs may contain *bile droplets*. They testify to the hepatocellular nature of the tumor. Cytoplasmic material may herniate into the nucleus and appear as nuclear inclusions in histological sections.

**Grading of HCC**. The degree of differentiation does not always correlate with the biological behavior of HCC. In addition, various grades of differentiation may occur in different areas in one and the same tumor. Therefore grading does not assume a major clinical significance in the management of patients with HCC although vascular invasion is seen more often in moderately or poorly differentiated HCCs than in well-differentiated tumors.

Occasionally it may be difficult to recognize the hepatocellular origin of tumor cells. Immunocytochemical staining with liver specific antibodies, for example Hep Par 1 and visualization of bile canaliculi with polyclonal anti-CEA or CD10 is often helpful in these cases (see Fig. 3.5) [129]. Most HCCs express cytokeratins 8 and 18 (hepatocytic markers). However, 50% also express cytokeratins 7 and 19 (cholangiocytic markers). Simultaneous expression of hepatocytic and cholangiocytic markers was demonstrated in 61.5% of cases in a recent study [110]. Thus cytokeratin immunostaining only contributes little to the differential diagnosis between HCC and cholangiocarcinoma [52]. Immunostaining for glypican 3 and survivin is more specific and may confirm the diagnosis of HCC [34, 149]. Clathrin heavy chain and formiminotransferase cyclodeaminase have recently been described as novel immunohistochemical tumor markers for HCC [203]. Expression of $\alpha_1$-fetoprotein is observed in only approximately 25% of HCCs. Moreover, it often is weak and patchy in distribution. The specificity of $\alpha_1$-fetoprotein expression is relatively high, while its sensitivity is low. On the other hand most hepatoblastomas (see Section 102.3) show a strong $\alpha_1$-fetoprotein expression.

## Diagnosis

The diagnosis of HCC rests on laboratory findings, sonographic and radiologic techniques and on histopathology [221].

### *Clinical Manifestations*

HCC remains asymptomatic for long periods of time. If diagnosis would rely merely on clinical manifestations it would inevitably be made in an advanced stage of the disease. Symptoms are nonspecific and are those of the underlying chronic liver disease if HCC develops in the context of chronic liver disease. Patients with liver cirrhosis who, without an obvious reason develop ascites, hepatic encephalopathy, variceal bleeding or fever of unknown origin should be investigated for the presence of an HCC.

In advanced stages patients complain about a sense of pressure and fullness in the upper abdomen and report weight loss. Jaundice may be caused by bile duct compression or may be due to diffuse parenchymal tumor infiltration. Rarely a tumor may rupture into the peritoneal cavity causing an acute intraperitoneal hemorrhage and peritonitis. Over large, well-vascularized tumors a blood flow murmur may be heard in up to 25% of cases.

Rarely, a Budd–Chiari syndrome or obstruction of the inferior vena cava may be the initial manifestation of an HCC.

Occasionally patients with HCC develop *paraneoplastic syndromes*, such as hypoglycemia (consumption of glucose by the tumor; secretion of insulin-like growth factor II by the HCC in less than 5% of cases), erythrocytosis (production of erythropoietin by the HCC), hypercalcemia (secretion of parathyroid hormone-related protein; predominantly in the sclerosing variant of HCC), watery diarrhea (secretion of vasoactive intestinal peptide and other gastrointestinal peptides) or arterial hypertension (production of angiotensinogen by the HCC) [20].

The incidence of extrahepatic metastasis is approximately 13% [101].

### *Laboratory Findings*

The laboratory findings are nonspecific and usually the expression of the underlying liver cirrhosis (see Chapter 79). In addition to the routine parameters of liver function, the measurement of tumor markers assumes an important role in patients with hepatic mass lesions.

## $\alpha_1$-Fetoprotein

*$\alpha_1$-Fetoprotein* (AFP) is the most common serum tumor marker used. In countries with a high incidence of HCC serum concentrations of AFP are elevated in up to 90% of patients, occasionally >50,000 ng/mL. In countries with low or intermediate HCC incidence rates AFP levels are increased less often and serum concentrations are usually lower. AFP production by the tumor is age dependent, with younger patients showing higher AFP levels. Patients with an HCC that develops in the context of alcoholic cirrhosis usually have lower serum AFP levels than those in whom HCC is caused by viral liver cirrhosis. Up to 40% of patients with a small HCC have normal AFP levels. Patients with fibrolamellar HCC also usually have normal AFP levels (see Section 102.2). *The majority of patients with liver cirrhosis and slightly elevated serum AFP levels do not have a HCC*. However, AFP levels >500 ng/mL in a cirrhotic patient are virtually diagnostic of HCC, and AFP levels of approximately 100 ng/mL and lower, but steadily rising should arouse the suspicion of an underlying HCC. AFP concentrations do not correlate with the severity of clinical manifestations or with the size of the tumor. Possibly, however, AFP levels ≥1,000 ng/mL indicate a poor long-term prognosis after tumor resection (see below) [83]. The sensitivity of AFP is 39–64% and the specificity 76–91%. The specificity depends on the etiology of HCC and is higher in HBsAg-positive patients (78%) than in HBsAg-negative patients (50%) [12, 70, 75]. Even if the "best-case" estimates of AFP sensitivity and specificity are accurate, AFP has limited utility for detecting HCC. *AFP levels should be measured after a nodular liver lesion has already been detected with imaging techniques* [82].

The molecular structure of AFP is not uniform. Attempts at increasing the specificity of AFP in the diagnosis of HCC have been based on the different reactivities of its oligosaccharide side chains with lectins. The incidence of HCC in HCV-related cirrhosis was reported to be significantly higher in patients with elevated Lens culinaris agglutinin-reactive fraction of AFP (AFP-L3%) than in those with elevated "regular" AFP. The high sensitivity of approximately 91% for AFP-L3% persisted among patients with elevated AFP (20–200 ng/mL) suggesting that AFP-L3% may have clinical utility in patients with AFP concentrations of 20–200 ng/mL. Measurements of different AFP fractions, however, are still experimental and have not yet been adopted into clinical practice.

Increased AFP levels occur during pregnancy, in gonadal tumors and as an expression of liver regeneration following hepatic parenchymal necrosis.

*Hereditary persistence of AFP* may be due hepatocyte nuclear factor-1 mutations that result in AFP gene expression. Unexplained persistent elevated AFP levels should lead to family study and/or AFP gene promoter sequencing to avoid inappropriate explorations and treatment decisions [2].

## Des-γ-Carboxy-Prothrombin

Des-γ-carboxy-prothrombin (DCP) has been assumed to be more sensitive than AFP especially in small HCC. However, in approximately 20% of HCCs with a diameter of <3 cm serum levels of DCP were normal and recent studies even suggest that AFP is more useful than DCP in the diagnosis of small HCC [137, 166, 172]. Thus, currently the utility of DCP in the diagnosis of HCC is unclear and further prospective studies are needed [155]. Measurement of serum DCP levels has not gained access into clinical practice.

## Novel Markers

In view of the shortcomings of classical tumor markers, such as AFP, in the diagnosis of HCC the search for novel markers is proceeding at a fast pace. Immunocytochemical expression of heat shock protein 70, glypican 3 and glutamine synthetase has been described as helpful in distinguishing early HCC (grade 1) from dysplastic nodules arising in cirrhosis [56]. Glypican 3 also appears in serum and elevated levels have been reported in patients with HCC [31].

Endoglin, specific isoforms and cleavage products of endoplasmic reticulum proteins, the serum N-glycan profile and circulating levels of endothelial progenitor cells have recently been added to the list of potential HCC markers [40, 86, 140, 248]. Currently, however, all these potential new HCC markers are to be viewed as experimental and are not used in routine clinical practice.

Serum protein profiling using proteomic techniques is a promising novel approach to the diagnosis of HCC

in patients with chronic liver diseases [102, 179, 202]. At present, however, this technique is restricted to few highly specialized centers.

## Imaging Techniques

Imaging techniques are the cornerstone in the diagnosis of HCC. Architectural changes in the cirrhotic liver, however, make diagnosis difficult, since regenerative nodules are difficult to distinguish from neoplastic nodules.

The most important imaging tools are ultrasound (US), computed tomography (CT) and magnetic resonance imaging (MRI). The characteristic criteria for the diagnosis of HCC are arterial hypervascularity combined with washout in the portal-venous phase (for a detailed discussion of US, CT and MRI in the diagnosis of hepatic tumors see Chapters 37 and 38) [195].

Due to its wide availability US is usually the first technique employed. Most small HCCs are hypoechoic, but a hyperechoic pattern is by no means uncommon, particularly in patients under the age of 70 [191]. Contrast enhanced perfusional US (CEUS) allows the further characterization of the hypervascular pattern of most HCCs [74, 128]. The sensitivity of CEUS for the detection of HCC in a cirrhotic liver is 20–50%, its specificity is 92–96%. The sonographic detection rate of HCCs < 2 cm in a cirrhotic liver has been reported to be only 13% [221]. Thus, US is highly specific but insufficiently sensitive to detect small HCC in many cirrhotics [43].

Modern triphasic helical CT scans currently have a sensitivity of up to 90% in visualizing HCC and even small tumors measuring less than 1 cm may be delineated. The specificity of CT, however, especially for small tumors in a cirrhotic liver is much lower.

MRI has the advantage of a relatively minor risk of contrast-induced nephrotoxicity and is not associated with radiation exposure. Most HCCs are hypointense on T1-weighted, and hyperintense on T2-weighted images. The sensitivity of MRI for HCC measuring 1–2 cm is 80–85%.

Conventional angiography has lost its role in the diagnosis of HCC, but MRI angiography has been reported to be superior to helical CT for the detection of HCC ≥ 1 cm [24].

Overall CEUS, dynamic CT and contrast-enhanced MRI yield comparable results when performed by experienced and dedicated examiners. The differentiation of dysplastic nodules from early HCC remains unsatisfactory with all three methods, but contrast-enhanced MR examination of cirrhotic patients waiting for liver transplantation has been reported to be the best tool for the early detection of (pre)malignant lesions [134]. Small superficial (subcapsular) hepatic lesions represent a further diagnostic problem. They are often difficult to detect with all current radiological techniques. Diagnostic laparoscopy readily detects these subcapsular tumors and allows for biopsy under visual guidance (Fig. 102.5).

The sensitivity of $^{18}$F-fluoro-2-deoxy-d-glucose positron emission tomography (PET) in visualizing HCC lags far behind that of dynamic CT and MRI [108, 210]. PET is not able to detect HCCs smaller than 2 cm in diameter. However, PET plus CT fusion images may be useful in detecting extrahepatic metastases in HCC [163].

## Approach to a Hepatic Mass in a Cirrhotic Liver

The progress achieved over the last decades in detecting hepatic tumors with noninvasive imaging modalities is impressive. Findings may be quite characteristic, however, they are not pathognomonic for HCC.

Although the majority of arterially enhancing nodules measuring < 2 cm in patients with cirrhosis are

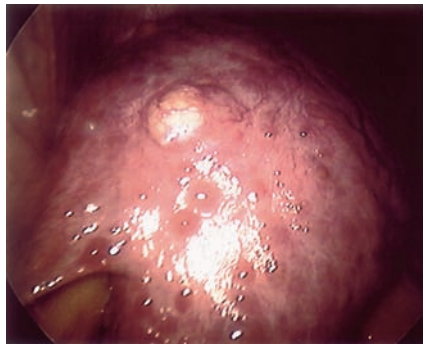

**Fig. 102.5** Laparoscopic appearance of a superficial hepatocellular carcinoma (approximately 1.5 cm in size) in a cirrhotic liver

benign, *any newly discovered nodule in a cirrhotic liver should be regarded as suspicious of HCC until proven otherwise* [14, 25, 178, 206]. Nodules larger than 2 cm are highly suspicious for HCC. Given the high pretest likelihood of HCC in a single liver nodule > 2 cm detected in a cirrhotic liver, current management *guidelines for HCC do not require biopsy in every patient to prove the diagnosis* [22, 65]. The diagnosis of HCC can be established without a positive biopsy if a conclusive vascular pattern (see above) is present on contrast enhanced imaging (US, MRI or CT) in combination with AFP serum levels. However, the sensitivity of these noninvasive criteria is 33% and absence of a conclusive pattern does not rule out malignancy [72]. Noninvasive morphologic criteria for the diagnosis of HCC, such as arterial hypervascularity combined with washout in the portal-venous phase, have been reported to be satisfied in only 61% of small nodules in cirrhosis. Relying on imaging techniques alone, diagnosis of HCC would be missed in up to 38% of cases in nodules < 2 cm in diameter [14]. Therefore, the AASLD guidelines suggest a greater role for image-guided biopsy of lesions greater than 1 cm clinically suspicious for HCC [9]. However, even liver biopsy findings may be inconclusive and it may be difficult to distinguish dysplastic lesions from highly differentiated HCCs.

The suggested sequence of tests used to diagnose HCC in a cirrhotic liver primarily depends on the size of the lesion. An algorithm for the evaluation of a liver mass in a cirrhotic liver according to the AASLD practice guidelines is reported in Fig. 102.6 [22].

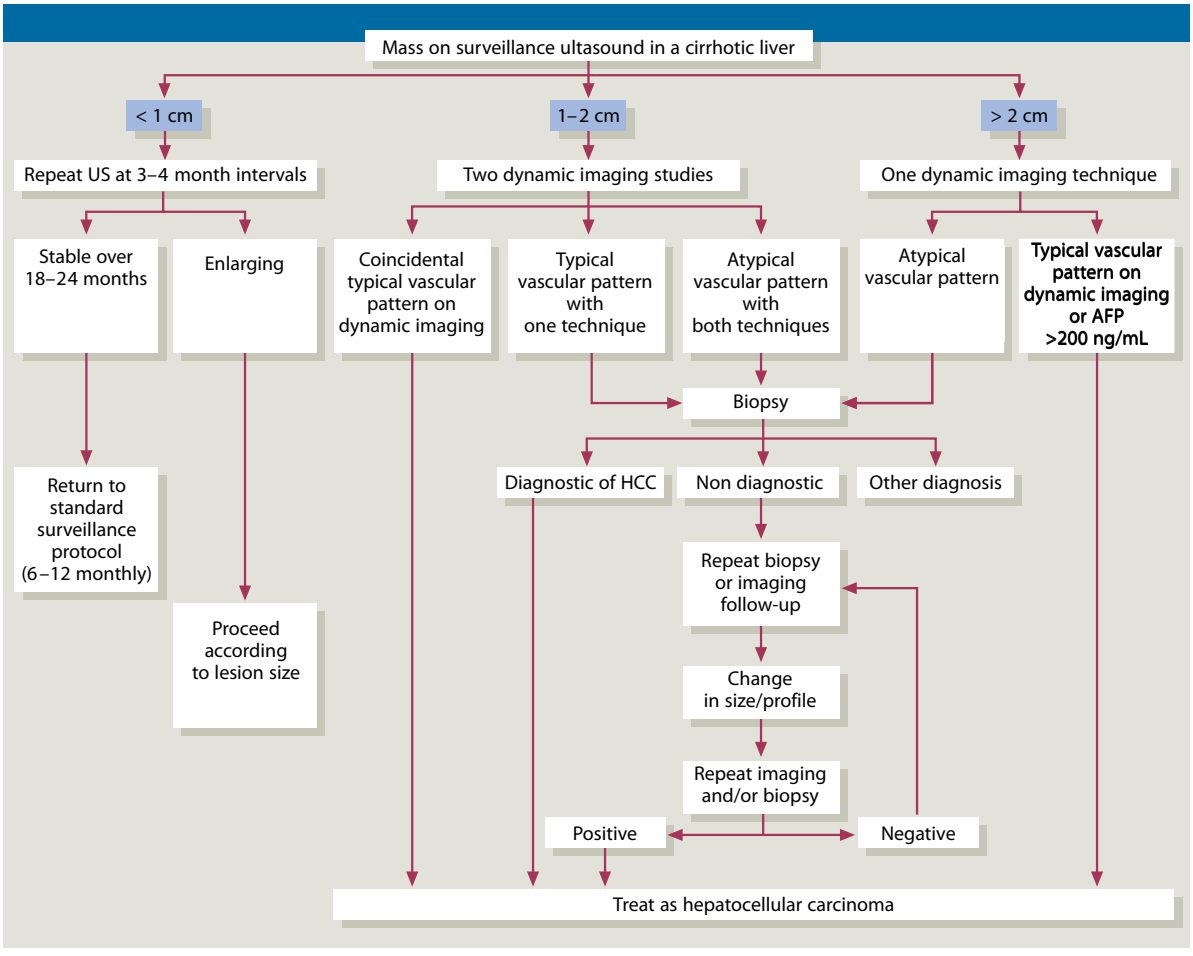


**Fig. 102.6** Algorithm for the evaluation a mass lesion in a cirrhotic liver (According to the AASLD Practice Guidelines [22]. With kind permission)

### Lesions Less Than 1 cm in Diameter

These lesions should be followed with US at intervals from 3 to 6 months. If there has been no growth over a period of up to 2 years, one can return to routine surveillance.

### Lesions 1–2 cm in Diameter

These lesions should be investigated further with two dynamic studies, either CT scan, contrast US or MRI with contrast. If the appearances are typical of HCC (arterial hypervascularity with washout in the portal-venous phase) in two techniques, then the lesion should be regarded as HCC and treated accordingly without the need of biopsy. If the vascular pattern is typical with only one or atypical with both techniques, the lesion should be biopsied with a fine needle. According to the European Association for the Study of Liver Diseases (EASL) recommendations these lesions should be biopsied irrespective of their vascular profile [21]. Patients with lesions 1–2 cm in diameter with a non-conclusive vascular profile and a negative biopsy should continue to undergo enhanced follow-up by repeat imaging and/or biopsy.

The characterization of a hepatic nodule < 2 cm by imaging techniques is more difficult and less reliable than of larger lesions. Up to 25% of these small nodules with arterial enhancement, but without venous washout, in a cirrhotic liver will remain stable or regress over time and thus are not HCC [22]. Biopsy is therefore important in these patients. However, fine needle biopsies of hepatic nodules < 2 cm also carry inherent problems. The false negative rate is 30–40%. This is due to sampling error, i.e. the lesion is so small that one cannot be certain that the sample does indeed derive from the lesion. Moreover, there is disagreement between pathologists as to the dividing line between dysplasia and well-differentiated HCC. Eighty percent of patients with high-grade dysplastic nodules, however, develop an HCC within 3 years. It may even be difficult to distinguish a well-differentiated HCC from normal liver on biopsy. An additional concern about needle biopsy is tumor cell seeding. Needle track seeding has been estimated to have a prevalence of up to 5% with systemic seeding potentially occurring even more often [217]. However, a recent systematic review and meta-analysis concluded that the incidence of needle tract tumour seeding following biopsy of an HCC is 2.7% overall, or 0.9% per year [211a].

### Lesions Larger Than 2 cm in Diameter

A nodule in a cirrhotic liver larger than 2 cm in diameter with the typical features of HCC on one dynamic imaging technique should be regarded as HCC. The positive predictive value of the clinical and radiological findings exceeds 95% and therefore this lesion does not require biopsy [21, 22]. Alternatively, if the AFP is >200 ng/mL liver biopsy is also not necessary. However, if the vascular pattern on imaging is atypical and the AFP is less than 200 ng/mL or if the nodule is detected in a non-cirrhotic liver, biopsy should be performed.

## Differential Diagnosis

The differential diagnosis of HCC includes all focal liver lesions, i.e. all benign and malignant tumors including metastases (see Chapter 101).

The imaging characteristics of focal liver lesions are discussed in Chapters 37 and 38. For the approach to the patient with a focal liver lesion see Chapter 51.

In the setting of chronic HBV infection (without cirrhosis) or cirrhosis of any etiology a focal lesion found incidentally or on screening has a high likelihood of being a regenerative or a dysplastic nodule or HCC.

## Prognosis and Staging

Spontaneous regression of HCC may occur, but is extremely rare and up to the year 2000 only 24 cases were reported in the English literature [220]. Overall HCC is usually detected late in its natural history and its prognosis is severe.

The mean survival time after diagnosis is approximately 6–20 months. In the United States the overall 1-year relative survival rate increased from 14% during 1977–1981 to 23% during 1992–1996. Between the same two time periods, less improvement was seen in the 5-year survival rates, which increased

from 2% to only 5%. The median survival increased slightly from 0.57 years during 1977 to 1981 to 0.64 years during 1992 to 1996 [62]. Significant racial variation in survival was reported which may be partly explained by a lower likelihood of receipt of therapy and more advanced HCC at diagnosis among blacks and Hispanics [49].

Survival times after diagnosis of HCC are shorter in high incidence areas than in low incidence areas. This, however, may be due to lead time bias, with HCC being detected earlier in its natural history (i.e. at an earlier stage) than, for example, in a high incidence area such as Africa.

The majority of Asian HCC patients are not suitable for curative surgical treatment. The overall median survival has been reported to be 3 months, and the 1-year survival only 7.8%. The median survival times of patients with Okuda stages I, II, and III (see below) were 5.1 months, 2.7 months, and 1.0 month, respectively. Independent prognostic variables were serum bilirubin, blood urea, serum alpha-fetoprotein, and Okuda stage [253]. The shorter survival of Asian patients may be due to the different etiology of HCC in Asia, where chronic HBV infection accounts for 70–80% of cases. HBV-related HCCs have a greater aggressiveness than HCV-related tumors; a high viral load prior to treatment is an adverse factor for survival [29, 131].

HCC mortality trends remain variable across Europe. In the early 2000s the highest HCC mortality rates in men were in France (6.8/100,000), Italy (6.7), and Switzerland (5.9), whereas the lowest ones were in Norway (1.0), Ireland (0.8), and Sweden (0.7). In women, a slight increase in overall HCC mortality was observed in Spain and Switzerland, while mortality decreased in several other European countries, particularly since the mid-1990s. In the early 2000s, female HCC mortality rates were highest in Italy (1.9/100,000), Switzerland (1.8), and Spain (1.5) and lowest in Greece, Ireland, and Sweden (0.3) [18]. Five-year relative survival in Europe was < 10%, ranging from 8% (southern Europe) to 5% (eastern Europe). The improved survival may be due to greater surveillance of cirrhotic patients. The survival gap between clinical and population-based series suggests patients with cirrhosis and HCC should be managed in centers of excellence [30].

Survival of untreated HCC patients strongly depends on baseline liver function (Child-Pugh class and MELD score) and on the presence or absence of prognostic factors, such as cancer-related symptoms and signs (ascites, bilirubin, portal vein thrombosis) or an invasive tumoral pattern [158]. Thus, the 3-year survival rate is 50% in asymptomatic patients with multinodular HCC, in contrast to only 8% in patients with either cancer-related symptoms or with portal vein thrombosis or extrahepatic spread (Fig. 102.7). In addition, there is increasing data to suggest that effective screening and surveillance, and the development of therapeutic interventions for HCC (see below), have led to improvements in the prognosis for HCC patients [158, 226].

*Molecular prognostication* by analysis of gene expression profiling emerges as a new technique to predict outcome of HCC, and will probably be adopted into clinical practice in coming years. HCC is a genetically heterogeneous tumor composed of several genetically homogeneous subclasses, each of which harbors characteristic genetic alterations [104]. Based on their genetic makeup HCCs differ in their biological aggressiveness. By analyzing the "genetic signature" survival and early recurrence after HCC resection can be predicted [103, 111, 122, 125, 229].

The *prognostic value of pretreatment tumor marker elevation* may differ according to the type of curative treatment [234]. AFP-L3 and DCP (see above) may have prognostic value only in patients treated with locoregional ablation. Elevated DCP and AFP-L3 levels pre- and post-ablation were reported to be significant predictors for HCC recurrence after curative ablation [225, 233]. These data, however, need further confirmation.

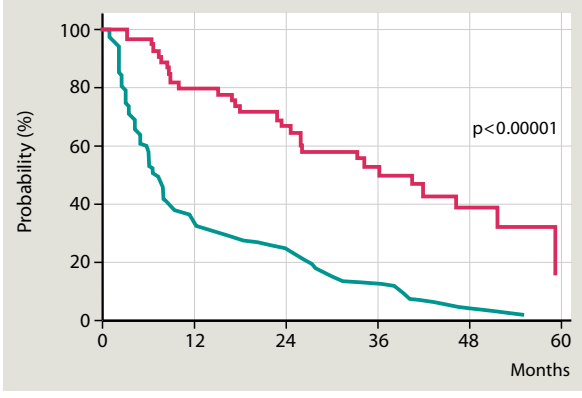


**Fig. 102.7** Overall survival of patients with HCC and absence of any adverse prognostic factor (red line) versus HCC patients with at least one adverse prognostic factor (blue line) (Reprinted from [145]. With permission)

AFP levels may predict recurrence of HCC after surgical resection and liver transplantation. AFP levels ≥ 1,000 ng/mL seem to indicate shorter postoperative survival times and a higher risk for postoperative recurrence.

*C-reactive protein* (CRP) was recently described as a prognostic factor for patients with HCC after surgical resection. The prognosis of highly sensitive-CRP-positive patients (≥3.0 mg/L) was poorer compared with highly sensitive-CRP-negative patients (<3.0 mg/L) [164].

*Circulating HCC cells* also indicate a higher risk of recurrence after resection [98, 240].

Elevated *vascular endothelial growth factor* (VEGF) levels in serum and tissues are related with a poor prognosis in patients with HCC. VEGF polymorphisms may be significant prognostic indicators for HCC patients [117].

*Diabetes mellitus* has been reported to be a risk factor for the recurrence of (HCV-related) HCC and to decrease the overall survival rates after surgical treatment [90, 116].

The *histopathological features* also have an impact on the course of HCC. Well-differentiated clear cell and fibrolamellar HCCs and tumors that are delimited by a capsule have a better prognosis. Histological grade of differentiation and macroscopic vascular invasion, as assessed on the explanted livers, are strong predictors of both survival and tumor recurrence in patients with cirrhosis who received transplants for HCC [99, 257].

The prognosis of HCC is related to *tumor stage* at presentation, and HCC stage guides treatment decisions. Staging includes assessment of local tumor extension (intrahepatic staging) and extrahepatic tumor spread. Although contrast-enhanced US is a valuable technique in the characterization of nodular lesions in a cirrhotic liver (see above) it has not resulted in any significant improvement in the ability of US to detect small tumor foci, since a comprehensive assessment of the whole liver parenchyma cannot be accomplished during the short duration of the arterial phase. Hence, CT or MRI are still mandatory for proper intrahepatic staging of HCC [127] Several *HCC staging systems* have been developed, but there is no worldwide consensus on the use of any given staging system [47, 144]. Among the major prognostic variables in untreated patients with nonsurgical HCC are constitutional symptoms, performance status, portal thrombosis, and extrahepatic spread (Table 102.2 and Fig. 102.7). The best predictors of survival are the presence of cancer-related symptoms (Performance Status Test [PST = 1–2 or constitutional syndrome) and the identification of an invasive pattern shown by the presence of vascular invasion or extrahepatic spread [144].

**Table 102.2** World Health Organization performance status grades [213]

| | |
|---|---|
| Stage 0 | Fully active, normal life, no symptoms |
| Stage 1 | Minor symptoms, able to do light activity |
| Stage 2 | Capable of self-care but unable to carry out work activities. Up for more than 50% waking hours |
| Stage 3 | Limited self-care capacity. Confined to bed or chair > 50% waking hours |
| Stage 4 | Completely disabled. Confined to bed or chair |

## TNM System

The TNM system classifies solid tumors according to the anatomic extent of the primary tumor (T), presence of lymph node metastasis (N), and distant metastasis (M) (Table 102.3). The TNM system does not take into account liver function and therefore does not have prognostic power. To estimate prognosis it should be combined with the Child-Pugh score and the clinical performance status of the patient (Table 102.2). The TNM system has not been validated prospectively in patients with HCC and is clearly inferior to the Okuda staging system, the Cancer of the Liver Italian Program Score and the Barcelona Clinic Liver Cancer Staging Classification.

## Okuda Staging System

The Okuda staging system takes into account the extent of the tumor and functional hepatic parameters, such as ascites, serum albumin and bilirubin levels. Vascular invasion and lymph node status are not assessed (Table 102.4). The mean survival times of untreated patients with Okuda-stages I, II, and III are 8.3 months, 2 months and 0.7 months, respectively [177].

**Table 102.3** TNM staging system for hepatocellular carcinoma and for intrahepatic cholangiocarcinoma

| | | | |
|---|---|---|---|
| | **Primary tumor (T)** | | |
| Tx | Spread of the primary tumor cannot be assessed | | |
| T0 | No primary tumor | | |
| T1 | Solitary tumor, ≤2 cm, no vascular invasion | | |
| T2 | Solitary tumor, ≤2 cm, vascular invasion | | |
| | or | | |
| | Multiple tumors in one liver lobe, each <2 cm, no vascular invasion | | |
| | or | | |
| | Solitary tumor, >2 cm, no vascular invasion | | |
| T3 | Solitary tumor, >2 cm, vascular invasion | | |
| | or | | |
| | Multiple tumors in one liver lobe, each <2 cm, vascular invasion | | |
| | or | | |
| | Multiple tumors in one liver lobe, smaller and larger than 2 cm, with or without vascular invasion | | |
| T4 | Multiple tumors in both liver lobes | | |
| | or | | |
| | Tumor involvement of a major portal vein branch or of liver veins | | |
| | or | | |
| | Invasion of neighboring organs (excluding gallbladder or perforation of visceral peritoneum) | | |
| | **Regional lymph nodes (N)** | | |
| Nx | Cannot be assessed | | |
| N0 | No regional lymph node metastases | | |
| N1 | Regional lymph node metastases present | | |
| | **Distant metastases (M)** | | |
| Mx | Cannot be assessed | | |
| M0 | No distant metastases | | |
| M1 | Distant metastases present | | |
| | **Stages** | | |
| Stage I | T1 | N0 | M0 |
| Stage II | T2 | N0 | M0 |
| Stage IIIA | T3 | N0 | M0 |
| Stage IIIB | T1 | N1 | M0 |
| | T2 | N1 | M0 |
| | T3 | N1 | M0 |
| Stage IVA | T4 | any N | M0 |
| Stage IVB | any T | any N | M1 |

Source: According to the American Joint Committee on Cancer Staging Manual, 5th edn. Lippincott-Raven, Philadelphia, 1997

**Table 102.4** Okuda staging system

| Criterion | Positive | Negative |
|---|---|---|
| **Tumor size**[a] | >50% | <50% |
| **Ascites** | Clinically present | Clinically absent |
| **Albumin i.s.** | <3 g% | >3 g% |
| **Bilirubin i.s.** | >3 mg% | <3 mg% |
| **Stage** | | |
| **I No criterion positive** | | |
| **II One or two criteria positive** | | |
| **III Three or four criteria positive** | | |

[a]Ratio between the maximal cross-section area of the tumor and i.s. In serum the maximal cross-section area of the liver

## *Cancer of the Liver Italian Program*

The Cancer of the Liver Italian Program (CLIP) combines tumor characteristics with the severity of cirrhosis (Table 102.5) [227]. A simple scoring system assigns linear scores (0, 1 or 2) to the covariates of the final model: the higher the final score, the worse the prognosis. Patients with HCC and a CLIP-score of 0 points have a median survival time of 36 months, which decreases to 3 months in patients with the maximal score of 6 points. The CLIP-score has been validated prospectively [228, 237]. Compared with the Okuda staging system, the CLIP-score gives more precise prognostic information, is statistically more efficient, and has a greater predictive power on survival. It is a very useful clinical staging system, is easy to calculate, and should be used in all patients with HCC [55]. The MELD-based modified CLIP systems may have a

**Table 102.5** The Cancer of the Liver Italian Program scoring system [227, 228]

| Variable | Score |
|---|---|
| **Child-Pugh stage** | |
| A | 0 |
| B | 1 |
| C | 2 |
| **Tumor morphology** | |
| Uninodular and extension ≤ 50% | 0 |
| Multinodular and extension ≤ 50% | 1 |
| Massive or extension > 50% | 2 |
| **$\alpha_1$-Fetoprotein** | |
| <400 ng/mL | 0 |
| ≥400 ng/mL | 1 |
| **Portal vein thrombosis** | |
| No | 0 |
| Yes | 1 |

better predictive ability than the original model for cancer staging [91].

## Barcelona Clinic Liver Cancer Staging Classification

The Barcelona Clinic Liver Cancer (BCLC) staging classification stratifies HCC patients into four major categories (early, intermediate, advanced, and end-stage) (Table 102.6). Based on the attribution of the patients to one of these categories, specific treatments are recommended. *Early stage (A)* includes patients with asymptomatic early tumors suitable for radical therapies, such as resection, transplantation or percutaneous treatments. Patients at the early stage represent 30% of the US and European patient populations. *Intermediate stage (B)* comprises patients with asymptomatic multinodular HCC. Patients at the intermediate stage represent 15–20% of the HCC population in the United States and Europe. *Advanced stage (C)* includes patients with symptomatic tumors and/or an invasive tumoral pattern (vascular invasion/extrahepatic spread). Patients at the advanced stage represent approximately 40% of the HCC population in the West. Stage B and C patients may receive palliative treatments/new agents in the setting of phase II investigations or randomized controlled trials. *End-stage disease (D)* (approximately 10% of the HCC population) contains patients with extremely grim prognosis that should merely receive symptomatic treatment [144, 145].

**Table 102.6** The Barcelona Clinic liver cancer staging classification [144]

| Stage | PST | Tumor status | Liver functional status |
|---|---|---|---|
| **Stage A:** | | | |
| Early HCC | | | |
| A1 | 0 | Single | No portal hypertension and normal bilirubin |
| A2 | 0 | Single | Portal hypertension and normal bilirubin |
| A3 | 0 | Single | Portal hypertension and abnormal bilirubin |
| A4 | 0 | 3 Tumors < 3 cm | Child-Pugh A–B |
| **Stage B:** | | | |
| Intermediate HCC | 0 | Large multinodular | Child-Pugh A–B |
| **Stage C:** | | | |
| Advanced HCC | 1–2[a] | Vascular invasion or extrahepatic spread[a] | Child-Pugh A–B |
| **Stage D:** | | | |
| End-stage HCC | 3–4[b] | Any | Child-Pugh C[b] |

*PST* Performance Status Test (see Table 102.2)
Stage A and B: All criteria should be fulfilled
Stage C: At least one criterion
Stage D: At least one criterion
[a]PST 1–2 or vascular invasion/extrahepatic spread
[b]PST 3–4 or Child-Pugh C

## Other Scoring Systems

The *Japan Integrated Staging score* (JIS score), which combines the Child-Turcotte-Pugh classification and tumor-node-metastasis staging, has been claimed to have better stratification ability and prognostic predictive power than the CLIP scoring system [119]. This assertion, however, needs further confirmation. Its predictive ability can be further improved when combined with the MELD-score [92].

The *Tokyo score* consists of four factors: serum albumin, bilirubin, and size and number of tumors. Five-year survival was 78.7%, 62.1%, 40.0%, 27.7%, and 14.3% for Tokyo scores 0, 1, 2, 3, and 4–6, respectively. It has been reported to provide good prediction of prognosis for Japanese patients with HCC requiring radical therapy [224, 231].

## Comparison of Different Staging Systems

Both CLIP and BCLC scores were said to be more effective than the Okuda score in stratifying patients into different risk groups with early-intermediate HCC [79]. The BCLC staging system includes aspects of performance status, tumor extent, liver function, and treatment, which are independent predictors of survival in patients with cirrhosis and HCC, and provides the best independent predictive power for survival when compared with the Okuda, CLIP, and JIS prognostic systems [41, 42, 156]. *Recently, the American Association for the Study of Liver Diseases (AASLD) and the European Association for the Study of the Liver (EASL) agreed on BCLC as a common staging system.*

## Prevention and Screening

Vaccination against hepatitis B is an effective measure to reduce the incidence of HCC. In Taiwan, where effective vaccination programs have been put in place, the incidence of HCC in children and young adults has been reduced significantly. Vaccination against HCV infection is not currently available. It may be anticipated that improvement in antiviral treatment with enhanced elimination of HBV and HCV will also contribute to a decreased incidence of HBV and HCV-associated HCC.

Detecting HCC at an early stage offers the prospect of intention-to-cure therapies thereby improving survival. Surveillance of patients at risk for HCC with ultrasound and measurement of serum AFP every 6 months is a practicable method that has been shown to be successful in detecting small HCC [13, 44, 45, 207, 208]. *Every patient at risk for HCC should undergo HCC screening and surveillance (ultrasound plus AFP measurements) every 6 months*. Measuring AFP alone is insufficient. Patients at risk for HCC are listed in Table 102.7. Most investigators agree that biannual screening is cost-effective, reduces HCC mortality and improves survival, although the prognosis in patients with advanced cirrhosis does not seem to be improved in all patients [132, 181, 182, 197, 232, 235, 246, 258]. The improved cumulative survival rate of patients whose HCC was detected during surveillance compared with symptomatic HCC cases is depicted in Fig. 102.8 [255]. Despite these data a Cochrane Review of 2004 concluded that there are still not enough high quality trials to support or refute screening of HBsAg-positive patients for HCC [247].

**Table 102.7** Surveillance is recommended for the following groups of patients

| |
|---|
| **Hepatitis B carriers** |
| Asian males ≥ 40 years |
| Asian females ≥ 50 years |
| All cirrhotic hepatitis B carriers |
| Family history of HCC |
| Africans over age 20 |
| For non-cirrhotic hepatitis B carriers not listed above, the risk of HCC varies depending on the severity of the underlying liver disease, and current and past hepatic inflammatory activity. Patients with high HBV-DNA concentrations and those with ongoing hepatic inflammatory activity remain at risk for HCC |
| **Non-hepatitis B cirrhosis** |
| Hepatitis C |
| Alcoholic cirrhosis |
| Hereditary hemochromatosis |
| Primary biliary cirrhosis |
| Although the following groups have an increased risk of HCC no recommendations for or against surveillance can be made because a lack of data precludes an assessment of whether surveillance would be beneficial |
| $\alpha_1$-Antitrypsin deficiency |
| Nonalcoholic steatohepatitis |
| Autoimmune hepatitis |

Source: Reprinted from [22]. With permission

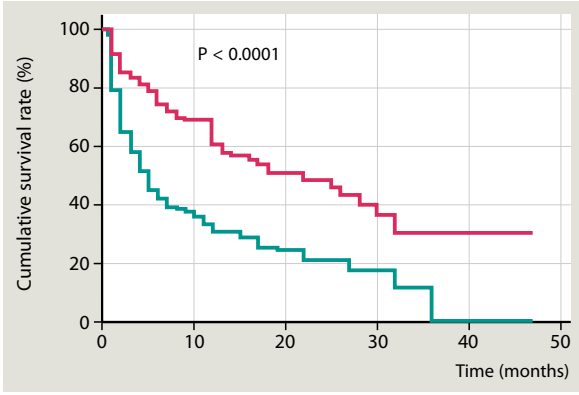


**Fig. 102.8** The cumulative survival rate in patients with subclinical HCC detected during surveillance (red line) and in patients with symptomatic HCC (blue line) (Reproduced from [255]. With permission)

## Therapy

Major advances have been made in the treatment of HCC, however, therapy is still unsatisfactory in many patients.

The treatment options in patients with HCC may be divided into

- Surgical procedures (resection or liver transplantation)
- Local ablative procedures (percutaneous or transarterial), and
- Pharmacological treatment

### *Surgical Resection*

Approximately 5–15% of patients with HCC are eligible for partial hepatectomy. Careful preoperative staging and selection of patients is essential for a good outcome. Some patients with HCCs that appear small on

preoperative US, CT and MRI may be found to have intrahepatic metastases at the time of surgery. Small (<1 cm) subcapsular tumors are often missed with the above imaging techniques. Therefore, a preoperative laparoscopy is advisable when planning resection, as it can easily detect these superficial lesions and may lead to a change in therapeutic strategy in up to 20% of cases.

Prognosis of HCC not only depends on the extension of the tumor, but also on the functional capacity of tumor-free liver tissue, i.e. whether HCC occurs in a non-cirrhotic or a cirrhotic liver. Patients without liver cirrhosis usually have a less complicated postoperative course and a better prognosis than those with a cirrhotic liver, and cirrhotic patients in stage Child-Pugh A have a better outcome than Child-Pugh C patients. Thus the expected postoperative functional reserve capacity of the liver is a major determinant of the extent of surgical resection. Unfortunately there are no reliable preoperative parameters to predict postoperative liver function (see also Chapter 104).

Surgical resection of HCC in most centers is restricted to small tumors (≤2–5 cm in diameter) or to tumors meeting the Milan criteria (see below) after their size has been reduced with neoadjuvant therapy, such as transarterial chemoembolization in non-cirrhotic patients or Child-Pugh class A cirrhotic patients. Patients with a HCC and Child-Pugh B or C cirrhosis are poor candidates for surgical resection [215]. Recurrences within 5 years after resection are observed in 40–70% of patients and the mean 5 year survival rates are approximately 30%. In carefully selected patients with small tumors (≤ 3 cm in diameter) and good liver function, 5 year survival rates of 50–90% have been observed.

Currently, postoperative adjuvant systemic chemotherapies that reduce recurrences effectively and prolong survival are not available.

## Liver Transplantation

Orthotopic liver transplantation (OLT) has an established role in the treatment of HCC, and every patient with newly diagnosed HCC should be referred to a specialized liver center [10]. OLT is primarily performed in cirrhosis-associated HCC, while surgical tumor resection is the treatment of choice in most patients with non-cirrhosis associated HCC. Careful adherence to stringent selection criteria is crucial for the outcome

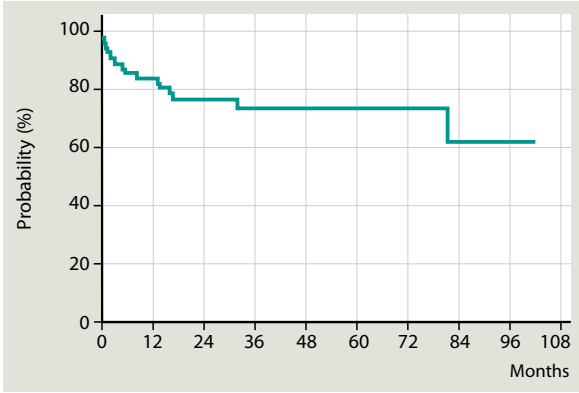


**Fig. 102.9** Actuarial survival of patients with HCC after orthotopic liver transplantation. The actuarial survival at 1, 3 and 5 years is 84%, 74% and 74%, respectively (Reproduced from [143]. With permission)

of OLT (see also Chapter 103). Patients with a HCC fulfilling the following criteria are eligible for OLT:

- Single tumor ≤ 5 cm, or
- Up to three tumors ≤ 3 cm each, and
- Absence of vascular invasion and lymph node or distant metastases [159]

Patients meeting these criteria ("Milan criteria") have 1, 5 and 10 year survival rates after OLT of 90%, 75% and 60%, respectively (Fig. 102.9) [143, 159]. The Milan criteria and a MELD score of ≥15 are currently used by UNOS[a] for listing or excluding patients with HCC for OLT. However, they are widely considered to be in need of revision, and in several centers the limit of transplantability has been pushed to a single HCC lesion of up to 6.5 cm or several tumors not larger than 4.5 cm each (University of California San Francisco [UCSF] criteria) [50, 250].

## Locally Ablative Techniques

The most common locally ablative techniques used include

- Transarterial chemoembolization (TACE)
- Radiofrequency ablation (RFA) and
- Percutaneous ethanol injection (PEI)

The principle of all locally ablative procedures is tumor destruction, either by chemical agents (chemotherapy,

[a]United Network for Organ Sharing

ethanol, acetic acid), thermal energy (radiofrequency, microwaves, laser, high intensity focused ultrasound) or radiation [214].

The percutaneous ablative techniques are very successful in tumors ≤3 cm in diameter. The smaller the lesion the more likely local ablation will be complete [71]. Initial complete tumor necrosis predicts survival and should be considered a therapeutic target irrespective of tumor size [196].

### Transarterial Chemoembolization

TACE is based on the transarterial injection of a cytotoxic agent (doxorubicin or cisplatin, with an emulsion of lipiodol) followed by blocking blood flow to the tumor by injecting microspheres. The combined effects of chemotherapy and ischemia result in tumor necrosis. TACE should only be considered in patients with unobstructed portal flow and sufficient hepatic functional reserve. Potential complications of TACE are pain, fever, fatigue, elevation of aminotransferase levels and liver failure. One report described reactivation of HBV by TACE [97]. Therefore, HBeAg-positive HCC patients receiving TACE should be closely monitored for HBV reactivation. There are no robust data to recommend pharmacologic suppression of HBV replication in all patients undergoing TACE.

TACE is mainly used in large unresectable HCCs and improves survival in carefully selected patients [147, 148]. Five-year survival rates of 26% for patients with unresectable HCC have been reported with TACE [219]. In addition, TACE may be used as a neoadjuvant treatment for down-staging HCC prior to surgical resection or in patients on the waiting list for OLT. It is associated with an excellent posttransplantation outcome. Pretreatment $\alpha_1$-fetoprotein levels of >1,000 ng/mL seem to predict treatment failure [78, 251]. Surgery is superior to TACE for patients with resectable HCC. In patients who refuse surgery, TACE can be considered [89]. Combining TACE with the sequential use of percutaneous ablation techniques, such as PEI (TACE-PEI) may improve results obtained by TACE alone [7].

### Radiofrequency Ablation

In RFA, needle electrodes are placed within the tumor under ultrasound or CT-guidance. They emit radiofrequency waves (480–500 kHz) that generate heat and lead to thermal tumor destruction. RFA may lead to a sustained complete response in small HCC (≤2–3 cm). *Results of RFA are equal to resection* in these patients [27, 142]. As with TACE, RFA may also be used as a bridge to OLT [151]. Recent studies suggest that RFA is superior to ethanol injection for HCC up to 4 cm [138, 139, 209]. RFA has surpassed PEI in the management of local tumor control and for overall patient survival [126, 139, 141, 209]. Currently, *RFA is considered the most effective percutaneous ablation therapy* [142]. A pilot study suggests that the effect of RFA may be enhanced with doxorubicin-eluting beads [127]. Increased risk of tumor seeding has been reported after percutaneous RFA [146].

### Percutaneous Ethanol Injection

In PEI, 1–10 mL of 96% ethanol is injected into the tumor through a needle under sonographic or CT-guidance. The highly concentrated alcohol denaturizes proteins and kills tumor cells. PEI is considered a reliable treatment for small HCC in terms of safety and efficacy, and complete necrosis of HCC < 3 cm may be achieved in 70–80% of tumors [57]. The advantage of PEI is its relative simplicity; its main disadvantage is its high local recurrence rate of up to 30% [109, 114]. Potential complications are seeding of cancer cells, peritoneal irritation, intraperitoneal hemorrhage, hepatic and renal insufficiency.

Percutaneous *acetic acid injection*, radiotherapy with *embolic $^{90}Y$ glass microspheres*, and *proton beam therapy* have also been tested in small numbers of patients and have been reported to be well-tolerated and effective [85, 120]. However, these methods have not yet been adopted into clinical practice, as further investigation is warranted.

*Cryoablation* through the insertion of a cryoprobe directly into the tumor has been most frequently applied intraoperatively, but has largely been abandoned in favor of RFA.

Percutaneous *radiation therapy* may lead to a reduction in size of large HCC but a survival benefit of radiation therapy has not been demonstrated. Potential complications of HCC radiotherapy are elevated liver enzymes, thrombocytopenia, duodenitis and gastroduodenal ulcers.

## *Medical Therapy*

### Systemic Chemotherapy

Although, in principal, a complete pathologic remission of inoperable HCC is possible with systemic combination chemotherapy, this is an exceptional event [130]. All randomized controlled trials which have been conducted over the past 25–30 years have failed to yield positive results of systemic chemotherapy for HCC.

### Hormonal Therapy

One third of HCCs express estrogen receptors, which was the rational to investigate the effects of *tamoxifen* on HCC. A Cochrane report found no data to support the use of tamoxifen for patients with HCC [173].

Cirrhotic liver and HCC express somatostatin receptors [11, 192]. However, there is no evidence that octreotide or other long-acting *somatostatin analogs*, such as lanreotide, have any beneficial effects in advanced HCC [8, 118, 256].

Androgen receptors occur in most HCCs, and the growth of HCC is thought to be dependent on androgens. However, no benefit in survival was found with *antiandrogenic treatment* (leuprorelin and flutamide) in male patients with advanced HCC [80].

### Molecularly Targeted Therapy

*Sorafenib* is a multitargeted tyrosine kinase inhibitor that inhibits Raf kinase, and also blocks the intracellular portion of the VEGFR. It acts in both tumor cells and endothelial cells.

Sorafenib has been shown to prolong survival of patients with end-stage HCC from a median of 7.9 to 10.7 months. The median time to radiologic progression was shown to be 2.8–5.5 months. There was no difference between sorafenib and placebo in the median time to symptomatic progression [150]. Sorafenib is administered at a dose of 400 mg p.o. bid. The main adverse effects are diarrhea and hand-foot syndrome. Sorafenib is the first systemic drug to be effective in patients with HCC, and currently is the new reference standard systemic treatment for advanced HCC.

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## 102.2 Fibrolamellar Carcinoma

### Definition

Fibrolamellar carcinoma (FLC) is a variant of HCC with distinctive histological and clinical features. It is rich in fibrous tissue and due to its epidemiological, pathological, clinical and prognostic characteristics it is justified to view FLC as a discrete entity [7].

### Epidemiology

FLC is a rare tumor in populations with a high incidence of HCC. In regions with low and intermediate incidences of HCC, however, approximately 5% of all HCCs fall upon the fibrolamellar variant. FLC is a tumor of adolescents and young adults [2]. In children and adolescents 15–40% of all HCCs are FLCs. Females are more commonly affected than males. Fewer than 10% of patients with FLC have signs of HBV and/or HCV infection.

In a retrospective population-based study in the US, 0.85% of all cases of primary liver cancer and 13.4% of all cases below the age of 40 were FLC. The age-adjusted incidence rate for FLC was 0.02 per 100,000 [3].

### Etiology and Pathogenesis

Both are unknown.

### Pathology

FLC nearly exclusively (96%) develops in a non-cirrhotic liver. The tumor is solitary and well demarcated, but not encapsulated in 56% of cases [7, 9]. Due to fibrous septa, or a central stellate fibrous scar, it may appear lobulated, resembling focal nodular hyperplasia macroscopically. On diagnosis its mean weight is 1 kg. It is predominantly localized in the left liver lobe and is easily resectable.

Histologically FLC consists of groups of large polygonal cells in a plate-like or, more rarely, in a microtrabecular arrangement separated by parallel wave-like bundles of fibrous lamellae. The cytoplasm of the tumor cells is densely eosinophilic and finely granular (oncocytic) due mitochondrial hyperplasia. It may contain inclusion bodies ($\alpha_1$-antitrypsin, fibrinogen, C-reactive protein, ferritin, albumin) and occasionally neuroendocrine granules [1]. The nuclei appear vesicular with prominent nucleoli. Immunocytochemical staining with polyclonal anti-CEA highlights the bile canaliculi between neighbouring tumor cells. An abundant fibrous stroma consisting of variously broad, parallel, hyalinized collagen bundles running between the tumor cells and containing thick walled vessels is typical of FLC. Occasionally calcifications of the fibrous stroma occur. The histological appearance of FLC is not uniform with areas resembling ordinary HCC or a cholangiocarcinoma being also present.

### Diagnosis

In its early stages FLC is asymptomatic. With increasing tumor size abdominal pain and weight loss occur. A mass can often be palpated in the upper abdomen.

AFP serum levels are elevated only very rarely. Occasionally CEA may be increased and a paraneoplastic hypercalcemia may be present. Due to synthesis by the tumor of a vitamin $B_{12}$ binding protein, vitamin $B_{12}$ levels in serum may be increased.

Since FLC develops in a non-cirrhotic liver, ultrasound, CT and MRI may visualize the tumor already in its early stages (≥1 cm in diameter). The definitive diagnosis, however, can only be established by histology.

### Differential Diagnosis

FLC must be differentiated primarily from ordinary HCC, especially from its sclerosing variant and from cholangiocarcinoma. The macroscopic appearance may be reminiscent of focal nodular hyperplasia due to fibrous septa and the central fibrous scar with thick-walled arterial vessels.

## Prognosis and Treatment

FLC has a better prognosis than HCC. The 5-year survival rates reported in the literature vary between 40% and 60% and correlate with tumor stage. In a recent study the 5-year relative survival rate was 31.8% for FLC, compared with 6.8% for HCC [3]. One reason for this difference may be the relative genomic homogeneity of FLC compared to HCC and the absence of clonal evolution [8].

FLC is resectable in approximately 60% of the cases. Only complete tumor resection and liver transplantation offer a chance of prolonging survival and of cure [4–6].

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# 102.3 Hepatoblastoma

## Definition

Hepatoblastoma is a malignant liver tumor originating from embryonal and fetal liver cells [4].

## Epidemiology

Hepatoblastoma accounts for approximately 5% of all malignant tumors, but for 25–45% of all primary liver tumors and 50–60% of all malignant liver tumors in childhood [4, 6]. Ninety percent of all hepatoblastomas occur during the first 5 years of life. Boys are affected twice as often as girls. With increasing age the gender difference equalizes. One third of children with a hepatoblastoma have a congenital anomaly. An association with familial adenomatous polyposis of the colon occurs particularly frequently.

## Etiology and Pathogenesis

Both are unknown. Numerous chromosomal alterations in patients with hepatoblastoma suggest the significance of genetic factors.

## Pathology

The tumor often has a remarkable size of up to 25 cm in diameter already at the time of initial diagnosis. It usually is solitary, partially lobulated by fibrous septa, well-vascularized and surrounded by a fine capsule. Histologically, hepatoblastomas may display an epithelial or a mixed epithelial-mesenchymal growth pattern. A fetal and an embryonal type are distinguished. The mesenchymal component often produces osteoid [2, 7]. Expression of AFP, cytokeratins (8, 18, 7, 19), vimentin, chromogranin A and S-100 by hepatoblastomas may be demonstrated by immunocytochemistry [2, 5].

## Diagnosis

### *Clinical Manifestations*

Growth retardation, fever, malaise, weight loss and a rapidly enlarging palpable upper abdominal tumor are among the leading signs and symptoms. Some patients develop signs of virilization due to paraneoplastic secretion of HCG.

### *Technical Investigations*

AFP levels in serum are markedly elevated in approximately 90% of the patients. The urinary excretion of cystathionine is increased in half of patients.

Tumor imaging is with ultrasound, CT or MRI. Calcifications occur frequently.

## Course and Prognosis

Hepatoblastoma is a rapidly growing tumor that most frequently metastasizes to the lungs, the abdominal lymph nodes and the brain. High AFP levels correlate with a large tumor mass. Tumor stage at diagnosis is prognostically relevant, while the histological type and the DNA-content do not significantly affect prognosis [1]. With timely diagnosis and rapid therapy, long-term survival may be achieved in 15–35% of cases.

## Therapy

Only surgical resection or liver transplantation offers a curative approach [3]. (Neo)adjuvant radiation and chemotherapy is applied in single cases, even though its efficacy is not proven.

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## 102.4 Angiosarcoma

### Definition

Hepatic angiosarcoma (HAS) is a mesenchymal malignant tumor originating from vascular or lymphatic endothelial cells [1, 3].

### Epidemiology

HAS accounts for only 2% of primary hepatic tumors, but it is the most common malignant mesenchymal tumor of the liver [3, 7]. The prevalence varies between 0.14 and 0.25 per million. HAS usually develops in the sixth and seventh decades of life. Men are affected three to four times more often than women. In childhood the female gender predominates.

### Etiology and Pathogenesis

Sixty to 75% of HASs are sporadic tumors. Twenty-five to 40% of cases are associated with the exposure to thorium dioxide ($Th^{232}$), polyvinyl chloride, arsenic, inorganic copper, or anabolic steroids [5, 8, 9, 10, 13, 16]. Radium, oral contraceptives and pesticides have also been implicated with the development of HAS, although a causal relationship has not been established. HASs have also been described in patients with hereditary hemochromatosis and von Recklinghausen's disease, but a causal relationship is questionable. The transition of infantile hemangioendothelioma to HAS has been described in isolated cases. Human herpesvirus 8, associated with Kaposi sarcoma, does not play a role in the pathogenesis of HAS.

In the past 7–10% of all HASs were associated with exposure to thorotrast. Thorotrast is a 25% colloidal solution of thorium dioxide ($Th^{232}$) and dextrin that was used as a radiographic contrast agent in the United States, Europe, and Japan from 1930 to 1960. $Th^{232}$ is a radioactive isotope that naturally emits α- and β-particles and γ rays. Ninety percent are α-particles. It has a half-life of 14 billion years. After intravascular injection, $Th^{232}$ is stored for life in the reticuloendothelial system, particularly in the liver (72%), spleen (12%), and bone marrow (8%) [3]. Although cholangiocarcinoma is most frequently seen, thorium-related angiosarcoma is characteristic for chronic α-radiation [8, 18]. The cumulative radiation dose that the liver is exposed to after a single application of thorotrast is 2,597 rad over 33 years. The latency period for the development of HAS is 20–42 years.

Vinyl chloride (VC) is a gas used in the plastics industry to produce polyvinyl chloride (PVC). VC is carcinogenic. It is rapidly metabolized and generates reactive metabolites that react with protein, DNA, and RNA. The mechanism of VC-induced carcinogenesis probably involves genetic mutations. Chloroethylene dioxide, a carcinogenic metabolite of VC causes mutations of the p53 gene. In sporadic HAS p53 mutations are rare [5, 15, 17]. The spectrum of VC-associated hepatic lesions encompasses liver fibrosis, cirrhosis, sinusoidal cell hyperplasia and nodular regenerative hyperplasia. The risk of developing HAS in persons having contact with VC (PVC industry) is 10–15 times higher than the risk in the general population.

Treatment with arsenic salts (Fowler's solution) may cause angiosarcomas after a latency period of up to 46 years [9, 16].

Several growth factors, including basic fibroblast growth factor, transforming growth factor, vascular endothelial growth factor, and growth hormone play a role in tumor angiogenesis. Nuclear and cytoplasmic expression of growth hormone receptors, as assessed by immunohistochemistry, is strongly enhanced in HASs, suggesting a role of growth hormone in the pathogenesis of HAS [11].

### Pathology

HAS typically has a multicentric growth pattern and, in 70% of all cases, the entire liver is infiltrated with masses ranging from a few mm to >15 cm in size. More rarely HAS presents as a solitary tumor [7].

*Macroscopically*, HAS appears as a greyish-white tumor with hemorrhagic and necrotic areas, blood-filled cavernous spaces of varying size and net-like fibrous strands. Thorotrast-induced HAS presents as a spongy, hemorrhagic lesion in which the thorotrast appears as a yellowish, calcareous material. A capsule is absent.

*Microscopically*, HAS consists of spindle-shaped or round, poorly differentiated malignant endothelial cells that exhibit severe nuclear atypia and frequent mitoses. The tumor infiltrates the surrounding liver parenchyma

and grows along preformed vascular structures, such as dilated sinusoids, terminal hepatic venules and portal vein branches. It leads to pressure atrophy of normal hepatocytes, separates liver cell plates and distorts the acinar structure of the liver. Tumor cells form irregularly anastomosing, blood-filled vascular channels. In contrast to hepatic epithelioid hemangioendothelioma (see Section 102.5), there is little stromal tissue. Occasionally epithelioid or spindle cells form solid tumor masses that may resemble fibrosarcomas. Foci of extramedullary hematopoiesis occur in the majority of cases. Factor VIII-associated and CD34-antigen may be demonstrated by immunohistochemistry, and positivity of endothelial markers is a clue for diagnosis, but may be variable within the tumor [6]. Thorotrast appears as a yellowish-brown to grey cristalline material that is predominantly deposited at the periphery of the tumor ("Thorotrast capsule").

## Diagnosis

### *Clinical Manifestations*

Symptoms of HAS are nonspecific. Patients complain of abdominal pain, fatigue, increasing weakness and weight loss. A rapid increase in abdominal girth may be the leading sign in children [2]. Hepatomegaly occurs in 50%, with jaundice and ascites seen in 25% of cases. A vascular murmur may be audible above the liver. Clinical presentation may also be acute with rupture and peritoneal bleeding in 15–27% of cases [12].

### *Laboratory Findings*

The laboratory findings are nonspecific and do not help in establishing the diagnosis. Thrombocytopenia is present in half of patients at the time of diagnosis. Tumor markers, such as AFP and CEA, are normal.

### *Imaging Techniques*

Thorotrast-induced HAS may already be discernible on plain abdominal radiography by visible deposits of the contrast agent. On ultrasound, CT and MRI HASs appear as multiple masses or as a heterogeneous dominant mass. The sonographic echopattern is irregular. On contrast-enhanced US, irregularly distributed vascular channels and blood deposits may be seen. On CT-scan the tumors are hypodense with irregular enhancement due to necroses and hemorrhages after administration of contrast [4]. MRI images essentially correspond to the CT findings with heterogeneous enhancement on the arterial and portal venous phase. MRI is useful to demonstrate the hemorrhagic and hypervascular nature of the tumor.

In the final analysis, all imaging methods, including angiography (nowadays very rarely employed), yield nonspecific findings that do not allow for the reliable distinction of HAS from large hemangiomas, hemangioendotheliomas and other vascular tumors.

A definite diagnosis can only be established by histological examination. The diagnostic puncture of angiomatous tumor masses, however, even with a fine needle is dangerous and may lead to massive, life-threatening bleeding in up to 16% of cases. Moreover, a false-negative result occurs in up to 50% of cases of fine needle biopsies, showing nonspecific, reactive inflammatory or fibrotic lesions. Therefore, it is suggested that liver tissue be obtained under laparoscopic guidance.

## Differential Diagnosis

The differential diagnosis includes all hypervascular hepatic tumors. Multicentric involvement of the entire liver with vascular masses, and the concomitant presence of splenic tumors and a hemoperitoneum argue for an angiosarcoma.

## Course and Prognosis

The prognosis of HAS is dismal. Most patients die within 6 months of diagnosis. Only 3% of patients survive longer than 2 years after diagnosis. Approximately 50% of patients die from hepatic failure. On autopsy, splenic and pulmonary metastases are found in two thirds of the deceased. The question of whether the tumor originated from the liver or whether it represents a primary, multicentric angiosarcoma often cannot be answered.

## Therapy

Since HAS progresses rapidly, and most angiosarcomas are multifocal, most cases are discovered at an advanced stage, and fewer than 20% of the patients receive surgery [3]. If possible, localized tumors should be resected.

In single cases a liver transplantation has been performed. Angiosarcomas have the poorest prognosis of all malignant liver tumors after hepatic transplantation. Recurrences occur in two thirds of patients, and all patients die within 2–3 years after transplantation. Thus, liver transplantation is not a valid therapeutic option in patients with HAS, and has consequently been abandoned [14].

Systemic chemotherapy is not effective in HAS.

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## 102.5 Epithelioid Hemangioendothelioma

### Definition

Hepatic epithelioid hemangioendothelioma (HEH) is a rare, usually multifocal neoplasm of vascular origin with a clinical course intermediate between benign hemangioma and malignant angiosarcoma.

### Epidemiology

Since its first description by Weiss and Enzinger in 1982, approximately 450 cases have been published until 2008 [6, 9]. The incidence is <0.1 per 100,000. The mean age of patients with HEH is 42 years (range: 3–86 years), and the male-to-female ratio is 2:3 [4, 6]. Children are affected very rarely [7].

### Etiology and Pathogenesis

Causative factors are unknown. In contrast to many other primary liver tumors, HEH does not arise in the context of chronic liver disease. According to one hypothesis, HEH originates from a primitive reticuloendothelial stem cell that is capable of differentiating into endothelial and dendritic cells.

### Pathology

Most commonly, the entire liver is infiltrated by multiple, hard, grey-white lesions varying in size from a few mm to >10 cm. The nodular tumor masses tend to coalesce, ultimately affecting the entire liver.

The tumor cells grow along preformed vascular structures. Despite extensive infiltration of the liver, however, the portal tracts and terminal hepatic venules usually remain discernible, allowing for the delineation of acinar structures. Intraacinar tumor growth leads to atrophy of hepatocytes and finally to the loss of trabecular structure (Fig. 102.10). Papillary tumor proliferations may invade venous vessels. The neoplastic cells have a dendritic, spindle-shaped, irregular appearance with numerous interdigitating extensions, or they are epithelioid, rounded with abundant cytoplasm. Intracytoplasmic vacuoles that may contain red blood cells occur commonly. Immunohistochemical demonstration of endothelial markers, such as factor VIII-associated and CD34 antigen is positive. The fibrous stroma is rich in sulfated mucopolysaccharides which, in places, impart a myxoid, hyalinized appearance. With progression

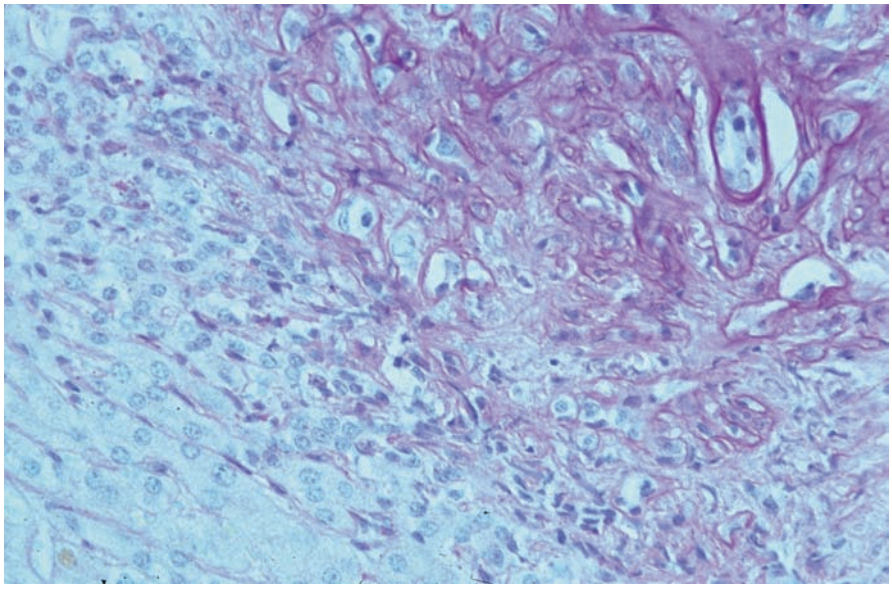

**Fig. 102.10** Epithelioid hemangioendothelioma. PAS staining after diastase digestion (×400)

of the lesions fibrosis increases and calcifications appear, so that it may be difficult to identify the tumor cells in the sclerosed areas [3, 5].

## Diagnosis

### *Clinical Manifestations*

Approximately 20% of patients are asymptomatic at the time of diagnosis and the tumor is discovered incidentally. The remainder present with right upper quadrant pain (50%), hepatomegaly (20%) and weight loss (16%) [6]. Jaundice, fever and signs and symptoms of portal hypertension occur more rarely. A Budd-Chiari like syndrome may occur in isolated cases, possibly due to tumor invasion or fibrous obliteration of terminal hepatic venules and sublobular veins [8].

### *Laboratory Findings*

16% of patients do not show any laboratory abnormality, and in the remainder findings are nonspecific. Increased alkaline phosphatase (70%), γ-glutamyl transpeptidase (45%), aminotransferases (23–29%), and bilirubin (20%) are the most prominent changes. Most tumor markers (e.g., $\alpha_1$-fetoprotein, carcinoembryonic antigen, and CA 19-9) are in the normal range.

### *Imaging Techniques*

US, CT and MRI are helpful in delineating discrete tumor nodules, and in visualizing confluent tumor masses, thus allowing for the appreciation of the intrahepatic growth pattern of HEH. Imaging studies (with and without contrast enhancement), however, cannot provide a specific diagnosis. Positron-emission tomography scan currently plays no role in the diagnosis of HEH. The contrast between numerous intrahepatic tumors and a good clinical condition, the slow course of the disease, and the presence of intratumoral calcifications, are suggestive of HEH [1].

The definitive diagnosis requires a histopathological analysis. A large tumor sample should be obtained since the rate of missed diagnoses may amount to 75% on small needle biopsy specimens, and HEH may be misinterpreted for scar tissue, cirrhosis, veno-occlusive disease, metastatic signet ring cell carcinoma, sclerosing hepatocellular carcinoma, cholangiocarcinoma, chondrosarcoma, leiomyosarcoma or angiosarcoma.

## Differential Diagnosis

The differential diagnosis includes all, especially hypervascular, liver tumors. Metastatic adenocarcinoma and various tumors with a fibrous stroma, such as cholangiocarcinoma, scirrhous HCC, or sclerosed hemangioma, should also be considered.

## Course and Prognosis

HEH has a highly variable behavior and there are no clinical parameters that allow for the estimation of its prognosis in the individual patient. The tumor has a relatively low malignant potential and many patients have a slowly progressive course, surviving for decades. However, a rapidly fatal evolution is also possible. Histopathological criteria, such as nuclear polymorphism and mitotic activity, also do not yield useful prognostic information [3, 5].

Irrespective of the type of treatment (or no treatment), approximately 40% of patients survive for more than 5 years after diagnosis. Single cases with survival times of >20 years have been documented.

Approximately every fourth patient develops metastases, predominantly in the lungs, abdominal, mediastinal and portal lymph nodes, as well as in the mesentery, peritoneum, spleen and bones.

## Therapy

Due to its multicentric nature, surgical resection usually is only possible in approximately 10% of patients. If only isolated tumor nodules are present, liver resection is the treatment of choice, with 1-year and 5-year survival rates of 100% and 75%, respectively.

The most common management is liver transplantation (45% of patients), with 1-year and 5-year patient survival rates of 96% and 55%, respectively.

Systemic chemotherapy or radiotherapy are only moderately effective, with 1-year and 5-year survival rates of 73% and 30%, respectively [2, 6].

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# 102.6 Embryonal Sarcoma

## Definition

Embryonal sarcoma (ES) of the liver is a rare primitive neoplasm, unique to the liver.

## Epidemiology

Twenty-five percent of all childhood liver tumors are ESs. Eighty-eight percent of the tumors occur before the age of 15 years [3, 4].

## Etiology and Pathogenesis

Both are unknown. Aberrations of chromosome 19q have been described both in mesenchymal hamartomas and in ES, which gave reason to the hypothesis that ES may originate from malignant transformation of mesenchymal hamartomas [1].

## Pathology

Macroscopically, ES usually are single, large, globular masses with solid and cystic gelatinous areas, measuring up to >20 cm in size. Seventy-five percent of the tumors are localized in the right liver lobe.

Microscopic features included spindle, oval, stellate, epithelioid or multinucleated cells loosely or densely arranged in a myxomatous matrix. Entrapped bile ducts and hepatic cords are often present at the periphery of the tumours. Microvesicular fat vacuoles, intracellular and extracellular eosinophil, periodic acid-Schiff-positive, diastase-resistant hyaline globules are commonly present. Atypical mitoses are frequent [2–5, 7]. ES may invade adjacent organs and metastasize to the lungs and to the abdomen.

## Diagnosis

### *Clinical Manifestations*

An abdominal mass and right upper quadrant pain are the most frequent findings at diagnosis. Further symptoms, such as fatigue, nausea, vomiting, fever and jaundice may supervene.

### *Laboratory Findings*

Liver enzymes are usually normal. AFP is not elevated.

### *Imaging Techniques*

US, CT and MRI disclose a hepatic mass with solid and cystic areas. On CT-scan approximately 90% of the tumor has the density of water.

ES of the liver may undergo pluripotential differentiation. Diagnosis is based on histological features of large tumor samples. Immunohistochemistry has no specific or diagnostic relevance [7].

## Differential Diagnosis

The differential diagnosis encompasses all solid hepatic masses. Tumors with cystic components, such as cystadenomas and cystadenocarcinomas, parasitic cysts, and posttraumatic hematomas should all be included in the differential diagnosis.

## Course and Prognosis

ESs grow fast. Pulmonary metastases and recurrences after resection occur frequently. Only approximately one-third of patients survive 3 years after diagnosis [5].

## Therapy

Whenever possible, complete surgical resection with or without adjuvant chemotherapy should be attempted. A response to chemotherapy has been observed in isolated patients in whom tumor resection was not possible. Doxorubicin, platinum, cyclophosphamide, dacarbazine, 5-fluorouracil and vincristine have been used [5, 6].

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## 102.7 Primary Hepatic Malignant Lymphoma

The liver is one of the organs most commonly involved in malignant lymphomas. In patients with Hodgkin's lymphoma the liver is involved in 5–10%, in Non-Hodgkin's lymphoma in 15–40% of cases.

On the other hand, primary hepatic malignant lymphomas (PHML) are very rare and account for 0.4–1% of all extranodal lymphomas at most. Currently, fewer than 200 cases of PHML have been reported in the literature [3, 8, 10, 18, 20]. A primary hepatic presentation of Hodgkin's lymphoma is extremely rare [19].

### Epidemiology

PHML occurs in all age groups, but predominantly in men older than 50 years. Immunocompromised patients, such as those with HIV-infection, are predominantly affected [6, 17].

### Pathogenesis

PHML probably originates from portal lymphocytes or from Kupffer cells. They usually do not develop in the background of an underlying chronic liver disease. However, there are single case reports of the development of PHML in patients with primary biliary cirrhosis [15, 16]. Viruses (for example, EBV) could play a pathogenetic role especially in immunocompromised patients. Hepatitis C virus is known to be a lymphotropic virus that can sustain clonal B-cell expansion, and increasing evidence points toward a role of HCV infection in the etiology of malignant lymphomas [7, 14]. A causal relationship between chronic HCV infection and PHML is not established. However, a small series of PHML has been reported, suggesting a nonfortuitous association with HCV infection [9].

### Pathology

PHML may occur as a solitary lesion, as a multicentric tumor or as a diffuse infiltration of the liver (in 16% of cases). Patients with AIDS usually present with multicentric PHML.

*Microscopically* the lymphomatous infiltrates typically occur in the portal tracts. They may, however, involve the adjacent acinar parenchyma. A lymphomatous infiltration of the sinusoids favors hepatic involvement in extrahepatic lymphoma rather than PHML [1, 4, 20].

Approximately two thirds of PHML are B-cell, and 25% are T-cell lymphomas. Primary low-grade B-cell lymphomas of MALT-type occurring in the liver have also been described [11, 12]. They are characterized by a dense lymphomatous infiltrate of the portal tracts with lymphoepithelial lesions of the bile ducts and may mimic a chronic hepatitis or an inflammatory cholangiopathy.

*Hepato-splenic γ/δ T-cell lymphomas* represent a subgroup of peripheral T-cell lymphomas that in addition to the liver also affect the spleen and bone marrow. They are aggressive tumors, occurring usually in young adults and commonly develop in the context of chronic immunosuppression or long-standing antigenic stimulation, especially after organ transplantation. In contrast to most B-cell lymphomas, γ/δ T-cell lymphomas diffusely infiltrate the sinusoids.

### Diagnosis

#### *Clinical Manifestations*

Symptoms are nonspecific with right upper quadrant pain, hepatomegaly, and jaundice. Fever, night sweats and weight loss (B symptoms) occur in 50% of patients. γ/δ T-cell lymphomas usually present with signs of systemic disease. Rarely, occlusion of the portal vein with signs of portal hypertension or a cholestasis due to lymphomatous infiltration of the bile ducts may be the leading clinical signs. Very rarely PHML may present as acute liver failure. Histologically these patients usually show a massive infiltration of sinusoids, portal tracts and vessel walls with lymphoma cells.

#### *Laboratory Findings*

There are no specific laboratory findings. Aminotransferases and alkaline phosphatase may be elevated.

### *Imaging Techniques*

Depending on the growth pattern US, CT and MRI show a tumor mass, a multifocal or a diffuse process. Contrast enhancement does not increase the diagnostic specificity. Due to their high glycolytic activity PHML take up fluorodeoxyglucose on PET scan [5].

The diagnosis is established by image-guided needle biopsy with no need to proceed to surgical biopsy [2]. Immunocytochemical examinations are mandatory in order to not misinterpret the lesions as poorly differentiated carcinoma.

## Differential Diagnosis

PHML is a very rare tumor, not very significant in the differential diagnosis of hepatic mass lesions. If, however, solitary or multiple liver lesions develop in immunocompromised patients PHML should be considered.

A rare differential diagnosis is reactive lymphatic hyperplasia (pseudolymphoma) which may appear as a space occupying lesion. It is seen occasionally in patients with underlying immunologic disorders, such as chronic thyroiditis [13].

## Course and Prognosis

The median survival time of patients with PHML is approximately 1.5 years. Patients without an underlying disease have a slower progression and longer survival times. Prognosis is especially severe in immunocompromised patients.

## Therapy

Solitary nodules have a relatively favorable prognosis after surgical resection. Further treatment options are systemic chemotherapy and radiotherapy. Controlled trials as to the best treatment regimen are not available.

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## 102.8 Various Rare Tumors

### Malignant Fibrous Histiocytoma

Primary hepatic malignant fibrous histiocytoma (MFH) is a rare entity with ill-defined clinicopathologic characteristics. MFH usually occur in muscle and in the retroperitonal soft tissue. At present, only approximately 35 cases of primary hepatic MFH have been reported in the literature [2, 5, 6].

The precise nature of MFH has been debated for years. Currently, a histiocytic lineage of the tumor cells is no longer favored [1].

The average age at diagnosis is between 50 and 60 years. There is no gender predilection. Symptoms are nonspecific with abdominal pain, fever, malaise, anorexia, and weight loss.

Macroscopically, hepatic MFH usually appears as a solitary mass measuring 5.5–20 cm. Histologically, a storiform-pleomorphic pattern with variable degrees of necrosis predominates. A myxoid pattern is seen in only 10–15% of cases [3–5].

Laboratory findings and imaging techniques (US, CT, MRI) yield nonspecific findings. The diagnosis is made by histology.

The prognosis of hepatic MFH depends primarily on tumor size and stage at the time of diagnosis. No effective therapy for hepatic MFH is available and the mean survival time is only approximately 3 months. Systemic chemotherapy and transarterial chemoembolization are not effective. Whenever possible surgical resection should be performed. Possibly ezrin expression in tumor cells can provide additional prognostic information. Isolated patients with a low ezrin immunoreactivity score (<1) survived >4 years after surgery [5].

### Kaposi's Sarcoma

Kaposi sarcoma (KS) is induced by human herpes virus 8. Hepatic KS occurs only in AIDS patients and is usually associated with KS lesions in other organs. Hepatic KS lesions are seen in 12–25% of fatal cases in this population, but do not appear to contribute to morbidity and mortality of the disease [7].

Involvement of the liver with KS is typically multicentric. The tumor nodes vary in size from a few mm to approximately 7 cm. Macroscopically, the nodes appear red-brown, spongiform, and hemorrhagic. Histologically, spindle-shaped tumor cells containing hyaline globules with large, irregular nuclei occur in the portal tracts, but occasionally may infiltrate the adjacent parenchyma. Spindle cells express endothelial cell markers CD31, CD34, and also VEGF receptor 3 and podoplanin [9]. Slit-like vascular spaces and hemosiderin accumulation are typical, and peliotic foci are often seen at the periphery of the tumors [8]. The latter must be differentiated from bacillary peliosis hepatis, occurring also more often in AIDS patients.

## Other Rare Tumors

In addition to the hitherto described tumors hepatic leio- and rhabdomyosarcomas, squamous cell carcinomas, malignant teratomas, carcinoids, fibro- and liposarcomas may originate primarily in the liver [10].

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# Liver Transplantation: Indications, Preoperative Evaluation and Posttransplantation Management

# 103

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## Chapter Outline



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Perhaps more than any other field in medicine, transplantation relies on a utilitarian ethic. Far fewer organs are available than there are patients who need them. Liver transplant physicians and surgeons must decide whom the optimal candidates for transplant are and when they should be transplanted. Resources in medical care are generally plentiful and physicians make most decisions about modalities of care without concern about the relative or absolute availability of the modality. The transplant physician must choose those patients who are most like to benefit from receiving a new liver and who will thrive after transplant. The choice of which patient to transplant is difficult and requires rigorous evaluation of the patient, the patient's support system, progression of both liver disease and comorbidities, and prospects for recovery. Mathematical models that assess risk by combining medical facts with disease epidemiology such as the Model for Endstage Liver Disease (MELD) score in use in the United States have assisted with these decisions. In the end, the transplant enterprise rations organs and care to those who are perceived to benefit most. This chapter focuses on indications for transplantation, the evaluation and management of the patient prior to transplant, and the common challenges of managing the patients in the posttransplant period.

## History

Although transplantation as a safe, effective therapeutic modality has come into its own only in the past 50 years, the concept of the successful replacement of human organs has captivated scientific discourse for millennia. One need only to look at the mythologic chimera to appreciate a subtext of appreciation of the hypothesis of grafting non-self parts onto a patient to achieve both function and tolerance of the organ.

Starzl et al. performed the first human liver transplant on March 1, 1963 in a child with biliary atresia [90]. The child died intraoperatively. The second and third patients, both adults, died 22 and 7.5 days postoperatively respectively of complications from extensive pre-transplant malignancy. By the end of 1963, a total of seven patients had received liver transplants [20, 63, 91, 93]. Due to the poor results being achieved a voluntary worldwide moratorium on transplantation was imposed until 1967 so that the issues involved with transplantation could be studied further.

Later series of patients proved more successful. During the interval period, it was learned that patients awaiting transplant who were severely ill and could not wait for a matched organ would not suffer tremendously by receiving an unmatched one [39, 91]. The next advance was the appreciation that a "triple drug cocktail" of antithymocyte globulin introduced in combination with azathioprine and prednisone could achieve better results [39, 92].

The next major advance was the discovery of cyclosporine in 1973 and its implementation into clinical practice in 1983. By the 1990s, liver transplantation had become an accepted treatment for cirrhosis with morbidity and mortality in carefully selected patients, which were similar to those for other complex, tertiary procedures.

## Philosophy

By necessity, transplantation decisions are guided by a utilitarian philosophy. In the best of all possible worlds, a treatment modality would be without risk and available to all those who would benefit from it. The treatment of hepatic encephalopathy with lactulose, for example, is essentially without risk, inexpensive, and widely available to those who need it.

On the other hand, some therapies are so difficult to tolerate, so dangerous to the patient, and so cost ineffective that they are rarely dispensed. Until the advent of antibiotic therapy, pneumonectomy was the mainstay of therapy for tuberculosis. The procedure, of course, was poorly effective and had high morbidity and mortality. Antituberculous medical therapy is for more efficacious with significantly less morbidity and mortality. Most treatment decisions are in the middle where physicians balance the risks and benefits of several management strategies.

A liver transplant is a far more nuanced decision for both the physician and the patient. It is the only effective therapy for end stage liver disease yet it is not available to all due to organ shortage. And the operation carries both significant risk and substantial benefit. End stage liver disease is *cured* but replaced by a new and different chronic condition by way of a major surgical procedure. The patient must be ill enough to derive a benefit from transplant. The patient must not be too ill in order to have a reasonable chance to

survive through and thrive after the transplant. Moreover, one patient's transplant affects all the other patients awaiting transplant. While the patient receiving a new liver benefits from the transplant, patients on the remainder of the waiting list who did not receive the graft are still at risk for adverse pre-transplant outcomes until such time as they themselves receive a liver transplant.

Utilitarian philosophy dictates that resources should be focused on where they would do the most good. Maximal good at minimal cost is a common mantra of utilitarian thinkers. Rationing a limited supply of organs to a large pool of eligible recipients becomes a utilitarian enterprise.

## The Evaluation Process

Once the patient's primary medical care team suspects that a patient would potentially benefit from liver transplantation, the patient is usually referred to a specialist who is trained in recognizing patients who meet criteria for possible transplant listing. Frequently, it is a gastroenterologist or a hepatologist in the community who refers the patient to a liver transplant center. Primary care physicians or other specialists may also pursue the referral directly. Physicians who are not specialists in transplantation need to be educated in deciding when a patient with liver disease is a candidate for transplant evaluation.

After a referral is made, the patient is seen and evaluated by the liver transplant center. What is the patient's risk for surgery? Does the patient have preexisting medical conditions unrelated to their liver disease that would make transplantation unnecessarily risky? Does the patient have psychosocial barriers to transplant? Does the patient have the social support structure in place to adequately ensure that they will be compliant with the rigorous medical regimen required of a liver transplant recipient?

These questions all need to be answered. Another crucial issue is whether the patient's liver disease is at a sufficiently early stage that they would derive no survival benefit from and thus should not be listed for transplantation. Conversely, the patient's liver disease should not have progressed to such an advanced state that surgery would be overwhelmingly risky. The patient has to be evaluated for coexisting medical conditions that would make transplantation surgery or the post-operative course too risky.

Once the psychosocial and medical evaluations have been completed, the transplant center must take this information and make a decision whether the patient should be placed on the transplant list. This usually involves an objective evaluation and discussion by a committee composed of transplant surgeons, hepatologists, psychiatrists, social workers, transplant coordinators, nurses, and others who decide whether patients would benefit from listing for transplant.

## Listing for Transplantation

The subsequent discussion focuses on the process of listing for transplantation in the United States. European and other systems function differently but the themes of the American model are illustrative of systems in place in other countries to list patients for transplant. Once a transplant center decides to place a patient on the waiting list for transplant, the patient is registered with the United Network for Organ Sharing (UNOS) Organ Center. UNOS runs a centralized computer network that includes every transplant hospitals' waiting list and links all organ procurement organizations (OPOs). The system currently in place is called UNet (see "http://www.unos.org") and involves a password-protected data entry system where each candidate needs to be verified by at least one other person with UNet access. Once entered onto a transplant center's waiting list, the patient is made part of the national organ allocation infrastructure from which decisions about organ allocation are made. Organs are allocated locally within the local organ distribution unit (usually 1–3 OPO's coverage area) first. If no appropriate recipient is found locally it then proceeds to the level of local OPO's UNOS region, and then nationally to the other ten UNOS regions for all patients with chronic liver disease. Broader regional sharing arrangements are available patients with acute liver failure in most regions and now to allow regional sharing before local allocation to patients with MELD scores <15. Though prioritization and allocation methods differ, similar systems exist in Europe such as the central European system Eurotransplant, which allocates organs in six countries, and Scandiatransplant, which performs the same function for the five Nordic nations.

## Scoring Systems and Prioritization on the Waiting List

Organ allocation systems are structured upon evidence-based outcome measures and principles of justice and equity, maximal utility, and transparency[12]. The goal is to optimize the allocation and distribution of organs. In prior years in the US, organ allocation was predicated principally on location (at home, hospitalized or ICU-bound) and on waiting time. It is now a risk-based system using the MELD model.

For over 30 years, the Child-Pugh classification system has been used to predict mortality and morbidity in liver disease [72]. Although variations of the Child-Pugh classification have been critical in stratifying patients for liver transplantation, it poorly prioritizes patients on the liver transplant list[100].

Previously, the Child-Pugh Class combined with location and waiting time determined organ allocation. While the system did adequately differentiate very sick patients from others, it was not very good at determining medical urgency, and thus was largely a waiting time based system.

Organ allocation is now based on the Model for End stage Liver Disease (MELD) and the Pediatric End stage Liver Disease (PELD) model, mathematical regression models that more accurately predict short-term mortality on the waiting list and benefit from transplantation. MELD is based on a logarithmic transformation of the recipient's international normalized ration (INR), bilirubin, and creatinine in a validated mathematical model. PELD includes the albumin, bilirubin, INR, age (<1, >1 year old) and the presence of growth failure to risk-stratify candidates [59].

Perhaps the greatest difference between MELD and PELD and the Child-Pugh score is that MELD contains only easily verifiable objective, rather than objective plus subjective variables (degree of encephalopathy and ascites). MELD also more accurately predicts the patient's need for transplant, temporal likelihood of transplantation, and, most importantly, short-term prognosis while waiting for transplant. By allowing the physician involved in the care of the pre-transplant patient a mechanism to calculate a score through a simple formula, MELD has provided the physician with a simple tool to link the patient's prognosis along with their probability of transplantation. This becomes invaluable when selecting patients for transplantation.

MELD is based on a patient's laboratory parameters at a single point in time. As a result, risk of death while on the transplant risk is perhaps more accurately predicted by serial than by single scores. The change in MELD or *delta MELD* does add additional predictive accuracy to the MELD in estimating survival likelihood. It provides valuable information regarding the progression of disease while at the same time prognosticating on the patient's course [61]. Delta MELD has been proposed to enhance prioritization for transplantation but difficulties in defining standard intervals for delta MELD have hampered its implementation. Other proposed additions to MELD have attempted to quantify the degree of portal hypertension using encephalopathy, ascites, or the presence of hyponatremia. Of these, the latter has had the most support due to its objective nature and is currently under study.

## Impact of MELD on Outcomes and Transplant Benefit

Donor organs should be allocated to patients with the highest likelihood of transplant benefit. After the implementation of MELD, the survival benefit of liver transplantation within 1 year of transplantation has been concentrated among patients at the highest risk of pretransplant death. Consequently, patients who are at the lowest risk of pre-transplant death and have little or no demonstrable benefit from transplantation continue to wait on the waiting list [60].

Since the introduction of the MELD-based allocation system, there has been a direct relationship with risk of death while waiting for transplant and MELD score with a decrease in the overall pretransplant mortality rate. Patient and graft survival have also improved since the implementation of MELD [60]. Since MELD and PELD scores provide physicians with a snapshot of the patient at a single time point, they must be updated periodically to provide the physician with a continuous picture of the patient's status.

Since MELD is based on objective laboratory tests and results in a large, continuous scale of severity, it better estimates the short-term prognosis of patients with end-stage liver disease [42, 52]. For patients with a low MELD score, mortality without transplant is not high enough that it outweighs the morbidity and mortality risks of surgery and the vast majority of patients with low MELD scores tend to remain stable in the short-term. Transplantation of patients with low MELD scores (<15) has been associated with a higher mortality post-transplant

compared with patients with higher MELD scores, suggesting that transplant of patients with low MELD scores is not the best use of the donor pool [60].

On the other hand, MELD has proven poor at predicting post-transplant outcomes [61] The purpose of the MELD score is to assist in selection of patients who will benefit most from transplantation when compared with their mortality risks from living with their end-stage liver disease. MELD alone does not provide a highly accurate measure of the patient's chances for survival after liver transplantation. Interestingly post-transplant survival is only modestly decreased at very high (>30) MELD scores. This is a fortunate finding as survival benefit would be diminished if patients with high MELD scores had prohibitive risk with or without transplantation.

## Indications for Transplantation

Only patients who are felt to be capable of surviving the perioperative period should be considered for organ replacement therapy. They also must have the ability to comply with the intense medication regimen and follow-up required of transplant recipients. They must be able to abstain from addictive behavior such as alcohol or drug abuse. Most importantly, they should have no other major medical illnesses significantly curtailing life expectancy.

Any process intrinsic to the liver, which leads to decompensation and liver failure is theoretically an indication for liver transplant. The list of indications for liver transplantation is as extensive as the spectrum of liver disease itself. Generally, any form of end-stage liver disease that is irreversible and curable with liver transplantation is considered an indication. If the disease is a systemic disease involving the liver, systemic cure must be achieved with liver transplantation or the systemic effects must not be severe in nature.

### *Minimal Listing Criteria*

Due to concerns about regional disparities in the types of patients waiting for transplant and about significant variations in criteria for placing a patient on the waiting list for transplantation, the American Society of Transplant Physicians and the American Associations for the Study of Liver Diseases formulated recommendations for minimal listing criteria for placement of adults on the liver transplant waiting list in 1997 [53]. The major thrust of the guidelines were recommendations that placement on the waiting list indicates that both the center and the patient are ready to proceed with transplant immediately should an organ become available.

The patient's expected survival should be less than or equal to 90% within 1 year without transplantation to qualify for listing. Exceptions to this rule were recommended for some patients. In particular, patients who do not meet these survival criteria but have other indications for immediate transplant such as impaired quality of life should be listed. Examples of this would be patients with refractory ascites, difficult to control encephalopathy, and intractable pruritus.

Based on the panel's review of published data, they recommended minimal Child-Pugh scores for listing. A score greater than or equal to 7 (Child's class B or C cirrhosis) was recommended as a cutoff as this score correlates with a survival rate at 1 year of less than or equal to 90%. This reflects the morbidity associated with decompensation as manifested by symptoms such as ascites, jaundice, hepatic encephalopathy, and bleeding. Patients with a Child-Pugh score of greater than or equal to 7 or with the presence of bleeding associated with portal hypertension should be listed for transplant.

The most common diagnoses for adult patients waiting for liver transplantation are post-necrotic cirrhosis, alcoholic liver disease, hepatitis B and C, hepatocellular carcinoma, and cholestatic liver disease (e.g., primary sclerosing cholangitis and primary biliary

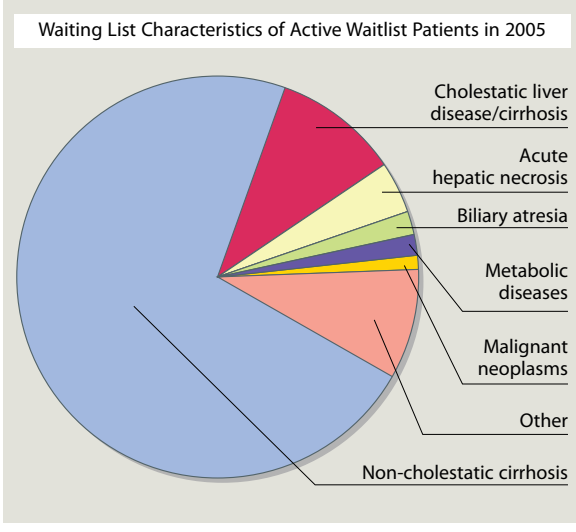


**Fig. 103.1** Indications for liver transplantation (Annual Report of the U.S. Organ Procurement and Transplantation Network and the Scientific Registry of Transplant Recipients)

cirrhosis) (Fig. 103.1). What follows is a discussion regarding the issues surrounding the more common indications for liver transplantation.

## *Alcoholic Liver Disease*

(See also Chapter 88).

A history of alcohol use is very common in patients evaluated for liver transplantation and needs to be distinguished from alcohol-induced liver disease. Alcohol use frequently appears in patients transplanted for other reasons (e.g. Hepatitis C). In these cases, alcohol frequently acts a cofactor in the development of end-stage liver disease.

Concerns about recidivism following a period of abstinence from alcohol prior to transplantation dominate discussions about transplant in the patient with alcoholic liver disease. Does a personal tendency towards alcohol addiction predispose patients to resume alcohol use even after a period of abstinence prior to transplant? Will alcohol use following transplantation affect the new organ and if so, how much and to what degree? Data on the adverse effects of recurrent alcoholic liver disease are much more limited than for recurrence of HCV or other liver diseases, further clouding the picture.

Because of these questions, alcohol use, abuse, and dependence all touch on the utilitarian ethical issues central to candidate selection for liver transplantation. In addition, given the stigma associated with alcoholism and addiction in general, this issue is highly charged within many transplant teams and in the public eye.

It is difficult to predict which alcoholics will develop alcoholic liver disease. Some risk factors do emerge such as drinking outside of meal times, drinking on a daily basis, and consuming multiple drinks at a single sitting. Obesity, advancing age, and genetic factors also play a role in the advance toward end stage liver disease [65].

As discussed, the survival benefit from transplantation for alcoholic liver disease is greatest in the sickest patients. Patients with Child-Pugh class B and C cirrhosis and patients with intractable encephalopathy, history of variceal bleeding, poor quality of life, and a life expectancy of less than a year seem to benefit most from transplantation [65].

Current recommendations for minimal listing criteria for patients with alcoholic liver disease include a Child-Pugh score greater than or equal to 7, portal hypertensive bleeding or an episode of spontaneous bacterial peritonitis. It is crucial for the transplant team to involve a specialist in addiction in the decision to transplant the patient with alcoholic liver disease. The specialist must deem the risk of recidivism to be low. Most specialists recommend that the patient have concurrent 6-month period of continuous abstinence to be listed. Some recommend referral to a regional review board if the program feels that the patient is a candidate for transplantation prior to not achieving 6 months of abstinence [53]. Others evaluate on a case-by-case basis when the patient is too ill to survive 6 months or participate in a rehabilitation program. Other than abstinence duration, a strong social support system and ability to alter the environment in which drinking occurred, good insight into alcoholic behavior, shorter duration of addiction, and immediate cessation without relapses upon recognition of an alcohol related health or social problem are predictors of ability to maintain abstinence. These same abstinence criteria should be applied to patients with dual liver disease diagnoses that include alcoholic liver disease.

Because many patients with alcoholic liver disease do not stop drinking or die soon after being diagnosed, and others who become abstinent at the time of diagnosis may improve their liver function to a point where transplant is not required, most patients with alcoholic liver disease do not undergo orthotopic liver transplant [98]. Abstinence for 6 months, in effect, excludes patients with reversible liver failure from alcoholic hepatitis from being transplanted. While the key primary concern when considering patients with alcoholic liver disease for transplant remains whether or not they will relapse into their prior alcohol use patterns, a secondary thornier question is whether reversion to prior deleterious behaviors will actually prove detrimental to the transplanted liver.

Eighty-five percent of US and European liver transplant programs require some period of abstinence prior to listing patients for transplantation [24]. The generally accepted period of abstinence required by many centers is 6 months. The rationale for requiring a period of abstinence by patients prior to transplantation is based on ensuring that the patient's liver is not reversible upon abstaining from alcohol thus obviating the need for transplantation, identifying those patients who are at high risk for relapse to drinking, and allowing time for alcohol addiction and relapse prevention therapy prior to transplant [65].

The 6-month period is not based on established epidemiologic evidence nor has abstinence been shown to adequately predict post-liver transplantation abstinence. Longer periods of abstinence, though, do correlate with lower rates of recidivism. Most surprisingly, scant evidence exists to support the fact that abstinence impacts patient or graft survival [65].

While it is sensible to follow the 6-month rule, strict enforcement clearly hurts those patients who die during the abstinence period or experience complications of their liver disease before the 6-month period is completed. Decisions regarding alcohol use should therefore be guided on a case-by-case basis [48].

Additionally, alcohol use is a powerful cofactor in the progression of other liver disease. Use of alcohol following transplantation could impact negatively, for example, on the survival of the graft in patients transplanted for cirrhosis primarily related to hepatitis C [65].

## *Viral Hepatitis*

(See also Chapter 63).

### Hepatitis C

Viral hepatitis remains a leading cause of end-stage liver disease. In addition to overall severity of cirrhosis, the transplant team needs to take into account the degree of viral replication at the time of evaluation. Treatment of the virus should be tailored accordingly to promote graft survival following transplantation. It has been shown, for example, that higher hepatitis C viral load prior to transplantation correlates with lower survival rates post-transplant. An HCV-RNA titer of $\geq 1 \times 10^6$ copies/mL before transplant had a cumulative 5-year survival of 57% versus 84% for those with HCV-RNA titers of $<1 \times 10^6$ copies/mL ($P = 0.0001$) [16].

Owing to the near-universal recurrence of viremia following transplantation, hepatitis C virus is a particularly vexing problem for the transplant physician. Patients transplanted with HCV have overall lower graft survival rates when compared with patients transplanted for most non-viral liver disease. Evaluation, therefore, requires not only detecting those patients who would benefit most from transplantation, but should also focus on developing anti-viral strategies to reduce or eliminate the viral burden prior to transplantation and reduce the risk for post-transplant recurrence. Pretransplant viral load, advanced recipient age, hyperbilirubinemia, elevated INR, pretransplant CMV status, and advanced donor age impact adversely on patient survival after transplant in patients transplanted with HCV [17].

Recurrence of hepatitis C virus after transplant is near universal. Recurrent HCV viremia is often asymptomatic and has minimal early clinical implications. Unfortunately, HCV has an accelerated post-transplant course compared to the non-transplant population. Recurrent cirrhosis and liver failure may ensue. Controversy exists regarding the issue of whether to retransplant patients with graft failure due to recurrent hepatitis C. Should these patients be retransplanted? Should those who need a second transplant receive higher or less of a priority than those having their first? The criteria for retransplantation for recurrent HCV vary from center-to-center. Some centers no longer perform retransplantation for HCV due to poor patient and graft survival.

Patients retransplanted for recurrent HCV have worse outcomes than primary transplants. On the other hand, the outcomes are is not clearly worse than retransplantation for other causes. It does not seem reasonable, then, to systematically exclude patients with recurrent HCV from retransplantation. However, patients with early aggressive recurrence with graft failure within the first year following their primary transplant have very poor outcomes with retransplantation. As with primary transplants, patients with very high MELD scores carry higher retransplant risks with them than do patients with lower MELD scores. These two groups of patients should not undergo repeat transplantation except under selected conditions.

### Hepatitis B

Historically, hepatitis B patients requiring transplant had suffered a worse fate as patients transplanted for HCV, with virtually universal severe recurrence of disease and graft failure. This led to the conclusion that HBV was a contraindication to OLT in the late 1980s due to poor outcomes. Recurrence of HBsAg in the serum was associated with fibrosing cholestatic hepatitis, an extremely aggressive form of HBV ending in graft loss and a 1-year mortality of over 50%. Risk of recurrence was related to the degree of viremia; patients who were HBeAg positive

and those with detectable HBV DNA by solution hybridization (>$10^6$ copies/mL) had high rates of recurrence. Patients with fulminant HBV, Delta coinfection, and HBeAg negative patients all had lower pre-transplant viral loads and lower rates of recurrence.

In the modern transplant era, recurrence of HBV following transplantation is uncommon and much less severe than recurrence of HCV following transplant for hepatitis C cirrhosis. Prophylaxis with Hepatitis B immune globulin (HBIg) in combination with oral nucleoside or nucleotide therapy can prevent reinfection of the graft in over 80% of patients [3, 22, 105].

Significant HBV recurrence after transplant on this prophylactic regimen is rare. This has revitalized transplant for HBV. Questions remain as to the optimal duration of HBIg and the ideal antiviral therapy combination. The use of nucleoside monotherapy with lamivudine post-transplant had over a 20% recurrence rate. Most programs now use HBIg for at least 1 year and some programs use HBIg indefinitely in combination with either single or combination nucleoside/nucleotide therapy. Levels of HBsAb > 500 IU/mL are associated with very low rates of recurrence when used with or without oral antiviral agents. Our center uses combination therapy indefinitely at the current time as long-term data on emergence of resistant HBV on antiviral therapy without HBIg is lacking.

## Cholestatic Liver Disease

(See also Chapters 73–77).

Patients transplanted for cirrhosis due to primary biliary cirrhosis and primary sclerosing cholangitis, the benign cholestatic liver diseases have some of the best long-term outcomes in liver transplantation. To assess severity, the Mayo models for primary sclerosing cholangitis (PSC) and primary biliary cirrhosis (PBC) are frequently used. Patients with a Child-Pugh score greater than or equal to 7 or the advent of portal hypertensive bleeding are eligible by current criteria for listing for transplant. Alternatively, patients with a Mayo model risk score predicting greater than 10% mortality at 1 year should be listed for transplant [53].

The patient with PSC and recurrent episodes of cholangitis presents special challenges to the liver transplant team. These patients may have MELD scores that inadequately reflect mortality since morbidity and mortality are more commonly due the development of resistant bacterial or fungal infections. These patients may be underserved by their MELD scores. They also suffer from greatly impaired quality of life due to the need for frequent endoscopic or percutaneous procedures and frequently require external drainage.

Added priority for OLT for these patients may be obtained in some cases by petitioning the regional review board (RRB) for additional MELD points. A similar approach can be taken for other types of patients in whom their degree of quality of life impairment is not reflected by MELD. Patients with PBC and intractable pruritus or severe metabolic bone disease in patients may petition for additional MELD points. This is controversial as it diverts livers away from patients with higher mortality risks and these exceptions are decreasing in frequency. An alternate solution is to view these complications as indication for proceeding to living donor liver transplantation if an adequate donor volunteers.

## Malignant Diseases of the Liver

Because extrahepatic spread of hepatobiliary tumors brings with them a poor prognosis, selection of patients with malignant disease for liver transplant is complicated. Theoretically, the primary malignant diseases of the hepatobiliary tract, hepatocellular carcinoma (HCC) and cholangiocarcinoma, when confined to the liver, could be cured with surgical resection. Surgical resection, however, often would result in insufficient hepatic reserve remaining for the patient. As a result, patients will often need a transplanted organ to replace the resected organ, due to either to bilobar disease or severe liver disease in the remnant that would lead to liver insufficiency post-resection. Additionally even with adequate reserve and a curative resection, the liver remnant retains malignant potential and thus is at high risk for *de novo* and recurrent cancer. Thus transplant has evolved into a crucial therapy for these diseases.

Due to the difficulty in predicting whether the malignancy has spread to extrahepatic sites and rendered transplantation futile, criteria have been developed to stratify the risks of recurrence of tumor. These criteria are based on size and other tumor characteristics. These criteria are then used to decide whether to list patients with a malignancy for transplantation.

## Cholangiocarcinoma

(See also Chapter 116).

Cholangiocarcinoma is a highly malignant neoplasm arising from the bile duct epithelia. Sixty percent occur in the perihilar region at the confluence of the right and left hepatic ducts (Klatskin tumor). Primary sclerosing cholangitis is the most common known risk factor for the development of cholangiocarcinoma. Currently, there are no effective medical therapies for cholangiocarcinoma. After diagnosis, the median survival is 9–12 months, with long-term survival being infrequent.

Even with resection, local recurrence is common. Patients in whom the cholangiocarcinoma arises in the context of PSC present special issues. Patients with PSC and cholangiocarcinoma have disease that is more frequently multifocal and are more likely to have fibrotic/cirrhotic liver disease that makes the risk of liver failure and death following resection prohibitive [33].

Historically, cholangiocarcinoma recurred in most patients and was viewed as a contraindication to transplantation. Reported long-term survival rates following transplantation ranging from 20% to 30%, significantly lower than those for patient transplanted for cirrhosis [87]. The treatment for cholangiocarcinoma, however, has changed dramatically over the past decade and with it the survival prospects following transplantation.

While prior studies showed poor outcomes and high recurrence rates in patients with cholangiocarcinoma who had been transplanted, some caveats should be kept in mind regarding this data. First, this older data originated from patients in varying stages of disease. Next, selection criteria of candidates for transplantation were poorly defined. Finally, in no cases were adjuvant and neoadjuvant chemotherapy was given.

In 1994, a new management protocol involving preoperative chemoradiotherapy followed by liver transplantation for patients with localized perihilar cholangiocarcinoma and regional lymph node negative disease was introduced at the Mayo Clinic. With this new protocol, 92% of patients were alive at 1 year following transplant and 82% were alive at 5 years [74]. These data suggested that the use of neoadjuvant therapy in these patients selects a group of patients with cholangiocarcinoma who will likely achieve a clear survival benefit from transplantation. We and others have modified the protocol using different chemotherapeutic agents with more activity in pancreatic and biliary tract tumors in combination with brachytherapy and/or external beam radiation. Better treatment strategies and improved detection with MRCP, ERC, intraductal ultrasound and biopsy, and FISH of biliary aspirates can further improve outcomes. However, this indication remains controversial. Transplantation should only be performed in the context of well-defined clinical studies at centers with experience in this type of protocol.

## Hepatocellular Carcinoma

(See also Chapter 102.1).

Primary cancer of the liver, hepatocellular carcinoma presents similar, but less vexing, difficulties. Like cholangiocarcinoma, HCC is frequently tied to primary liver disease in this case coexisting cirrhosis or viral hepatitis. Unlike cholangiocarcinoma, a large body of data has shown that small HCC can be treated with liver transplant with a low recurrence rate. Controversy in this area exists is as to the size limit of the tumor(s) for transplantation and acceptable recurrence rates. Current recommendations are that liver transplantation is indicated in patients with small HCC (Stage I–II, i.e., T1-2, N0, M0 disease) with no evidence of invasion of large vascular structures or extrahepatic disease [26]. The prognostic value of high alpha-fetoprotein (AFP) levels is controversial. Recurrence is higher for patients with elevated AFP values, especially those >1,000–2,000. These patients are more likely to have either more extensive disease in the liver than noted on pre-operative imaging or occult extrahepatic disease. Though no cutoff of AFP is recommended to exclude patients from OLT, caution and planned exploration with an identified back-up candidate prior to OLT may be prudent for patients with AFP > 1,000, especially if the number has risen despite locoregional therapy. If patients clearly have no evidence of extrahepatic spread and the tumor appears small enough that metastasis would seem unlikely, then transplantation can offer high long-term cure rates with low recurrence rates post-OLT.

The optimal diagnostic criteria for hepatocellular carcinoma as well as the role of pre-transplant therapy remain controversial. While lesions above a certain size can be diagnosed as hepatocellular carcinoma based on imaging only, smaller lesions present diagnostic challenges. At one extreme are some experts

who have called for biopsy with histologic confirmation of all lesions being evaluated for transplantation. Some advocate biopsy only for nodules less than 2 cm in size, the protocol supported by the guidelines put forth by the European Association for the Study of Liver Disease (EASL) [82]. Others do not feel that biopsy is needed when characteristic imaging is obtained, largely due to concerns about false-negative results with biopsy. Given rates of "incidental" HCC on explants in HCV patients of up to 20%, it is prudent to go with the assumption that lesions that appear as HCC on imaging performed with liver specific protocols should be assumed to be HCC even without a biopsy.

We advocate biopsy of all lesions in patients who have no other independent indication for OLT, e.g. a patient with Child A cirrhosis, a small lesion (<2 cm) and a low AFP. Even in these cases, serial imaging and close follow-up is required as some will turn out to have HCC even with a negative biopsy. In patients with decompensated disease and a new hypervascular lesion >2 cm that is characteristic of HCC, however, the risk of core biopsy as well as the risk of needle-track spreading and false-negative results make a presumptive diagnosis of HCC an appropriate choice. It is important to note that fine needle aspiration does not provide the architectural detail required for diagnosis of a well-differentiated HCC and core needle biopsy is required.

Tumor size, AFP level, presence of vascular or capsular invasion, bilobular location, and number of nodules are poor prognostic factors and independently predict mortality from and recurrence of HCC. AFP is a strong predictor of recurrence and AFP greater than 300 ng/dL indicates a high chance of recurrence [26].

Since it is based on a postoperative scoring system rather than pre-OLT imaging, the TNM system of staging cannot be used with 100% accuracy to evaluate for transplant. Utilizing an international registry of hepatic tumors in liver transplantation, Klintmalm et al. showed that tumor size greater than 5 cm, presence of vascular invasion and a poorly-differentiated histologic grade of the tumor are strong negative predictors of patient survival after transplantation for HCC [44].

If selection of candidates is limited to patients with solitary tumors less than 5 cm or to subjects with up to three nodules, each under 3 cm in size, then the 5-year survival rate may exceed 70% and the recurrence rate is usually less than 15% following transplantation [10, 41, 50, 58, 83]. These criteria, known as the Mazzaferro or "Milan" criteria remain the standard criteria used to select patients with HCC for OLT and are used to adjust priority for OLT at UNOS. Other more liberal size criteria (e.g. UCSF criteria) have also been proposed based on retrospective studies showing good results. The UCSF criteria show similar survival rates for patients with single tumors less than 6.5 cm or two lesions less than 4.5 cm to those within the Milan criteria [101].

*Priority on the waiting list for OLT for HCC*. Because many patients with HCC have relatively well-compensated cirrhosis and are therefore underserved by scoring systems which emphasize both clinical and biochemical markers of end stage liver disease, corrective measures have been developed to ensure that HCC patients are not disadvantaged in organ allocation. The current MELD-based scoring system used by UNOS, for example, allows for an exception to be made for patients with hepatocellular carcinoma. These patients receive special "exception points" if they meet certain diagnostic parameters for their HCC. The rationale behind this rule is that patients with HCC may not have cirrhosis or intrinsic liver disease. They would have lower MELD scores based on laboratory scores that do not accurately predict their risk of mortality on the waiting list due to tumor growth. They would consequently have less chance of transplantation and more progression of their tumors on the waiting list.

Allowing for a correction to the MELD score for patients with HCC has compensated for these patients being underserved by their "physiologic" MELD score. The low priority for HCC in the prior allocation scheme had led to a high dropout rate secondary to tumor progression. Since patients with HCC would derive a very large benefit from early transplantation prior to metastasis or growth of the lesion, the allocation system was altered to take this into account. Initially, T1 lesions were given a MELD score of 24 and T2 lesions a score of 29. The priority was increased every 3 months. No priority was given to tumors that exceeded these guidelines. The result was a marked increase in the number of HCC patients being transplanted along with very short waiting times and a decrease in the dropout rate to close to zero.

Surprisingly, up to 25% of patients transplanted in the United States with HCC after the initial implementation of MELD exception points did not have evidence of HCC in the explanted liver, suggesting incorrect interpretation of the findings or imaging results which led to the diagnosis of HCC. This finding has led some

to postulate that encouragement of early transplant in the MELD era might provide incentive for limited workups in these patients [30, 83]. Most of these were T1 lesions, less than 2 cm. Additional modification has led to a reduced MELD priority for T2 lesions (24 points and now 22 points) with no additional priority for T1 lesions. This has reduced the total proportion of HCC patients among transplant recipients and should reduce the rate of HCC cases without histologic confirmation in the explant.

Living donor liver transplantation offers distinct benefits for the patient with HCC. With LDLT, the transplant can be performed earlier, before the HCC has metastasized. Those patients with single tumors less than 5–7 cm in size, or with three or fewer nodules, and no evidence of extrahepatic disease or vascular invasion can be considered for LDLT. In fact, this may be the best option for the growing number of patients with cirrhosis and small tumors [95, 96]. LDLT results in a better outcome for patients with HCC, with a lower risk of death (RR = 0.35). LDLT patients are younger and tend to have lower MELD scores. Furthermore, by limiting use of the donor pool, LDLT allows more patients to undergo transplantation [51]. However, enthusiasm for LDLT as an ideal option for HCC patients has been tempered by recent data showing increased recurrence when compared with DDLT recipients [27].

Since many patients with HCC have HCV and owing to the slow growth of the HCC, it had been thought that these patients may be the ideal candidates for efforts to eradicate HCV prior to transplant. Since patients with decompensated cirrhosis oftentimes cannot tolerate ribavirin and pegylated interferon, therapy had previously not been an option in many cases. Everson et al. recently reported the use of a Low Accelerating Dosage Regimen of interferon and ribavirin (LADR) whereby patients are begun on low doses of the medication with doses being escalated as tolerated [25]. They showed that viral eradication on therapy can be achieved in up to 40% of patients. Patients transplanted on therapy for as little as 12 weeks with negative HCV RNA in the serum have only an approximate 20% risk of viral recurrence post-transplant. This will likely provide a substantial survival benefit by decreasing post-OLT morbidity from HCV recurrence. Since patients with HCC often have no decompensation and oftentimes do not have urgent needs to be transplanted owing to the relatively slow-growing nature of HCC and success of locoregional therapy, they may be ideal candidates for this regimen. It is likely that only LDLT candidates and those with HCC MELD upgrades would be able to tolerate LADR and undergo transplantation while still on antiviral therapy.

*Therapy for HCC pre-OLT*. The role of pre-transplant locoregional or systemic therapy is unclear. The goal of pre-transplant therapy is to reduce tumor burden in order to prevent recurrence and to prevent progression on the waiting list. Theoretically, necrotic tumor would be less likely to be spread during mobilization and removal of the liver during the transplant.

Various forms of locoregional therapy, as detailed in other chapters, exist. In the past, approaches have included chemotherapy directed at lesions via an intraarterial pump, transcatheter embolization of the arterial supply of a lesion, and radiofrequency ablation. All these modalities have been used with varying degrees of success. Transarterial chemoembolization (TACE) has been used routinely to prevent progression of HCC during the waiting period.

Prior to the 1990s, therapy toward unresectable HCC was seen as palliative, not curative. In 1995, Venook et al. showed that patients who receive preoperative chemoembolization followed by OLT can achieve long-term survival. Ten of 11 patients with unresectable, histologically-confirmed HCC who received preoperative chemoembolization and later underwent OLT remained tumor-free at a median time of 40 months [99]. More recently, chemoembolization or TACE, whereby a chemotherapeutic agent is injected along with a compound that limits circulation to the tumor, has shown some effectiveness even without transplantation.

Randomized data on the efficacy of pre-transplant TACE is limited, both in the transplant and non-transplant populations. However, lower posttransplant recurrence rates have been seen in chemoembolized patients compared with patients with incidental tumors, despite larger tumor size in the chemoembolization group. This may represent an effect of TACE at lowering recurrence rates although it may also reflect differences in tumor biology in the two groups. Since TACE treats entire lobes of the liver, it also treats tumors that might be too small to be seen on imaging.

Percutaneous ethanol or acetic acid injection and radiofrequency ablation (RFA) are being used increasingly for a wide variety of malignant diseases of the liver. RFA and acetic acid can be used in lesions up to 5 cm; RFA can also be performed in the operating

room with laparoscopy or laparotomy, usually with ultrasound guidance. For larger lesions, directed ablation usually achieves a higher degree of necrosis than TACE. Needle track spread can occur and is a concern. Recurrence risk after percutaneous catheter ablative therapy is less well studied and cannot be predicted [26, 82]. Often a combination of approaches is used particularly if the lesion is larger (e.g. beyond the UNOS criteria for additional priority) or waiting time is predicted to be long.

Data suggest that neoadjuvant therapy for HCC improves posttransplant survival. A study looking at pretransplant ablative therapy (ethanol injection, embolization, TACE, and RFA) among patients registered in the International Registry of Hepatic Tumors in Liver Transplantation showed a dramatic increase in recurrence-free survival after OLT among patients with HCC who had undergone ablative therapy. 69% of patients were alive free of tumor in the ablation group as compared with 31% of patients in the group that did not receive ablation therapy ($P < 0.0001$) [44]. Interestingly, the positive impact of therapy increased as tumor characteristics worsened with the survival benefit increasing as the number of nodules in the tumor grew.

## Metabolic Liver Disease

The metabolic liver diseases, including Wilson's disease, hereditary hemochromatosis, and $\alpha_1$-antitrypsin disease, all are indications for transplantation since they all can lead to irreversible damage in the liver. Since these diseases are all characterized by having systemic effects outside of the liver, the pretransplant workup must include a coordinated workup to allow for identification of systemic disease that would preclude transplantation.

Hemochromatosis can lead to iron deposition in the myocardium resulting in an irreversible cardiomyopathy or conduction system damage with arrhythmia. Cardiac dysfunction is a primary reason for higher mortality after transplant in patients with hemochromatosis than those with other indications. $\alpha_1$-antitrypsin deficiency can lead to pulmonary emphysema, requiring careful cardiopulmonary evaluation to exclude pulmonary insufficiency or pulmonary hypertension. Occasionally, combined liver–lung transplant is required but end-stage liver and lung disease rarely coexist.

## Vascular Disease of the Liver

Vascular abnormalities such as Budd–Chiari syndrome or veno-occlusive disease frequently result in acute hepatic failure because of the abrupt nature of the disease development. Portal vein thrombosis is much better tolerated and rarely causes liver failure. Portal vein thrombosis is usually the result of chronic liver disease and presents with sudden worsening of portal hypertension. Because of its frequent association with HCC, careful screening for HCC should occur with all new cases of portal vein thrombosis.

Thrombosis may in fact be a harbinger of other vascular or hematologic disease. As vascular thrombosis is generally associated with some underlying coagulation disturbances, the workup for all patients with vascular thrombosis must include evaluation for a hypercoagulable state or underlying malignancy that could have lead to the disease. Many of these patients have underlying hematologic disorders, e.g., polycythemia vera, and, in addition to blood testing, bone marrow biopsy is frequently needed.

Any hypercoagulable state that is found must be corrected. This aspect is crucial to the success of the transplant. Patients transplanted for Budd–Chiari need to be treated with anti-coagulation medication post-OLT for life due to the high rate of recurrent clotting in this cohort.

## Fulminant Hepatic Failure

Fulminant hepatic failure, the rapid development of encephalopathy, coagulopathy and jaundice in those without a history of chronic liver disease, is an indication for liver transplantation. While patients may progress rapidly to a state of critical illness and die prior to transplant, those who do undergo transplantation have excellent outcomes. Transplantation should be considered in any patient who presents with acute liver failure but must be actively pursued upon the onset of stage II encephalopathy [53]. The criteria for the definition of fulminant hepatic failure include the development of hepatic encephalopathy within 8 weeks of the onset of symptoms in a patient without a history of liver disease. Subacute hepatic failure is defined as the development of hepatic encephalopathy within 6 months but after 8 weeks of the onset of symptoms in a

patient not previously know to have liver disease. Patients with subacute failure have a virtually universal mortality without organ replacement and all should be considered for transplant [53]. Acetaminophen toxicity is the most common cause of fulminant liver failure. Viral etiologies follow in frequency. Drug toxicity is the most common form of subfulminant liver failure when the etiology is known.

Patients with fulminant hepatic failure need to be cared for in a monitored setting with close observation. A number of patients who meet criteria for the diagnosis will recover full liver function without therapy [66]. It is therefore critical to understand the chances of recovery in patients with fulminant hepatic failure prior to a decision to accept an organ for transplantation. If the patient has a clear opportunity for full recovery, transplant may become unnecessary. On the other hand, waiting too long may lead to irreversible cerebral edema and death. The use of the King's College criteria and other prognostic scoring systems, as well as transjugular liver biopsy to look for massive or submassive necrosis, may assist clinicians in differentiating patients with a low chance of spontaneous recovery from those who should undergo expedited transplant.

A quandary arises when one is presented with acute liver failure secondary to acute alcoholic hepatitis. As discussed above, alcoholism presents significant psychosocial barriers to transplantation, particularly if the transplant is performed in a patient without a history of abstinence from alcohol. If a patient has a known drinking history and the liver failure is clearly secondary to alcoholic hepatitis after a binge drinking episode, the transplant team is faced with a dilemma as to whether to transplant a liver into an individual with a known predilection for alcohol abuse without knowing the patient's chances for recovery from addiction. In most centers alcoholic hepatitis is viewed as a contraindication to OLT. Exceptions are occasionally made on a case-by-case basis in acute settings particularly in young patients or unclear etiology (e.g. alcohol plus acetaminophen toxicity).

## Indications for Liver Transplant in the Child

Special consideration must be paid to the pediatric patient with liver failure. Their disease patterns can be unique. The stakes are very high, particularly in infants. Living donor liver transplantation is more common as parents will often volunteer to donate for their children. These issues make pediatric liver transplantation distinct from adult liver transplantation.

The indications for pediatric liver transplantation are similar to those for adult liver transplant but vary in prevalence; the most common indication is cholestatic diseases (predominantly biliary atresia), metabolic diseases, fulminant hepatic failure, chronic active hepatitis, malignancy, Budd–Chiari syndrome, parenteral nutrition induced cirrhosis, trauma, and Caroli's disease (UNOS Policy 3, Appendix 3B) [97]. Like adults, children should be listed for transplantation if decompensation has already occurred. Unlike most adult cases, transplantation in children may be also pursued if liver failure is considered inevitable even in the absence of any signs of disease as most disease are cured with transplant, will not recur and earlier transplant may allow for more normal growth and development. Decompensation includes intractable cholestasis, portal hypertension with or without variceal bleeding, multiple episodes ascending cholangitis, failure of synthetic function including coagulopathy or low serum albumin or low cholesterol, failure to thrive, growth failure or malnutrition, intractable ascites, encephalopathy, unacceptable quality of life including school failure or intractable pruritus, metabolic defects for which liver transplantation will reverse life-threatening complications, prevent irreversible central nervous system damage, or life threatening complications of stable liver disease such as hepatopulmonary syndrome. The failure of growth or normal development is an indication for transplantation. Liver transplant will usually reverse growth failure with significant catch-up growth if performed early in the course of disease.

### *Pediatric Cholestatic Diseases*

Cholestatic disease is the most common indications for liver transplant in children. Obstructive cholestasis includes biliary atresia and sclerosing cholangitis. Biliary atresia alone accounts for 60–70% of childhood liver transplants. Though potentially curative when performed in the first 45 days of life, the Kasai procedure (hepaticoenterostomy) to treat congenital biliary atresia does not completely obviate the need for transplantation in most cases. Even with timely performance of the procedure, around 75% of patients will

develop cirrhosis with portal hypertension and require a transplant. Children who have undergone the Kasai procedure should be listed for transplant when any indication for transplant occurs or if the surgeon deems the operation to have a high probability of failure. Without the Kasai procedure, death is inevitable before age two and children not undergoing the procedure or who have significant portal hypertension precluding Kasai should be listed as soon as the diagnosis is made (UNOS Policy 3 Appendix 3B) [97].

Mild and severe forms of intrahepatic cholestasis may require transplant. Even in mild cases, pruritus may be so severe as to require transplantation. Severe forms of intrahepatic cholestasis, such as Byler's disease and Alagille syndrome can lead to cirrhosis requiring transplant. With Alagille syndrome, cardiomyopathy is a frequent association and may make transplantation impossible. Careful cardiac evaluation is therefore warranted (UNOS Policy 3 Appendix 3B) [97].

### *Pediatric Metabolic Liver Diseases*

Metabolic diseases of childhood which are indications for transplant include urea cycle defects, Crigler–Najjar syndrome, tyrosinemia, Wilson Disease, and cystic fibrosis. The presence of any metabolic defect in the pediatric patient with liver disease needs to be investigated prior to transplant as some metabolic defects have extrahepatic manifestations that may not be reversible with liver transplant. Therefore, liver transplant should be considered only if the defect is localized to the liver or, if it occurs in extrahepatic tissue, does not involve the central nervous system. The defect needs to be corrected by a normally functioning liver. Finally, the extrahepatic manifestations of the metabolic defect should not be so severe so as to preclude liver transplant. Tyrosinemia is associated with a high rate of HCC and this needs to be investigated carefully.

### *Viral and Non-viral Hepatitis in Children*

Owing to the amount of time needed to go from inflammation to cirrhosis, viral hepatitis is a relatively infrequent indication for transplant in children. Nevertheless, children should be listed for transplant if one or more of the general indications for liver transplant are met. Autoimmune hepatitis, often with overlap with sclerosing cholangitis (overlap syndrome or autoimmune cholangiopathy) may require transplantation, especially in adolescence, if immunosuppressive therapy is unsuccessful at controlling the inflammation.

### *Hepatic Malignancy in Children*

Hepatoblastoma (see Section 102.3) is the most frequent primary liver malignancy in children. Unlike HCC, hepatoblastoma is locally invasive with late distant metastases and a better long-term prognosis and children should be immediately listed if the disease is confined to the liver but unresectable or if recurrent tumor is found after resection (UNOS Policy 3 Appendix 3B) [97]. Neoadjuvant chemotherapy is frequently used pre-transplant for patients with extensive disease. HCC is a rare indication for transplant in children except as a complication of metabolic liver disease (e.g., tyrosinemia).

### *Retransplantation in Children*

Hepatic artery thrombosis post-OLT is the most common indication for retransplantation in children and occurs at a higher frequency, particularly with reduced size grafts than in adult OLT. It may present very acutely and require urgent retransplantation [19]. Other indications for retransplantation include acute rejection (rare), primary non-function, chronic rejection, and recurrent disease. Chronic rejection is frequently due to medication noncompliance during adolescence and requires careful psychosocial evaluation to ensure compliance post-retransplantation.

## Evaluation and Listing

Once the decision to evaluate a patient for transplant is made and the patient has been referred to a transplant center for evaluation, the pretransplant workup is begun (Table 103.1). Although the protocol and requirements vary from hospital to hospital, most programs require a basic battery of laboratory work examining the patient's general chemistry and hematologic

**Table 103.1** Pretransplant workup

| | |
|---|---|
| **Triage** | Review records and medical history |
| **Pathology** | Review biopsy if available |
| **Consults** | Transplant coordinator |
| | Transplant hepatologist |
| | Transplant surgeon |
| | Social worker |
| | Psychiatrist |
| **Radiology** | Doppler ultrasound to assess portal vein patency |
| | MRI/MRV/MRCP |
| | EKG |
| | Bone density scan |
| | Chest x-ray (PA and lateral) |
| | Bone scan (if patient with hepatocellular carcinoma) |
| | Chest CT (if patient with hepatocellular carcinoma) |
| **Cardiology** | 2D Bubble echocardiogram |
| | Adenosine stress test (patient > 45 years old or with risk factors for coronary artery disease |
| | Coronary angiography (if positive stress test or high risk for coronary artery disease) |
| **Pulmonary** | Room air arterial blood gas |
| | Pulmonary function testing with DLCO |
| | Arterial blood gas on 100% oxygen (if bubble echocardiogram is positive) |
| **Neurology** | |
| Patients over 60 or with history of coronary artery disease | Carotid Doppler ultrasound |
| Any patient with history of seizures, CVA, or neurological disorders | MRI brain |
| | Neurology consult |
| **Gastrointestinal** | Colonoscopy (if over 50 or with elevated CEA) |
| | Upper endoscopy (screen for varices) |
| | ERCP (if history of PSC) |
| **Gynecology** | PAP |
| | Mammogram |
| **Consults (optional)** | Anesthesia |
| | Dental |
| | Nutrition |
| | Infectious disease |
| | Ophthalmology |
| | Physical rehabilitation |
| | Interventional radiology |
| | Oncology |
| **Laboratory studies** | |
| Chemistries | Electrolytes (N, K, Cl, $CO_2$, Mg, $PO_4$), BUN, creatinine, glucose, albumin, total protein, AST, ALT, total bilirubin, alkaline phosphatase, LDH, GGTP, ferritin, Iron (transferrin saturation), AFP, cholesterol, TSH, uric acid |
| Hematology | CBC with differential and platelet count, PT, PTT |
| Serologies | Hepatitis B (SAg, DNA, CAb, EAg, EAb) |
| | Hepatitis C (anti-HCV, RNA quantitative, genotype) |
| | RPR, varicella titers |
| | EBV (IgG and IgM) |
| | CMV antibody |
| | HIV antibody |
| **Other** | ABO with antibody screen (x2) |
| | PPD with anergy panel |

counts. In addition, laboratory examinations frequently include elements of the liver disease workup that have not been previously performed.

The workup also includes a search for occult infection, which could be reactivated with immunosuppression. Chest x-ray and PPD are performed to ensure that the patient does not have latent tuberculosis. RPR testing for evidence of latent syphilis and HIV testing is performed. Any infections, chronic or otherwise, should be treated or under control to prior to transplant.

A thorough workup includes an evaluation of the patient's general medical condition and fitness for major surgery. EKG, chest radiography, echocardiogram (frequently a saline contrast or "bubble" echocardiogram to exclude intrapulmonary shunting due to hepatopulmonary syndrome), cardiac stress testing, and pulmonary function testing with DLCO and arterial blood gas are common elements of the workup. Close coordination with a referring physicians and specialists is required to best understand the patient's preoperative risk.

Imaging of the abdomen and hepatic parenchyma and vasculature is routinely performed on all pre-transplant patients. Ultrasound can be used to assess the patency of the portal and hepatic vasculature and to determine liver size. CT or MRI are frequently used to exclude HCC or for abnormalities on ultrasound. Additional tests, e.g. right heart catheterization to exclude pulmonary hypertension, should be used selectively based on the results of the aforementioned workup.

As mortality and morbidity following transplant may be related to compliance with medication, a thorough psychiatric evaluation of the patient and his or her capabilities is paramount [102]. Resumption of addictive behavior following transplantation could adversely affect survival and must be treated beforehand. Since the patient's psychosocial status and social support may impact on compliance and outcome, a thorough exclusion or correction of any psychiatric or socioeconomic barriers is crucial and should include evaluation by a psychiatrist and/or social worker.

Forty-three percent of candidates for liver transplantation have at least one psychiatric disorder, so an understanding of the patient's psychiatric state is crucial to assessing suitability for transplant [76]. As a result, dedicated social workers and psychiatrists are integral members of the transplant team and their evaluation is critical in transplant selection and management.

Socioeconomic barriers to compliance can negatively impact graft survival. If immunosuppressive medications cannot be obtained due to insurance, work or cultural issues, failure of the graft is likely. Socioeconomic barriers can make follow-up difficult or lead to recidivism to addictive behavior. A thorough evaluation can detect future challenges that could be avoided. Financial coordinators assess adequacy of transplant insurance coverage. Social workers assist the patients in obtaining additional financial support for transplantation and appropriate follow-up. The goal should be to provide care to all that require it. If these resources are not readily available, then the social work team should attempt to identify adequate resources for patients to undergo transplant successfully.

## Contraindications to Liver Transplant

A basic tenet of dealing with a scarce resource like a donated organ is that one should only transplant those candidates in whom a reasonable chance of graft and patient survival is predicted. There are individuals in whom, for one reason or another, transplantation is contraindicated. Some contraindications are relative. If ameliorated before transplant, then the transplant can proceed. Others are absolute. In these cases, there is no reasonable expectation that either the patient or the graft will survive with an acceptable quality of life for an acceptable period of time post-transplant, or justice prevents allocation to an individual who is unlikely to ever meet the criteria for OLT.

It is important to note that relative contraindications, particularly for living donor liver transplants, vary from center to center. Most contraindications to liver transplant relate to comorbid conditions [95, 96]. Relative contraindications to transplant include alcohol or illicit drug use within 6 months in a patient with a history of alcohol or substance abuse, extrahepatic malignancy in the past (not including non-melanoma skin cancer or those that meet oncologic definition of cure), and systemic sepsis [53].

### *Age*

Advancing age is an important relative contraindication to liver transplantation. Data suggest that in order to have good outcomes, patients above the age of 70

need to undergo liver transplant in a relatively well-compensated state. If these patients are allowed to wait until their MELD score is high, their chances of debility or of developing comorbid medical conditions that would preclude transplant rise significantly [81].

## Psychosocial Contraindications

As discussed, alcohol abuse is a relative contraindication to liver transplantation. Although some patients do maintain good graft function if they continue to drink following transplant, recurrence of disease with resumption of alcohol use remains a concern. Unfortunately, there are few reliable predictors of relapse in alcohol patients making the identification before the transplant of those patients at greatest risk for relapse following transplantation difficult [8, 48].

## Cardiopulmonary Contraindications

The presence of pulmonary disease, particularly pulmonary hypertension, can be an absolute contraindication to liver transplantation as severe pulmonary hypertension or severe hypoxemia from hepatopulmonary syndrome makes the patient an unacceptable operative risk. During the transplant procedure, hemodynamic challenges are manifest, particularly during the time of vena caval clamping and reperfusion of the newly implanted graft following restoration of caval flow. These changes may overwhelm the patient whose right ventricular function is impaired. A mean pulmonary artery pressure of greater than 35 mmHg is considered by many to be a contraindication if it is impossible to lower with medications pre-transplant. Right heart catheterization should follow a suggestion of pulmonary hypertension on echocardiography [18].

The presence of hepatopulmonary syndrome predicts morbidity and mortality in patients with end stage liver disease. Patients with the hepatopulmonary syndrome have nearly twice the 1-year mortality of other transplant recipients. However, early hepatopulmonary syndrome is reversible with OLT and can be viewed as an indication to expedite transplantation. Diagnosis is difficult as there are no strict definitions for the diagnostic criteria [4]. Criteria currently accepted by most centers include the presence of liver disease combined with a $pO_2$ of 70 mmHg or less while breathing room air, and evidence of intrapulmonary vascular dilatation or shunting.

Response to breathing 100% oxygen and calculation of shunt ratios does not adequately predict outcomes for patients with hepatopulmonary syndrome [56]. Because Doppler echocardiography has a poor predictive value in estimating pulmonary artery pressures, right heart catheterization is recommended to assess for the presence of portopulmonary hypertension in patients at risk [4].

## Infectious Contraindications

### HIV Infection

It was previously thought that infection with the human immunodeficiency virus (HIV) was an absolute contraindication to liver transplantation. Though it remains controversial, with many centers still regarding transplantation of HIV infected individuals as experimental, other centers have transplanted HIV positive patients with acceptable results. Thus, HIV is now at most relative contraindication to OLT. Concerns have included the cumulative impact of virologic and pharmacologic immunosuppression, inability to control HIV viremia, and decreased life expectancy after transplantation. In the era of highly active anti-retroviral therapy (HAART), however, these ideas have been reassessed. Transplantation of selected HIV positive patients with suppressed HIV viral load is now being performed in selected patients and is the subject of a NIH-sponsored multi-center study. It has been shown, for example, that HIV patients with hepatitis B virus who receive prophylaxis against recurrence of HBV following transplantation have excellent short and long-term survival rates [104]. Various centers have published results that show 1-year survival rates that are no different for HIV positive and negative patients [31, 73].

Caution needs to be taken during the transplant evaluation to ensure the absence of occult infection or other HIV-associated comorbidities that would contraindicate transplantation. The current absolute contraindications to liver transplant in patients with HIV disease include uncontrollable HIV due to multidrug

resistance, chronic renal failure, leukoencephalopathy, advanced malnutrition, requirement for life support, and the presence of opportunistic infections.

Requirements for life support pre transplant and advanced malnutrition correlate with poor survival following transplantation. Patients should not have had a recent opportunistic infection within 6–12 months, previous Kaposi's sarcoma, and infection with JC polyoma virus. Of note, history of pneumocystic carinii pneumonia is not a contraindication to transplant [31].

Since the cirrhotic patient usually has hypersplenism, their CD4 counts may appear to be low due to their liver disease and not secondary to infection with HIV. Lower CD4 counts can be accepted if based on comparison with the absolute neutrophil count [31].

Another relative contraindication for patients with HIV disease is a noncompliance with HIV medications. Patients who are ineligible for HAART owing to intolerance of the medication because of severe liver dysfunction should be considered for transplant if they have shown prior responsiveness to HAART [31]. Also unclear are the contraindications for patients with HIV disease who are characterized as long-term nonprogressors and have low, stable viral counts in the absence of therapy with HAART.

If they meet certain criteria, therefore, HIV patients should be considered for transplant. Patients with HIV disease should only be transplanted at centers with particular expertise in peri-transplant HIV management and where a transplant pharmacologist is involved who has particular expertise in drug-drug interactions given the complexity of managing immunosuppressive drugs and concomitant HAART. A transplant infectious disease specialist who has worked with issues involving HIV and transplant is also highly recommended [31].

#### Other Infectious Contraindications

Patients with active, uncontrolled infection cannot be transplanted. Infection presents a significant risk to the transplanted patient given the high dose immunosuppression required of patients acutely following transplantation. Most immunosuppressive protocols involve high dose steroid pulses immediately following transplantation. Patients who have spontaneous bacterial peritonitis need to be treated adequately prior to transplantation. Infection with drug resistant bacteria or fungi may require delisting or deactivation until these pathogens are eradicated as the outcome of transplantation can be poor in this setting (see delisting criteria below). Though rigorous data are lacking, recommendations for treatment of resistant bacteria (methicillin resistant *Staphylococcus aureus* and vancomycin resistant enterococci being the most common) or fungi are 4–6 weeks of appropriate antibiotics/antifungals with surveillance cultures and documentation of eradication.

## The Waiting List

### *Management on the Waiting List*

While on the waiting list, it is crucial that the transplant team remain invested in the care for the patient's end-stage liver disease. Deficiencies in attending to the pre-transplant medical needs of patients are common. Screening rates for HCC are poor. Standard preventive procedures for patients with end-stage liver disease, including endoscopic screening for esophageal varices and primary or secondary prophylaxis against variceal bleeding may not be followed [55].

MELD scores need to be updated regularly, every 3 months on average or when there is any change in the patient's clinical condition. Adjusting the patient's MELD score regularly ensures that the patient will be at a place on the waiting list appropriate to their condition.

### *Timing of Transplantation*

Transplantation should be timed so that the patient derives the maximum benefit from receiving a new liver. If the transplant is performed too early, before the patient develops liver failure, for example, then the morbidity and mortality from transplant will outweigh the benefits. On the other hand, if the transplant team waits too long to list a patient for transplant, then the risks of the procedure can overshadow the benefits of the transplant.

It is important to remember that MELD foretells pre, not posttransplant survival. It is a powerful tool for determining who those patients are who would most benefit from transplant. Data suggest that patients with

MELD scores less than 15 do not derive a survival benefit in the first year, particularly those with scores less than 12. For MELD scores above 15, the survival benefit increases with each increase in MELD [60]. In a separate study by Desai and colleagues, MELD score was found to be a relatively poor predictor of post-transplant outcomes in all but patients with the highest 20% of MELD scores [21].

If a patient is moribund due to end-stage liver disease, comatose, in renal failure, and/or on a ventilator, then the risks of dying regardless whether he or she is transplanted or not outweigh the now-diminished benefit of the transplant. Analysis of UNOS data showed that hospitalization in the intensive care unit prior to transplant, retransplantation, female donor to male recipient, age greater than 44 years, and recipient race increased the rate of allograft failure, specifically the need for retransplantation, among patients receiving adult-to-adult living donor liver transplant predicted poor outcome [1].

There is no absolute MELD cut-off for transplant futility. Though the post-transplant risk of dying increases by 50% at high MELD scores (>30) and there is a higher rate of waiting list removal for the reason of "death/too sick," outcomes in a selected group sickest patients are still reasonable, especially given the over 300-fold increase in mortality pre-transplant in high MELD candidates.

Ideally, the optimal timing for transplant is when patients begin to show evidence of decompensation or achieve a MELD score of 15 or greater. Synthetic dysfunction, malnutrition, or experiencing the first complication of cirrhosis indicates the onset of decompensation. It is expected that patients will survive the waiting time required to procure an organ based on their MELD score. Patients with HCC should be referred for transplant evaluation as soon as the tumor is discovered. Children should be referred for transplant evaluation at the point that they fall off their growth curve [14]. Though there is no longer an advantage to early referral in terms of waiting time, early referral allows the team to address pre-transplant problems and optimize management and timing of transplantation.

### *Delisting Criteria*

If the patient's condition has worsened to the point that the procedure's risks would now outweigh benefits or the patient's condition has improved significantly, transplant may no longer be the best option for the patient. Delisting or temporarily deactivating patients from the transplant list involves a calculation that the patient would not derive a survival benefit from the transplant. There have been calls for the development of absolute minimum cutoff survival rates for acceptable predicted post-transplant survival. These rates would likely range from 40% to 60% [67]. In response to calls for the development of an intermediate status such as a temporary deactivation status or a modification of the patient's MELD score with a decrease in points for critical complications, patients can be temporarily suspended on the waiting list as a Status 7 without losing priority on reactivation [67].

Mechanical ventilation, requirement for hemodialysis, fungal or resistant bacterial infections, and a prior transplant all adversely impact likelihood of post-transplant survival. If several of these factors are present, the post-transplant risk becomes prohibitive and transplant should be deferred. Current research is directed at developing evidence-based recommendations for delisting.

Patients should be removed from the transplant list if their condition deteriorates to the extent that it is unlikely that they would survive the procedure. All previously discussed absolute contraindications to listing are reasons for delisting if they transpire after listing while a patient is awaiting transplant. This includes resumption of alcohol use while awaiting transplantation [65].

## Living Donor Liver Transplantation

First performed by Yamaoka et al. in Japan in 1994, centers in the United States have performed over 1,700 living donor liver transplants (LDLT) since 1989 in children and 1998 in adults (Source: UNOS). Because living donation permits the timing of transplantation independent of waiting time and severity of liver disease, criteria for transplantation in these patients are somewhat different than for patients waiting for deceased donor organs.

Timing of LDLT is a great deal more flexible. The transplant team can select a transplant time that is individualized for the particular patient. The living donor organ has significantly less cold ischemia time than the deceased donor organ as a consequence of moving immediately from the donor to the recipient. Owing to

rigorous screening of the living donor, the organ should be coming from a healthy individual. For these reasons, living donor livers hypothetically of better quality than deceased donor livers. On the flip side, the living donor allograft has significantly less hepatic mass than a full-sized deceased donor organ.

To date outcomes of living donation and deceased donor transplantation have been similar. There is a potential benefit from LDLT to the patients awaiting deceased donor liver transplant as well. Every transplant with a liver obtained from a living donor potentially frees up a deceased donor organ for transplant into another recipient as living donor recipients are not part of the deceased donor recipient pool [96]. Thus living donation potentially benefits everybody on the transplant waiting list.

Patients receiving a living donor organ have a reduced waiting period. This decreases the risks of decompensation or death prior to transplantation thus improving the chances for success. As a result, patients receiving living donor grafts have improved survival on the waiting list and overall survival from the time of listing [9]. Mortality on the waiting list for living donor recipients is half that of those listed for DDLT only [49, 80]. Since the transplant is performed on an elective basis, the operation can proceed immediately after the workup is performed and the donor is deemed a good candidate. Secondary to the flexibility of the waiting period prior to transplant in living donor recipients, the team has time to stabilize the patient's comorbid conditions, further improving the chances for success [95, 96].

LDLT grafts have tremendous growth potential; the graft doubles in size within 4 weeks of transplantation, generating over 150,000 hepatocytes every second for the first week following transplant [6, 57]. Questions have risen as to whether there are any negative implications of the accelerated growth rate found in the liver segment transplanted in living donor liver transplants. It has been postulated, for example, that this growth potential may predispose to more aggressive recurrence of HCV in patients transplanted for HCV cirrhosis. Several recent large studies have shown that the incidence and severity of HCV recurrence were not different between DDLT and LDLT recipients [79].

Patients with decompensated cirrhosis with Child-Pugh scores greater than or equal to 10 are the most appropriate patients for LDLT. Acute hepatic failure is also an acceptable indication but concerns regarding coercion and adequacy of the donor workup given the time constraints on donor workup have made this indication somewhat controversial. Recipients should meet standard indications for OLT and not have any contraindications. Using the current donor and recipient criteria, on a national level only 5% of patients listed for deceased donor liver transplants currently undergo living donor transplants mainly related to lack of appropriate donors [95].

As with deceased donor transplantation, the ideal candidate for living donor liver transplant is one who is sick enough to derive a benefit from transplantation but not so sick as to incur potentially high post-transplant mortality risk. A simple rule of thumb is that an appropriate LDLT candidate is a patient you would transplant today if organs were unlimited. The MELD score can help identify candidates for living donor liver transplantation who are not likely to benefit because they are either too sick or too well to undergo transplant [29].

In a study by Trotter et al., between 1997 and 1999, 51% of 100 potential LDLT recipients evaluated at one center were rejected [96]. The most frequent reasons for donor rejection included medical comorbidity, high-risk psychosocial issues, obesity, financial issues, and procurement of a deceased donor organ during the evaluation. Overall in experienced LDLT programs, it appears about one-third of adult patients on the waiting list may have a potential donor and half of these will undergo the procedure, indicating that LDLT may be applicable in up to 15% of patients on the waiting list [96].

## The Posttransplant Period

### *The Perioperative Period*

The transplant recipient is usually very ill at the beginning of the procedure. He/she may be profoundly coagulopathic, anemic, encephalopathic, and vasodilated owing to the hyperdynamic circulatory status of the cirrhotic patient. These factors, in addition to the technical challenges of the procedure itself, make perioperative setting very challenging.

Patients usually remain intubated immediately following the procedure. Most often, they are brought directly to a surgical intensive care unit where recovery

from anesthesia is supervised by a team of anesthesiologists, surgical intensivists, transplant surgeons, and transplant hepatologists. Hypovolemia, owing to both intraoperative bleeding and fluid losses, is common and oftentimes requires assessment with a pulmonary artery catheter.

Particular attention needs to be paid to the pulmonary artery pressures. Owing to vena caval and aortic manipulation during the operation, pulmonary pressures may vary widely during the surgery. Patients are oftentimes loaded with fluid in the intravascular space. Poor renal function before the transplant may preclude the patient from properly mobilizing this fluid. The team managing the patient postoperatively must be attentive to pulmonary arterial pressures, wedge pressures, and cardiac output to properly manage the patient's fluid status.

Patients should be extubated as soon as feasible. Lines should be discontinued as soon as possible as they remain niduses of infection in immunocompromised host. Patients should have their diets advanced as soon as bowel function returns.

At our center, patients are transferred to the transplant ward once they are extubated and hemodynamically stable. Once on the floor, the focus of care shifts to more routine postoperative management. Patients' diets are advanced. They are encouraged to ambulate. Wounds are given the proper care.

In addition, immunosuppressive regimens must be properly established and patients should be educated about their medical regimens prior to being discharged from the hospital. A team often comprised of a pharmacist, nurse or nurse practitioner, and physician assistant provides medication teaching. They are taught about the side effects and doses. We use a book with pictures of individual pills and a brief quiz to assess their knowledge. Successful completion of the teaching is a prerequisite for discharge home.

## *Immunosuppression*

Together with advances in surgical technique, advances in immunosuppression have permitted rapid advances in liver transplantation over the past two decades. Modern immunosuppression ideally precludes the patient from recognizing the allograft as nonself while at the same time minimally affecting the body's ability to respond to other nonself antigens, primary infective pathogens. Immunosuppression can broadly be divided into four categories; steroids, calcineurin inhibitors, cell cycle inhibitors, and monoclonal antibodies.

Discovered by Jean-François Borel in 1973 in the soil fungus Tolypocladium inflatum, the FDA approved *cyclosporine* in 1983, which revolutionized transplantation. By inhibiting T-cell activation, cyclosporine selectively suppresses cell-mediated immunity. Cyclosporine binds to cytoplasmic receptor protein known as cyclophilin. It then inhibits the calcium and calmodulin-dependent phosphatase calcineurin. Calcineurin is integrally involved in the transcription process that activates IL-2 and other cytokines. Produced by T-helper cells, these cytokines play key role in the cellular recruitment that alters the cellular milieu of the graft and precipitates acute cellular rejection.

As with all calcineurin inhibitors, cyclosporine has numerous side effects including nephrotoxicity and neurotoxicity. Between 10% and 27% of patients may experience neurotoxicity from cyclosporine. Effects may range from mild tremors to seizures and frank neuropsychiatric disturbances.

The effects of calcineurin inhibitors on the development of hyperlipidemia, hypertension, and diabetes mellitus will be discussed later on this chapter. The pharmacokinetics of calcineurin inhibitors is affected by other medications that induce or inhibit cytochrome P450 activity, some of which may be commonly used in the transplant setting. Azole antifungals, for example, will raise the levels of calcineurin inhibitors.

Earlier formulations of cyclosporine required emulsion in a fatty-substance such as whole milk. Owing to both the difficulty of taking this liquid form and to the unreliable absorption associated with this, a newer microemulsion form of cyclosporine was developed. This form of cyclosporine is the predominant form in use today.

The newer calcineurin inhibitor, *tacrolimus* (also commonly referred to by its clinical trial number FK506), is a 100-fold more potent than cyclosporine. Similar to cyclosporine it inhibits transcription of cytokines and chemotactic factors critical to the immune response. However, tacrolimus binds to different cellular receptor, FK binding protein (FKBP12), to exert its effects. The complex then enters the cytoplasm to inhibit calcineurin and the production of IL-2, 3, 4, 8 and other chemotactic factors.

Since it is more potent than cyclosporine, therapeutic tacrolimus levels are roughly one tenth those of cyclosporine. It has a similar side effect profile and

pharmacokinetics to cyclosporine. Tacrolimus has supplanted cyclosporine as the predominant first-line immunosuppressive agent in most programs in the US, though there is debate as to which has superior outcomes in patients with hepatitis C and HCC.

*Corticosteroids* have broad effects on the immune system and were the original immunosuppressants used in transplantation. They block T cell and antigen presenting cell- induced cytokine expression. They predominantly block production of IL 1, 2, 3, and 6. Unlike calcineurin inhibitors, which are used primarily as maintenance immunosuppressants, steroids can be used as both maintenance agents and for the treatment of acute cellular rejection.

Steroids are typically given in high doses (up to 1 g) intraoperatively and then tapered. They are used as induction therapy perioperatively and are eventually tapered in most patients. Similar doses are used in the treatment of acute cellular rejection. Because high dose steroids accelerates the progression of recurrent hepatitis C post transplant, their use for the rejection in patients transplanted for hepatitis C virus is approached with caution and avoided whenever possible in some programs.

*Azathioprine*, an imidazole derivative of mercaptopurine antagonizes purine metabolism. This inhibits the synthesis of the DNA, RNA, and proteins central to the maturation of immune cells. A corollary to this property is the fact that a central side effect of their use is myelosuppression. In rare cases, azathioprine can cause significant hepatotoxicity.

*Mycophenolate mofetil* (MMF) is a more potent alternative cell cycle inhibitor. MMF is metabolized to mycophenolic acid, which inhibits de novo purine nucleotide synthesis via disruption of production of inosine monophosphate dehydrogenase and guanosine nucleotides. These actions prevent the DNA replication required in both B and T lymphocytes which are unable to use alternative salvage pathways to produce purines. The most common side effect of MMF is GI upset and diarrhea, which may improve in some cases with decreased dose or change to an enterically coated formulation of mycophenolic acid. A proton pump inhibitor is frequently used to prevent GI ulceration from MMF.

Cellular immune machinery can also be targeted directly through the use of *monoclonal* and *polyclonal antibodies*. The polyclonal antibodies include antithymocyte globulin (Thymoglobulin, ATG) which is directed against multiple T-cell epitopes. These include CD 2, 3, 4, 8, 16, 28, and the T cell receptor. They exert their effects on natural killer cells and macrophages. The oldest polyclonal antibody, ATG's use has been augmented by the use of more specific monoclonal antibodies which may not lead to as many wide-reaching and long-lasting effects on the immune system.

The monoclonal antibodies include the anti-T cell antibody OKT3 (muronomab), and anti IL-2 receptor antibodies daclizumab (Zenapax), and basiliximab (Simulect). OKT3 binds to the CD3 receptor thus inactivating the T cell receptor. Basiliximab and daclizumab bind to and block binding of IL-2 to the IL-2 receptors to inhibit antagonize T cell proliferation. Antibody preparations are used either to treat steroid-refractory rejection or to spare the use of steroids or calcineurin inhibitors. Induction with delayed calcineurin inhibitors are frequently used in patients with renal failure or coma peri-transplant. OKT3 and thymoglobulin are used primarily to treat acute cellular rejection, but are also used for induction. Daclizumab and basiliximab are usually used for induction in steroid-free or calcineurin-sparing protocols.

*Rapamycin is* similar to tacrolimus in that it binds to FKBP12 and the response of T and B cell activation by cytokines. It also prevents cell-cycle progression and proliferation. Interestingly, rapamycin has a second binding site known as the TOR receptor (target of rapamycin). By blocking this receptor, rapamycin blocks progression of the cell cycle. Owing to the association of its use with hepatic artery thrombosis in two clinical studies, the US FDA has issued a black box warning against its use in liver transplant recipients. Other unique side effects of rapamycin include hyperlipidemia and impaired wound healing.

Immunosuppression regimens can broadly be categorized as induction, maintenance, or anti-rejection regimens. Perioperatively, recipients usually receive bolus doses of steroids and begin calcineurin inhibitors once they are able to take oral medications. Oftentimes, *monoclonal antibodies* such as basiliximab or daclizumab are used in lieu of calcineurin inhibitors if the patient has shown signs of renal insufficiency prior to transplant in order to offset the nephrotoxic effects of these drugs. Since monoclonal antibodies may exert their effects for weeks, calcineurin inhibition can be delayed. Cell cycle inhibitors are also given during induction.

By the time the patient is ready for discharge from an uncomplicated liver transplant (usually on the order of 1–2 weeks postoperatively), the patient is usually discharged on a maintenance regimen of a calcineurin inhibitor kept at relatively higher therapeutic levels, a steroid taper, with or without a cell cycle inhibitor. One

**Table 103.2** Effects of important drugs on cyclosporine and tacrolimus levels

| Increase | Decrease |
|---|---|
| Azole antifungals | Rifampin |
| Macrolides | Carbamazepine |
| Calcium channel blockers | Phenytoin |
| Amiodarone | Fosphenytoin |
| Protease inhibitors | Phenobarbital |
| | Rifabutin |
| | St. John's Wort |

must remain cognizant of the numerous interactions other medications may have with calcineurin inhibitors which may raise or lower calcineurin levels (Table 103.2).

The treatment of acute cellular rejection is dependent on the patient's reason for transplant. Usually a biopsy is undertaken if rejection is suspected to evaluate for other sources of abnormal liver chemistries such as recurrent viral hepatitis, ductular obstruction, or new viral infection from pathogens such as cytomegalovirus. Once rejection is established, the first line therapy is usually a steroid bolus and maximization of existing immunosuppression. If this regimen fails and a second biopsy still shows rejection, then one often moves on to the use of monoclonal antibody preparation such as Thymoglobulin or OKT3.

## *Rejection*

Graft loss due to acute cellular rejection is a rare occurrence due to the ability of aggressive treatment to treat rejection. While aggressive maintenance immunosuppressive regimens clearly have prevented acute cellular rejection and are responsible for long-term graft survival, their adverse effects can be difficult to manage.

Acute cellular rejection biopsies are given a score ("Banff" score) indicating a combination of venous and bile duct damage and portal inflammation. This score determines the severity of the rejection episode (Table 29.10) [38].

## *New Onset Diabetes Mellitus Following Transplant*

New onset diabetes mellitus is common following transplant. It has been reported to occur with an incidence of 13.4% [34]. This number is significantly higher in patients receiving tacrolimus which has been reported to have an incidence of new onset diabetes mellitus associated with it use from 10.4% to 22.7% and is higher in HCV positive recipients than in patients without HCV [34, 84].

Cyclosporine therapy may increase insulin secretion in patients with a high pretransplant risk of new onset diabetes mellitus. Impaired pretransplant glucose tolerance and a body mass index greater than 25 were important risk factors for new onset diabetes mellitus [85]. Recipient age greater than 45 and high rates of steatosis in the allograft have also been correlated with the incidence of post transplant new onset diabetes mellitus [62].

The use of methylprednisolone boluses is clearly associated with impaired glucose tolerance and diabetes. One report estimated that each steroid bolus increases the risk for the development of diabetes mellitus by 9% [5]. Conversion to cyclosporine from tacrolimus improves glycemic control and reduces insulin requirement [23]. Among oral hypoglycemic agents, pioglitazone, rosiglitazone, and sulfonylureas have all been tried with success [54, 85].

Most patients are tapered to lower doses of steroids fairly quickly following transplant. Those patients who do develop diabetes mellitus are at increased risk for poor posttransplant outcomes. It is associated with a threefold increase in 10-year mortality, much of which is due to its association with infections [5]. Recipients with diabetes mellitus have increased rates of cardiovascular events, infections, and ophthalmologic complications [40]. A review of the United Network for Organ Sharing database revealed impaired 5-year patient and graft survival compared with controls. Type I diabetic patients had the most impaired survival post transplant [103].

Patients with the metabolic syndrome have evidence of increased insulin resistance, obesity, hypertension, and hyperlipidemia. More than half of patients post liver transplant have been reported to develop the metabolic syndrome. These patients have a more than threefold increased risk for vascular events and are 50% more likely to develop frank diabetes mellitus [47].

## *Arterial Hypertension*

Occurring in more than half of patients following liver transplant, arterial hypertension is the most common

cardiovascular complication following liver transplant [13, 32, 86]. Elevations in plasma endothelin-1 and increased arterial stiffness from various causes are thought to be important in the pathogenesis of posttransplant hypertension [64]. Hypertension is usually treated with calcium channel and beta- blockers posttransplant owing to concerns about enhancement of calcineurin-induced nephrotoxicity with ACE inhibition.

## Hyperlipidemia

Hyperlipidemia is also common following liver transplant. As many as 45% of patients will develop elevated serum lipid levels post-OLT [45, 69, 94]. Cyclosporine seems to preferentially dispose to hyperlipidemia and conversion to tacrolimus from cyclosporine has been shown to reduce hyperlipidemia [77].

## Biliary Complications

Biliary complications are identified in up to one quarter of patients, 45% of whom had strictures and 18% of whom had bile leaks in one series. Endoscopic therapy via endoscopic retrograde cholangiopancreatography (ERCP) was successful in most patients with strictures and virtually all patients with bile leaks [75].

Patients with anastomoses of the bile duct directly to a roux-limb of small bowel usually require percutaneous transhepatic cholangiography due to the difficulty of inserting an endoscope so deep into the bowel to find the anastomotic site for endoscopic retrograde cholangiography. However, a recent series showed that ERCP can be performed in selected patients with a roux limb by experienced endoscopists, though it depends on the length of the roux limb [15].

Bile leaks are easily identified on ERCP as 87% of patients have their leak site identified via this modality. Furthermore, these leaks can be treated in over 80% of patients. Bile duct obstruction relief via ERCP is slightly less successful with only 71% of patients achieved relief [71].

Bile duct cast syndrome is an uncommon entity whereby the small ducts of the transplanted liver fill with secretions and residue. This admixture hardens and forms cast-like structures, which can lead to obstruction. ERCP is only successful in 25% of patients in treatment of biliary casts [71]. Many patients will require retransplant.

MRI has emerged as an important modality in diagnosing biliary obstruction and leaks following transplant. Using ERCP as a reference standard, one study showed that MRCP had a sensitivity and negative predictive value of 100% and a positive predictive value of 92.9% in detecting strictures [43].

## Vascular Complications

The most common vascular complication is hepatic artery thrombosis, which can be catastrophic in the early post-transplant period. Bile ducts are exquisitely sensitive to ischemia and derive all their blood supply from the arterial circulation immediately post-OLT. Hepatic artery thrombosis may lead to severe diffuse ischemia that can result in acute liver failure or biliary complications including leaks, biloma collections, subsequent infections, sepsis, or diffuse structuring and eventual graft failure.

Hepatic artery thrombosis is usually diagnosed on Doppler ultrasound, which is frequently performed during the first few days following transplant. When identified, hepatic artery thrombosis can be repaired surgically or via vascular interventional radiology procedures with good results. It is key to identify this complication early.

It occurs in 3–9% of transplants and acute graft loss is possible. One 10-year series showed that it occurred in 4.9% of patients. It was associated with Roux-en-y anastomosis, prolonged cold ischemia, operative time, use of blood products, retransplant, reoperation, and the use of aortic conduits [88].

## Recurrent Disease

The Achilles heel, so to speak, of the liver transplant enterprise has been recurrent disease, particularly recurrent hepatitis. Thanks to more aggressive prophylactic intervention and effective oral therapy, hepatitis B virus recurrence is prevented in the majority of patients. Recurrence of hepatitis C, however, continues to be a problem for the transplant hepatologist and is the leading cause of long-term graft loss.

Recurrence of hepatitis C virus vexes the transplant physician. While recurrence of viremia is near universal, recurrence of clinically significant viral hepatitis is fortunately an occurrence in a minority of patients. Yet it is well established that the use of steroid boluses is associated with recurrence. For this reason, the immunosuppressive protocols of many institutions usually reserve steroid boluses as a second-line regimen after all other therapeutic changes such as maximizing current calcineurin levels or conversion to the other calcineurin (e.g. changing cyclosporine to tacrolimus) have been exhausted.

Patients who develop recurrent HCV are particularly challenging to manage. Thus far, no effect prophylactic regimen is available to prevent recurrence of HCV. For those who do progress to clinically significant chronic hepatitis, treatment can be extremely difficult. Retransplantation is an option but patients retransplanted for recurrent HCV have high mortality postoperatively [2]. Some transplant programs will not retransplant patients for recurrence of HCV. A number of case series have shown some encouraging results with treatment of patients with pegylated interferon and ribavirin regimens following transplant (see Section 63.3 "Hepatitis C"). Concerns about precipitation of acute cellular rejection, patient tolerance, and effectiveness in bringing about sustained virologic responses have hampered their widespread use.

## *Infection*

Posttransplantation infections can generally be broken down into three different periods, the immediate postoperative period (from hospital discharge through the first month), from the first through 6 months, and from 6 months onward (Fig. 103.2). Infections perioperatively generally are aggressive forms of the nosocomial and surgical infections common in patients who have had major procedures or who have been hospitalized for long periods of time. Infections over the next 5 months are generally opportunistic infections such as EBV, Cryptococcus, aspergillus, and CMV. Infections after 6 months are mostly community acquired or persistent infections such as urinary tract infections, human herpesvirus, influenza, and community acquired pneumonias [78].

Bacterial infection generally occurs in the immediate posttransplant period and is associated with nosocomial

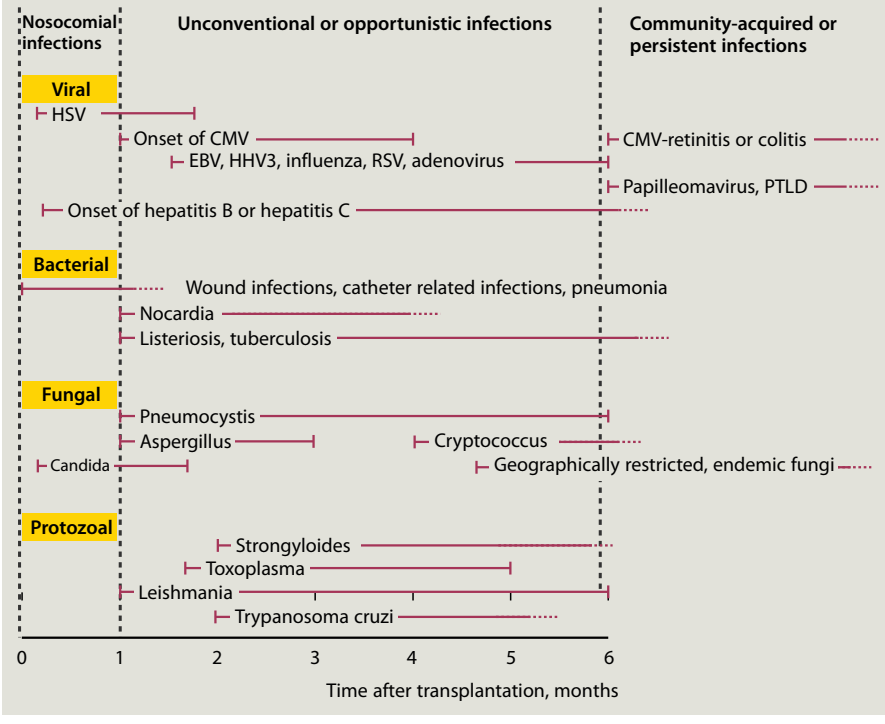


**Fig. 103.2** Posttransplantation infections according to time after transplantation (Source [81]; reproduced with permission)

infections. A recent series showed that 62.7% of infections in the period from 1998 to 2003 were bacteremia. This represented a rapid increase from prior intervals. Interestingly, the proportion of bacteremias that were due to infections with gram positive organisms fell from 75% to 48.3% and the proportion due to infection with gram negative organisms rose from 25% to 51.8%. The most common pathogens in bacteremic patients were methicillin-resistant *Staphylococcus aureus*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa* [11, 89].

Cytomegalovirus (CMV) infection is a common cause of late posttransplant complications with a peak incidence at 6–7 weeks post-OLT. CMV is unique in that it can precipitate episodes of acute cellular rejection. It is thought to directly affect antigen and cytokine expression and its effects are protean including a flulike illness, vasculitis-like syndromes including pneumonitis, nephritis, colitis, and hepatitis, and predisposition to other opportunistic infections as well as rejection and graft dysfunction [28].

Fortunately, CMV does not affect overall patient survival. It occurred in one series in 65.8% of patients [36]. Interestingly, a decline in CMV infection was noted between 1998 and 2003 [89]. Since CMV usually is due to reactivation from the donor graft or the recipient, those at highest risk for CMV infection posttransplant are those patients who were CMV negative prior to transplant and received a CMV positive graft ("D + R-"). Those CMV negative recipients receiving CMV negative grafts ("D-R-") are at lowest risk. Decisions about whether to perform surveillance for CMV with PCR and use preemptive treatment or to use selected or universal prophylaxis vary widely and are often based on the donor and recipient CMV status.

As with other viral infections, risk factors for CMV infection include aggressive immunosuppression. In one study, patients on cyclosporine-based regimens and on three-drug (calcineurin, steroids, mycophenolate mofetil) were at increased risk for CMV [35]. Other risk factors for CMV infection within 3 months posttransplant include recipient CMV status, donor age, and recipient lymphocyte count [7].

Epstein–Barr virus is significant in that it is associated with an uncommon entity found in the posttransplant population known as posttransplant lymphoproliferative disorder (PTLD). This is a polyclonal or monoclonal lymphoid proliferation of rapid onset. It is more common in children who have received extensive rejection treatment, and is usually extranodal. One series found its incidence in 22 of 621 transplant recipients [46]. The treatment is to minimize immunosuppression and use antiviral therapy to allow the immune system to clear EBV.

Fungal infections can be catastrophic in liver transplant patients [70]. Fungal peritonitis is extremely difficult to sterilize and fungal pneumonia is associated with very poor outcomes. Systemic fungal infection, in particular aspergillosis is also difficult to clear.

For these reasons, the question of antifungal prophylaxis had been considered. Some patients can be identified as low-risk for invasive fungal infections and routine posttransplantation antifungal prophylaxis is unnecessary.

Candidiasis is a frequent cause of posttransplant fungal infection. Use of antibiotic prophylaxis against spontaneous bacterial peritonitis prior to transplant, posttransplant hemodialysis, and retransplantation all are predictive of invasive candidiasis. Candida albicans is the most frequent species followed by candida glabrata. Invasive candidiasis carries with it a mortality of greater than 30%. Non-albicans candida species and prior antifungal prophylaxis are associated with poorer outcomes [37].

Invasive aspergillus infections are usually fatal. Once aspergillus is established in the body enough to manifest itself clinically, it is usually impossible to eradicate. The aspergillus galactomannan test oftentimes is effective in detecting aspergillus infection prior to any clinical manifestations. One series of living donor liver transplant recipients identified risk factors for invasive aspergillosis including preoperative intensive care unit stays and preoperative steroid use [68].

## Conclusion

With adequate post-transplant care, 1-year survival post-OLT can approach 90%. Impressive gains in surgical technique, immunosuppression, management of complications, and aggressive pretransplant care are first and foremost responsible for good outcomes following a liver transplant. Appreciation of the full spectrum of patient care includes attention to the patient's psychosocial needs and general health. Rigorous screening of patients pretransplant allows for the selection of those patients who will benefit most from a liver transplant. Finally, new mathematical models for patient survival

pretransplant are altering organ allocation systems worldwide and allow for organs to be transplanted into those patients who will derive the greatest survival benefit from receiving a liver transplant.

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# Risk of Surgery in Patients with Liver Disease

# 104

Patrick S. Yachimski and Lawrence S. Friedman

## Chapter Outline



Patients with liver disease may face increased risk when undergoing surgical procedures. Appropriate care of these patients includes preoperative assessment and identification of those at increased risk, optimization of medical therapy for liver disease before surgery, and postoperative management of manifestations of decompensated liver disease.

The magnitude of risk for an individual patient is a function of both the severity of the underlying liver disease and the nature of the surgical procedure. In addition, perioperative factors, including hemodynamic perturbations during surgery and use of systemic anesthetic or sedative medications, can have pronounced physiologic effects in patients with compromised hepatic function.

This chapter outlines an approach to the assessment and management of patients with liver disease preparing for surgery and reviews methods of risk stratification. Liver transplantation is not a subject of this chapter and is addressed elsewhere in the textbook (see Chapter 103).

## Effects of Surgery on the Liver

### *Liver Biochemical Tests*

Minor elevations in serum aminotransferase, bilirubin, and/or alkaline phosphatase levels occur commonly following surgical procedures [25, 30]. For instance, in a series of patients undergoing laparoscopic cholecystectomy and in whom preoperative liver biochemical test results were normal, elevation of aminotransferase levels to approximately two times normal was detected in more than 70% of patients postoperatively [35].

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Such elevations are typically transient, are not indicative of hepatic dysfunction, and therefore are of negligible significance. Higher aminotransferase elevations (aspartate aminotransferase [AST] and alanine aminotransferase [ALT] levels ≥3 times normal) in the postoperative setting are suggestive of hepatic injury, in many cases resulting from ischemia.

In patients with known underlying liver disease, postoperative liver biochemical test elevations may be clinically significant and merit careful attention. For example, marked hyperbilirubinemia may be indicative of hepatic decompensation and should prompt evaluation for encephalopathy, coagulopathy, and other manifestations of decompensated disease.

## Hepatic Blood Flow and Hemodynamic Effects

The liver has a dual afferent blood supply consisting of portal venous and hepatic arterial blood flow. Perfusion from either of these sources may be compromised in patients with cirrhosis and portal hypertension, because of portal venous hypertension and impaired arterial autoregulation. Moreover, patients with cirrhosis may have alterations in the systemic circulation, due to arteriovenous shunting, and reduced splanchnic inflow. As a consequence of these circulatory derangements, the liver is particularly susceptible to ischemia in patients with cirrhosis and portal hypertension (see Chapter 62). Ischemic insults may be the result of frank episodes of hypoperfusion, such as systemic hypotension in the setting of hemorrhage or sepsis. Other insults may be more subtle. For example, positive pressure mechanical ventilation and surgical techniques such as pneumoperitoneum during laparoscopic surgery [72] or traction on abdominal viscera may cause alterations in hepatic perfusion.

In addition, administration of most volatile anesthetic agents results in decreased hepatic blood flow [29]. This is particularly the case with halothane and enflurane, which exert a negative inotropic effect and reduce systemic vascular resistance [7, 63]. Animal data suggest that isoflurane causes relatively less perturbation in hepatic arterial blood flow [29], and this agent is often preferred for patients with liver disease. Desflurane and sevoflurane are similarly thought to have little effect on hepatic arterial blood flow.

## Hypoxemia

Pulse oximetry may demonstrate low oxygen saturation in approximately 20% of patients awaiting liver transplantation [58]. The overall prevalence of hypoxemia in patients with liver disease cannot be estimated precisely, however, because pulse oximetry may overestimate arterial oxygen tension in patients with cirrhosis [1]. Nonetheless, among the population of patients with liver disease awaiting general surgical procedures, the potential for significant intraoperative hypoxemia may be anticipated in those with specific manifestations of decompensated disease (Table 104.1). For instance, pulmonary mechanics may be compromised by the presence of either ascites or hepatic hydrothorax, which may impede mechanical ventilation. Ascites, encephalopathy, and the use of sedative anesthetics all may contribute to an increased risk of perioperative aspiration.

Hepatopulmonary syndrome is characterized by liver disease and an increased alveolar-arterial gradient in the presence of an intrapulmonary shunt caused by dilated ateriovenous communications (see Chapter 80). Clinical clues to hepatopulmonary syndrome include platypnea (increased dyspnea in the upright posture) and orthodeoxia (oxygen desaturation in the upright posture). A substantial rate of early postoperative mortality due to cardiopulmonary complications has been reported in patients with hepatopulmonary syndrome undergoing liver transplantation [5]. Elective surgery should be avoided in patients with hepatopulmonary syndrome.

Portopulmonary hypertension describes pulmonary hypertension in the presence of advanced liver disease (see Chapter 80) and may be suspected on the basis of echocardiographic findings, although right heart catheterization may be required for precise estimates of pulmonary arterial pressures [17, 45]. Severe pulmonary hypertension refractory to medical therapy is associated with a high operative mortality rate in patients

**Table 104.1** Causes of hypoxemia in patients with liver disease

| |
|---|
| Hepatopulmonary syndrome |
| Portopulmonary hypertension |
| Moderate or severe ascites |
| Hepatic hydrothorax |
| Aspiration |
| Comorbid illness (e.g., chronic obstructive pulmonary disease) |

undergoing liver transplantation [47] and is therefore a contraindication to transplant listing. Although published data regarding surgery in patients with portopulmonary hypertension are limited, pulmonary hypertension of any etiology has been shown to correlate with postoperative mortality among patients undergoing noncardiac surgical procedures [49], and patients with portopulmonary hypertension should be considered to be at high (if not prohibitive) surgical risk.

### *Hepatic Metabolism of Anesthetic Agents*

Genetic polymorphisms of cytochrome P450 2E1 have been implicated in occasional episodes of fulminant hepatitis following halothane exposure [44], and halothane is now used rarely in adults (see Chapter 93). Beyond this notable exception, however, there are few data to suggest that either the choice of anesthetic agent or mode of administration (inhaled or spinal) influences outcome in patients with liver disease [64, 91].

One study of patients undergoing coronary artery bypass surgery reported a higher frequency of postoperative serum aminotransferase elevations among patients receiving propofol compared with sevoflurane anesthesia, but the difference was likely of no clinical or physiologic significance [53]. In fact, although propofol is metabolized by hepatic glucuronidation, its serum half-life remains relatively short even in patients with cirrhosis and it is an effective and appropriate anesthetic agent in patients with liver disease [77].

Alterations in the action of neuromuscular blocking agents used in induction of anesthesia may be more pronounced among patients with liver disease. Particularly for agents with an expanded volume of distribution, such as vecuronium and rocuronium, the onset of and recovery from anesthesia may be prolonged [43,50]. Atracurium and cisatracurium are considered the preferred muscle relaxants in patients with liver disease because neither the liver nor the kidney is required for their elimination. Further, the duration of neuromuscular blockade produced by atracurium is not affected by decreased levels of plasma cholinesterase, which is synthesized by the liver. Doxacurium, which is eliminated primarily by the kidney, is preferred for prolonged procedures such as liver transplantation.

## Operative Risk in Patients with Liver Disease

### *Challenges in Estimating Operative Risk*

In a retrospective study of 733 cirrhotic patients undergoing surgery at the Mayo Clinic between 1980 and 1991, the 30-day operative mortality rate was 11.6%, and the overall complication rate was 30% [92]. These figures encompass patients undergoing a broad range of surgical procedures, from excision of skin lesions to laparotomy, surgery on the thoracic cavity, or intracranial surgery. In addition to the severity of liver disease (see later), morbidity and mortality in this cohort was associated with indicators of comorbid illness, including chronic obstructive pulmonary disease, perioperative infection, and American Society of Anesthesiologists (ASA) class (Table 104.2) [92]. Such variables may be predictive of risk in patients without liver disease and may be applicable in any global assessment of fitness for surgery.

The particular challenge in assessing operative risk among patients with liver disease is to identify patients at risk and characterize the risk with use of a prediction algorithm. This process begins with recognition of the presence of liver disease, followed by grading of the severity of liver disease with attention to specific manifestations of decompensated disease. Both the Child-Turcotte-Pugh (CTP) score (Child class) and Model-for-Endstage-Liver-Disease (MELD) score have been applied as a means of discriminating low-risk from high-risk patients (see Chapters 79 and 103).

The majority of published studies describing operative risk in patients with liver disease derive from

**Table 104.2** American Society of Anesthesiologists (ASA) classification

| Class | |
|---|---|
| I | Healthy patient |
| II | Patient with mild systemic disease without functional limitation |
| III | Patient with severe systemic disease with functional limitation |
| IV | Patient with severe systemic disease that is a constant threat to life |
| V | Moribund patient not expected to survive >24 h with or without surgery |
| E | Emergent nature of surgery (added to classification I–V as above) |

**Table 104.3** Operative mortality in patients with cirrhosis

| Author, year (Reference) | N | Type of surgery | Overall mortality rate[a] | Mortality rate stratified by Child class or MELD score |
|---|---|---|---|---|
| Garrison 1984 [27] | 100 | Biliary<br>Peptic ulcer<br>Other abdominal | 30% | Child A: 10%<br>Child B: 31%<br>Child C: 76% |
| Mansour 1997 [56] | 92 | Cholecystectomy<br>Hernia repair<br>Other abdominal | 26% | Child A: 10%<br>Child B: 30%<br>Child C: 82% |
| Suman 2004 [81] | 44 | Elective cardiac | 16% | Child A: 3%<br>Child B: 41%<br>Child C: 100% |
| Perkins 2004 [70] | 33 | Cholecystectomy | 6%[b] | MELD <8: 0%<br>MELD ≥8: 6% |
| Farnsworth 2004 [22] | 40 | Abdominal<br>Genitourinary<br>Head/neck | 17.5% | Child A: 15%<br>Child B: 9%<br>Child C: 60%<br>MELD ≤8: 8%<br>MELD 9–16: 10%<br>MELD ≥17: 57% |
| Hayashida 2004 [40] | 18 | Cardiac | 17% | Child A: 0%[c]<br>Child B: 50%<br>Child C: 100% |

[a]30-day mortality rate, unless otherwise specified
[b]90-day mortality rate
[c]Cardiopulmonary bypass patients only. *Child* Child class; *MELD* Model for Endstage Liver Disease

single-center, retrospective cohorts. These data are susceptible to limitations, including small cohort size, the potential for selection bias, and lack of external validation. Subjects in one cohort may differ in important respects from subjects in another cohort (or from patients encountered in an individual clinical practice), and comparing results between studies performed in different centers or across different time periods may be difficult. Furthermore, the data largely do not apply to patients with noncirrhotic chronic liver disease.

Nonetheless, the results of studies describing operative risk in patients with liver disease have been remarkably consistent (Table 104.3). As one might expect, operative morbidity and mortality increase with increasing severity of liver disease, whether measured by CTP or MELD score. In general, patients with compensated cirrhosis have a low overall risk, and the risk increases for patients with advanced or decompensated disease.

## *Preoperative Screening*

Chronic liver disease, including disease attributable to common etiologies such as viral hepatitis or nonalcoholic fatty liver disease (NAFLD), may be subclinical and asymptomatic until the disease has reached advanced stages. Therefore, chronic liver disease cannot be excluded reliably on the basis of the absence of specific symptoms. Nonetheless, thorough preoperative history taking should include questioning about symptoms including prior jaundice, pruritus, and fatigue. An exposure history should also be elicited, specifically including risk factors for chronic viral hepatitis such as blood transfusion prior to the late 1980s, injection drug use, and needle exposure through tattoos or body piercing. An alcohol and medication history, including use of over-the-counter and herbal supplements, should be elicited.

In some instances, physical examination findings may suggest the presence of underlying liver disease. Either hepatomegaly or a small, nodular liver contour may be clues to liver disease. Splenomegaly may be detected in the presence of portal hypertension. Hormonal abnormalities in the setting of cirrhosis may result in gynecomastia, testicular atrophy, or cutaneous findings such as palmar erythema or spider angiomata. Documentation of any of the above findings should prompt further evaluation.

Obtaining liver biochemical tests for screening purposes in asymptomatic persons is neither routine

nor recommended in the preoperative setting. As many as 0.5% of asymptomatic young adults may have baseline serum aminotransferase elevations, as documented in a study of nearly 20,000 United States military personnel [48]. Moreover, among apparently healthy blood donors with an elevated aminotransferase level, the elevation may represent a one-time occurrence in up to one third of cases [24]. On the other hand, normal liver biochemical test results do not exclude cirrhosis.

In instances when liver disease is suspected on the basis of physical examination findings or persistent liver biochemical test abnormalities (aminotransferase level and/or alkaline phosphatase level >1.5 times the upper limit of normal on at least two occasions), elective surgery may need to be delayed so that further investigation can be undertaken, including biochemical and serologic testing for viral hepatitis, autoimmune liver disease, and metabolic disorders. Abdominal ultrasonography or magnetic resonance cholangiopancreatography may be considered when there is suspicion of cholestasis or biliary obstruction (Table 104.4). Abdominal computed tomography or magnetic resonance imaging may reveal a liver size and contour suggestive of cirrhosis or may detect intraabdominal varices and splenomegaly compatible with portal hypertension. However, abdominal imaging cannot reliably identify hepatic fibrosis or cirrhosis. If establishing the presence or absence of cirrhosis is essential for operative planning or predicting surgical risk, or in instances when noninvasive evaluation fails to reveal the specific cause of abnormal liver biochemical test results, a liver biopsy may be necessary.

**Table 104.4** Evaluation of liver biochemical abnormalities based on predominant pattern: noninvasive testing options

| |
|---|
| **Hepatocellular pattern (elevated serum aminotransferase levels)** |
| Hepatitis B surface antigen and antibody |
| Hepatitis C antibody |
| Serum iron studies (iron, ferritin, total iron binding capacity) |
| Celiac sprue serology (tissue transglutaminase antibody) |
| Antinuclear antibody |
| Serum ceruloplasmin level |
| Serum alpha-1 antitrypsin level |
| Abdominal ultrasound |
| **Cholestatic pattern (elevated serum alkaline phosphatase and/or bilirubin levels)** |
| Antimitochondrial antibody |
| Abdominal ultrasound |
| Magnetic resonance cholangiopancreatography |

## *Conditions for Which Elective Surgery is Contraindicated*

### Acute Hepatitis

Acute hepatitis may be caused by viral infection, autoimmune or genetic disorders, or toxin-mediated injury (including prescription medications, over-the-counter medications such as acetaminophen, herbal supplements, and alcohol [see next section]) (see Chapter 49). The diagnosis of acute hepatitis can be made and the etiology determined in almost all instances through noninvasive means such as a history, physical examination, and serologic testing.

Patients with acute hepatitis are thought to have an increased operative risk, particularly for abdominal surgical procedures [30]. This conclusion comes from studies reported in 1958 and 1963, describing operative mortality rates of 9.5–13% in patients with acute hepatitis [39,80]. In the former series, 42 patients with viral hepatitis and 16 patients with drug-induced hepatitis were identified retrospectively over a 12-year period. In the majority of instances, exploratory laparotomy had been performed for suspected extrahepatic biliary obstruction, and the operative mortality rate among those discovered to have viral hepatitis was 9.5% [80].

Although the diagnosis of acute viral hepatitis as well as surgical techniques have evolved since these studies were published, the standard recommendation remains that elective surgery should be deferred in patients with acute hepatitis. This recommendation is particularly appropriate when considering that most cases of acute hepatitis are self-limited and improve clinically and biochemically with supportive care.

### Acute Alcoholic Hepatitis

Acute alcoholic hepatitis occurs when excessive alcohol consumption leads to necroinflammatory changes in the liver, including hepatocyte swelling, an inflammatory infiltrate, and hepatocyte necrosis (see

Chapter 88). Presenting symptoms may include jaundice, fever, and hepatomegaly. Some patients present with manifestations of decompensated hepatic disease, including ascites, encephalopathy, and gastrointestinal bleeding. The 30-day mortality rate may exceed 50% for patients with severe acute alcoholic hepatitis.

Acute alcoholic hepatitis must be distinguished from acute cholecystitis. Fever, right upper quadrant tenderness, and leukocytosis can be present in both diseases, but the pronounced jaundice and hyperbilirubinemia seen in alcoholic hepatitis are uncommon in cases of cholecystitis.

Laparotomy performed in a patient with unsuspected or undiagnosed acute alcoholic hepatitis may have serious consequences, as is underscored by data from a retrospective series of patients with alcoholic hepatitis who underwent either open or percutaneous liver biopsy [32]. The mortality rate was 58% among the 12 patients who underwent open biopsy, compared with 10% among the 39 patients who underwent percutaneous biopsy. Because liver biopsy was performed in addition to a second surgical procedure during exploratory laparotomy in 9 of the 12 patients, and only one death in this group was clearly related to intraabdominal hemorrhage, laparotomy itself, rather than liver biopsy, appears to have been responsible for the high mortality rate in this group [32].

Acute alcoholic hepatitis is a contraindication to elective surgery. Abstinence from alcohol for 6–12 weeks generally results in resolution of hepatic inflammation and hyperbilirubinemia. Occasionally, however, alcoholic hepatitis may persist for months despite prolonged abstinence from alcohol. We recommend a 12-week delay, as well as a thorough reassessment of hepatic function, before elective surgery following a diagnosis of acute alcoholic hepatitis.

### Acute Liver Failure

Acute liver failure describes acute liver injury with the development of coagulopathy and hepatic encephalopathy within 26 weeks of the onset of illness in a patient without known preexisting liver disease (see Chapter 78). Patients are severely ill and have a grave prognosis without liver transplantation. They are therefore unlikely to survive any surgical procedure other than liver transplantation.

## *Surgery in Patients with Chronic Liver Disease*

### Chronic Viral Hepatitis

Chronic hepatitis is characterized by liver inflammation of greater than 6 months' duration. The differential diagnosis includes viral, autoimmune, and genetic disorders. The most common cause is viral hepatitis caused by hepatitis B virus (HBV) and hepatitis C virus (HCV) infection (see Chapter 63). Chronic hepatitis may be asymptomatic without obvious physical findings and may persist for decades. Diagnosis is often based on the detection of persistently elevated aminotransferase levels. Liver biopsy is often indicated to identify the presence and extent of hepatic fibrosis.

Literature before the identification of HCV suggested that the risk of surgery is increased in patients with chronic hepatitis. A report from 1970 described two patients (both female and in their teens with hepatomegaly and jaundice of 3–4 months' duration who might be considered cases of acute hepatitis or acute liver failure by current criteria) who underwent exploratory laparotomy and died postoperatively, at 18 and 27 days respectively, of hepatic coma [38]. However, in the absence of hepatic fibrosis or cirrhosis, there are no compelling data to suggest that surgical risk is increased in patients with chronic viral hepatitis. A study of nearly 2,500 patients in a Veterans Affairs cohort who underwent surgery over a 5-year period reported a mortality rate of 0.7% in HCV-seropositive patients, as compared with a mortality rate of 2.5% in HCV-seronegative patients [15]. Patients in the HCV seropositive group were younger, had a lower mean ASA class, and had a shorter mean duration of surgery than patients in the HCV-seronegative group. Each factor predicted mortality in a multivariate analysis, and HCV serostatus was not significantly associated with mortality. Similarly, there was no observed difference in complication rates between the two groups [15].

Postoperative liver biochemical test abnormalities can be expected in patients with chronic viral hepatitis. In a series of patients undergoing laparotomy, HCV-seropositive patients had higher mean postoperative serum AST and alkaline phosphatase elevations than matched HCV-uninfected controls [61]. However, resolution of these changes and correlation with any adverse outcome were not described in this study, and it is

uncertain whether these liver biochemical abnormalities were of any clinical significance [61]. Similarly, patients with chronic HBV infection but without significant hepatic necroinflammatory activity or fibrosis should face no increase in operative risk.

Patients with chronic viral hepatitis may require long-term antiviral therapy. Nucleoside/nucleotide analog therapy for chronic HBV should not be interrupted in the perioperative period; hepatitis flares may follow cessation of therapy, and interruption of treatment may raise concerns about viral resistance when therapy is resumed. Patients receiving therapy for HCV may experience myelosuppression due to peginterferon and hemolytic anemia due to ribavirin. Leukopenia, when present, may contribute to functional immunosuppression. Treatment-induced thrombocytopenia, if severe, may contribute to operative bleeding. In general, however, peginterferon and ribavirin therapy should not be discontinued without consulting the patient's treating gastroenterologist or hepatologist.

## Nonalcoholic Fatty Liver Disease (NAFLD)

Nonalcoholic fatty liver disease is associated with overweight and obesity and is the hepatic manifestation of the metabolic syndrome (see Chapter 89). NAFLD describes a range of hepatic disease activity, ranging from bland steatosis, to nonalcoholic steatohepatitis (NASH), to steatohepatitis with fibrosis and, ultimately, cirrhosis. Ultrasound imaging may detect increased echogenicity indicative of fatty liver, but a liver biopsy is necessary to distinguish steatohepatitis with or without fibrosis from bland steatosis.

Abdominal surgery in patients with NAFLD has come under increased attention due to the prevalence of this condition in the bariatric surgery population. Up to 6% of patients who undergo gastric bypass surgery will be found intraoperatively to have cirrhosis, and more than 90% of morbidly obese patients who undergo bariatric surgery will have histologic evidence of hepatic steatosis [11, 54]. Patients with NAFLD in the absence of advanced hepatic fibrosis do not appear to be at increased operative risk for elective surgical procedures, but the presence of NAFLD may influence operative management. Hepatomegaly due to NAFLD in some instances may require conversion from a laparoscopic to an open approach to Roux-en-Y gastric bypass [75]. Some investigators have suggested modification of the length of the Roux limb, from a distal to a more proximal Roux-en-Y gastric bypass, when cirrhosis is found on an intraoperative frozen section of the liver [78].

Patients with NASH who achieve weight loss following successful bariatric surgery may experience histologic improvement in steatohepatitis and perhaps even fibrosis [8, 26, 51]. Excessively rapid weight loss, however, may paradoxically worsen steatohepatitis and even precipitate hepatic failure, as has been observed following biliopancreatic diversion and jejunoileal bypass [4, 14, 33, 89].

Individual patients with NAFLD may have other comorbidities of the metabolic syndrome such as insulin resistance, hypertriglyceridemia, and hypertension, and are thus at significant risk for cardiovascular morbidity [36, 82]. Overall survival is lower among patients with NAFLD compared with matched controls, with cardiovascular disease as the leading cause of mortality [20]. The increase in mortality underscores the need for thorough and attentive preoperative cardiac risk stratification in patients with NAFLD who undergo elective surgery.

## Other Causes of Chronic Liver Disease

Patients with *hemochromatosis* may have myocardial iron deposition and are at risk for cardiomyopathy and cardiac conduction abnormalities, which may manifest in the perioperative period. In the past, outcomes were poorer following liver transplantation in patients with hemochromatosis, but with improved preoperative management, outcomes are now similar to those for patients with liver disease of other causes.

Manifestations of *Wilson's disease* may include neuropsychiatric disturbances. When present, the patient's ability to participate in informed procedural consent and clinical decision-making may be impaired. In addition, surgical procedures may precipitate neuropsychiatric symptoms. D-penicillamine, used as chelation therapy for Wilson's dose, impairs collagen cross-linking and may subsequently impair wound healing, and the dose should therefore be reduced in the preoperative and early postoperative periods.

Parenchymal damage may accumulate in the lungs as well as liver in patients with *alpha-1 antitrypsin deficiency*. Thorough history taking and physical examination with attention to respiratory function should be

conducted in such patients; pulmonary function testing should be performed before elective surgery when emphysema is suspected.

*Autoimmune hepatitis* in remission is not a contraindication to elective surgery in a patient with compensated hepatic function. However, patients receiving chronic glucocorticoid therapy for either autoimmune hepatitis or an autoimmune hepatitis-overlap syndrome may have adrenal suppression and should receive appropriate perioperative "stress" doses of glucocorticoids to avert adrenal crisis.

## Cirrhosis

### Operative Risk Stratification by Child Class

Cirrhosis is characterized by hepatic parencyhmal necrosis with fibrosis and nodular regeneration (see Chapter 79). As a result of fibrosis and disorganized hepatic architecture, sinusoidal pressure increases and may lead to portal hypertension. Major clinical sequelae of cirrhosis are generally the result of impaired hepatic synthetic function (such as hypoalbuminemia or prolongation of the prothrombin time) or the effects of portosystemic shunting (gastroesophageal varices, splenomegaly with hypersplenism), or a combination of the two (encephalopathy).

Surgical risk is increased in patients with cirrhosis, and the magnitude of perioperative risk correlates with the degree of hepatic decompensation. Clinical factors that are not specific to hepatic disease, such as ASA class, presence of chronic obstructive pulmonary disease, and elevated serum creatinine level, increase the risk of perioperative complications in patients with cirrhosis [92]. In addition, liver-specific indices, specifically the CTP score (Child class) and MELD score correlate with mortality.

General consensus has held that elective surgical procedures are well tolerated by patients with Child class A cirrhosis, permissible with thorough preoperative evaluation and medical optimization for patients with Child class B cirrhosis (with the exception of extensive hepatic resection or cardiac surgery [see later]), and contraindicated in patients with Child class C cirrhosis. Operative risk increases further in cases of emergent surgery. Data in support of the correlation between the Child class and operative risk derive from retrospective series since the 1970s.

In a study published in 1984, Garrison and colleagues reported a retrospective series of 100 cirrhotic patients who underwent abdominal surgery. The 30-day operative mortality rate was 30%, with a stratified mortality rate of 10% for patients with Child class A cirrhosis, 31% for those with Child class B cirrhosis, and 76% for those with Child class C cirrhosis [27]. Mortality rates stratified by type of surgical procedure were 21% for biliary tract surgery, 35% for peptic ulcer surgery, and 53% for colonic resections. The vast majority of deaths (87%) were attributed to sepsis or multiorgan failure, or both. In multivariable analysis (excluding the Child class), the presence of ascites, infection, and coagulopathy each independently predicted operative mortality [27].

Mansour and colleagues reported nearly identical results in 1997 [56]. Among 92 patients undergoing abdominal surgery including cholecystectomy and hernia repair, the 30-day mortality rate was 26%: 10% (5/48) for patients with Child class A cirrhosis, 30% (10/33) for those with Child class B cirrhosis, and 82% (9/11) for those with Child class C cirrhosis. Emergency surgery was associated with a higher mortality rate than nonemergent surgery: 22% versus 10% for patients in Child class A; 38% versus 30% for those in Child class B; and 100% versus 82% for those in Child class C [56].

For some cirrhotic patients, the degree of portal hypertension may be disproportionate to the severity of hepatic parenchymal dysfunction. Therefore, even among patients with Child class A cirrhosis, the presence of portal hypertension may be associated with operative morbidity, particularly postoperative ascites. Transjugular intrahepatic portosystemic shunt (TIPS) therapy for portal decompression has been reported as a bridge to abdominal surgery in small series of selected Child class A patients with portal hypertension [6, 31].

No study has prospectively validated the predictive value of the CTP score or Child class in assessing operative risk. Moreover, the above data must be interpreted in light of the evolution of surgical indications and techniques since the original studies were conducted. For example, with the advent of effective gastric acid suppression and therapy to eradicate *Helicobacter pylori*, as well as endoscopic hemostatic therapy, surgery for peptic ulcer disease has become much less common.

## Operative Risk Stratification by MELD Score

The MELD score is based on the patient's serum bilirubin level, creatinine level, and International Normalized Ratio (INR). The score was initially developed as a means of predicting survival in patients undergoing TIPS [55] and was subsequently validated as a predictor of mortality in patients awaiting liver transplantation. The MELD score has supplanted the CTP score as the principal criterion for donor liver allocation in the United States and elsewhere (see Chapter 103).

In predicting operative risk among cirrhotic patients undergoing non-transplant surgery, the MELD score appears to be at least as accurate as the Child class and may allow more precise numerical estimates of operative mortality for individual patients [66]. Among a retrospective cohort of 140 surgical patients with cirrhosis undergoing gastrointestinal, genitourinary, cardiovascular, thoracic, and musculoskeletal procedures, increasing MELD score correlated with an increasing mortality rate in a linear fashion. The mortality rate increased approximately 1% with each increase in MELD score of one point within the range of 5 to 20 and 2% with each increase in MELD score of one point for MELD scores above 20 [65]. The overall perioperative mortality rate was 16.4%, with all deaths occurring in patients who underwent gastrointestinal, cardiovascular, or thoracic procedures and no deaths in those who underwent genitourinary or musculoskeletal procedures [65].

The MELD score may also be used in a dichotomous fashion to predict operative outcomes. In a case-control study of patients who underwent cholecystectomy, two deaths occurred in patients with a MELD score ≥8, as compared with no deaths in patients with a MELD score below 8 [53]. A MELD score ≥8 demonstrated a sensitivity of 91% and specificity of 77% in predicting 90-day postoperative morbidity. A relatively high complication rate (17%) was observed in the control group in this study [70].

The usefulness of the Child class and MELD score for stratifying operative risk has been compared in a number of studies [9, 22]. In a series of 40 patients who underwent abdominal, genitourinary, or head and neck surgery and had an overall 30-day operative mortality rate of 17%, the correlation between the Child class and MELD score in predicting mortality was 0.76 [22]. Specific mortality rates were 15% (2/13) for patients in Child class A, 9% (2/22) for those in Child class B, and 60% (3/5) for those in Child class C, compared with 8% (1/12) for those with a MELD score ≤8, 10% (2/21) for those with a MELD of score 9–16, and 57% (4/7) for those with a MELD score ≥17 [22]. Higher 30-day and 90-day mortality rates were evident in patients who underwent emergent (n=16) as compared with elective (n=24) surgery [22].

The most compelling data in support of the MELD score for operative risk stratification come from a large retrospective study of operative outcomes in cirrhotic patients published in 2007 by Teh and colleagues at the Mayo Clinic [84]. A total of 772 subjects underwent surgery during two time periods, 1980–1990 and 1994–2004. Five hundred eighty-six subjects underwent digestive surgery, 107 underwent orthopedic surgery, and 79 underwent cardiovascular surgery. For patients with a MELD score >8, each one-point increase in the MELD score resulted in a 14% increase in both 30-day and 90-day mortality rates and a 15% increase in the 12-month mortality rate (Fig. 104.1). In multivariate analysis, MELD score, age, and ASA class were significant predictors of risk. ASA class appeared to be the strongest predictor of 7-day mortality, whereas the MELD score appeared to be the strongest predictor of mortality beyond 7 days [84].

A clinical prediction rule derived from this cohort is available at the website http://www.mayoclinic.org/meld/mayomodel9.html. On providing a patient's age, ASA class, INR, serum bilirubin level, and serum creatinine level, numeric estimates of the patient's 7-day, 30-day, 90-day, 1-year, and 5-year mortality rates are generated (Table 104.5). This prediction rule has not yet been validated prospectively, and its performance, therefore, may be somewhat less robust than its performance in the derivation cohort.

At present the MELD score appears to be the best tool for estimating perioperative risk in patients with cirrhosis. Use of the MELD score and Child class for this purpose need not be mutually exclusive. The Child class remains a useful strategy for rapid bedside assessment of disease severity and risk stratification.

## Operative Risk Associated with Specific Types of Surgery

Except where specifically mentioned earlier, much of the available data focuses on patients who underwent general abdominal surgery. Factors to be considered in

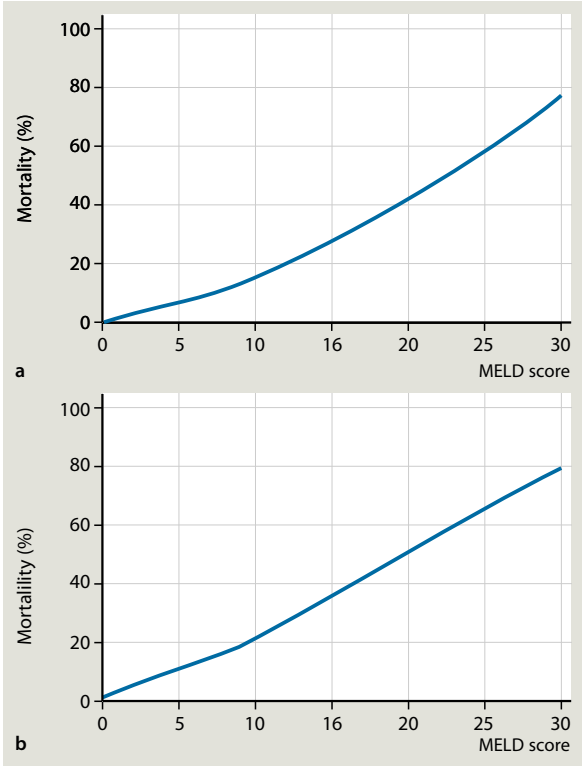


**Fig. 104.1** Relationship between operative mortality and MELD score in 772 subjects with cirrhosis who underwent surgery in 1980–1990 or 1994–2004. Panel A shows 30-day mortality; Panel B shows 90-day mortality. For patients with a MELD score > 8, each one-point increase in the MELD score resulted in a 14% increase in both 30-day and 90-day mortality rates. (From [84. With permission.) *MELD* Model for Endstage Liver Disease

**Table 104.5** Risk factors for postoperative mortality in patients with cirrhosis according to the Mayo Clinic Model

| **Input variables** |
|---|
| Age |
| ASA Class (see Table 104.2) |
| Serum bilirubin level (mg/dL) |
| Serum creatinine level (mg/dL) |
| International normalized ratio |
| Etiology of cirrhosis (alcoholic or cholestatic versus viral/other) |

From http://www.mayoclinic.org/meld/mayomodel9.html. ASA, American Anesthesiologists Association

cirrhotic patients undergoing abdominal surgery include decreased hepatic blood flow due to traction on abdominal viscera, delayed wound healing, and the development of incisional hernias, particularly in patients with ascites. Further modifications in risk assessment, and attention to specific elements of operative morbidity, may be appropriate in cirrhotic patients undergoing nonabdominal surgery or specific abdominal operations.

**Biliary tract surgery and obstructive jaundice.** Biliary tract surgery, including cholecystectomy, can present unique challenges in patients with cirrhosis. Increased vascularity of the gallbladder bed in the context of portal hypertension can result in bleeding during dissection of the gallbladder. An increased risk of bleeding should be anticipated in patients with advanced cirrhosis as demonstrated by a prolonged prothrombin time or thrombocytopenia, although the actual risk of bleeding does not correlate with coagulopathy [73, 85]. In general, laparoscopic cholecystectomy is permissible for patients with Child class A cirrhosis and selected patients with Child class B cirrhosis [18, 71, 90]; however, an open approach is recommended if cholecystectomy is necessary in patients with Child class C cirrhosis or a MELD score >8 and portal hypertension. In a patient with decompensated cirrhosis who presents with cholecystitis and requires an acute biliary intervention, percutaneous cholecystostomy may be preferable to cholecystectomy.

Cirrhotic patients may present with jaundice caused by extrahepatic biliary obstruction rather than parenchymal liver disease. In a series of 373 patients presenting with acute biliary obstruction between 1976 and 1981, Dixon and colleagues reported a 30-day mortality rate of 9.1% [19]. In this cohort, 92 (25%) underwent a Whipple resection, hepaticojejunostomy, or creation of another type of biliary-enteric anastomosis; the remainder underwent cholecystectomy, with or without intraoperative bile duct exploration. Not surprisingly, the mortality rate was significantly higher among patients presenting with malignant versus benign biliary obstruction (26.1% versus 3.7%, $p < 0.0005$). Additional factors predictive of mortality included age greater than 60 years, anemia (hematocrit value <30%), and marked hyperbilirubinemia (>11 mg/dL) [19].

Perioperative morbidity in patients with acute biliary obstruction may be due to hemodynamic and circulatory insults as well as renal dysfunction, particularly in patients with sepsis due to suppurative cholangitis. A possible mechanism for these systemic complications includes translocation of gut micoflora into the systemic circulation and resulting endotoxemia. Intestinal barrier integrity may be compromised in patients with obstructive jaundice and in those with cirrhosis, leading to increased permeability to microorganisms [42, 69].

Limited animal and human data suggest that gut decontamination by oral administration of lactulose may attenuate the risk of endotoxemia, but it is not clear that any clinical benefit is directly attributable to lactulose administration, as opposed to administration of antibiotics and careful attention to the patient's hemodynamic and volume status [46, 67 87]. Therefore, although lactulose therapy is appropriate for the perioperative management of cirrhotic patients with encephalopathy, administration of lactulose to cirrhotic patients prior to biliary surgery is not standard practice. Hemodynamic and volume support with crystalloid or colloid, with monitoring of urine output, as well as avoidance of nephrotoxic drugs such as aminoglycoside antibiotics, constitute appropriate management in such cases.

There are no data to suggest that preoperative biliary decompression in patients with obstructive jaundice leads to a decrease in operative morbidity or mortality. However, endoscopic (or percutaneous) biliary therapy is preferable to surgery in patients with specific causes of biliary obstruction, such as choledocholithiasis and biliary stricture. In a small retrospective series of cirrhotic patients with acute cholangitis due to choledocholithiasis, mortality rates were 100% (2/2) in those undergoing emergent surgery but only 7% (1/15) in those undergoing emergent endoscopic therapy [16]. On the other hand, a larger retrospective study identified cirrhosis as an independent risk factor for complications among patients undergoing endoscopic retrograde cholangiopancreatography (ERCP) (multivariate adjusted odds ratio 2.93, 95% confidence interval 1.48–9.50); the increased risk was attributable principally to hemorrhage following sphincterotomy [23]. Endoscopic papillary balloon dilation has been recommended as an alternative to sphincterotomy, with a lower risk of hemorrhage (but possibly a higher risk of pancreatitis), in cirrhotic patients with coagulopathy [68].

**Cardiac surgery**. Cardiac surgery and procedures requiring use of cardiopulmonary bypass carry higher risks than intraabdominal or other general surgical procedures in patients with cirrhosis. Factors contributing to an increased risk of hepatic decompensation include total time on bypass, use of pulsatile rather than nonpulsatile bypass flow, and administration of vasopressor agents for hemodynamic support. Cardiopulmonary bypass may adversely affect platelet function and fibrinolysis, further exacerbating coagulopathy.

In two retrospective series of patients who underwent surgery requiring cardiopulmonary bypass, relatively low mortality rates were observed in those with Child class A cirrhosis: 0% (0/10) and 3% (1/31), respectively, but rates were markedly increased in those with Child class B (44–50%) and C (100%, n=2) cirrhosis. More than 75% of Child class B and C patients experienced hepatic decompensation perioperatively [40, 81].

The Child class and MELD score appear to be comparable in predicting operative risk in cirrhotic patients undergoing cardiac surgery [81]. In the above cohort described by Suman and colleagues, a CTP score of >7 demonstrated 86% sensitivity and 92% specificity for predicting mortality and 66% sensitivity and 97% specificity for predicting hepatic decompensation. Similarly, a MELD score of >13 demonstrated 71% sensitivity and 89% specificity for predicting mortality and 67% sensitivity and 97% specificity for predicting hepatic decompensation [81].

These limited data suggest that cardiac surgery may be accomplished safely in patients with a low CTP score (Child class A and carefully selected class B patients) or low MELD score. Cardiac surgery should otherwise be avoided, and less invasive options considered, in patients with advanced cirrhosis. Portal decompression with TIPS placement as a bridge to cardiac surgery in patients with portal hypertension has been described in individual case reports [76]; however, because a TIPS may increase right ventricular preload, it may be contraindicated in patients with impaired cardiac function.

**Hepatic resection**. Not uncommonly, resection of a hepatic lesion may be considered, among other options, in a patient with underlying liver disease (particularly a patient with cirrhosis in whom a focal hepatocellular carcinoma [HCC] develops). Increasingly, liver transplantation has been preferred over resection in patients with either one lesion <5 cm in diameter or up to three lesions each ≤3 cm. However, the shortage of donor livers often precludes timely liver transplantation in these patients. Nonsurgical options for treating HCC include radiofrequency ablation, microwave ablation, ethanol injection, and chemoembolization.

When hepatic resection is considered in such cases, the question often arises as to whether the remnant liver will provide adequate functional hepatic capacity. Inadequate hepatic reserve following resection can have disastrous consequences, including acute liver failure. Mortality rates as high as 25% are reported following hepatic resection in patients with cirrhosis [60]. Data on estimating perioperative risk associated with hepatic

resection in patients with cirrhosis are limited. Computed tomography scans with volumetric analysis grading of cirrhosis, as well as indocyanine green dye tests, have been used to estimate functional hepatic reserve [41, 86]. Clinical scoring systems such as the Child class and MELD score also appear to be useful for risk stratification. In an analysis of 82 cirrhotic patients undergoing hepatic resection, perioperative mortality was 29% for patients with a MELD score ≥9 but zero in patients with a MELD score ≤ 8 [83]. However, another study identified Child class and ASA class, but not MELD score, as significant predictors of outcome following liver resection. In this study, the mean MELD score was low (6.5), which may have limited the ability of MELD to discriminate between low-risk and high-risk groups [74]. Mortality rates were higher for patients undergoing resection of primary (16.3%) as opposed to metastatic (5%) liver tumors, presumably because of the higher prevalence of cirrhosis in the former group [74].

The presence of chronic viral hepatitis in patients with cirrhosis and compensated hepatic function does not appear to have an adverse impact on outcome. In a series of 172 subjects with HCV-related HCC who underwent hepatic resection (98 with cirrhosis and 4 without cirrhosis), the outcome was best predicted by tumor-related factors, including the serum alpha-fetoprotein level and tumor vascular invasion [37]. The influence of specific viral factors, such as the level of serum viremia or degree of hepatic necroinflammatory activity, was not reported. Child class (A versus B) was not a significant predictor of outcome on multivariate analysis [37]. Another study has suggested, however, that the risk of HCC recurrence may be higher and overall survival may be poorer following hepatectomy for patients with HCC and chronic viral hepatitis as compared with patients who have HCC unrelated to viral hepatitis [62].

### Endoscopic Procedures

Patients with cirrhosis routinely undergo upper gastrointestinal endoscopy for surveillance for esophageal varices and treatment of active variceal hemorrhage, among other indications. Cirrhosis in and of itself is not a contraindication to endoscopy, although manifestations of decompensated disease such as encephalopathy and coagulopathy may influence the approach to procedural sedation and endoscopic tissue acquisition (biopsy and polypectomy), respectively.

Additional caveats must accompany certain endoscopic techniques. Percutaneous endoscopic gastrostomy should be avoided in many patients with cirrhosis, because enlarged abdominal wall or visceral blood vessels due to portal hypertension may be inadvertently punctured during blind percutaneous trocar placement. If gastrostomy tube placement is necessary in a patient with portal hypertension, an open surgical approach is advised. Gastrostomy tube placement for nutritional support should also be avoided in patients with moderate or severe ascites. The issue of endoscopic sphincterotomy in cirrhotic patients with coagulopathy was discussed earlier.

## Perioperative Care

### *Coagulopathy*

Coagulopathy in patients with liver disease may result from clotting factor deficiency due to hepatic synthetic dysfunction, depletion of vitamin K stores due to malnutrition, and thrombocytopenia due to portal hypertension and splenic sequestration of platelets (see Chapter 80). The prothrombin and partial thromboplastin times may be prolonged, and low-grade disseminated intravascular coagulation, consistent with fibrinolysis, may be present.

Administration of vitamin K will correct coagulopathy due to nutritional or bile salt deficiency but not due to hepatic synthetic dysfunction. Transfusion of fresh frozen plasma and platelets may be necessary preoperatively or intraoperatively in patients with marked coagulopathy or thrombocytopenia, respectively, in order to permit safe surgery. Additional blood products such as cryoprecipitate and pharmacologic agents such as desmopression (1-deamino, 8-D arginine-vasopressin, or DDAVP) are typically reserved for treatment of active bleeding that does not respond to standard measures.

Recombinant factor VIIa (rFVIIa) has been introduced as an additional option for the prevention and treatment of bleeding due to coagulopathy in cirrhotic patients undergoing surgery. In a randomized controlled trial of cirrhotic patients undergoing orthotopic liver transplantation, patients randomized to perioperative rFVIIa were less likely to require packed red blood cell transfusions than patients randomized to placebo [52]. A similar study in cirrhotic patients undergoing

partial hepatectomy demonstrated no beneficial effect of rFVIIa, although the majority of patients had Child class A cirrhosis with relatively mild coagulopathy [79]. The risk of thromboembolic events was not increased in patients receiving rFVIIa in the above studies but remains a theoretical concern [52, 79]. Despite case reports documenting rFVIIa infusions for hemostasis in cirrhotic patients undergoing a range of procedures, from colonoscopic polypectomy to dental extraction, the data are insufficient to recommend rFVIIa routinely in cirrhotic patients undergoing non-transplant surgery [3, 10].

## Ascites

Ascites may increase the risk of wound dehiscence and abdominal wall herniation following abdominal surgery. Moreover, respiratory mechanics may be compromised due to either impaired diaphragmatic excursion or hepatic hydrothorax. For these reasons, efforts to control or eliminate ascites should be undertaken before elective abdominal surgery. Some patients with decompensated cirrhosis may have refractory ascites despite dietary sodium restriction and diuretic therapy (see Chapter 54). In such cases, ascites can be drained at laparoscopy or laparotomy, although ascitic fluid will typically re-accumulate within days.

In some instances, an umbilical hernia resulting from ascites may represent an indication for surgical intervention in a patient with cirrhosis. Umbilical hernias can be at risk of incarceration or spontaneous rupture. Elective surgical umbilical hernia repair, either with or without mesh prosthesis, may be performed safely in carefully selected patients with decompensated cirrhosis [57]. Urgent repair following umbilical hernia rupture may require concomitant placement of a TIPS to control ascites [21].

## Renal Dysfunction

Patients with advanced liver disease may develop complex circulatory derangements, not limited to portal hypertension, that include a hyperdynamic circulation with peripheral vasodilalation and augmented splanchnic inflow (see Chapter 80). Renal consequences of these changes include excessive activation of the renin-angiotensin-aldosterone system and development of the hepatorenal syndrome. The prognostic significance of renal dysfunction in patients with cirrhosis is underscored by the presence of the serum creatinine level as a component of the MELD score.

In cirrhotic patients undergoing liver transplantation, risk factors for postoperative renal failure include baseline renal dysfunction (manifested by a low glomerular filtration rate, which may be underestimated when calculated from the serum creatinine value alone in cirrhotic patients with malnutrition and reduced muscle mass), intraoperative hypotension, prolonged vasopressor requirements, and long duration of surgery [12, 88]. Volume status, urine output, and systemic perfusion should be monitored assiduously in the perioperative period. Intravenous infusions of salt-poor albumin are widely used in lieu of crystalloid fluid replacement in patients with liver disease, despite a lack of data supporting an advantage of this approach except for reducing mortality in patients with spontaneous bacterial peritonitis. Equally important, iatrogenic nephrotoxins should be avoided whenever possible, including but not limited to aminoglycoside antibiotics, nonsteroidal anti-inflammatory agents, and intravenous contrast agents.

## Encephalopathy

Clinically overt hepatic encephalopathy can typically be treated effectively with either lactulose or nonabsorbable antibiotics (see Chapter 80). Elective surgery should be deferred until encephalopathy has been controlled.

Even for patients without overt hepatic decompensation, some degree of encephalopathy may be encountered following surgery, because precipitants of hepatic encephalopathy invariably occur in the postoperative period, including volume contraction, hypokalemia, infection, and use of sedative or psychoactive medications. However, there are no compelling data to suggest a role for propyhylactic therapy to prevent encephalopathy in patients undergoing non-hepatic, non-portosystemic shunt surgery.

Avoiding the use of sedative or psychoactive medications can be a particular challenge in the postoperative period. Whenever possible, short-acting medications should be used at the lowest effective dose.

Among patients with alcoholic liver disease and ongoing alcohol use at the time of surgery, the differential diagnosis of postoperative change in mental status must include alcohol withdrawal. When alcohol withdrawal is suspected, pharmacologic therapy should be initiated to prevent delirium tremens.

### *Nutrition*

Malnourishment and protein-energy malnutrition are common among patients with chronic liver disease, particularly alcoholic liver disease. Nutritional deficiencies among these patients may be underdiagnosed. Clinical clues to nutritional deficiencies include muscle wasting, ascites (presumably malabsorption is caused in part by intestinal edema), and hypoalbuminemia, which may not be attributable solely to hepatic synthetic dysfunction [13].

Poor nutritional status has an adverse impact on outcome among cirrhotic patients in general and those undergoing surgical procedures in particular [2, 34, 59]. The patient's nutritional status should be addressed before elective surgery (see Chapter 91). Oral nutritional support should be the initial and preferred mode of delivery for patients without a contraindication to oral intake. Percutaneous gastrostomy, as previously mentioned, is contraindicated in patients with ascites or suspected abdominal wall varices. Central venous catheterization for parenteral support carries a risk of septic and bleeding complications and should be avoided.

## Postoperative Monitoring

Following surgery, patients with liver disease should be monitored closely for signs of hepatic decompensation, which may include encephalopathy, jaundice, ascites, coagulopathy, and renal dysfunction. When any of these indicators of decompensation is present, supportive therapy should be initiated, as described in the preceding sections.

Serum bilirubin levels and the INR are the best laboratory measures of hepatic function to follow in the postoperative period. Elevations in the serum bilirubin level are common following surgery in patients with liver disease, particularly following blood transfusion or complex surgical procedures.

In patients with severely impaired hepatic dysfunction due to either advanced cirrhosis or fulminant hepatic failure, impaired hepatic gluconeogenesis may contribute to hypoglycemia. Postoperative serum glucose levels should be monitored regularly in such instances, with intravenous administration of dextrose supplementation as necessary.

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# A Look to the Future: Gene Therapy in Liver Diseases

# 105

Hubert E. Blum

## Chapter Outline



Molecular biology and recombinant DNA technology increasingly contribute to the diagnosis, therapy and prevention of human diseases. Molecular methods allow the early and/or specific detection of inherited, infectious and malignant liver diseases. In addition, such analyses increasingly lead to a better understanding of the pathogenesis of the various liver diseases which in turn had an impact on patient management, including the presymptomatic identification of patients at risk, the correct staging of the disease and the follow-up of patients undergoing therapy. Thus, molecular biology is increasingly becoming an integral part of basic as well as clinical hepatology. In the following chapter we will briefly review current concepts and potential applications of gene therapy for the treatment or prevention of various liver diseases.

## Genetic Classification of Diseases

Genetically, human diseases can be classified into three major categories: (1) Hereditary monogenic diseases that are caused by a single gene defect and inherited by the classical Mendelian rules. There are more than 4,000 monogenic diseases described. For an increasing number of these diseases the genetic basis is being identified. (2) Acquired monogenic diseases are infections as well as malignancies that are caused by the mutation or epigenetic modification of a single gene. (3) Complex genetic diseases are associated with mutations of several genes that are acquired and frequently accumulated during life-time. Several common human diseases belong to this category, such as most malignancies [2, 27, 37, 74].

Gene therapy is defined as the introduction of genetic material into human cells with a therapeutic or

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preventive benefit. In a broader definition, cell or organ transplantation are included. In the following discussion the basic concepts of gene therapy as well as some therapeutic and preventive applications for liver diseases will be reviewed [2, 27, 37, 74, 78, 79, 89].

## Gene Therapy of Liver Diseases

Based on the genetic classification of diseases detailed above, the principle of gene therapy involves six therapeutic concepts: gene repair, gene substitution and cell therapy for hereditary monogenic diseases, block of gene expression and DNA vaccination for acquired monogenic diseases and gene augmentation and DNA vaccination for complex genetic diseases (Table 105.1). For clinical applications, gene therapy is explored with the aim to either provide novel therapeutic strategies for diseases for which there is no effective treatment available to date or to replace and in some cases complement existing therapeutic modalities, thereby increasing their efficacy and/or their reduce adverse events.

### *Gene Repair*

An increasing number of liver diseases have been molecularly defined as a defect of a single gene (Table 105.2). In this context, one therapeutic concept is the *in vitro* or *in vivo* repair of the defective gene. Indeed, in the Gunn rat model of the Crigler Najjar syndrome type I Kren et al. were able to partially correct the genetic defect underlying the UDP-glucuronosyl transferase deficiency by the intravenous injection of a cyclic normal/wild-type chimeric oligonucleotide [59]. While these findings have not been independently confirmed or extended to other hereditary monogenic (liver) diseases, the data suggest that it is in principle possible to repair a gene defect *in vivo*. Further, it has been shown that cellular RNA species can be modified by *trans*-splicing group I ribozymes. Such ribozymes may in principle allow for the treatment of a variety of inherited diseases at the RNA level [50, 65, 93].

**Table 105.1** Concepts of gene therapy

| Type of disease | Gene therapy |
|---|---|
| Hereditary monogenic diseases | Gene repair<br>Gene replacement<br>Hepatocyte transplantation |
| Acquired monogenic diseases | Block of gene expression<br>DNA vaccination |
| Complex genetic diseases | Gene augmentation<br>Immune therapy |

**Table 105.2** Hereditary monogenic liver diseases (selection)

| Gene | Disease |
|---|---|
| UDP-glucuronosyl transferase | Crigler Najjar syndrome type I |
| Alpha-1-antitrypsin | Liver cirrhosis, emphysema |
| CF transmembrane regulator | Mucoviscidosis, cystic fibrosis |
| Factor VIII | Hemophilia A |
| Factor IX | Hemophilia B |
| Fumaryl acetoacetate hydrolase | Tyrosinemia type 1 |
| LDL receptor | Familial hypercholesterolemia |
| Ornithine transcarbamylase | Hyperammonemia |

### *Gene Substitution*

The targeted substitution of a defective cellular gene by the normal/ wild-type homologue with production of the physiological gene product is another approach to correct a hereditary or acquired monogenic gene defect. Indeed, in an animal model of hereditary tyrosinemia type 1 (HT1), a liver disease caused by a deficiency of fumarylacetoacetate hydrolase (FAH), multiple injections of a retroviral vector carrying the FAH gene resulted in a gene transfer efficiency of >90% of hepatocytes and the restoration of a normal liver function [33, 83]. In patients, examples for gene substitution are the partial correction of severe hemophilia A by the *ex vivo* transduction of autologous skin fibroblasts with the normal/wild-type factor VIII gene, followed by laparoscopic implantation of the genetically modified fibroblasts into the omentum majus or of hemophilia B by adenovirus-associated vector (AAV)-based gene transfer [55, 90].

In rare situations in which a hepatocelluar carcinoma (HCC) is caused by the mutation of a tumor suppressor gene, e.g., the p53 gene, the substitution of the mutated by the normal/ wild-type gene *in vitro* can reduce the number of tumor cell colonies and restore cisplatin sensitivity [116, 119].

## Hepatocyte Transplantation

Allogeneic or ex vivo genetically modified autologous hepatocyte transplantation is a promising strategy to treat hereditary monogenic liver diseases. In patients with familial hypercholesterolemia (FH) that is caused by various mutations in the low density lipoprotein (LDL) receptor gene, apart from orthotopic liver transplantation, liver-directed gene therapy has been performed in a pilot study in five patients [6, 10, 34, 35, 45]. Autologous liver cells, prepared from a surgical biopsy, were transduced *ex vivo* with a recombinant retrovirus expressing the normal LDL receptor. These *ex vivo* genetically modified hepatocytes were transplanted by portal infusion and resulted in significant and prolonged reductions in LDL cholesterol in 3/5 patients for at least 4 months, demonstrating the feasibility of engrafting a limited number of *ex vivo* transduced hepatocytes. Also, allogeneic hepatocyte transplantation has been successfully used in patients to partially correct Crigler-Najjar syndrome type I and glycogen storage disease type I [26, 80].

## Block of Gene Expression or Function

For diseases caused by the expression of an acquired gene or the overexpression of an endogenous gene, blocking gene expression can be an effective therapeutic approach. Several strategies can be employed (Fig. 105.1): interference with the transcription of genes by binding of transcription factors to nucleic acids introduced into or synthesized in the cells (decoy strategy), by binding of single-stranded nucleic acids to double-stranded DNA, forming a triple helix structure, hybridization of RNA molecules possessing endonuclease activity (ribozymes) to RNA, resulting in its sequence-specific cleavage, RNA interference (RNAi) by small inhibiting RNA (siRNA) or microRNA (miRNA), block of translation by antisense oligonucleotides and the intracellular synthesis of peptides or proteins, interfering with their normal counterpart, termed dominant negative (DN) mutant strategy (Fig. 105.1) [14, 23, 40, 42–44, 56, 100–102, 112]. These different strategies have been applied to a number of malignant and infectious diseases. In particular ribozymes, siRNAs, antisense oligonucleotides and DN mutants have been experimentally explored to treat hepatitis B virus (HBV) and hepatitis C virus (HCV) infections.

### Ribozymes

Ribozymes ('ribonucleic acid enzymes') were originally discovered as naturally occurring RNA molecules that catalyze the sequence-specific cleavage of RNA and RNA splicing reactions [40, 101]. This catalytic activity is the major attraction of the ribozyme concept since one ribozyme can cleave many target RNAs. Ribozymes that cleave RNA are being developed as inhibitors of gene expression and viral replication. Several studies have clearly demonstrated that hammerhead ribozymes can specifically cleave HBV RNA or HCV RNA *in vitro* [5, 66, 91, 107]. *In vivo*, however,

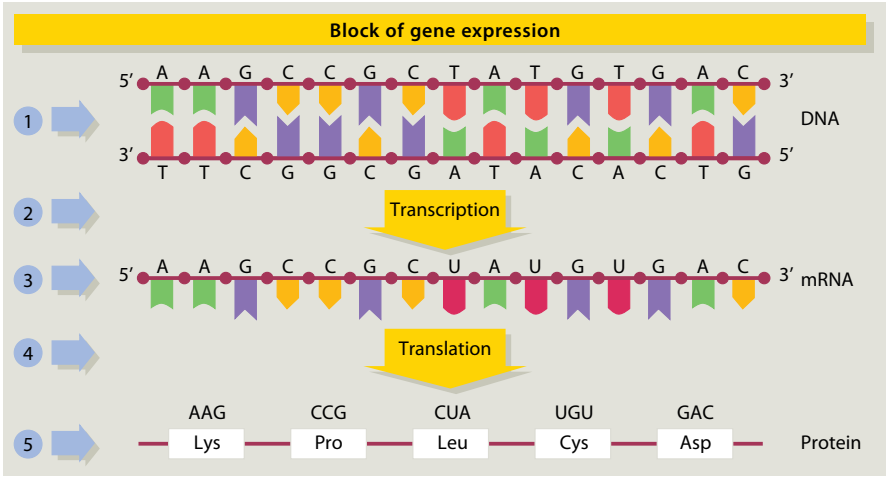


**Fig. 105.1** Strategies aimed at blocking gene expression

an efficient ribozyme-mediated cleavage of HBV RNA could not be demonstrated to date. For HCV infection, the elimination of HCV RNA in infected hepatocytes by ribozymes has also been reported [66, 111].

### Small Interfering RNA

siRNA interference is a recently discovered basic intracellular mechanism that has been explored also for the inhibition of HBV and HCV infection (Fig. 105.2) [23, 56, 100, 112]. For HBV, inhibition of viral gene expression and replication has been shown *in vitro* and in different mouse models *in vivo* [29, 32, 57, 58, 71, 99, 104, 118]. For HCV, inhibition of viral gene expression and replication has been shown *in vitro* in the replicon system [54, 88, 114]. While effective in blocking viral gene expression and replication, *in vivo* oversaturation of celluar miRNA/ short hairpin RNA (shRNA) pathways can result in lethal hepatotoxicity. For future siRNA-based strategies in animals or humans, these findings indicate that the control of intracellular shRNA expression levels through optimizing shRNA dose and sequence will be key to reduce the risk of oversaturating endogenous small siRNA pathways.

### Antisense Oligonucleotides

Antisense nucleic acids are designed to specifically bind to RNA or mRNA, resulting in the formation of RNA–DNA (antisense oligodeoxynucleotides) or RNA–RNA hybrids (antisense oligoribonucleotides) with an arrest of RNA replication, reverse transcription or mRNA translation [14, 42, 43, 102, 109]. Antisense effects can be potentiated by degradation of RNA in RNA-DNA hybrids by cellular RNases H. While conceptually simple, it is clear now that not all desired as well as undesired effects are caused by the target sequence specific antisense action of the oligonucleotides or the cellular enzymes mentioned above [9, 22].

The antisense strategy has been successfully applied *in vitro* to HBV infection [8, 30, 81, 115] and to HCV infection [1, 39, 75, 98, 105, 110]. In addition, studies in nude mice, in the duck hepatitis B virus (DHBV) and the woodchuck hepatitis virus (WHV) model of HBV infection demonstrated the *in vivo* applicability of this approach [4, 82, 117]. While no toxic effects have been observed in these experiments, the contribution of non-antisense effects to the inhibition of viral replication or gene expression has not been systematically assessed in most studies. Independent of the antisense or non-antisense mechanism of the biological effects, an *in vitro* screening procedure for the identification of functionally active oligonucleotides should greatly facilitate the design of oligonucleotide based antiviral therapies [9, 103].

### Interfering Peptides or Proteins

The intracellular synthesis of interfering peptides or proteins, including single chain or whole non-secreted antibodies, is aimed at the specific interference with the assembly or function of viral structural or non-structural proteins and represents a type of intracellular immunization. This approach has been shown for block mammalian and avian hepadnavirus gene expression and replication *in vitro*. For example, the fusion of different polypeptides of various lengths to the carboxy-terminus

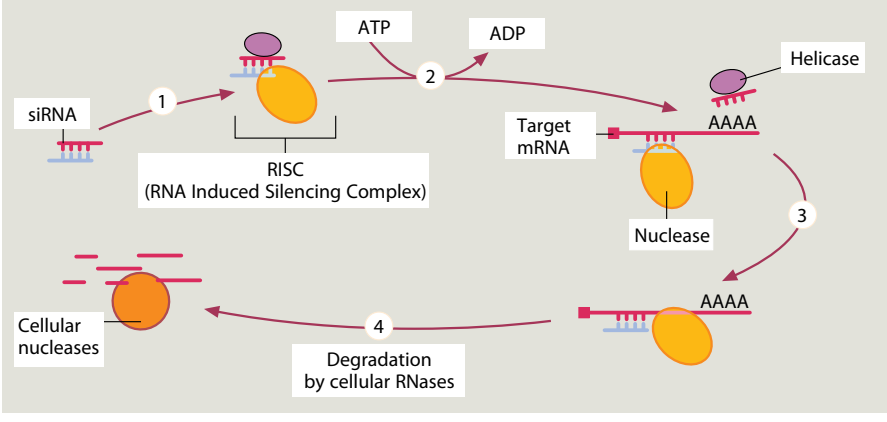


**Fig. 105.2** Principle of RNA interference [56]

of the viral core protein yields DN mutants [21, 94, 95, 108]. These DN mutants are species-specific and suppress viral replication by at least 90% at an effector to target ratio of 1:10. Moreover, the non-secretory form of the hepatitis B e antigen (HBeAg) was shown to effectively inhibit viral replication and may indeed act as a natural regulator of HBV propagation [11, 36, 96]. The potential advantage of DN mutants over ribozymes or antisense oligonucleotides is their relative independence from viral sequence variations, minimizing the risk of selecting or accumulating 'therapy escape' mutants.

## DNA Vaccination

A novel approach is DNA vaccination resulting in the manipulation of the immune system by introduction of expression vectors into muscle cells or dendritic cells and long lasting cellular and humoral immune responses. The direct gene transfer into muscle [7] represents an exciting new development and elegant application of gene therapy [7, 72, 84]. The therapeutic DNA vaccine acts by the intracellular plasmid-derived synthesis of a viral protein which enters the cell's MHC class I pathway [72]. Only proteins that originate within the cell can be processed by MHC class I molecules that carry fragments of the protein to the cell surface. There they stimulate $CD8^+$ cytotoxic T cells, resulting in cell-mediated immunity. In principle, this strategy is applicable to the treatment of acquired genetic diseases, associated with the expression of disease-specific antigens serving as targets for $CD8^+$ cytotoxic T cells.

Therapeutic DNA vaccination has been experimentally explored for HBV as well as HCV infection and holds great promise as an effective molecular therapy for these viral diseases [20, 62, 64, 69, 70, 97, 106]. In this context, the coexpression of HBsAg and interleukin-2 was shown to greatly increase humoral as well as cellular immune response [16].

## Gene Augmentation

Complex genetic diseases are among the most prevalent clinical problems. In this situation, gene augmentation is aimed at the local expression of a therapeutic gene product that is physiologically not expressed or expressed at therapeutically insufficient levels. This strategy is explored among others for the treatment of hepatocellular carcinoma (HCC) (Fig. 105.3).

### Suicide Gene Therapy

An interesting strategy to treat HCCs is genetic prodrug activation therapy *via* the introduction of a 'suicide gene' into malignant cells followed by the administration of the prodrug. This concept has been experimentally explored in HCC cells *in vitro* and *in vivo*, e.g., for the HSV-tk gene [47, 52, 53, 87, 113], the gene encoding cytosine deaminase (CD) that converts the prodrug 5-fluorocytosine to 5-fluorouracil which inhibits RNA and DNA synthesis during the S-phase of the cell cycle [51], the gene encoding purine nucleoside phosphorylase that converts purine analogs into freely diffusible toxic metabolites [61, 77] as well as the gene encoding cytochrome P450 4B1 [76, 77]. A significant bystander effect of cell killing caused by suicide gene

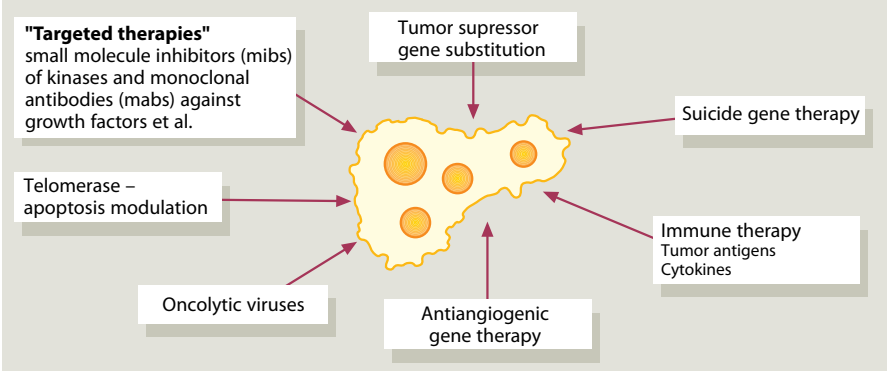


**Fig. 105.3** HCC treatment: experimental strategies, including gene therapy

expression could be demonstrated *in vitro* and *in vivo*, based on cell-cell contact rather than release of cytotoxic substances from the transduced cells [63]. At the same time, the bystander effect may also affect non-malignant dividing cells in the target tissue, potentially limiting the application of this strategy.

#### Antiangiogenic Gene Therapy

This concept has been experimentally explored in a HCC mouse model using the angiostatin gene. Angiostatin gene transfer resulted in reduced tumor volume and vascular density [48].

#### Oncolytic Viruses

This new and elegant approach uses p53 mutations for selective, adenovirus-mediated lysis of tumor cells. Thus, an adenovirus mutant was engineered that replicates selectively in p53-deficient human tumor cells [41, 68]. Other examples are the adenoviral introduction of Smac antagonizes the inhibitor of apoptosis proteins in HCC tumor cells and enhances tumor cell death and tumor-specific replication-restricted adenoviral vectors [38, 86]. Further, the intravascular administration of a replication-competent genetically engineered herpes simplex virus (HSV)-1 resulted in oncolysis of a diffuse HCC [85]. More efficient HSV-1-based vectors have been developed [67].

### *Immune Therapy*

In the process of malignant transformation new antigenic surface proteins can be expressed (tumor antigens), oncofetal proteins can be re-expressed, for example alpha fetoprotein (AFP) and cytokine genes can induce cytolytisis of tumor cells.

#### AFP-Specific Immune Therapy

This strategy has been explored in mice and humans. Vaccination with an AFP-expressing DNA construct resulted in tumor rejection and prolonged survival in a mouse model [31]. Also in patients AFP-specific T cells could be detected [12, 13]. Since AFP is not only expressed by tumor cells but also by regenerating liver cells and in liver cirrhosis immunization against AFP carries the risk of autoimmune hepatitis, as has been experimentally shown in mice [28].

#### Immune Therapy with Antigen Presenting Cells

The use of antigen presenting cells (APC) is another strategy that has been explored employing dendritic cells (DC) exposed to tumor lysates, peptides or *ex vivo* tranduced with tumor antigen expressing DNA constructs. While this strategy is conceptually very interesting, to date there are no data available that demonstrate its clinical efficacy [49].

#### Cytokine Gene Therapy

Cytokine genes, such as tumor necrosis factor (TNF)-alpha, granulocyte macrophage colony stimulating factor (GM-CSF), interferon (IFN)-alpha or IFN-gamma, interleukin (IL)-2, -4, -6, -7, -12 and -18, B7–1, CD40 ligand and others have been explored in preclinical HCC animal models and in some patients with gastrointestinal tumors. Complete regression of a HCC was demonstrated *in vivo* by TNF-alpha, IL-2, IL-12 and an activatable interferon regulatory factor-1 in mice [3, 15, 46, 60]. Gene transfer was achieved *in vivo* by delivering retroviral or adenoviral vectors systemically, directly into the tumor or into the peritoneal cavity [15, 46]. A pilot study in patients with gastrointestinal tumors exploring the intratumoral injection of an adenoviral IL-12 expression construct showed only marginal efficacy, however [92].

## Molecular Prevention of Liver Diseases

DNA-based prophylactic vaccination against HBV infection, for example, is possible by intramuscular injection of a plasmid expressing hepatitis B surface antigen (HBsAg). HBsAg is taken up by cells *via* phagocytosis or endocytosis, processed through the major histocompatibility complex (MHC) class II system and primarily stimulates an antibody response through $CD4^+$ helper T cells with the production of anti-HBs [17–19, 72, 73, 84]. While the DNA-based

vaccination against HBV infection induces anti-HBs antibodies and prevents HBV infection, DNA-based vaccination against HCV infection of chimpanzees has been shown not to prevent infection but to result in the resolution of acute HCV infection through an effective vaccine-induced cellular immune response [24, 25].

## Summary and Perspectives

Molecular analyses have become an integral part of biomedical research as well as clinical medicine. The definition of the genetic basis of many human diseases has led to a better understanding of their pathogenesis and has in addition offered new perspectives for their diagnosis, therapy and prevention. Genetically, human diseases can be classified as hereditary monogenic, acquired monogenic and complex genetic diseases. Based on this classification, gene therapy is based on six concepts: gene repair, gene substitution, cell or organ transplantation, block of gene expression or function, gene augmentation and DNA vaccination. While the recent developments in gene therapy for liver diseases are promising, various delivery, targeting and safety issues need to be addressed before these strategies will enter clinical practice. Nevertheless, gene therapy will become part of the management of patients with liver diseases, complementing existing therapeutic and preventive strategies.

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# Gross and Microscopic Anatomy

# 106

Ulrich Beuers and Henryk Dancygier

## Chapter Outline



## Embryonic Development

The gallbladder and bile ducts are of endoblastic origin and develop, like liver parenchyma, from the hepatic diverticulum (see also section I) [4]. This liver bud derives from a thickening of the endoblastic epithelium in the anterior (ventral) foregut wall between days 18 and 22 of embryogenesis [7]. The human gallbladder anlage can be identified as early as day 29 of embryogenesis as a dilatation along the distal half of the hepatic diverticulum. The extrahepatic bile ducts are formed during withdrawal of the duodenum from the septum transversum and, thereby, drawing out of the stalk of the hepatic diverticulum. Two nearby buds form the pancreas [4].

## Gallbladder

The gallbladder is a distensible muscular pear-shaped sac closely attached to the undersurface of the right liver lobe. Its size varies from 6 to 12 cm in length and 2.5–5 cm in width and it may contain 20 up to 50 mL of bile. The gallbladder consists of a fundus, a corpus, an infundibulum and a neck which opens up to the cystic duct (Fig. 106.1). After emptying, the gallbladder wall forms multiple villous formations separated by crypts which may extend through the muscularis and form a network of "Rokitansky-Aschoff sinuses". These sinuses may be a source of inflammation in case of bacterial stasis, for example in cholecystitis due to gallstone disease.

The gallbladder wall is composed of a mucosa, a lamina propria, a muscularis, and a serosa (Fig. 106.2). The mucosa contains a surface epithelium with a single

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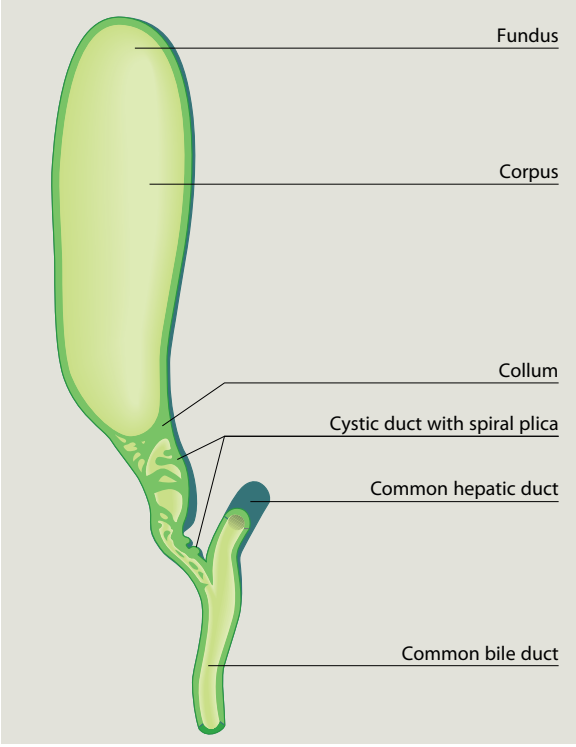


**Fig. 106.1** Gallbladder opened through a longitudinal section (According to [6])

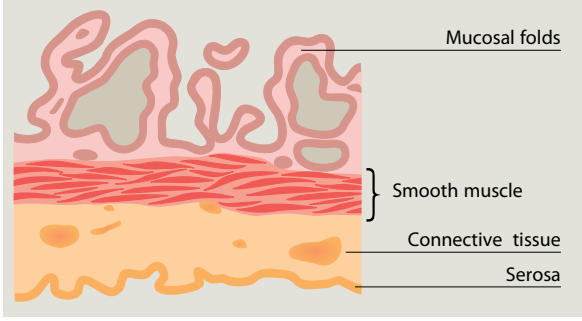


**Fig. 106.2** Cross section through the gallbladder wall with the typical mucosal foldings (According to [6])

layer of tall columnar cells. The nuclei are ovoid and situated at the basal pole. The apical membrane forms multiple microvilli. Tubuloalveolar mucus-forming glands are localized in the wall of the gallbladder neck where the mucous membrane forms a spiral valve (of Heister) which contributes to regulation of bile flow in and out of the gallbladder. The surface epithelium is attached to a lamina propria which contains blood and lymph vessels. A muscularis mucosae and a submucosa are lacking. The muscularis propria is composed of smooth muscle bundles which are oriented in longitudinal, circular and oblique directions. The adventitia is a layer of connective tissue containing blood and lymphatic vessels, nerve fibers, and diverse cell types including ganglion cells, lymphocytes, and lipocytes. In the wall attached to the liver, small bile duct structures, possibly of embryonic origin, and directly connected to intrahepatic bile ducts can sometimes be identified ("Luschka's bile ducts"). These may become a cause of biliary leakage after cholecystectomy.

The gallbladder is supplied by the cystic artery, an end artery, which commonly originates from the right hepatic artery. The cystic vein drains blood from gallbladder and cystic duct usually to the portal vein. Lymphatic vessels of the gallbladder drain into a lymph node at the neck of the gallbladder. Sympathetic (s) and parasympathetic (ps) nerve fibers accompanying the hepatic artery and the portal vein form a network in the gallbladder wall and are relevant for transmission of pain (s) and gallbladder contraction (ps) and relaxation (s).

## Extrahepatic Bile Ducts

The extrahepatic bile ducts consist of the right and left hepatic ducts which may show wide variations in form and number, and converge at the hilum 1–2 cm outside the liver surface to form the common hepatic bile duct. The cystic duct connects the S-shaped gallbladder neck over a length of about 4 cm with the common hepatic duct. Together, they form the common bile duct which passes dorsal to the upper duodenum to the pancreatic head, and enters the duodenal wall after fusion with the pancreatic duct (90% of cases; separate openings of bile duct and pancreatic duct in 10%) to form a common channel (Y-pattern) or just a common opening (V-pattern) [1]. The duodenal opening of the common bile duct and the pancreatic duct at the papilla of Vater is tightly controlled by a circular muscle, the sphincter of Oddi (Fig. 111.1) [5].

The wall of the extrahepatic ducts consists of a columnar epithelium of cholangiocytes, a fibromuscular layer, and a serosa. The muscularis of the human extrahepatic bile ducts contains thin, mainly longitudinally oriented layers of smooth muscle which may contract sporadically, but in contrast to some

animal species, appear unable to exhibit effective peristaltic activity [8].

A dense network of sympathetic, parasympathetic and peptidergic nerve fibers surrounds the bile ducts in submucosal and subserosal layers. The sphincter of Oddi in particular is innervated by a dense network of cholinergic and peptidergic neurons.

The common bile duct and the hepatic ducts are lined by simple columnar epithelium. Tubulo-alveolar intramural glands are a regular finding. In the terminal common bile duct the epithelial cells sit on a loose layer of connective tissue, the tunica fibromuscularis that becomes more and more dense as it approaches the hilum of the liver (Fig. 106.3). The ampullary epithelium forms crypt-like folds (Fig. 106.4). The various *cell types* of the human extrahepatic bile ducts have been studied on transpapillary biopsies obtained during endoscopic retrograde cholangiography [2, 3]. The superficial epithelium of the extrahepatic bile ducts contains five different cell types (Table 106.1).

**Table 106.1** Cell types in the epithelium of human extrahepatic bile ducts

- Main epithelial cells
- Goblet cells
- Tuft cells
- Endocrine/paracrine cells
- Lymphocytes

The most frequent cell type occurring in extrahepatic human bile ducts is the *main epithelial cell*. This cell has a regular polygonal or cylindrical form. Beneath the microvillous brush border there is a terminal web. In the supranuclear region many mitochondria are present. Lysosomes and peroxisomes are found less frequently. Many cells have a prominent Golgi apparatus. Secretory vacuoles, as a further sign of their metabolic activity, are frequently found in the cytoplasm. The vesicles of the smooth endoplasmic reticulum are often grouped together in separate cell compartments. The rough endoplasmic reticulum is only poorly developed. The epithelial cells are in close contact with one another. This is accomplished by indentations of neighboring cell membranes as well as by desmosomes (Fig. 106.5).

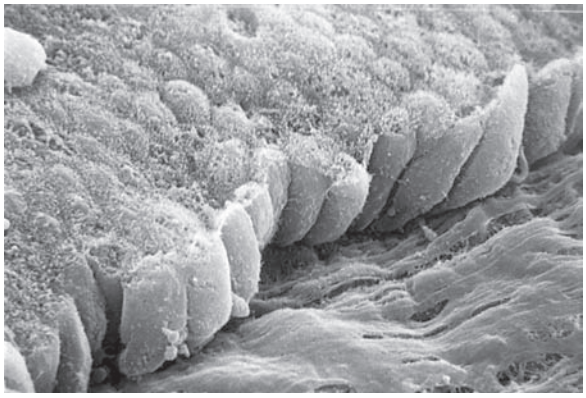

**Fig. 106.3** Endoscopic transpapillary biopsy of the common bile duct. The tall columnar epithelial cells of the common bile duct rest on a dense fibromuscular layer. Scanning electron microscopy (From [3])

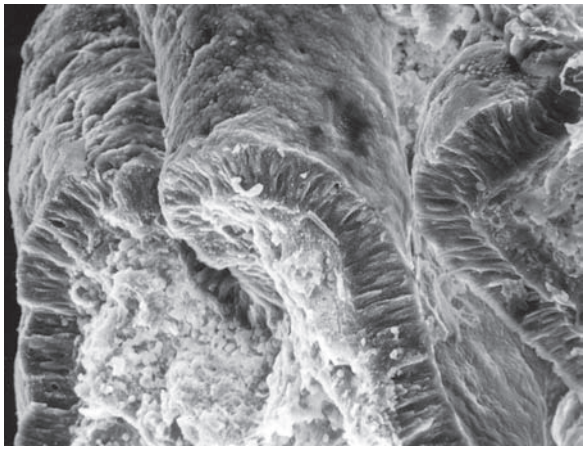

**Fig. 106.4** Ampullary biopsy. The epithelium forms crypt-like invaginations. Scanning electron microscopy (From [3])

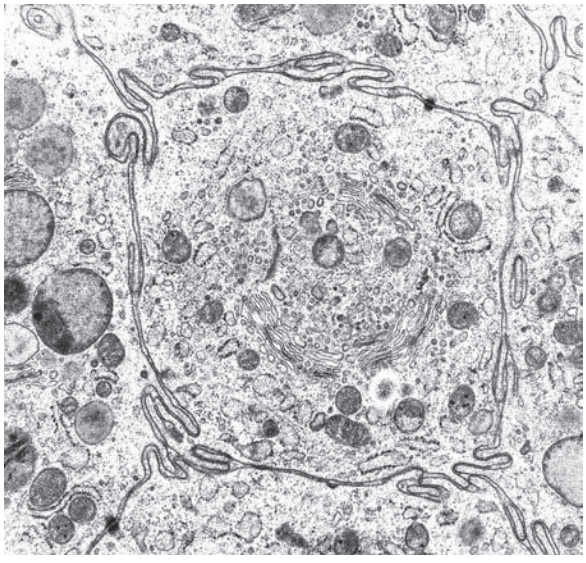

**Fig. 106.5** Cross-section of surface epithelial cell of common bile duct displaying a well-developed Golgi apparatus and indentations of cell membranes of neighboring cells. Transmission electron microscopy (From [3])

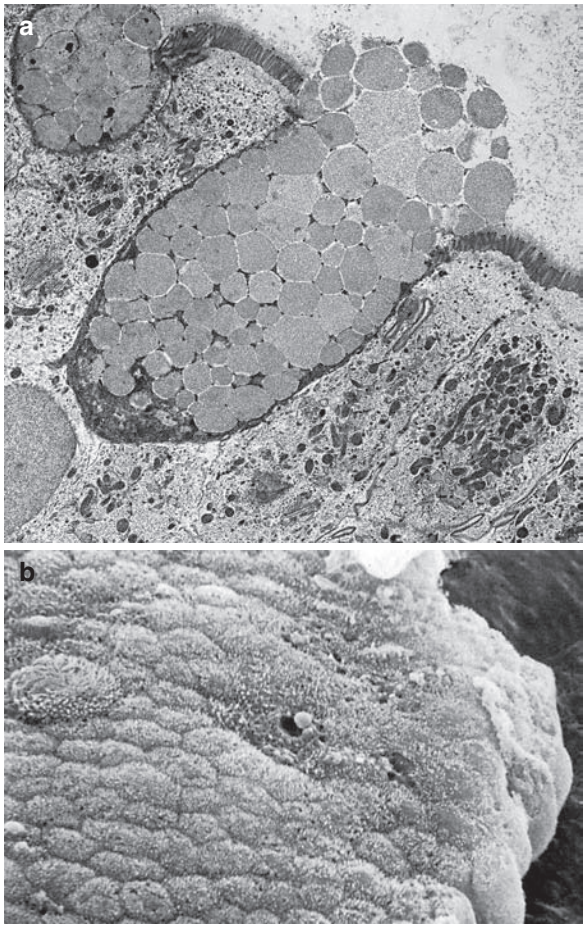


**Fig. 106.6** (**a**) Goblet cell in common bile duct. Transmission electron microscopy. (**b**) Endoscopic transpapillary biopsy of common hepatic duct. A single goblet cell discharging its mucous content is seen at the center of the micrograph. The epithelial cells of the hepatic duct present a well-developed brush border. Scanning electron microscopy (From [3])

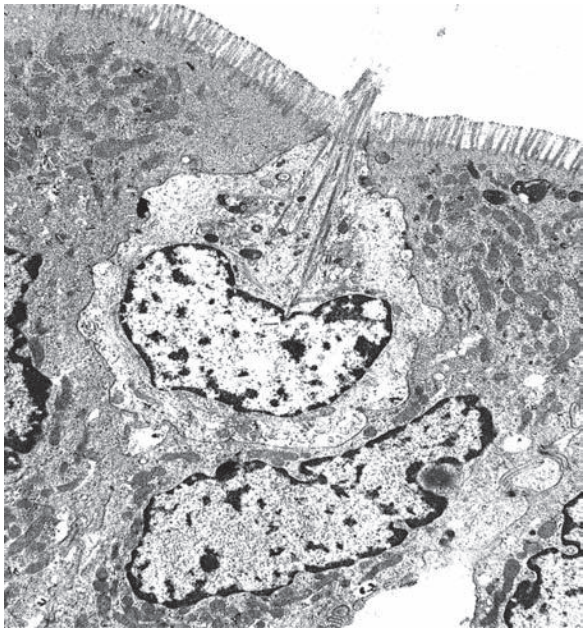

**Fig. 106.7** Tuft cell (From [3])

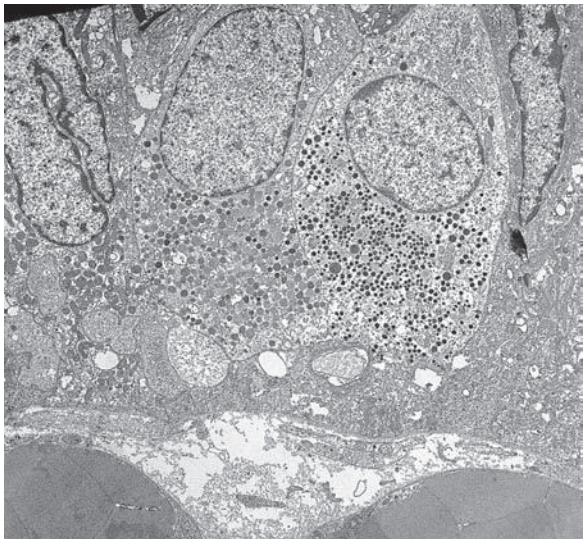

**Fig. 106.8** Two endocrine/paracrine cells with characteristic infranuclear granules. The cell on the left is a somatostatin-storing D-cell (From [3])

Scattered *goblet cells* are found only rarely in normal bile ducts (Fig. 106.6). In chronic cholangitis their number may increase markedly.

The so called *tuft cell* is a very rare intraepithelial cell type that occurs in the ampullary epithelium (Fig 106.7). This cell is round to oval, its cytoplasm is less osmophilic than that of neighboring epithelial cells, and it contains some lysosomes and few mitochondria. As compared with the main epithelial cell, fewer organelles are present. The nucleus is situated basally and is oval in shape. Typical for this cell type is the microvillous tuft that protrudes beyond the level of the brush border of neighboring cells into the duct lumen. The microvilli possess thick central microfilaments that reach deep down into the

cytoplasm where they are anchored in the microfilamentous cytoskeleton.

*Endocrine/paracrine cells* are a rare but regular constituent of the extrahepatic bile ducts (Fig. 106.8). They are most prevalent in the epithelium of the ampulla and cystic duct, but are scattered throughout the entire extrahepatic bile duct system. These cells contain various amines and peptides, such as serotonin and somatostatin [2].

Vasoactive intestinal peptide (VIP) immunoreactivity is found throughout the entire extrahepatic bile duct system and is confined to nerve fibers running beneath the epithelium and within the muscle layer. The corresponding nerve cell bodies are located mainly in the wall of the cystic duct.

*Lymphocytes* belonging to the mucosa associated lymphatic tissue are found throughout the entire extrahepatic bile duct system. Intraepithelial lymphocytes are predominantly T helper cells, while intramural lymphocytes mostly belong to the suppressor/cytoxic subtype. Approximately 1–2% of intraepithelial lymphocytes are large granulated lymphocytes, i.e. cells exhibiting natural killer activity

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# Physiology of the Gallbladder and the Extrahepatic Bile Ducts

# 107

Ulrich Beuers

## Chapter Outline



## Physiology of the Gallbladder

### *Bile Storage and Excretion*

In humans, the gallbladder serves as a storage reservoir for bile secreted by liver cells and for bile modified by cholangiocytes. Approximately 50% of hepatic bile is taken up by the gallbladder, with the remainder directly released into the duodenum. During interdigestive storage, bile is effectively concentrated to 10–20% of the original volume entering the gallbladder. Removal of electrolytes and water from gallbladder bile is mediated by active and passive mechanisms, among which coordinated $Na^+/H^+$ and $Cl^-/HCO_3^-$ exchange via a highly expressed type 3 $Na^+/H^+$ exchanger (NHE-3) and the $Cl^-/HCO_3^-$ exchanger 2 (AE2) may play a pivotal role for transepithelial transport of NaCl to the serosal side. Water absorption is assumed to occur secondary to NaCl transport across the mucosa mainly via aquaporins. Solute transporters like the apical bile salt transporter, ASBT, and the organic anion transporting protein, OATP-A, may mediate uptake of conjugated bile acids and other organic anions, and conjugate export pumps may mediate apical transport of various organic anions into the gallbladder lumen (MRP2) and basolateral transport into the blood (MRP3). However, their exact contribution to solute exchange across human gallbladder mucosa is yet unclear.

Bile acids as the major organic solutes of bile (by weight, 68% bile acids; 22% phospholipids, 4% cholesterol, 4.5% proteins, 0.5% bilirubin) reach concentrations up to more than 100 mmol/L in the gallbladder and can, thereby, be secreted into the intestinal lumen in a highly concentrated form [5]. Bile acids have a critical function in digestion and mucosal defense. They are mediators of intestinal lipid absorption by forming mixed

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micelles with dietary lipids in the intestinal lumen. Bile acids exert signaling properties at physiological concentrations in all cell types equipped with bile acid transporters such as hepatocytes, cholangiocytes and intestinal mucosal cells. Thereby, they may modulate expression and function of proteins involved in biotransformation and transport of exogenous and endogenous substrates on a transcriptional level via interaction with nuclear receptors, e.g. the farnesoid X receptor, FXR. In parallel, bile acids interact with a wide range of cellular signaling cascades including cytosolic free $Ca^{++}$, protein kinase C (PKC) isoforms, mitogen-activated kinases (MAPK) Erk1/2, p38MAPK, and JNK, phosphoinositol-3-kinases (PI3K), protein kinase B (PKB), protein kinase A (PKA) and numerous others involved in short-term posttranscriptional regulation of biotransformation and secretion [2, 3]. Bile acids act also as modulators of intestinal peptide hormone secretion such as fibroblast growth factor 19 (FGF19). FGF19 is involved in the regulation of bile acid metabolism and gallbladder filling.

**Table 107.1** Agents affecting gallbladder motility

| Stimulus | Gallbladder contraction | Gallbladder relaxation |
|---|---|---|
| Acetylcholine | + | |
| Cholecystokinin (CCK) | + | |
| Fibroblast growth factor (FGF19) | | + |
| Gastrin releasing peptide (GRP) | + | |
| Histamine | | |
| (via H1) | + | |
| (via H2) | | + |
| Motilin | + | |
| Neuropeptide Y | + | |
| Neurotensin | | + |
| Pancreatic polypeptide | | + |
| Peptide YY | | + |
| Secretin (with CCK) | + | |
| Somatostatin | | + |
| Substance P | + | |

## *Gallbladder Motility*

Contraction and relaxation of the gallbladder are under complex neurohumoral control and are intimately related to the tone of the sphincter of Oddi. Cholecystokinin (CCK) is a potent stimulus for a contractile response of the gallbladder. CCK is released upon ingestion of a fat- or protein-rich meal from the proximal small intestine. Serum levels correlate with the contractile response of the gallbladder. CCK induces a slow contraction of the gallbladder with markedly enhanced gallbladder bile secretion into the duodenal lumen over a period of 20 min. In addition, gastrin releasing peptide (GRP), histamine (via H1 receptors), motilin, neuropeptide Y, secretin (together with CCK), and substance P stimulate gallbladder contraction. In contrast, FGF19, histamine (via H2 receptors), neurotensin, pancreatic polypeptide, peptide YY, and somatostatin are among the signaling agents which counteract contraction and induce gallbladder relaxation (Table 107.1).

Vagal stimulation may lead to gallbladder contraction whereas stimulation of sympathetic nerves may lead to gallbladder distension. Postprandial gallbladder contraction is controlled by central nervous, gastric and duodenal stimuli and may result in >75% bile expulsion. Central nervous effects may be responsible for up to 40% of gallbladder emptying as has been shown in subjects after a virtual dinner. Gastric distension and duodenal stimulation of CCK release after meal ingestion then increases gallbladder contraction to more than 75%. In the interdigestive phase, the gallbladder partially contracts at regular intervals to release up to 40% of its contents [4].

## Physiology of the Extrahepatic Bile Ducts

Human bile duct epithelia contribute a volume of 25% (–40%) of an alkaline, bicarbonate-rich fluid to daily bile secretion. It is assumed from animal studies that mainly cholangiocytes of "large" ductules and ducts with a diameter > 15 µm are responsible for bicarbonate-rich fluid secretion. Cholangiocytes are equipped with a wide variety of apical and basolateral transport proteins which allow for transport of solutes from bile to blood ("cholehepatic shunt" of solutes like bile acids secreted into bile and returning to the liver via cholangiocytes and the peribiliary plexus) and secretion of electrolytes, e.g. $Cl^-$ and $HCO_3^-$ into bile (see also Chapter 52). The apical cAMP-sensitive $Cl^-$ channel CFTR and the apical $Cl^-$/ $HCO_3^-$ exchanger 2 (AE2) play a pivotal role for alkalinization of bile. In addition, apical $Ca^{++}$-sensitive $Cl^-$ channels may contribute to $HCO_3$-rich choleresis. Biliary $Cl^-$ secretion may cause paracellular $Na^+$ movement

into the bile duct lumen followed by transcellular water movement along an osmotic gradient via water channels (aquaporins), further enhancing cholangiocellular fluid secretion. Basolateral $Na^+$ uptake is mediated by $Na^+/H^+$ exchange via NHE1 and the $Na^+/K^+/2Cl^-$ cotransporter NKCC1, whereas apical $Na^+$ uptake is mediated by the $Na^+/H^+$ exchanger NHE2.

The apical sodium bile acid transporter, ASBT, together with the basolateral bile acid transporters, OSTα/OSTβ and MRP3, may mediate "cholehepatic shunting" of conjugated bile acids from bile to the peribiliary plexus. Unconjugated bile acids (e.g., C24 dihydroxy and C23 nor-dihydroxy bile acids) may enter cholangiocytes via the apical membrane in exchange for $HCO_3^-$.The role of a basolateral truncated ASBT in basolateral bile acid secretion is less clear. It is also unclear whether relevant amounts of bile acids are shunted under physiological conditions across cholangiocytes from bile to the peribiliary plexus via the above-mentioned mechanisms or whether these mechanisms only provide an "escape strategy" under conditions of biliary obstruction.

Interestingly, receptor-mediated regulation of cholangiocyte secretion occurs both via the basolateral and apical membrane indicating that blood- as well as bile-derived signaling agents may affect cholangiocyte function. Stimuli of cholangiocyte $HCO_3^-$ secretion include secretin together with CCK, acetylcholine, ATP, or VIP. Inhibitors of cholangiocyte $HCO_3^-$ secretion include endothelin, insulin, or somatostatin. Purinergic (P2γ) receptors, insulin receptors and insulin-like growth factor-1 (IGF-1) receptors have been identified on apical cholangiocyte membranes. They support the concept of *hepatocyte-cholangiocyte crosstalk* via e.g. ATP, insulin or IGF-1 in bile. Cholangiocyte-hepatocyte crosstalk via cholangiocyte-derived IGF-1, nerve growth factor (NGF) or vascular endothelial growth factor (VEGF) secreted into the peribiliary plexus back to the liver has also been described [1].

Bile acids – like various hormones and cytokines – may exert regulation of cholangiocyte secretion in a way similar to their modulation of hepatocellular secretion [3]. The therapeutic bile acid ursodeoxycholic acid (UDCA) is known to stimulate $HCO_3^-$ secretion into bile both in man and in experimental animals. It has been speculated that UDCA might stimulate cholangiocyte $HCO_3^-$ secretion through $Ca^{++}$-dependent mechanisms via activation of a $Ca^{++}$-dependent $Cl^-$ channel different from CFTR and concomitant stimulation of $Cl^-/HCO_3^-$ exchange via AE2

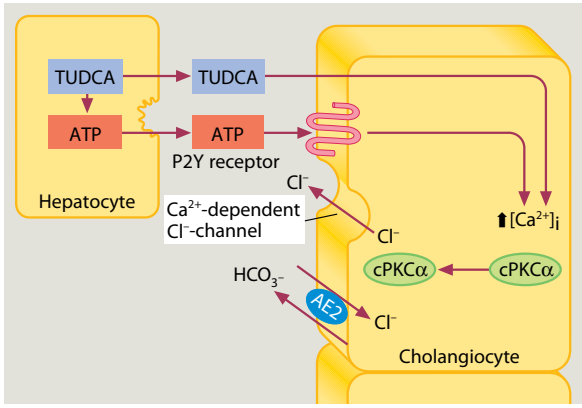


**Fig. 107.1** Model of a crosstalk between hepatocytes and cholangiocytes: modulation of cholangiocyte secretion by taurine-conjugated ursodeoxycholic acid (TUDCA) [3]. For details, see text

(Fig. 107.1). UDCA conjugates increase the concentration of cytosolic free $Ca^{++}$ in cholangiocytes and increase membrane binding of $Ca^{++}$-sensitive cPKCα, mechanisms similar to those observed in hepatocytes [3]. In addition, UDCA conjugates might also increase the concentration of cytosolic free $Ca^{++}$ in cholangiocytes indirectly by stimulating hepatocellular ATP secretion into bile which then might induce $Ca^{++}$-dependent $Cl^-$ secretion via apical P2γ ATP receptors (Fig. 107.1).

## Physiology of the Sphincter of Oddi

The sphincter of Oddi controls release of bile (and pancreatic juice) into the duodenum [4]. A remarkable species variability exists in motility and function of the sphincter of Oddi. The human sphincter of Oddi exerts a basal pressure 3 mmHg above the low pressure in the bile ducts and the pancreatic ducts. Superimposed on the basal pressure, phasal contractions are observed at a rate of about 4/min (see also Chapters 47 and 111). Bile flow after a meal is promoted by both a reduction of the phasic contractions and a fall in the sphincter of Oddi basal pressure. Various hormones and a dense network of nerves tightly control the function of the sphincter of Oddi. Among these, met-enkephalin, morphine, neuropeptide Y, nitric oxide, and substance P have been shown to stimulate sphincter of Oddi contraction whereas CCK, calcitonin gene-related peptide (CGRP), glucagon, peptide YY, somatostatin, and tramadol inhibit sphincter of Oddi activity [4].

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# Anomalies of the Gallbladder and the Cystic Duct

# 108

Michael A. Kern and Peter Schirmacher

## Chapter Outline



Anomalies of the gallbladder and extrahepatic bile ducts can occur in relation to number, position or shape. Several of these anomalies have clinical significance. The knowledge of such anomalies is essential not only for the diagnosis of biliary disease but also with respect to the growing abundance of minimally invasive therapeutical strategies in the treatment of gallbladder and extrahepatic bile duct diseases.

## Numerical Anomalies of the Gallbladder

*Agenesis of the gallbladder* occurs in up to 0.13% of the population and it is the result of a failure of the cystic bud to develop. Patients with this anomaly fall into one of three broad categories: children with multiple congenital anomalies, asymptomatic adults in whom the abnormality is first discovered at autopsy, and symptomatic adults who have cholelithiasis. In the case of gallbladder agenesis, the cystic duct is also almost invariably absent and the surface of the liver may not contain the usual corresponding depression [3, 16, 25]. Other congenital anomalies that have been associated with agenesis include polycystic kidney disease, tracheoesophageal fistulae, cardiac anomalities, annular pancreas, Kippel–Feil syndrome, and gastrointestinal anomalies, including imperforate anus, duodenal atresia, and shortening of the ascending colon [13, 17, 29]. This anomaly may also accompany congenital hypoplasia of the right lobe of the liver.

A *hypoplastic gallbladder* may occur when the caudal bud undergoes incomplete development or when the solid stage of the bud is not recanalized. This anomaly may be found in association with congenital

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biliary atresia and in cystic fibrosis. This condition should be differentiated from an acquired postinflammatory, fibrotic shrinkage of the gallbladder. Although an absent gallbladder usually remains asymptomatic, the risk of developing cholelithiasis is increased. Through the preoperative determination of an absent gallbladder, extirpation of a presumably symptomatic gallbladder can be avoided [9].

*Duplication of the gallbladder* is uncommon (4 out of 1,000 cases) (Fig. 108.1). Both gallbladders may drain into their own separate cystic ducts or via one bifid duct. There are rare examples of cases in which one of the cystic ducts drains directly into either the left or right hepatic duct or even into the duodenum. Duplication of the gallbladder may also be associated with abnormalities of vascular supply, including an anomalous anterior displacement of the right hepatic artery [9].

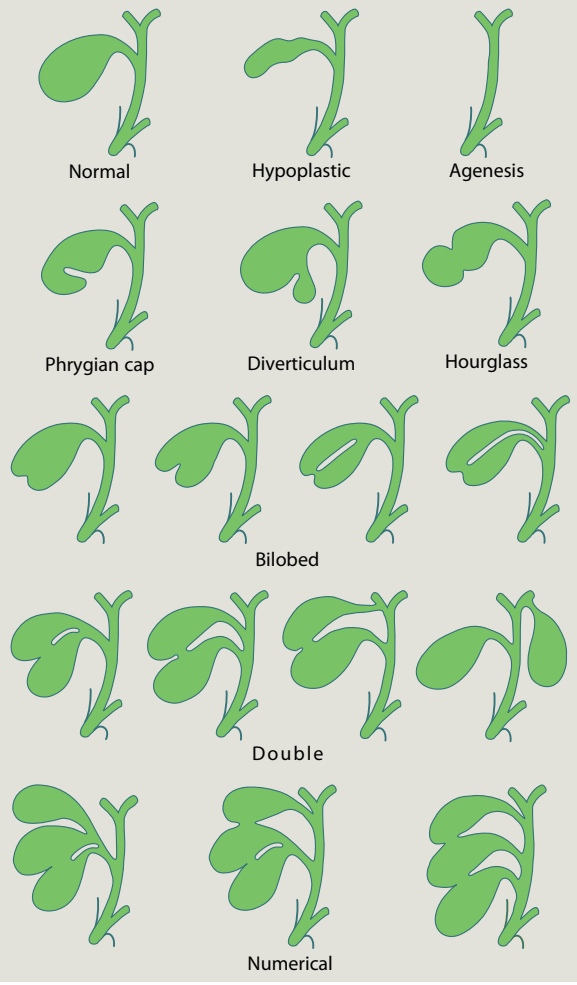


**Fig. 108.1** Variations of the gallbladder

An incomplete duplication of the gallbladder (vesica fellea divisa), may be differentiated form a complete duplication (vesica fellea duplex). A *vesica fellea divisa* is characterized by a dividing wall containing all wall components (in contrast to a septated gallbladder, vesica septa, see below), which separates the fundus and, in some cases, the corpus of both gallbladders as well. The neck portion of the gallbladder is not duplicated and only one cystic duct is present. The external shape of the gallbladder may be completely normal. In the case of a *vesica fellea duplex*, which is approximately five times more frequent, two main forms are differentiated: the Y-type and the H-type (the most frequent variation). The Y-type is characterized by the confluence of both cystic ducts to a common end portion whereas the H-type denominates two complete gallbladders and two separate cystic ducts, which either enter the common hepatic duct separately or they enter the common hepatic duct as well as the right or left hepatic duct. The location and size of these gallbladders can vary. A triplicate of the gallbladder is an extreme rarity [9, 10 15, 19, 22, 30].

## Positional Anomalies of the Gallbladder

Minor malpositionings of the gallbladder, which are usually of little clinical significance, include an intrahepatic gallbladder and a floating gallbladder (i.e. one that is distracted from the surface of the liver by its own mesentery). *Intrahepatic gallbladders* rarely cause specific clinical problems, although basically they are subject to diseases commonly encountered in normally positioned organs. These diseases include inter alia cholecystitis, cholelithiasis, and abscess formation. Problems may, however, be encountered at cholecystectomy, when liver tissue may need to be excised to allow for adequate exposure of the gallbladder. In most instances, however, the gallbladder is not deeply buried in the liver. Intrahepatic gallbladders may also cause diagnostic problems on imaging studies if the possibility of a malpositioned organ is not considered.

In the case of the *floating gallbladder*, the gallbladder may be completely covered by peritoneum, and the fixation of the organ is achieved through the

supplying vessels as well as a string-like fold of the peritoneum ending at the ventral edge of the liver. This anomaly is pathologic, as a rotation of the vessels can cause ischemia and subsequent gangrene of the gallbladder. The presence of a floating gallbladder has also been associated with hypoplasia of the left lobe of the liver.

Rarely, one will find a *left-sided gallbladder*, in which the cystic duct joins either the common hepatic duct or the right or left hepatic ducts. Equally seldom is a retro-duodenal position of the gallbladder [9, 12].

## Internal and External Morphological Anomalies of the Gallbladder

A *multiseptate gallbladder* is a congenital anomaly that is readily diagnosed by ultrasound examination. The lumen is partitioned by thin septa covered by normal mucosa and consisting of a muscle layer and a lamina propria in continuity with the bladder wall. The septa may be fenestrated or noncommunicating (Figs. 108.1 and 108.2).

Both the body and the fundus of the gallbladder can display a congenital kink. In its complete form the kinking includes the serosa, and in its incomplete form it is sub-serous in nature and the surface of the gallbladder appears normal. If the fundus of the gallbladder shows kinking the condition is called a *"phrygian cap."* This anomaly accounts for approximately 5% of cases and there are no associated pathophysiological processes [9, 32].

A congenital *stricture of the gallbladder* has also been described. This anomaly can be present in various parts of the gallbladder. When the gallbladder is constricted around the middle of the organ as the result of an anomaly in the gallbladder bed it is referred to as an "hourglass" gallbladder. Rarely, a true congenital gallbladder diverticulum can be found, in which the complete organ wall exists within the diverticulum. The usual location for such diverticula are the fundus and the neck (Fig. 108.1).

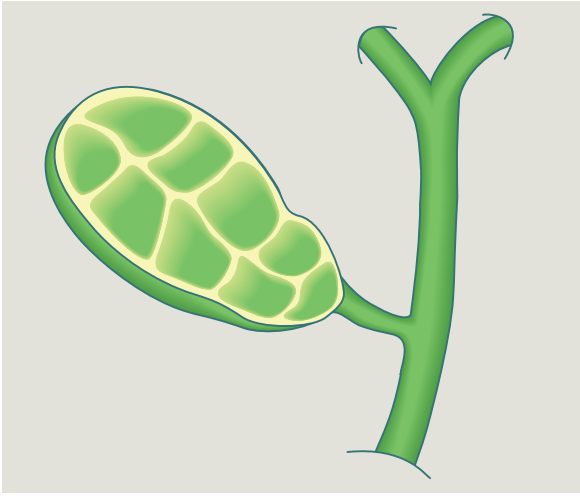

**Fig. 108.2** Multiseptate gallbladder

## Anomalies of the Cystic Duct

Knowledge of the variations of the cystic duct is important, especially to avoid injury when performing laparoscopic and open cholecystectomy. Approximately 13% of individuals show some variations of the bile ducts, including abnormalities of the cystic or common bile ducts and the presence of accessory hepatic ducts (Table 108.1).

Usually the cystic duct, 4 cm long on average, joins the common hepatic duct at an acute angle from the right side approximately 3 cm distal to the confluence of the right and left hepatic ducts. There are many variations, for which the frequencies have been reported in wide ranges. The union with the common bile duct may occur abnormally high or low. The union may occur from the left instead of from the right side, after crossing the front or from behind. It is also possible for the cystic duct to join into the right or, very rarely, the left hepatic duct. Even if only one gallbladder is present, two cystic ducts may connect the gallbladder with the common hepatic duct. A few cases of cystic dilation of the cystic duct, with or without the presence of stones, have been reported. An isolated duplication of the cystic duct is extremely rare and, when present, it can be associated with a highly variable union of both ducts (Fig. 108.3) [1, 6, 7].

**Table 108.1** Anomalies of the extrahepatic biliary tract

| |
|---|
| Stenosis and atresia |
| Cystic disease of the common bile duct |
| Accessory hepatic duct |
| Malpositioning and reduplication of the common bile duct |

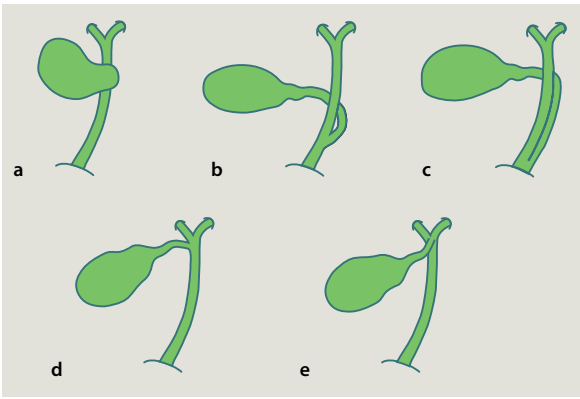


**Fig. 108.3** Examples of anomalies of the cystic duct. (**a**) Absent cystic duct. (**b**) Cystic duct joining common bile duct at lower level. (**c**) Long cystic duct fused to the common bile duct. (**d**) Cystic duct joining the right hepatic duct. (**e**) Cystic duct joining at the junction of both hepatic ducts

## References

for Chapter 108 are listed at the end of Chapters 110 (page 1437)

# Anomalies of the Extrahepatic Bile Ducts 109

Michael A. Kern and Peter Schirmacher

## Chapter Outline



Variations in the course, form and number of extrahepatic bile ducts are frequent. Thus, the differentiation between a variation of a normal and pathological state is not always easy. The knowledge of such variations is especially important with regard to operative and non-operative bile duct interventions.

## Anomalies in the Number and/or Course of the Extrahepatic Bile Ducts

The right hepatic duct can join the cystic duct, which is a clinically important anomaly. If, intraoperatively, this duct is ligated distal to the union, the bile flow from the right liver lobe will be blocked. The right hepatic duct can also drain directly into the gallbladder. Both hepatic ducts can join the duodenum directly without first uniting, whereby in these cases, the right hepatic duct usually incorporates the cystic duct [8, 18].

Duplication of the common bile duct, i.e. splitting after the union of both hepatic ducts, is very rare. Both ducts may then join the gastrointestinal tract in a highly variable pattern along different portions of the GI-tract (see below).

Course anomalies of the bile duct are merely variations in location of the junction with the gastro-intestinal tract. This usually occurs in the middle third of the descending part of the duodenum, but it can vary both in an oral and aboral direction. Rare cases may include a proximal shift as far as the stomach or a distal shift as far as the ileum or even the colon [20].

True accessory (i.e. additional) or aberrant bile ducts with connection to the gallbladder or the cystic duct are generally rare but usually have normal bile drainage capacity; however, when present, they have important surgical considerations because, if overlooked,

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they can cause bile leakage as well as bile drainage disturbances.

A true rarity is the congenital bronchobiliary fistula, i.e. an inborn connection between the respiratory tract and the biliary system.

While the Sphincter of Oddi usually includes both the distal bile duct as well as the segment of the pancreatic duct that is in close proximity to the duodenum, the union of the bile and pancreatic ducts may occur above the sphincter and lead to the formation of a "common channel". This anomaly, which has also been discussed as a possible cause of a bile duct cyst, has been shown to increase the risk of malignancy. When a bile duct cyst is present, the malignancy usually occurs in this duct or in the gallbladder; however, when a bile duct cyst is not present, a carcinoma can only be found in the gallbladder.

## Form Anomalies of the Extrahepatic Bile Ducts

### *Congenital Bile Duct Cysts*

Idiopathic (congenital) bile duct cysts refer to dilatations of the extrahepatic biliary tract and are therefore not cysts in an anatomical sense. They vary in size and location and can be present singularly or in multiplicity (Fig. 109.1) [2, 4, 6]. The separation of the common hepatic duct or the cystic duct can also be affected by this dilation; the transition to the rest of the biliary tract is always prominent. Because this dilation can occur proximal to the junction of the cystic duct with the common hepatic duct, the term "biliary cyst" should be used strictly for this case, contrary to common clinical terminology. This term also includes intrahepatic biliary cystic lesions. According to the Todani classification, *5 types of cystic biliary lesions* can be differentiated [33]:

- Type Ia: solitary, fusiform (spindle-shaped), extrahepatic cystic transformation of the bile duct with cystic duct involvement (about 80% of cases)
- Type Ib: segmental, extrahepatic dilation
- Type Ic: diffuse or columnal extrahepatic dilation
- Type II: saccular-like, extrahepatic supraduodenal cysts
- Type III: intraduodenal diverticulum (choledochocele)
- Type IVa: intra- and extrahepatic fusiform cysts
- Type IVb: multiple extrahepatic cysts
- Type V: solitary or multiple intrahepatic cysts (Caroli syndrome) (see Chapter 56)

Depending on the size of the cysts, compression of neighboring structures is often the consequence and a chronic bile stasis is often found proximally to the cysts. All cysts (with the exception of choledochoceles) histologically demonstrate scattered islets of columnar epithelium; typical biliary epithelium is not present. The relatively thick wall contains predominantly connective tissue with a small amount of smooth muscle. Depending on the age of the patient, inflammatory changes of the wall can be seen with increasing frequency. Choledochoceles are usually lined by duodenal mucosa or, more rarely, by biliary or mixed mucosa.

The clinical presentation is typically characterized by one or more of the following symptoms: jaundice, swelling, and/or pain in the right upper quadrant. If the disease manifests itself in the first few weeks of life, jaundice is often the only symptom. If the biliary cyst first becomes symptomatic after the first decade of life, it may cause abdominal pain occasionally accompanied by intermittent jaundice. Whether the development of biliary cysts can pathogenetically lead to a related wall weakness, inflammatory processes, or a distal drainage blockade, has not yet been decided. The

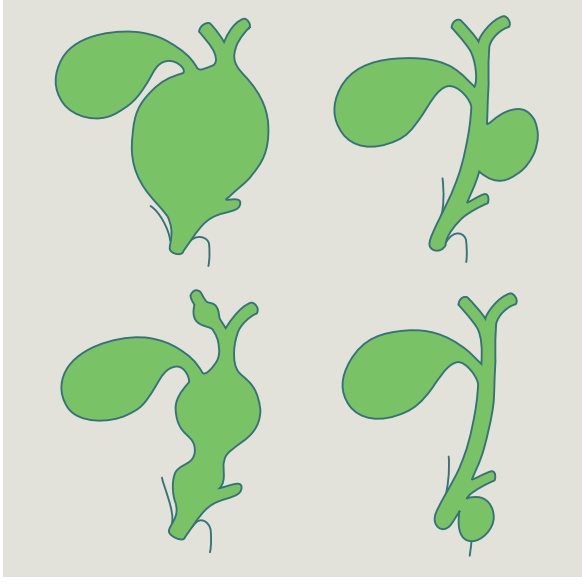

**Fig. 109.1** Different types of congenital cystic dilatations of the common bile duct

anomalous junction of the bile duct with the pancreatic duct distally to the duodenum with a long "common channel" is most frequently associated with the common form of biliary cysts (Type I), as it is most often seen in association with these cysts. Because the union lies above the Sphincter of Oddi, pancreatic secretions can flow into the bile duct without hindrance, which can promote inflammatory destructive biliary tract alterations even *in utero*. The clinical importance of biliary cysts lies in their association with increased stone formation and cholangitis. This may lead to secondary biliary cirrhosis and an increased frequency of carcinoma within the area of the dilatation in approximately 20% of cases. Histologically, these are adeno-, squamous cell- and undifferentiated carcinomas. Therapeutically, operative draining methods (i.e. choledochocystojejunostomy) have been replaced by methods involving the removal of the dilated biliary segment and bilioenteric anastomoses (usually a hepaticojejunostomy). The advantage of resection lies in the low rate of stone formation and subsequent recurrent cholangitis, as well as the decreased risk for carcinoma. Choledochoceles can be differentiated from the other choledochal cysts histologically (see above). To achieve free drainage, an opening to the duodenum or an endoscopic sphincterotomy is performed [27, 34, 35].

## Congenital Biliary Atresia and Stenosis

Congenital biliary atresia describes a wide spectrum of inborn obliterative cholangiopathies and ranges from complete absence of the entire intra- and extrahepatic biliary system to a singular, localized stenosis of the bile duct (see also Chapter 56) [5]. Altogether, its incidence is approximately 1/10,000 live births. The cause of the differing forms of biliary atresia is not clear. Several acquired etiologies have been discussed, especially exposure to viruses in the later stages of pregnancy. Metabolic causes or genetic defects may also be involved. Histologically, fibrosis-inducing, inflammatory and angioproliferative changes are found in areas of structural bile duct remnants. In approximately 25% of cases this condition is associated with extrabiliary malformations.

The diagnosis is usually made within the first weeks of life after clinical symptoms including persistent jaundice, acholic stools and hepatomegaly prompt further diagnostic evaluation. These diagnostics include, in addition to radiologic imaging, liver biopsy. Characteristically, portal edema, ductular reaction, ductular bile thrombi and swelling of hepatocytes with the formation of giant multinucleated hepatocytes and detection of neutrophil granulocytes is observed.

The respective malformation is characterized by the location of the atretic lesion; that is, whether the common bile duct, hepatic duct, cystic duct and gallbladder as well as the intrahepatic biliary tract are involved. Clinically, the Kasai classification is used for extrahepatic biliary atresia (Fig. 109.2) [21]. Without therapy, there is a risk of hemorrhage as the result of vitamin K deficiency (from a resorption deficiency), infection, and liver failure. In the case of solitary extrahepatic atresia, proper intrahepatic biliary structures can be demonstrated histologically. In the more unfavorable forms of the disease, the intrahepatic biliary tract is involved in the destructive process, which can advance to progressive liver failure even after a successful Kasai's portoenterostomy. While an operative correction is not possible in the case of complete atresia, extrahepatic atresia can be successfully treated before irreversible liver damage results if diagnosed early

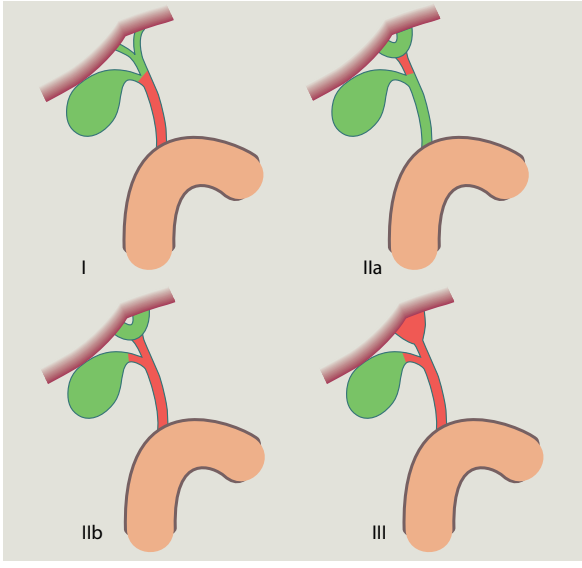


**Fig. 109.2** Classification of biliary atresia according to Kasai. Type 1: closure of the common bile duct; Type 2a: closure of the common hepatic duct; Type 2b: closure of the common hepatic duct, the common bile duct and the cystic duct as well as cystically dilated biliary ducts in the region of the portal vein; Type 3: like Type 2b plus the absence of anastomosis-capable biliary tracts in the portal vein

enough (preferably before the 8th week of life). Depending on whether an anastomosis-compatible biliary tract is present (rare), either a biliodigestive anastamosis (Roux-en-Y) or a Kasai's portoenterostomy is performed. In this operation, atretic tract remnants are removed and a jejunal loop disconnected via Roux-en-Y is anastomosed with the cut surface of the liver hilus, so that the small, connected biliary ducts can drain. In the case of an inoperable atresia or after an unsuccessful operation, the last therapeutic option is a liver transplantation, which has become increasingly more successful in recent years. Biliary atresia is presently the most frequent cause for transplantation in childhood [11, 14, 24, 31].

## References

for Chapter 109 are listed at the end of Chapters 110 (page 1437)

# Benign Strictures of the Extrahepatic Bile Ducts

# 110

Michael A. Kern and Peter Schirmacher

Strictures, or scarred narrowing, of the extrahepatic biliary tract can have several etiologies. One must distinguish this condition from extraluminal stenosis resulting from, for example, compression of the distal biliary tract in chronic pancreatitis. Additionally, the possibility of a malignant stricture must always be considered and investigated. Benign strictures in the biliary tract most frequently occur postoperatively, especially after laparascopic cholecystectomy. Direct intraoperative biliary tract injuries or clips placed too close to the bile duct can form a stricture via ischemia and/or scarring. Biliary tract strictures occur with significant frequency after liver transplantation and are mostly of ischemic nature, while those due to primary sclerosing cholangitis are mostly inflammatory (see Chapters 52, 75 and 103).

A suspicion of the diagnosis is possible through an indicative clinical history combined with the clinical and laboratory evidence of cholestasis (with or without jaundice). Sonographically, the biliary tract proximal to the stricture is dilated. Diagnosis is confirmed by endoscopic retrograde cholangiography, during which therapy can be performed [28]. Through the combination of dilatation of the stricture and stenting, the mechanical obstruction can be permanently eliminated in many cases. This procedure is especially successful in early postoperative stenoses located below the bifurcation. Anatomically difficult cases (e.g. after Billroth II procedure) may be approached with percutaneous transhepatic cholangiographic methods in the hands of skilled interventional gastroenterologists or radiologists. If dilatation and stenting are unsuccessful, the possibility of a neoplastic process must be evaluated and an operative revision is usually necessary [23, 26].

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# Motility Disorders of the Bile Ducts and Postcholecystectomy Syndrome

# 111

Hans-Dieter Allescher

## Chapter Online



## Postcholecystectomy Syndrome

### *Definition and Epidemiology*

Cholecystectomy is an efficient and effective way to treat symptomatic gallbladder stones or cholecystitis with an excellent postoperative outcome. However, in a small subgroup of patients biliary type symptoms persist even after removal of the gallbladder, and in some patients new, colicky symptoms in the right upper quadrant develop. For the subgroup of patients with persistent or new complaints after cholecystectomy the term "postcholecystectomy syndrome" has be coined. Postoperative complaints can occur in 2–15% of patients, though only a very small portion of these patients suffer from a functional disorder of the papillary region (sphincter of Oddi dysfunction), which can be related either to an organic stenosis of the papillary region or to a motility disorder of the sphincter of Oddi (sphincter of Oddi dyskinesia) (see below).

### *Etiology and Pathogenesis*

In patients with suspected postcholecystectomy syndrome, it is mandatory to evaluate and distinguish different possible origins of their symptoms. Besides a biliary etiology, the symptoms can be related to organic disorders of the upper GI-tract or to functional bowel disorders such as functional dyspepsia or irritable bowel syndrome. Patients with functional bowel disease should ideally be identified prior to cholecystectomy because even though they may have cholecystolithiasis, the likelihood of cholecystectomy resulting in a durable improvement in their symptoms is low.

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Sometimes these patients with false indications for surgery are also categorized as postcholecystectomy syndrome.

In a relatively small subgroup of patients (5–15%), symptoms originate in the biliary system and the biliary or pancreatic sphincter. These patients show certain abnormalities of the biliary or pancreatic system, which can be identified by means of different diagnostic tests (e.g. ultrasound, laboratory tests, ERCP, hepatobiliary scintigraphy, sphincter of Oddi manometry).

The etiology of sphincter of Oddi dyskinesia (SOD) is not completely understood. Some data suggest that it is caused by disturbances of the autonomic neural innervation of the sphincter region (Fig. 111.1). Cholecystokinin octapeptide (CCK) induces the release of other inhibitory mediators (VIP, NO) in the sphincter muscle, which usually inhibits SO motility. In patients with SOD, CCK induces a characteristic paradoxical excitatory contractile response of the sphincter segment. This could indicate that in SOD patients a similar disorder of the inhibitory nerves is present as it is in achalasia. In addition, there is experimental evidence that a reflex pathway exists between the gallbladder, duodenum and sphincter of Oddi, which could be severed during cholecystectomy. In this case SOD would be a consequence of the operative procedure. There are some patients, however, who have SOD even with their gallbladder in situ. As with other functional disorders, the symptoms could be a reason for the operative removal of the gallbladder. In this case SOD would be the cause and not the consequence of the operation.

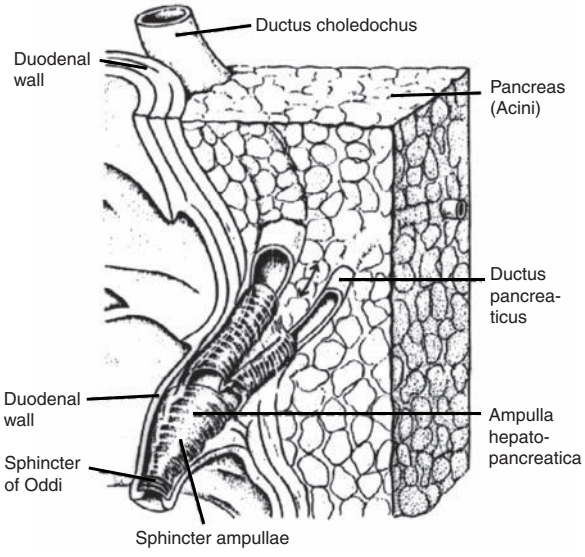



**Fig. 111.1** Anatomy of the sphincter of Oddi region

## Clinical Presentation and Diagnosis

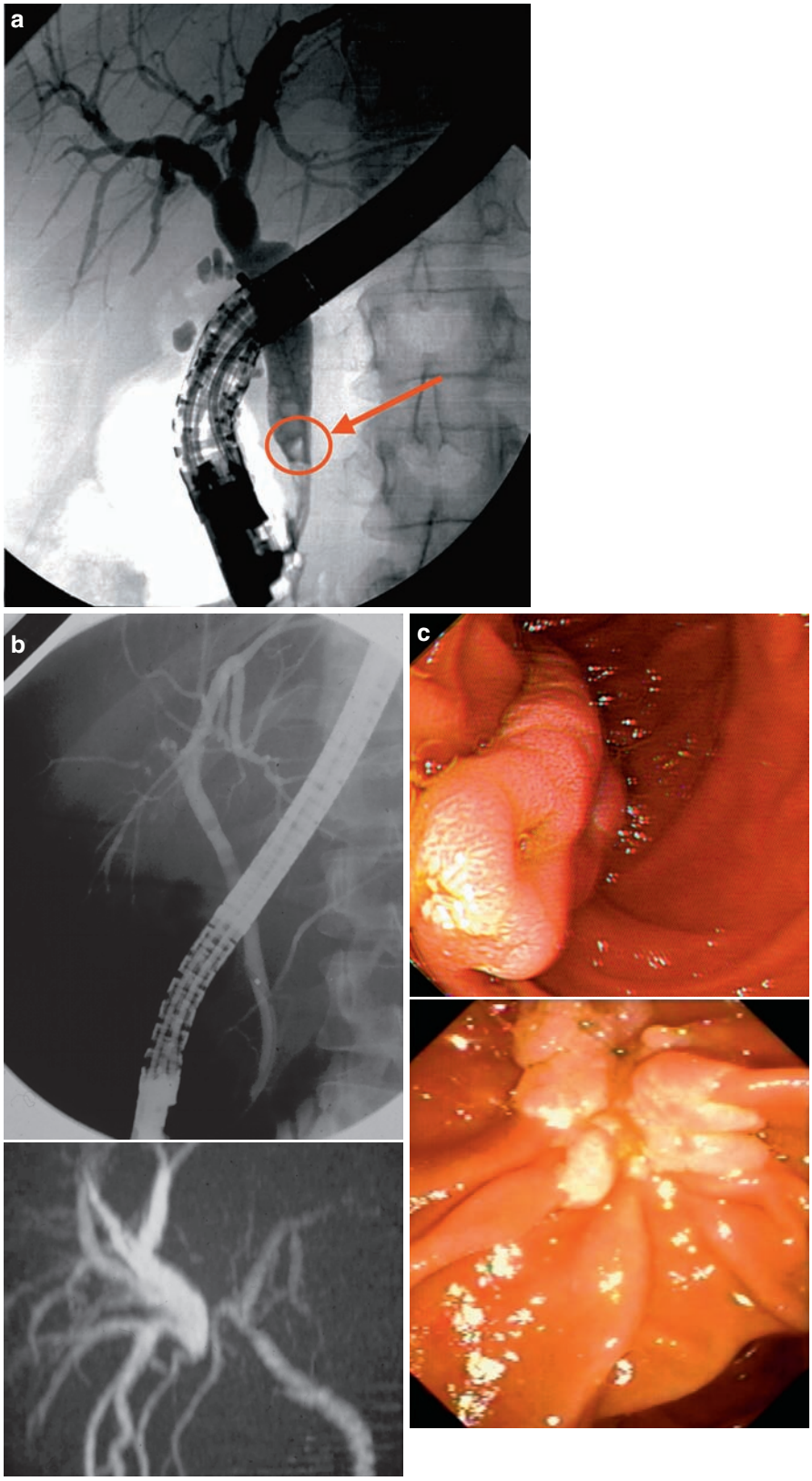
Patients with a biliary origin of postcholecystectomy complaints suffer from typical colicky, postprandial right upper quadrant pain. The pain often radiates to the back and to the right shoulder, and can last for several hours. These symptoms can be associated with elevations of cholestatic liver enzymes. In contrast, intermittent cholestasis, slight jaundice or fever as well as acholic stool is atypical for functional disorders of the sphincter of Oddi and is more likely due to a mechanical obstruction or stenosis [7].

When the functional disorder involves the pancreatic segment, either isolated or in combination with the biliary sphincter, recurrent attacks of mild pancreatitis or elevation of pancreatic enzymes can be observed.

As a first step it is important to rule out pathologic structural or anatomical causes of the symptoms (e.g., stones, tumors of the papilla). This includes laboratory tests (liver enzymes, alkaline phosphatase, γGT, bilirubin, lipase, amylase) and upper GI endoscopy as well as an abdominal ultrasound. To rule out retained bile duct stones transcutaneous abdominal ultrasound, MRCP or endoscopic ultrasound can be used [1]. When MRCP is unavailable or a distal CBD stone is suspected, endoscopic ultrasound is preferred as it has been shown to have the best negative predictive value with lowest complication rate. Using MRCP or ERCP additional etiologies of postoperative biliary complaints such as benign bile duct strictures, a long cystic duct with retained stones, or bile duct or papillary tumors can be identified (Fig. 111.2). If these causes of the biliary symptoms can be ruled out, dysfunction of the papilla has to be considered.

## Differential Diagnosis

In the following overview different etiologies of upper abdominal complaints following cholecystectomy are summarized.

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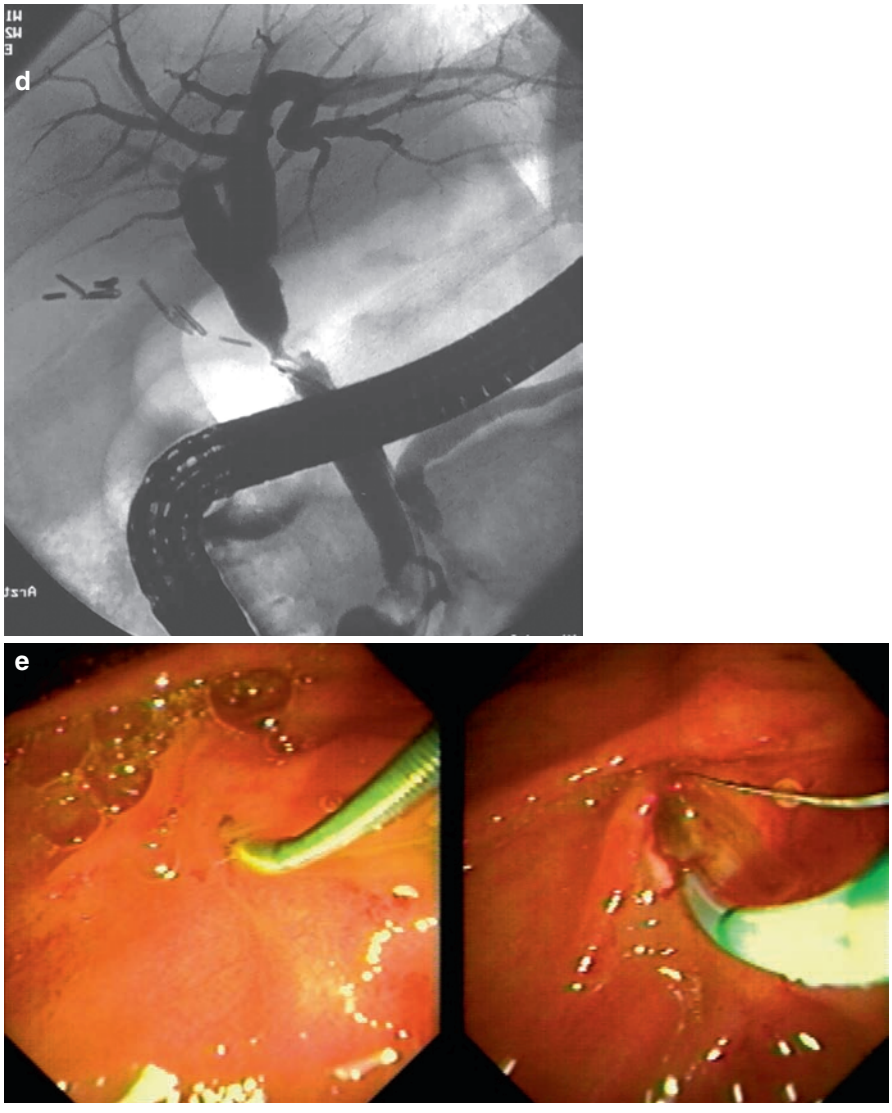


**Fig. 111.2** Different pathologic findings in patients with postcholecystectomy syndrome. (**a**) Retained stone in the common bile duct, (**b**) dilated segmental right intrahepatic bile duct; visualized only on postoperative MRCP (*lower panel*), (**c**) adenoma of the papilla of Vater, (**d**) postoperative common bile duct stricture, (**e**) stenosing papillitis. Endoscopic sphincterotomy via a guidewire

Reasons for typical right upper quadrant biliary-type pain after cholecystectomy are

- Bile duct stones
- Bile duct injuries
- (Postoperative) scarring of the bile duct
- Structural abnormalities of the bile duct (choledochocele)
- Long cystic duct with retained stone(s)
- Mirizzi syndrome
- Tumors of the bile duct or the papilla
- Sphincter of Oddi dysfunction
  - Stenosis of the papilla
  - Sphincter of Oddi dyskinesia
- Other organic reasons in the upper abdomen (e.g. peptic ulcer)
- Functional bowel disorders
  - Functional dyspepsia (FD)
  - Irritable bowel syndrome (IBS)

### Bile Duct Stones

A retained and overlooked stone in the bile duct following cholecystectomy has to be considered as cause of a post-cholecystectomy syndrome (1–6%) and should be ruled out with percutaneous ultrasound, endoscopic ultrasound, MRCP or ERCP. Even intraoperative cholangiography via the cystic duct does not completely rule out a retained stone, as stones can still be found in up to 4% of patients.

Most of the patients with a retained stone develop symptoms shortly after the operation. However, in some patients a symptom-free postoperative interval of several weeks or longer can be present. Similar to the acute situation, endoscopic ultrasound and MRCP are the diagnostic methods of choice to rule out a retained stone. These methods have widely replaced ERCP as they bear no risk of postinterventional pancreatitis in the diagnostic setting. If a stone is identified, ERCP and endoscopic sphincterotomy followed by stone extraction is the therapy of choice.

### Long Cystic Duct with Retained Stone

A long cystic duct has been discussed for a long time as a possible cause of postoperative complaints. A remaining dilated proximal cystic duct remnant with Heister's spiral valve or a retained stone can cause symptoms [21, 30]. If the stone is retained in the more distal part of the cystic duct, a compression of the closely related bile duct system can occur (Mirizzi-Syndrome). The results of interventional studies are contradictory. Whereas some series report an improvement of symptoms after removal of the long cystic duct, others failed. Another possible cause for symptoms in patients with a long cystic duct remnant is the formation of neuromas and granulomas due to suturing material following conventional cholecystectomy.

### Bile Duct Strictures

In cases of complicated intraoperative situations with severe inflammation, bleeding or injuries of the duct system, postoperative scarring of the tissue can lead to a stricture or even obstruction of the bile duct system. The incidence of such scarring is reported in the literature to be in the range of 5–10%, mostly involving the middle part of the bile duct [9].

The clinical presentation is characterized by progressive jaundice and cholangitis, and can thus be distinguished from SO dysfunction. This situation can be suspected by abdominal ultrasound showing a typical dilatation of the biliary system proximal to the stenosis, and can be verified by either MRCP or ERCP. The treatment is based on endoscopic dilatation of the stricture and long term stenting with large diameter stents and/or multiple stents for several months. In cases that are refractory to endoscopic therapy, surgical intervention with a biliary bypass procedure should be considered.

### Stenosis and Tumors of the Papilla

Scaring of the papilla, adenomas and malignant tumors of the papilla can lead to papillary obstruction with subsequent biliary symptoms. These conditions can be present prior to cholecystectomy, and symptoms persist or even worsen after removal of the gallbladder. Papillary adenomas and tumors can usually be identified by endoscopic inspection or biopsy of the papilla with a side-viewing instrument. Sometimes an endoscopic sphincterotomy is necessary for correct identification and diagnosis. Scarring of the papilla with a papillary stenosis (e.g. postinflammatory, papillitis stenosans) shows a considerable similarity and overlap with sphincter of Oddi dyskinesia (see below).

## Sphincter of Oddi Dysfunction

Sphincter of Oddi dysfunction is responsible for "post-cholecystectomy syndrome" in only a minority of cases (5–15%) Altogether this abnormality is observed in fewer than 1% of all patients who have undergone cholecystectomy. The symptoms are characterized by typical colicky right upper quadrant pain or by recurrent attacks of pancreatitis [11, 24, 29]. In the latter situation, intermittent obstruction of the pancreatic outflow tract leads to recurrent attacks of pancreatitis.

### *Diagnostic Procedures*

The diagnosis of sphincter of Oddi dyskinesia is primarily based on the exclusion of other organic disorders of the bile duct system (see above) and the surrounding

**Table 111.1** Classification of sphincter of Oddi dyskinesia (SOD) based on clinical criteria (Milwaukee criteria) [7, 11]

| | Typical right upper quadrant pain | Elevation of AP, γGT AST or ALT 2–3 times ULN | Dilatation of the bile duct (ERCP) > 12 mm | Drainage of contrast medium from bile duct > 45 min |
|---|---|---|---|---|
| **SOD type I** | + | + | + | + |
| **SOD type II**[a] | + | +/–[a] | +/–[a] | +/–[a] |
| **SOD type III** | + | – | – | – |

[a]At least one of three criteria positive

**Table 111.2** Classification of sphincter of Oddi dyskinesia (SOD) of the pancreatic sphincter based on clinical criteria

| | Typical right upper quadrant pain | Elevation of amylase or lipase > 1.5–2 times ULN | Dilatation of the pancreatic duct (ERCP) > 6 mm | Drainage of contrast medium from pancreatic duct > 9 min |
|---|---|---|---|---|
| **SOD type I** | + | + | + | + |
| **SOD type II**[a] | + | +/–[a] | +/–[a] | +/–[a] |
| **SOD type III** | + | – | – | – |

[a]At least one of three criteria positive
*ULN* Upper limit of normal

organs. Laboratory tests show recurrent abnormalities of cholestatic enzymes (alkaline phosphatase, gamma-glutamyltransferase) or of aminotransferases. Usually an increase of two to three times the upper limit of normal can be observed (see Tables 111.1 and 111.2, and introduction to SOD). These laboratory findings can be used to differentiate organic disorders as they usually present with higher and more persistent elevation of laboratory values.

Abdominal ultrasound or MRCP can be used to evaluate the bile duct system, including the measurement of extrahepatic bile duct diameter (normal: <7 mm for patients with gall bladder in situ, <9 mm for patients after cholecystectomy). Additionally the pancreatic duct width before and after a stimulatory meal can be evaluated.

Historically, a standard diagnostic procedure has been ERCP as this allows for a direct visualization of the bile duct system which can identify or rule out organic abnormalities. Several radiologic phenomena can be identified which indicate the presence of a sphincter of Oddi dyskinesia such as dilatation of the bile duct (common bile duct >12 mm) and delayed drainage of the contrast medium from the bile duct system (>45 min) (Table 111.3). In some patients a characteristic pain response upon intubation or manipulation of the papilla can be observed, which is regarded by some groups as a separate clinical entity of a "hypersensitive bile duct system". The presence of biliary pain, pathologic laboratory and ERCP findings (or, subsequently, noninvasive imaging) has been used to subdivide patients with SOD in different groups according to the Milwaukee classification (see below) [7, 11].

**Table 111.3** Tests for the diagnosis of sphincter of Oddi dyskinesia

| | |
|---|---|
| **Pharmacological provocation tests** | Codeine test (50 mg)<br>Morphine (0.12 mg/kg)-neostigmine (0.012 mg/kg) test (Nardi-Test)<br>Fentanyl test<br>CCK-test (20 ng/kg i.v.)<br>Ceruletide 5 µg i.v.<br>Secretin test (1 U/kg) |
| **ERCP** | |
| **MRCP** | |
| **Ultrasound with provocation tests** | *Bile ducts*: fat rich meal (American breakfast, Lipomul 1.5 mg/kg); CCK-OP 10–20 ng/kg/3 min i.v.); ceruletide (50 ng/kg i.v.)<br>*Pancreas*: secretin (1 U/kg i.v.) |
| **Hepatobiliary scintigraphy** with or without provocation tests | Morphine (0.04 mg/kg i.v.)<br>CCK-OP (10–20 ng/kg/3 min i.v.)<br>Oral nitrates |
| **Probatory stent placement** | See text |
| **Botulinum toxin injection** | See text |
| **Sphincter of Oddi manometry** | See text |

### Sphincter of Oddi Manometry

See Chapter 47. Introduction of a manometric catheter device during ERCP allows a functional evaluation and manometric characterization of the sphincter of Oddi. After having established normal manometric findings and normal pressure values in the sphincter of Oddi, several manometric abnormalities have been described in patients with sphincter of Oddi dyskinesia, which could be diagnostic for this disorder [7, 17, 26]. These findings include:

1. Increased contraction frequency (tachyoddi) > 10/min
2. Increased proportion of retrograde contractions >20%
3. Increased contraction amplitudes (>180 mmHg) with prolonged duration (>7 s)
4. Increased basal sphincter pressure (>40 mmHg)
5. Paradoxical contractile response upon pharmacological stimulation with cholecystokinin octapeptide (CCK-OP)

Based on these motility phenomena the presence of sphincter of Oddi dyskinesia has been postulated. In the clinical setting sphincter of Oddi manometry is the gold standard for the diagnosis of SOD. Based on a prospective therapeutic study, the finding of increased basal sphincter pressure has gained acceptance for the diagnosis of SOD and a sphincter Oddi pressure of >40 mmHg is used for discrimination [7]. The clinical relevance of other findings is still a matter of debate. An increased basal sphincter pressure is not only used for diagnosis of SOD, but can also be used to predict the response rate of endoscopic sphincterotomy in these patients (see below).

Sphincter of Oddi manometry, however, is technically difficult and demanding, as an atraumatic intubation of the papilla should be performed to avoid artifacts and complications. Also, spasmolytics such as butylscopolamine or glucagon, cannot be used as they can suppress the phasic activity of the sphincter. Additionally, the analysis and evaluation of the manometry tracings requires manometric knowledge and experience with this method and interpretation is complicated by possible artifact due to movement of the catheter and kinking of the probe. As SO manometry is also associated with considerable complications, its clinical role is not without controversy and the procedure should only be performed in specialized centers by experienced specialists. Even in these specialized centers SO manometry is not feasible in 10–15% of patients due to failed intubation of the papilla, and is associated with considerable side effects (acute pancreatitis) in 5–15%.

Besides sphincter Oddi manometry, a series of pharmacological provocative tests have been suggested and introduced for diagnosis of SOD (see "overview on provocative tests"). Even though some of these tests can suggest the presence of SOD without manometry by inducing typical clinical symptoms, the sensitivity and specificity of these tests is too low to be useful in clinical practice. Another diagnostic approach to SOD uses either hepatobiliary scintigraphy (e.g. HIDA-scintigraphy) with or without pharmacological provocation or use of spasmolytics. Similarly, abdominal ultrasound evaluation of the bile and pancreatic duct with provocative stimulation of biliary flow by a fat-rich meal or CCK-OP has been suggested. These methods can be used to identify a possible functional obstruction in the papillary region, however they also need profound experience and have a low positive predictive value. In a recent study, symptomatic improvement following a probatory intrasphincteric injection of botulinum toxin was evaluated as a discriminative diagnostic test for patients with SOD [32].

### Pharmacologic Provocation Tests

For a positive diagnosis of SOD, a series of pharmacological stimulation tests have been suggested, which should induce and reproduce the typical clinical symptoms [5, 14, 23]. However none of these tests has been shown to have sufficient clinical sensitivity and specificity for routine clinical use. Some of these tests are used in combination with imaging methods such as hepatobiliary scintigraphy, abdominal ultrasound or endoscopic ultrasound (see below).

### ERCP, MRCP

ERCP is the fundamental diagnostic procedure in a patient with suspected SOD, especially to rule out structural abnormalities and other disorders or diseases (Figs. 111.3 and 111.4). The increasing use of MRCP, however, qualifies this non-invasive imaging method as a possible first choice prior to ERCP. However, no prospective or interventional studies on patients with SOD are available so far. MRCP can identify and rule out significant structural abnormalities. However, whether

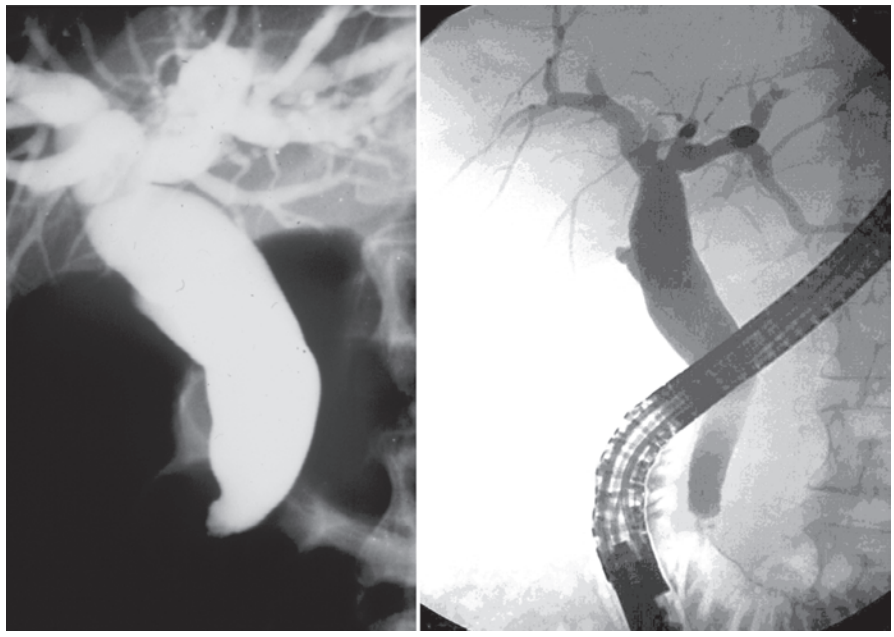

**Fig. 111.3** ERCP in type I SOD. Markedly dilated common bile duct

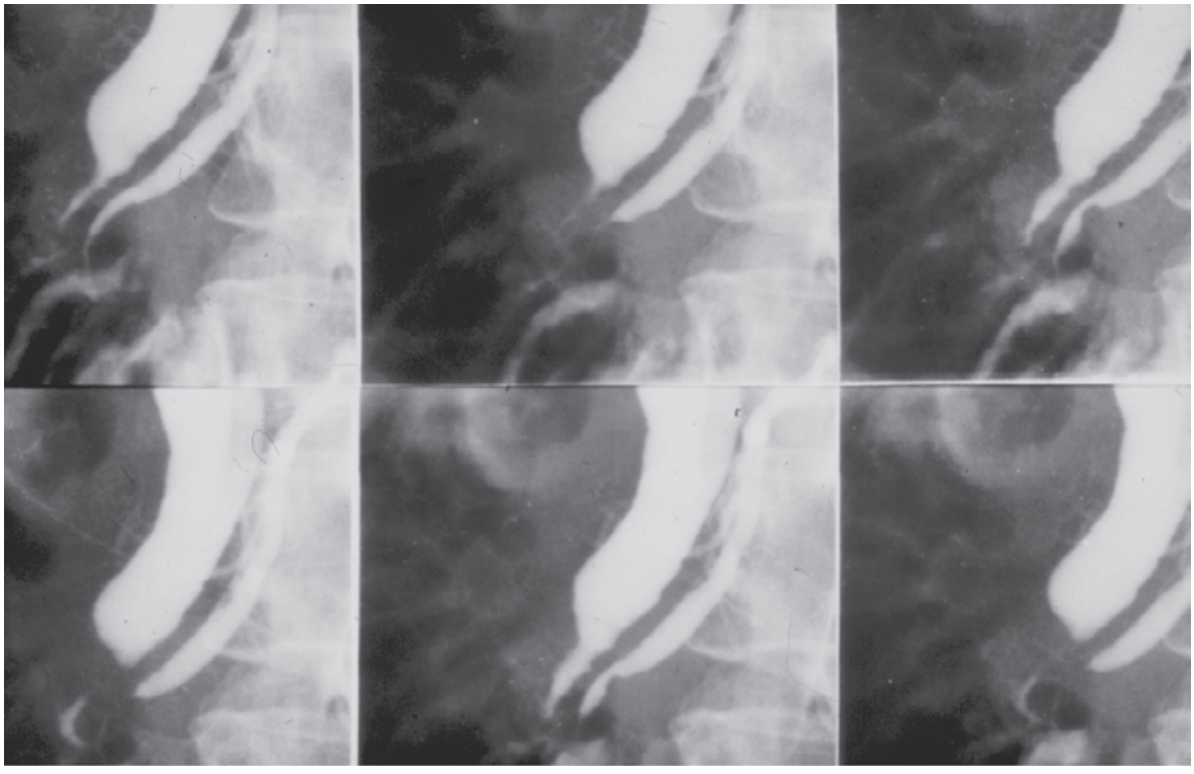

**Fig. 111.4** Normal papillary function during ERCP

MRCP adds significant additional information in patients with normal laboratory findings and sonographically non dilated bile ducts remains to be shown. As MRCP cannot add additional information on bile duct emptying or papillary movements, the role of MRCP in SOD (type II and III) remains experimental.

### Abdominal Ultrasound with Provocation Tests

Evaluation of the bile duct and pancreatic duct diameter before and after a provocative stimulus (fat-rich meal, CCK-OP, ceruletide, secretin) was evaluated and used for differentiation of sphincter of Oddi dysfunction.

Abdominal ultrasound before and after a provocative meal (fat-rich American breakfast, Lipomul) or CCK-OP or its agonist ceruletide allows the functional evaluation of the biliary system and the biliary sphincter [3]. A provocative meal releases endogenous CCK which causes a contraction of the gallbladder and a relaxation of the papillary sphincter leading to increased bile flow in healthy people. Thus, despite the contractile effect on the gallbladder CCK normally causes a reduction or at least only a small increase (<0.5 mm) in the diameter of the bile duct, irrespective of whether the gallbladder is in situ or removed. In patients with SOD a paradoxical response of the sphincter region to CCK results in an increase of the bile duct diameter. An increase of more than (1-)2 mm is pathologic. For standardization, the bile duct diameter is determined in a subcostal longitudinal section in which the hepatic artery crosses the portal vein. In a small prospective study in patients with SOD the clinical value of ultrasound with provocation with ceruletide was evaluated. A satisfactory accuracy was reached (sensitivity 85%, specificity 100%) only in patients with a dilated bile duct and elevated liver and cholestatic enzymes. In contrast, in patients with normal bile duct diameter and normal laboratory findings abdominal ultrasound with ceruletide provocation was not satisfactory (sensitivity 27%, specificity 27%). Thus ultrasound with provocative testing seems not to be useful in those patients with suspected SOD (type II and III), in whom pre-selection for manometry would be desirable.

For pancreatic testing, secretin (1 U/kg i.v.) induces a powerful stimulation of pancreatic secretion with a subsequent slight pressure elevation of the sphincter of Oddi, which induces a slight dilatation of the pancreatic duct even in healthy people (<1 mm). In patients with SOD this effect can be significantly enhanced and an increase of more than 2 mm is clearly pathologic. In several prospective studies a pathologic result of the ultrasound secretin test was a relatively good predictor of symptomatic improvement following endoscopic sphincterotomy. Thus this test could be used in patients with recurrent pancreatitis and suspected isolated dysfunction of the pancreatic part of the sphincter to substantiate the need for SO-manometry or endoscopic sphincterotomy.

### Hepatobiliary Scintigraphy

Hepatobiliary scintigraphy evaluates the kinetic of mostly Tc99m labeled compounds, which are excreted into the biliary system (e.g. 99mTc-BISIDA, 99mTc-DISIDA). With this method, both the excretion into and the clearance from the biliary system into the duodenum can be analyzed (Fig. 111.5) [6]. By use of typical regions of interest (ROI) in the right and left liver, along the bile duct, the gallbladder, and the duodenum, a quantitative clearance kinetic of the tracer can be constructed. From the various parameters analyzed, the half maximal isotope clearance (T1/2) and the percentage of isotope clearance in 45 min (<60–63%) are regarded the most sensitive parameters for the evaluation of a sphincter of Oddi dysfunction.

Similar to other imaging methods, scintigraphy has also been used with various pharmacological stimulation tests. Besides morphine sulphate and CCK-OP, there have been attempts to differentiate organic fixed stenosis from functional motility disorders with the use of spasmolytics.

The sensitivity of this method is only in the range of 65–70% and even a combination with abdominal ultrasound methods reaches only a sensitivity of 80%. However, similar to the ultrasound test hepatobiliary scintigraphy can be used as a pretest prior to SO-manometry. Whether it increases the diagnostic and therapeutic yield remains to be shown.

### Probatory Stent Placement

Several authors suggested that the clinical response to a probatory stent placement over several weeks could be

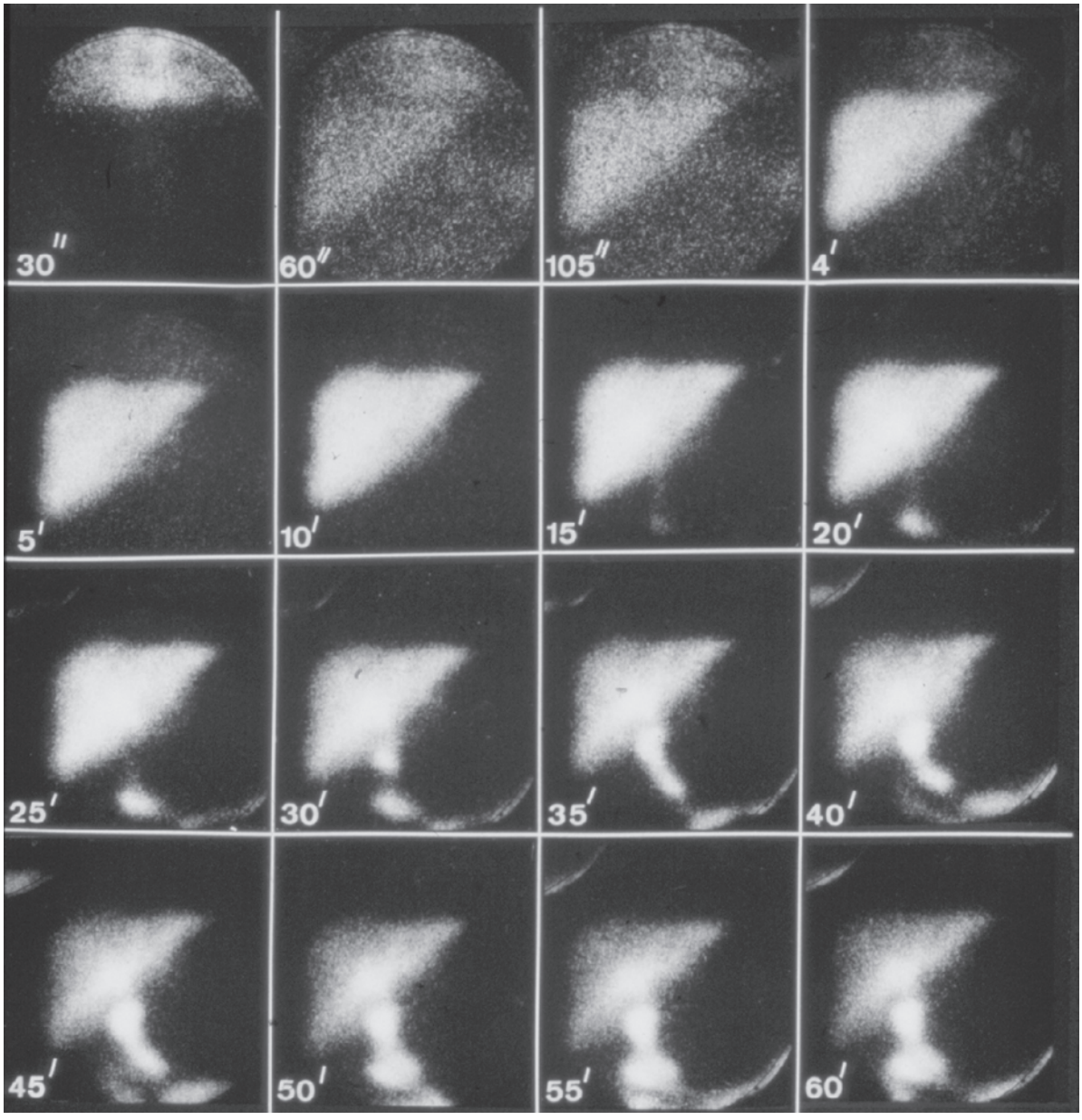


**Fig. 111.5** Hepatobiliary scintigraphy (normal findings)

used to decide whether or not endoscopic sphincterotomy could be helpful [18, 25]. This cannot be recommended in the pancreatic system, due to possible short term ductal changes induced by the stent. In the bile duct, stent placement without prior sphincterotomy was associated with a high complication rate (30% pancreatitis with difficult and complicated course), thus this procedure cannot be recommended as diagnostic test.

### Botulinum Toxin Injection (BoTox)

Wehrmann et al. suggested the use of intrasphincteric injection of botulinum toxin (BoTox) to differentiate patients with suspected sphincter of Oddi dyskinesia and in patients with idiopathic pancreatitis [31, 32]. They evaluated the clinical response of patients with SOD type III with increased basal pressure (>40 mmHg) to

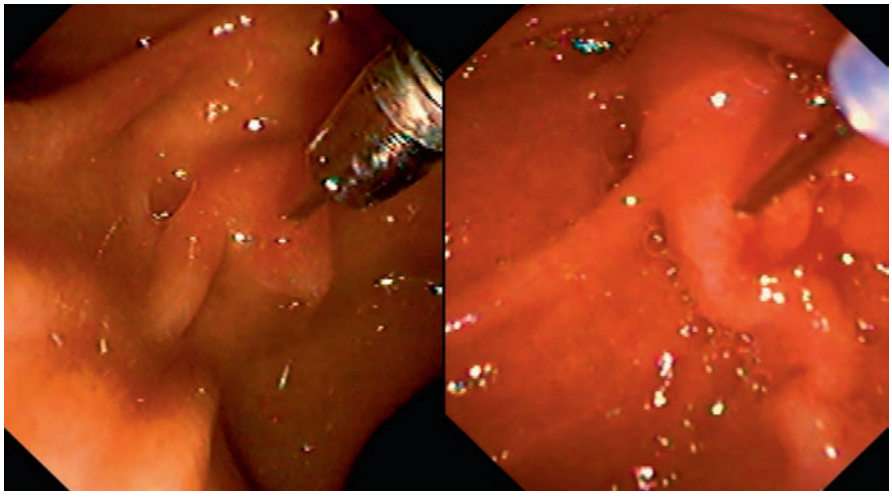

**Fig. 111.6** Endoscopic appearance of botulinum toxin injection into the roof of the papilla

intrasphincteric injection of botulinum toxin (Fig. 111.6). All patients who responded initially to the injection and developed symptoms again after 6 months showed improvement or complete symptom relief following endoscopic sphincterotomy. In contrast, patients who did not respond to BoTox injection showed a significantly lower response rate to sphincterotomy (20%). If this data can be verified by a prospective study, BoTox injection could help to discriminate and identify patients with SOD type III, who currently pose the largest problem in the clinical decision making process (see below) [32].

Similar to its use in the bile duct sphincter, BoTox injection can also ameliorate symptoms which originate in the pancreatic sphincter. In patients with recurrent idiopathic pancreatitis and suspected SOD of the pancreatic sphincter, injection of BoTox resulted in clinical improvement in 80% of the treated patients. However, further data are needed.

## Sphincter of Oddi Dyskinesia

In order to determine the clinical role of sphincter of Oddi dyskinesia it is mandatory to answer the question whether this disorder and its various types as suggested by the Milwaukee classification really exists as a separate entity or whether it constitutes a continuum from organic papillary stenosis (SOD type I) to functional bowel disorder (SOD type III) (Tables 111.1 and 111.2) [1]. To answer this question, several studies on the diagnosis and treatment of these disorders have been reported, which clearly support the existence of different forms of sphincter of Oddi dyskinesia.

### *SOD Type I*

In patients who are categorized as SOD type I (typical clinical complaints, 2–3-fold elevated laboratory values, dilated bile duct and delayed drainage of contrast from the biliary system), there is mostly a clear indication for endoscopic sphincterotomy during the initial diagnostic work up. Patients with this constellation are strongly suspected of having an organic papillary stenosis, which is best diagnosed with ERCP (and biopsy if indicated) and treated with endoscopic sphincterotomy. In fact, in the majority of these patients an organic stenosis can be found and when manometry is performed it shows an increased basal pressure (>40 mmHg) in the majority (70–100%) of the cases. Sometimes the opening of the papilla is constricted and narrowed, so that cannulation is technically difficult or impossible. If manometry is performed in this situation the manometric catheter (diameter 1.7 mm) is compressed in the stenotic sphincter region, which results in the recording of an increased basal pressure; however, this is not actually due to an active muscular tone. This can only be differentiated by using a smooth muscle relaxant, which should reduce or abolish the increased basal pressure. It was postulated that endoscopic sphincterotomy might only be clinically helpful in patients with papillary stenosis [28].

As for other manometric procedures in SOD patients, patients have to be informed that manometry alone

already bears a considerable risk for complications (pancreatitis) even without ERCP. This risk is even further increased if the manometry also involves the pancreatic sphincter segment or if the manometry is not followed by a drainage procedure of the pancreatic systems such as placement of a protective stent or sphincterotomy. On the other hand, in SOD type I most patients will benefit symptomatically from endoscopic sphincterotomy. Thus in type I SOD patients who have typical clinical symptoms, abnormal laboratory values, and structural abnormalities, there is usually no need for sphincter of Oddi manometry; rather, these patients can proceed directly to endoscopic sphincterotomy. Similarly, functional tests (see above) and pharmacological stimulation can be helpful, as they are sensitive in this subset of patients, but their clinical use is limited as the indication is already based on the other aforementioned abnormalities.

## SOD Type II

Patients with suspected SOD type II constitute an inhomogeneous group. The Milwaukee classification is relatively arbitrary, and a wide range of patients is included in this group.

In this group of patients only a subgroup (40–86%) shows pathological results of sphincter of Oddi manometry (increased basal pressure >40 mmHg). If further management and treatment is based on these results, then patients with increased basal sphincter pressure show a significantly higher symptomatic response (80–99%) to endoscopic sphincterotomy than patients without an increase of basal sphincter pressure (30–35%). In sham controlled studies patients with or without increased basal sphincter pressure were randomized to endoscopic sphincterotomy or sham treatment. Only the sphincterotomy group showed an increased symptomatic response in patients with increased sphincter of Oddi pressure, whereas the sham procedures showed no effect irrespective of whether the pressure was increased or not. Interestingly, only the increased basal pressure could be used to predict the treatment response to endoscopic sphincterotomy, whereas other manometric abnormalities showed no predictive value and no correlation with the treatment response [7, 8].

It has to be mentioned that in subsequent studies which confirmed this finding, a clear distinction between organic and functional stenosis was not always made. As mentioned, an organic stenosis can be differentiated from a functional sphincter of Oddi dyskinesia by pharmacologic interventions (spasmolytics, nitrates, CCK) during sphincter of Oddi manometry or hepatobiliary scintigraphy [12]. On the other hand, especially those studies which investigated spasmolytics during manometry or showed a symptomatic improvement of patients with SOD type II to intrasphincter botulinum toxin support and demonstrate that the symptomatic patients have a functional disorder of the sphincter of Oddi. However, due to the complication rate of the procedure, there is still some controversy whether sphincter of Oddi manometry must or should be performed prior to endoscopic sphincterotomy. New technical advances (aspirating catheter, prophylactic stenting, electronic manometric system) have significantly reduced the complication rate and might increase the clinical use of sphincter of Oddi manometry. Eventually the probatory injection of botulinum toxin could be useful in the clinical setting in order to indicate which patients will benefit the most from endoscopic sphincterotomy. In borderline cases additional use of ultrasound or scintigraphy with provocative testing may help to determine when to proceed with sphincter of Oddi manometry and/or endoscopic sphincterotomy.

## SOD Type III

The most difficult group of patients presents only with typical biliary right upper quadrant pain without laboratory or morphological abnormalities. Patients in this group outnumber those in the other subtypes and there is a considerable overlap with other functional disorders of the abdominal region (e.g. functional dyspepsia). Sphincter of Oddi manometry should therefore not be applied in general to all these patients. Thus, the difficulty is to select patients which will benefit from further diagnostic interventions.

The question is whether the different provocative tests can be used to differentiate these patients. As a first step ultrasound or scintigraphy with a provocative meal or stimulation could be used as they are not associated with major side effects or complications. If one

**Table 111.4** Prevalence of increased sphincter of Oddi basal pressure (>40 mmHg) in cholecystectomized patients with typical biliary symptoms

| | SOD type I | SOD type II | SOD type III |
|---|---|---|---|
| Toouli (1985) [27] | – | 20%<br>n = 26 | – |
| Geenen (1989) [7] | – | 55%<br>n = 42 | – |
| Lans (1991) [13] | 65%<br>n = 17 | 50%<br>n = 47 | 10%<br>n = 240 |
| Raddawi (1991) [16] | – | 16%<br>n = 19 | 0%<br>n = 15 |
| Meshkinpur (1992) [15] | 60%<br>n = 15 | 18%<br>n = 22 | 7%<br>n = 27 |
| Rolny (1993) [19] | 65%<br>n = 17 | – | – |
| Botoman (1994) [4] | – | 60%<br>n = 35 | 55%<br>n = 38 |
| Wehrmann (1996) [33] | 100%<br>n = 17 | 61%<br>n = 38 | 50%<br>n = 30 |

Source: Modified after [34]

**Table 111.5** Results of sphincter of Oddi manometry and of endoscopic sphincterotomy in patients with SOD type I, II and III

| | Increased SO basal pressure (>40 mmHg) | Clinical response to endoscopic sphincterotomy |
|---|---|---|
| **SOD type I** | 60–100% | 55–91% |
| **SOD type II** | 40–86% | 80–90% (>40 mmHg)<br>20–35% (<40 mmHg) |
| **SOD type III** | 20–55% | 8–56% (>40 mmHg) |

of these tests shows a positive result, further imaging with MRCP or ERCP could be indicated. In patients with SOD type III the percentage with a pathological manometric finding is significantly lower when compared to type I or II (see Table 111.4). Additionally, these patients also show a decreased symptomatic response rate to endoscopic sphincterotomy even if an increase in sphincter of Oddi pressure (>40 mmHg) is present (Table 111.5). Thus the use of sphincter of Oddi manometry is disputed in this patient group. In general, ERCP and endoscopic manometry are reserved for selected cases and should be performed in a specialized center.

Thus in these patients a trial of medical therapy should be used as a first step. Another possibility could be the probatory intrasphincteric injection of botulinum toxin as discussed above.

## Clinical Course and Prognosis

Only few data and studies are available on the long term outcome of patients with SOD. In a long term prospective study which followed patients for 4 years after endoscopic sphincterotomy, Geenen et al. showed that patients with elevated sphincter of Oddi pressure had a good and stable long term symptomatic response [8]. After endoscopic sphincterotomy there is the possibility of scarring and development of a stenosis of the orifice especially in the first month following sphincterotomy. If the sphincter muscle is completely cut through this usually attains no clinical relevance. However, in cases where symptoms reoccur after a symptom free interval this possibility has to be taken into account and diagnostic imaging and eventually even repeated manometry should be performed. As an alternative, repeat sphincterotomy can be performed, though it has an increased risk of bleeding and perforation. Alternatively, balloon dilatation of the papilla (6–8 mm balloon) or stenting of the papilla can be performed.

In some patients with elevated sphincter pressure there is no symptomatic relief following sphincterotomy. Several reasons have to be considered.

- Incomplete sphincterotomy with some fibers of the biliary or the pancreatic sphincter still intact
- Simultaneous dysfunction of the pancreatic sphincter (35–65%) which would require a simultaneous sphincterotomy of the pancreatic sphincter segment
- Other reasons for the pain attacks (chronic pancreatitis, retained stones, strictures, motility disorders of the upper GI tract, irritable bowel syndrome, functional dyspepsia)

In order to rule out a papillary origin in these non-responders, the completeness of the sphincterotomy has to be evaluated and pancreatic involvement has to be ruled out by manometry of the pancreatic sphincter segment if this was not carried out during the initial diagnostic manometry, as recommended.

## Therapy

The therapeutic alternatives in patients with sphincter of Oddi dyskinesia comprise medical therapy and endoscopic interventions.

### Medical Therapy

Biliary pain in SOD is thought to originate from spastic contractions of the sphincter of Oddi region. As for other spastic disorders, different spasmolytics and choleretics have been suggested and tested in patients with SOD. Besides glucagon and nitrates, which are mostly used for diagnostic purposes, anticholinergics (e.g., butylscopolamine) and $Ca^{2+}$ channel antagonists have been used for therapy [2, 10, 20, 22].

Two prospective placebo controlled studies evaluated the influence of nifedipine on the level and frequency of biliary pain in patients with SOD. Nifedipine reduced pain frequency in 75% of the patients. Even though these studies were performed with relatively few patients, empiric treatment with nifedipine is warranted in patients with SOD type III and in less severe cases of patients with SOD type II [20].

In another study, a progesterone containing preparation was used with clinical success. Other treatment options such as bile acids, pancreatic enzymes or choleretics have not been tested in prospective controlled studies. Botulinum toxin injection can be used in a subgroup of patients as a diagnostic and therapeutic tool.

### Endoscopic Interventional Therapy

In all patients with severe symptoms or overt obstruction of bile flow (SOD type I) and in all patients with proven sphincter of Oddi dyskinesia with increased basal sphincter pressure endoscopic sphincterotomy is the therapy of choice. Under these circumstances manometry can also be used to verify the completeness of the sphincterotomy.

The effectiveness of endoscopic sphincterotomy has been shown in a prospective trial in 47 patients with SO-dysfunction (SOD type II) and has meanwhile been shown in several other studies for all types of SOD. However, the success rates of endoscopic sphincterotomy in patients with elevated sphincter pressure decrease progressively from SOD type I to type II and III (Table 111.3).

Similar to sphincter of Oddi manometry, there is an increase in the complications rate with endoscopic sphincterotomy. Patients with SOD type II and III show a significantly higher rate of post sphincterotomy pancreatitis (12.5%) than patients with cholelithiasis (3.7%). Moreover, there seems to be an inverse relationship with bile duct diameter. While patients with markedly dilated bile duct showed a low rate of pancreatitis (>15 mm, 2.4%), there was a progressive increase in the pancreatitis rate with decreasing bile duct width (10–15 mm, 3.2%; 5–10 mm, 13.8%). Especially in patients with normal bile duct diameter (<5 mm), which is often found in patients with SOD type III, an extremely high pancreatitis rate (23.1%) was present.

A surgical intervention at the papilla or surgical sphincteroplasty is usually not indicated and should only be performed in rare exceptions. It is associated with higher morbidity and mortality (0.6–6%).

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# Gallbladder Stones

# 112

Ulrich Leuschner

## Chapter Outline



## Epidemiology

There are three different stone types: the cholesterol stone, the black pigment stone and the brown pigment stone, also called calcium-bilirubinate stone. So-called mixed stones contain the different components in different concentrations. A rare concrement, only observed in Japan, is the so-called pepper-and-salt-stone.

With a prevalence of 10–20%, gallstone disease in industrialized countries represents the most frequent disease after coronary heart disease and diabetes mellitus [31]. For Germany this means that about 8–16 million gallstone carriers exist. In females gallstones are found two to three times more often than in males.

The frequency of cholesterol gallstones of the gallbladder increases with age (Table 112.1). In children gallstones are found in approximately 5% (even babies may have gall-stones). Between the 30th and 69th year of life, the prevalence is 10% in men and up to 19% in women, and increases in 70–80 year old people to 30–40%. The annual incidence for men is 0.5% and for women 0.7%. The increasing figures in older age mirror a decreased contractility of the gallbladder, an increased cholesterol secretion associated with a decreased bile acid secretion and an augmented nucleation of the precipitated cholesterol. The speed of stone growth is 1–4 mm/year and seems to be the same in older and younger people.

While gallstones can be found in 10–20% of Europeans, 70–90% of female native American Indians living in the Southwest United States have gallstones. Very often gallstones are found in US-Mexicans, Chileans, Swedes, Czechs and Slovaks (Table 112.2). Gallstones are rare in the black population of Central Africa, but also in Blacks in the United States. They are rare in Panama, Japan, in Southeast Asia, Rumania, Greece and Iceland.

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**Table 112.1** Prevalence of gallstones in Europe

| Patient age (in years) | Male (%) | Female (%) |
|---|---|---|
| 0–9 | 0.0 | 0.0 |
| 10–39 | 1.5–7.2 | 2.5–6.9 |
| 40–49 | 4.4–10.0 | 10.0–17.0 |
| 50–59 | 6.2–14.7 | 14.7–26.4 |
| 60–69 | 9.9–15.9 | 25.0–31.2 |
| 70–79 | 15.2–23.3 | 28.9–33.0 |
| 80–89 | 17.9–26.2 | 30.9–42.8 |
| 90 | 24.4–42.9 | 35.4–45.7 |

Eighty to 90% of all gallbladder stones are cholesterol concrements. Ten to 20% are black pigment stones. Up to a size of 0.7 cm cholesterol- and pigment stones can migrate through the cystic duct into the common bile duct. These stones are called secondary bile duct stones. The so-called brown calcium-bilirubinate concrements are primary bile duct stones. They represent 10–20% of all bile duct stones and originate primarily in the biliary tree. Gallbladder stones and bile duct stones are found simultaneously in about 15% of cases.

The brown calcium-bilirubinate stones are the classic primary bile duct stones after manipulation of the bile ducts, for instance after endoscopic sphincterotomy, after operations at the Papilla of Vater or after cholecystectomy. Since in Europeans brown bilirubinate stones have never been observed before such interventions, they seem to be the result of therapeutic measures. In Asia, brown pigment stones are observed in about 20% of gallbladder stones, in India in 9% and in Iraq in 24%. In Europeans the brown stone of the gallbladder is extremely rare.

Black pigment stones are predominately found in the white and black population. Accounting for only 10–20% of all stones, they are less frequent than cholesterol stones. But in the United States during cholecystectomy black pigment stones have been found in 20–40%, and in patients older than 70 years of age even in 50%. In younger patients black pigment stones were only seen in about 10%. This means, that the frequency of black pigment stones in North America probably lies between 10% and 50% and increases with the age. In Japan, black pigment stones are found in 9% and in India in 33%. In native American Indians and in Mestizoes they are rare.

In Europeans intrahepatic concrements are rare. The relative prevalence amounts to only 1–8%. In South East Asia intrahepatic gallstones are more frequent, but in Japan the number is decreasing. Worldwide the prevalence of intrahepatic concrements is estimated to 1–15%. But the figures for intrahepatic stones are not very reliable (Table 112.3). As in cholesterol gallbladder stones the prevalence increases with the age. Eighty to 90% of the intrahepatic concrements are

**Table 112.3** Relative frequency of intrahepatic gallstones

| Country | Autopsy studies (%) | Surgery studies (%) |
|---|---|---|
| Taiwan | – | 47.4 |
| China | – | 38.0 |
| Hongkong | – | 21.2 |
| Korea | – | 18.3 |
| Singapore | – | 5.7 |
| Russia | 16.7 | – |
| Germany | 9.0 | – |
| Austria | 8.3 | – |
| Japan | 7.7 | – |
| Columbia | 7.7 | – |
| Norway | 7.2 | – |
| USA | 6.6 | – |

**Table 112.2** Frequency of gallbladder stones in the world population

| Extremely often (up to 70%) | Very often | Frequent (10–40%) | Rare | Very rare |
|---|---|---|---|---|
| US-Indians | US-Mexicans | US-Whites | US-Blacks | South-African Blacks |
| Pima | Chilean | Panama-Whites | Panama-Blacks | Egypt |
| Chippewa | Swedes | US-Puerto Rico | Japan | Sambia |
| Mic Mac | Czechs | South Africa-Whites | Singapore-Chinese | Nigeria |
| Sioux | Slovaks | Australia | Greece | |
| Navajo | | England | Iceland | |
| | | Italy | Romania | |
| | | Denmark | | |
| | | Germany | | |
| | | Norway | | |
| | | The Netherlands | | |
| | | Kashmir | | |

brown calcium-bilirubinate stones, 8% cholesterol stones, 2–3% black pigment stones. Etiologies include parasites, bacterial infections of the biliary tree, bile duct stenoses and strictures.

## Etiology and Pathogenesis

### *Cholesterol Stones*

By definition cholesterol stones are concretions which consist of at least 70% cholesterol (Fig. 112.1; Table 112.4). The contents of pigment, organic and anorganic compounds may vary. A diet rich in calories and cholesterol which induces obesity, a lack of physical training and activity, and factors of modern civilization play a major role in gallstone development. An interesting example has been described in Japan. Here, until the Second World War the brown calcium-bilirubinate stones predominated. Under the influence of increasing western food and of common prosperity, more and more cholesterol stones developed and pigment stones disappeared. Typically this observation was first made in big cities, later on in middle-sized towns, and finally on the country side. Additionally, the lack of dietary fibers favors the development of gallstones. A diet rich in fibers reduces the intestinal transit time, so that less cholic acid can be degraded to deoxycholic acid. Reabsorbed deoxycholic acid probably favors cholesterol supersaturation of bile. If deoxycholic acid is absent, bile remains poor in cholesterol. But since the development of cholesterol stones is a multifactorial process, it remains unclear which role this observation plays. In Table 112.5 the etiologic and pathogenetic factors involved in the development of cholesterol stones are listed.

Sugar, sweets and animal fat seem to favor the development of stones, but a correlation between serum concentrations of cholesterol and the frequency of cholesterol gallbladder stones does not exist in normal weight patients. Alcohol in smaller amounts and a fiber-rich diet seem to prevent the development of cholesterol stones. However, these few observations do not warrant to recommend alcohol for gallstone prophylaxis.

**Table 112.4** Composition of cholesterol, black and brown pigment stones

| Substance | Cholesterol stones | Black stones | Brown stones |
|---|---|---|---|
| Cholesterol | 70–98 | 1–13 | 2–28 |
| Calcium | 1 | 3–40 | 3–9 |
| Pigment | 3 | 10–90 | 28–79 |
| $Na^+$, $K^+$, $Mg^{++}$ | 0.05–1.0 | ? | ? |
| Ca-carbonate | – | 0–65 | – |
| Ca-phosphate | – | 0–32 | <1 |
| Bile salts | Few | 2 | 2 |
| Ca-palmitate-stearate | Very few | 0.3 | 11–67 |
| Glycoproteins | Few, changing | 10–30 | 5–15 |
| "Organic matrix" | Few, changing | 10–73 | 0–30 |
| Bacteria | None | None | Yes |

All figures are given as % of dry weight. Figures considerably vary in the literature. Glycoproteins are also included in the so called "organic matrix"

**Table 112.5** Etiological and pathogenetic aspects of the development of cholesterol gallbladder stones

| | |
|---|---|
| **Genetic, ethnic factors** | Genetic regulation of HMG-CoA reductase. High incidence and prevalence in Indians, low in Eskimos and in central Africa, Lith-genes |
| **Sex** | Females more often have stones than males |
| **Diet** | Diet high in calories, low in fibres |
| **Overweight** | Hypertriglyceridemia, lithogenic bile |
| **Diseases** | Crohn's disease, resection of the ileum of more than 1 m (?), Type 2-diabetes (?) |
| **Hormones** | More than two pregnancies, estrogen treatment |
| **Drugs** | Fibrates, somatostatin analogs |
| **Hypomotility of the gallbladder** | Stasis: reduces enterohepatic circulation of bile acids, stimulates nucleation |
| **Nucleating substances** | Total lipid concentration, $Ca^{++}$, IgA, biliary proteins, bile acids |
| **Mucus** | Mucins, glycoproteins |


**Fig. 112.1** Cholesterol gallstone

*Obesity* plays an important role in the development of stones especially in young women though it is less important in older women, and probably unimportant in men. In overweight persons the total body cholesterol increases, as does the amount in the liver. Overweight persons therefore do not only have increased cholesterol concentrations in the serum, but also have a lithogenic bile associated with a higher prevalence of gallstones. Since overweight persons often present with hypertriglyceridemia, a correlation between serum triglycerides and the development of gallstones has been suspected. A positive correlation exists with type-IV- and type-IIb-hyperlipoproteinemia. These two alterations of fat metabolism are characterized by triglyceride-rich very-low-density-lipoproteins (VLDL). High concentrations of high-density-lipoproteins (HDL) seem to prevent the risk of stone development. But whether triglycerides are indeed responsible for the development of gallstones remains to be determined. Rapid loss of weight in obese patients is associated with the development of gallbladder sludge or stones within 8 weeks in 25% of cases. A reduction of bile acid secretion and a decrease of gallbladder motility are responsible for this observation.

Additionally, *hormonal aspects* seem to play a role in gallstone development. So, for instance, young women have gallstones two to three times more often than men of the same age group. If in elder men female sexual hormones increase physiologically, the difference equalizes. That hormones play an important role in the development of gallstones can also be seen from investigations in Great Britain in the 1970s. These investigations have shown that women taking oral contraceptive pills had gallstones more often, and one decade earlier than women not taking the pill. These data, however applies only to the early contraceptive pills with a high hormone concentration. It was also believed that pregnancy favors the development of cholesterol stones. Epidemiological investigations, however, have shown that women with one or two children do not have gallbladder stones more often than women without children, but the prevalence increases after the third child. In pregnant women, in addition to many other unknown factors, hypomotility of the gallbladder and a hormonally-induced lithogenic bile probably plays an important role.

The situation is even less clear in diabetics. The prevalence of gallstone disease seems to be a little bit higher than in non-diabetics, but these observations were not uniformly corroborated. Besides gallbladder hypomotility and lithogenic bile, hyperinsulinemia is also being discussed as a potential etiologic factor.

*Genetic factors* probably play an important role. This is supported by the observation that Indians living in the Southwest United States, such as the Pima-, Chippewa-, Sioux-, Navajo-, and Mic-Mac-Indians, are gallstones carriers in about 70%, while gallstones are rare in Blacks living in Central Africa. But gallstone carriers of the above mentioned Indian populations very often were overweight, so that alimentary factors could play an additional role. Nevertheless, genetic aspects seem to be important, because a frequency of 70% as observed in Indians cannot be seen in overweight Europeans, Mexicans, or Chileans. The same has been observed in Eskimos living in Greenland. Although Eskimos for many decades have adopted a European diet and many of them are overweight, the prevalence of gallstone disease is still much lower than in Europeans. Thus, the role of genetics in gallstone formation still remains unclear and the reported accumulation of gallstones in families could not be proven statistically. Also, twin investigations were inconclusive, except for some individual reports on monocygotic twins. The frequent observation that gallstones run in families may be a result of the same diet, the amount of food, life-style and eating habits.

In the two inbred mice strains, A/J and AKR/J, it has been shown that a special diet rich in fat and cholesterol plus cholic acid increased accumulation of mucin gel and induced cholesterol supersaturation, however, only strain AKR developed gallstones [8, 17, 33]. Furthermore, QTL analysis identified three so-called Lith genes. Lith 1 is localized on chromosome 2 and another putative Lith gene (Lit 3) on chromosome 17. In men there is no evidence of an association between Lith genes and gallstone formation. Therefore, further investigations are needed.

Also *drugs*, such as clofibrate, somatostatin analogs and probably estrogens favor stone development. Aspirin seems to reduce the mucus layer in the gallbladder, though this is not absolutely proven. Chronic bile acid loss, as seen in ileal Crohn's disease, is associated with gallstones when bile acid synthesis in the liver is unable to compensate for bile acid losses in the stool. Rather improbable is that the resection of the terminal ileum induces the development of gallstones, at least if the resection is less than 100 cm in length. Nicotine has no influence on lithogenesis.

*Lithogenic bile*, nucleation factors, mucus of the gallbladder wall and gallbladder motility play important

roles in the *pathogenesis* of gallstones. The condition sine qua non for the development of cholesterol gallbladder stones is bile supersaturated in cholesterol, so-called lithogenic bile [2]. Lithogenic bile is caused by an increased activity of hepatic HMG-CoA reductase, the key enzyme of cholesterol synthesis, an increase of lipolysis in peripheral fat tissues, by the augmented uptake of cholesterol in the gut and finally by the reduced activity of 7α-hydroxylase, the key enzyme of bile acid synthesis. Changes of the activities of the two key enzymes could be genetically based, as was suggested in animal experiments. The result is bile supersaturated in cholesterol and undersaturated in bile acids. Sufficient bile acid concentrations are necessary to keep the water insoluble cholesterol dissolved.

But bile supersaturated in cholesterol alone does not induce gallstone formation by itself. Gallbladder contraction during and after meals would expel cholesterol supersaturated bile into the gut, which would prevent the formation of microcrystals and agglomeration of cholesterol crystals to form microliths. For the development of cholesterol stones, three additional factors are necessary. The predominance of *nucleation factors* compared to antinucleation factors, an increased secretion of gallbladder mucus and gallbladder hypomotility [1].

In bile of a healthy person, approximately 4–5 wt% of solid substances are cholesterol, 22% phospholipids and 67% bile salts. Although bilirubin is responsible for the color of bile, it represents only 0.3%. The relation between cholesterol and phospholipids plus bile salts characterizes human bile and can be demonstrated in a triangular coordinate system. The ratio of cholesterol to bile acids plus phospholipids is called saturation index. If the ratio between the three components switches in favor of cholesterol, then the index increases and cholesterol precipitates. If the milieu develops in favor of bile acids and phospholipids, cholesterol can be integrated into mixed micelles or phospholipid-cholesterol-vesicles. Bile is no longer lithogenic (see Chapter 7).

Approximately 70% of people present with a cholesterol supersaturated bile but without cholesterol crystals or gallstones. Nucleation studies have shown that in gallstone patients the time that passes until the first cholesterol crystals can be observed microscopically is less than 4 days, and in stone-free persons with the same bile composition 5–15 days. Since after a fasting period, for instance during the night, the morning bile is cholesterol supersaturated but in most persons gallstones do not develop, in the bile of stone carriers substances must exist which favor nucleation of cholesterol monohydrate crystals and the formation of microliths. These factors are called nucleation factors. Some years ago a glycoprotein of 130 kDa was isolated from hepatic and gallbladder bile. In *in vitro* investigations, this glycoprotein accelerated nucleation of cholesterol. This nucleation factor was especially found in patients with multiple gallstones, which could explain why these patients after chemical stone dissolution developed recurrent stones more often than patients with primarily solitary concrements. It has been discussed whether patients with solitary concrements suffer from a different disease than those with primarily multiple stones. Other promoters of nucleation are mucins, phospholipase C, con-A-binding proteins, calcium and other anions binding peptides, aminopeptidase N, IgG, IgM, haptoglobin, phospholipase A, fibronectin and alpha 1-antichymotrypsin.

Besides nucleation factors, another group of compounds has been described which prevent agglomeration of cholesterol crystals. These are, for instance, apolipoprotein A-I and A-II, IgA and some lecithin binding protein fractions. Whether these substances are really inhibitors of nucleation is not clear, since both lipoproteins have not only been found in stone-free persons but also in gallstone carriers.

Cholesterol monohydrate crystals develop from multilamellar cholesterol-rich vesicles, which represent a transport form for phospholipids and cholesterol (Chapter 7). The time which passes in a filtered or centrifuged bile till the development of cholesterol crystals is called nucleation time. *In vitro* nucleation time depends mainly on the influence of nucleation factors, but *in vivo* other aspects also play an important role (Table 112.5). The primary shape of the cholesterol crystals as well as the formation of first deposits seem to have an influence on stone growth. Gallstones usually grow 1–4 mm per year, though great variations have been observed [40].

*Gallbladder mucus* is also important for the development of gallstones. But it is possible that mucus mixed with bile has a different function during stone development than the more viscous mucus adherent to the gallbladder wall. Gallbladder mucus is a mixture of several glycoproteins, carbohydrates and chemically insufficiently defined “mucins”. Whether and how this mucus acts as a nucleation factor is not quite clear. On one hand it could change the surface of cholesterol

crystals in a similar way as typical nucleation factors do (mucin-nucleation factors), while on the other hand it could reduce the motility of the crystals and thus favor agglomeration. Dissolved mucin shortens nucleation time already at a concentration of 1–2 mg/mL. If the concentration is 10–40 mg/L then highly polymere aggregates develop, which are called mucin-gel. This gel covers as a 50–400 µm film the mucosa of the gallbladder wall and its crypts.

That there may be a difference between the mucus adherent to the gallbladder wall and the mucus mixed with bile fluid derives from two observations. At first it has been shown in animal experiments that the production of mucus by the gallbladder mucosa precedes cholesterol hypersecretion into bile, and second the cholesterol rich liquid crystals or multilamellar vesicles first intrude into the mucus of the gallbladder wall before they change to cholesterol monocrystals. This means, that gallstone development obviously starts in the crypts of the gallbladder wall and not in the lumen of the gallbladder or in bile fluid. Similar mechanisms probably occur during the formation of black pigment stones. How mucin formation and secretion is maintained is unknown. Prostanoids seem to play an important role. A diet rich in cholesterol results in the secretion of arachidonyl-lecithin, which promotes prostanoid synthesis.

An important aspect during gallbladder stone development is *gallbladder motility*. The formation of cholesterol crystals from unilamellar and multilamellar vesicles under the influence of nucleation and antinucleation factors is rather slow, so that gallbladder contractions during the meals should extrude all crystals from the gallbladder lumen and by this prevent agglomeration. But as investigations with radioisotopes have shown, gallstone patients have an increased fasting and residual volume after gallbladder contraction, so that crystals are only incompletely extruded from the gallbladder. This is especially the case in older patients, in whom stone formation additionally is caused by an augmented cholesterol secretion and expression of nucleation factors. Hypomotility as a contributor to gallstone development has been shown in animal experiments. It has been shown, for example, that glass beads located in the gallbladder of normal animals did not affect the motility of the gallbladder. Dilation of the gallbladder by stones does not cause or intensify hypomotility. And as has been shown with strips of the gallbladder wall, muscle fibres of stone patients respond to a lesser extent to cholecystokinin than those in healthy persons. Interestingly, this is different in a patient with black pigment stones, so that hypomotility in older patients obviously has a different meaning than in younger cholesterol stone carriers. Cholesterol supersaturation probably is in some way connected to gallbladder motility. Also in starving persons, during pregnancy, during complete parenteral nutrition and during the treatment with somatostatin analogs, gallbladder tone and gallbladder contractility are reduced which explains, why in these patients the development of sludge and gallstones is frequent.

This means, that during the development of cholesterol stones three complex aspects play an important role, (1) the disturbed thermodynamic equilibrium between cholesterol, phospholipids, bile acids, pigments and calcium, (2) kinetic aspects of nucleation, regulated by promoter- and inhibitor compounds, and (3) gallbladder motility, which all have to exist concomitantly over a certain period of time.

The growth of crystals, crystal agglomeration and the growth of gallstones in the following years has not been sufficiently investigated and therefore is not well understood. In most patients we find a complete set of equally sized concrements, which shows that all stones of this set originate from the same time period and grow to the same extent. A second and a third stone generation in the same gallbladder presenting with different stone sizes has been observed but is rare. If one investigates the sectional planes of the concrements of a single stone generation, they are identical: all stones present with radial or ring-shaped surfaces and other structural similarities. It is extremely rare to find cholesterol and pigment stones simultaneously in the same gallbladder.

In approximately 2% of the patients with very small (a few millimeters) gallstones, spontaneous dissolution may occur. But since calcium containing remnants persist in the gallbladder, spontaneous litholysis is incomplete.

## Black Pigment Stones

Etiology and pathogenesis of black pigment stones has not been investigated as intensively as that of cholesterol concretions [6]. Hemolysis, liver cirrhosis and the age of the patient play an important role. By definition pigment stones are concrements with a cholesterol

content of less than 25–30%. The most important substances in black pigment stones are bilirubin and bilirubin degradation products (bile pigments), free calcium, calcium carbonate, calcium phosphate and a so-called organic matrix of proteins, mucins and glycoproteins, in which the solid substances of the stones are integrated (Table 112.4).

Accounting for approximately 50% of the stone's weight, pigments are the most important compounds of this stone type. More than 98% of biliary bilirubin is bound to sugars as bilirubin di- and monoglucuronides (see Chapter 7). While bilirubin conjugates are very water soluble, unconjugated bilirubin and calcium salts of conjugated bilirubin are soluble to a much lesser extent. For the development of black pigment stones, a surplus of unconjugated bilirubin is most important. The relation between conjugated and unconjugated bilirubin rather than the total amount is important. Only in patients with hemolytic diseases is total bilirubin also increased. Probably the unconjugated bilirubin originates in the liver and not from deconjugation in bile. This is based on the observation in patients with Crigler–Najjar syndrome type I and in the Gunn-rat, in whom hepatic glucuronyl transferase genetically is absent.

Since in unconjugated bilirubin all hydrophilic groups of the molecule, which would render solubility possible to a certain degree even without a sugar component, are blocked by internal hydrogen bonds, the unconjugated bilirubin molecule is nearly insoluble at a pH of 7.4–8.4 even at the low concentration of only 0.3 mM. The molecule now presents with the so-called ridge-tile-form. In the ridge-tile-form the two halves of the molecule (pyrrole rings A, D vs. B, C) are arranged at an angle of >90° (see Figs. 7.7–7.9). Bile acids, bile acid phospholipid-micelles and lecithin-cholesterol vesicles are able to dissolve unconjugated bilirubin to a certain degree, while free cholesterol has no influence on solubility.

Calcium is important for the dissolution of bilirubin. Calcium is able to form unsoluble calcium salts with unconjugated bilirubin, and more than any other cation it induces web formation with pigments and fragments of pigment molecules to form the so-called black pigment. The black pigment, responsible for the name of this stone type, is a highly integrated network of bilirubin polymers and dipyrroles.

Nearly 50% of the dry weight of black pigment stones may be attributed to the so-called organic matrix. The organic matrix consists of a poorly defined group of mucins and of high- and low molecular glycoproteins, containing greater amounts of fructose and galactose. More than 50% the organic matrix consists of branch-chained polysaccharides, covalently bound to a central skeleton of polypeptides.

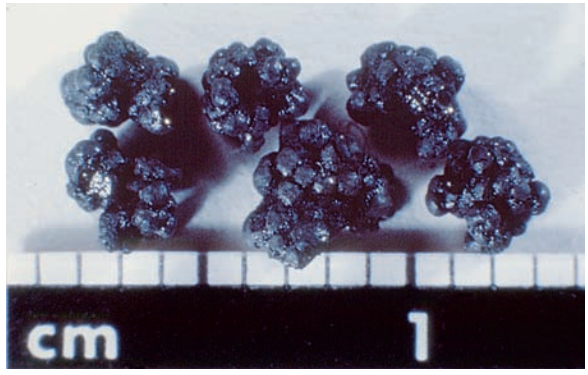


**Fig. 112.2** Mulberry shaped black pigment stones

It is unknown which role bacterial or abacterial cholecystitis, gallbladder motility, nucleation factors or bile lipids play in the development of black pigment stones. A diet rich in proteins is said to favor stone development. Hemolytic diseases may be associated with black pigment stones, but black stones are much more often observed in healthy older patients and patients with liver cirrhosis and especially with alcohol cirrhosis.

Little information exists on black pigment stone development and growth. Black pigment stones may be mulberry shaped, spiny or bizarre and seldom exceed 3–5 mm (Fig. 112.2). The stone center very often is calcified; a more diffuse calcification of the concrement is rarely seen.

## *Brown Pigment Stones*

Cholestasis, bacterial infection and parasites play an important role in the etiology of brown pigment stones. In Europeans brown pigment stones (also called calcium bilirubinate stones) mainly follow manipulations at the bile ducts, which show that bacterial contamination or infection indeed plays an important role. But neither infection nor cholestasiss per se induce gallstone formation. Only the combination of stasis and infection is responsible for stone development.

*Escherichia coli*, bacteroides and clostridia are most often seen in cholecystitis and cholangitis. All three bacteria have a high β-glucoronidase activity and can deconjugate bilirubin di-glucuronide. Water-insoluble bilirubin IX alpha develops. The bacterial enzymes can be antagonized by glucaro-1.4-lactone and bile acids, but obviously this mechanism is too weak and therefore unable to prevent the development of brown pigment stones. Deglucuronation of bilirubin can also be performed by biliary epithelial cells, and in addition to this mechanism a non-enzymatic hydrolysis exists. Besides β-glucuronidases, phospholipase A-I and bile salt hydrolases have been found in bile of pigment stone carriers, which metabolize lecithin to lysolecithin, glycerides to free fatty acids, and bile salt conjugates to free bile acids. Calcium salts of free fatty acids and bile acids precipitate, which decreases solubility of unconjugated bilirubin. As a result of lipid degradation palmitate and stearate form, which as typical bacterial degradation products are not detected in black concretions, which develop under sterile conditions.

Brown pigment stones contain less organic matrix than black stones. The composition of this matrix is similar to that of black pigment stones (Table 112.4). The consistency of brown pigment stones is crumbly and brittle, on the sectional plane different rings of growth indicate stone growth in intervals (Fig. 112.3). In many brown stones the center has a cholesterol nucleus which shows that the so-called primary pigment stones of the bile ducts can develop around cholesterol bile duct stones, which have migrated from the gallbladder into the common duct [20].

Only little is known about stone growth. Since an antlers-like branching into intrahepatic bile ducts has been observed, stone growth obviously occurs by apposition and simple sedimentation of bile pigments, cholesterol, calcium salts and mucus. This view is also supported by the observation of a diffuse stone calcification.

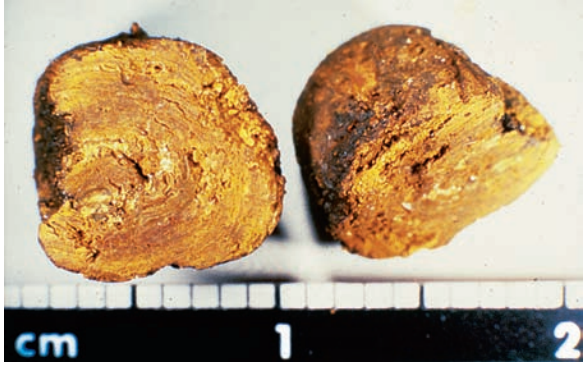


**Fig. 112.3** Brown pigment stones

Rare concrements are the so-called *pepper-and-salt-stones*. In these speckled concrements microspheres of black pigment are enclosed in a cholesterol rich matrix. This cholesterol-rich matrix is a mixture of cholesterol and several anorganic and organic compounds, similar to the organic matrix of black and brown pigment stones. The pepper-and-salt-stone was mainly observed in Japan. Further, there are very rare concrements composed of anorganic and organic calcium salts. They contain calcium carbonate, calcium phosphate and calcium palmitate. They are also called *fat-soap-stones*.

From these five stone types so-called mixed concrements have to be differentiated. This name should not be used any longer today, because it does not describe a certain stone type, but only a cholesterol concrement with different amounts of calcium salts and pigments, which on X-ray present as ring structures, calcified centers or with a diffuse calcification. Probably the different degree of calcification depends on recurring episodes of cholecystitis during which deposition of calcium bilirubinate or anorganic calcium salts on the stone surface occurs. When cholecystitis abates and cholesterol- rich bile creates a lithogenic milieu new cholesterol layers are laid down until the next inflammatory episode causes new rings of calcium to precipitate.

In summary, the three most important stone types, that is the cholesterol stone, the black pigment and the brown pigment stone, differ with respect to their pathogenesis as follows. The most important precondition for the development of a cholesterol concrement are metabolic disorders, during which a cholesterol supersaturated bile is secreted. Nucleation factors, mucus and gallbladder motility are additional factors that promote stone development. Precondition for the development of black pigment stones is a surplus of unconjugated bilirubin, its degradation products, of calcium and different calcium salts as well as a change in the pH of bile. Brown calcium bilirubinate stones develop after bacterial deconjugation of bilirubin diglucuronide, i.e. in the context of bacterial contamination or inflammation. The first two stone types develop in a sterile, the latter in an unsterile milieu. While cholesterol stones contain only smaller amounts of organic matrix, black pigment stones contain mucus from gallbladder mucosal crypts, and the brown stones from the

bile duct mucosa. In conclusion, all three stone types consist of identical compounds; they only differ in their relative composition.

### Gallbladder Sludge

Gallbladder sludge denotes a crumbly viscous mass in the gallbladder. Small gallbladder stones can hide in the sludge and therefore can be overlooked sonographically. Radiologically, sludge can be differentiated from chalk milk bile already on plain radiographs. Sludge is invisible, whereas chalk milk bile appears as a white, viscous radiopaque cloud. Sonographically sludge changes its shape when the patient moves, while chalk milk bile remains invisible on ultrasound.

Especially in the obese, but also in healthy slim persons, sludge can be detected after fasting periods. During total parenteral nutrition sludge is seen in 5% of the patients 4–6 weeks after onset of treatment, and in nearly 100% after 6 more weeks. Especially in children parenteral nutrition induces sludge formation. Approximately 40% of pregnant women develop sludge in the last trimester, which can still be detected after child birth. Twelve months later sludge exists only in 5%. Sludge has also been detected after treatment with ceftriaxone, with the amount of sludge being dose-dependent. Pathogenetically, emptying of the gallbladder probably plays the most important role.The bile acid pool is said to be enlarged, and the enterohepatic circulation of bile acids seems to be reduced.

Sludge consists of bilirubin-monoglucuronide, calcium-bilirubinate, lecithin, cholesterol crystals, cholesterol-monohydrate crystals and gallbladder mucin. Sludge after ceftriaxone treatment has the same composition, but calcium salts and the drug dominate.

Sludge may provide a matrix for the development of pigment- and mixed cholesterol-pigment-stones of the gallbladder. It may be asymptomatic but can also induce colicky pain, cholecystitis with all its complications as well as biliary pancreatitis.

## Diagnosis

See below, paragraph on asymptomatic and symptomatic cholecystolithiasis.

**Table 112.6** Ultrasound findings and limitations of sonography in gallstone disease

| Finding | Limitations |
|---|---|
| Stones < 2–3 mm | Criteria for gallstones often absent, stones sometimes hidden in gallbladder sludge |
| Ton-shaped stones (3–4 cm) | Minor reflexes on gallstone surface, lumen of the gallbladder not visible |
| Cystic duct stones | Immovable, no dorsal shadow |
| Stone number and size | Due to superposition difficult to determine |
| Stone age and stone type | No sonographic criteria available |
| Bile duct stones | Ultrasound waves are reflected at the bile duct wall, absence of stone criteria |

### Ultrasound

The gold standard in the diagnosis of gallbladder stones is ultrasound (see Chapter 37).

Sonography has no side effects and can be repeated, if necessary several times without burdening the patient. In addition, sonography provides useful information on the neighboring organs. The limitations of sonography are stones smaller than 2 mm in diameter, small stones hiding in gallbladder sludge, the difficulty in determining the exact number of stones present, the detection of stones in the cystic duct or so-called ton-shaped stones, and the diagnosis of bile duct concrements (Table 112.6) (see Chapter 113).

The sensitivity of sonography for the detection of gallbladder stones is greater than 90%. Plain radiography can be helpful in determining the particular type of stone. The sensitivity of ultrasound for detecting bile duct stones is much lower, on the order of 20–55%. The lower sensitivity for bile duct stones is in part due to the absence of typical findings for gallstones: the bile duct stone is immobile, there is not always a dorsal shadow, and sometimes the stone is not echogenic. Dilated bile ducts may be an indirect sign of stones but per se they are not proof of the presence of stones.

### Radiological Techniques

Nowadays, diagnosis of gallstones is commonly established by ultrasound. If chemical dissolution is planned, radiological investigations are necessary because only radiologic studies are able to differentiate between the three stone types. Radiological investigations include

plain X-ray of the gallbladder, cholecystography, computed tomography and endoscopic retrograde cholangio-pancreaticography (ERCP). In some cases, magnetic-resonance-cholangiography (MRC) or percutaneous transhepatic cholangiography (PTC) may be necessary.

*Plain X-ray* investigation is the oldest and cheapest method to diagnose calcified gallstones and very old concrements with gas clefts. Ten to 30% of all cholesterol stones of the gallbladder are calcified and therefore visible on plane X-ray. Black pigment stones of the gallbladder, age dependently seen in 5–30% of all gallbladder stones, are calcified in about 70%. Due to the small stone size, calcification in black stones can be demonstrated in most cases only after compression of the abdomen and the gallbladder. Most often, black pigment stones are calcified centrally, diffuse calcification is rare, and ring-shaped calcification even more infrequent.

Usually duct stones have migrated from the gallbladder into the common bile duct. Mostly these are smaller concrements, but up to a size of 0.7 cm they can still pass through the cystic duct. Because these stones are young they usually are radiolucent. In contrast, primary calcium bilirubinate stones of the biliary tree are calcified, and the calcium content amounts to 15–20% of stone weight but calcification in most stones is diffuse. Therefore only 2% of all bile duct stones are visible on plain X-ray.

Kidney stones, kidney cysts, nephrocalcinosis and calcification of the ribs have to be differentiated from calcified gallstones (Table 112.7). Inclusion of air in gallstones shows that the concrement is very old and that gas-filled clefts have developed. Aerobilia can be seen after endoscopic sphincterotomy, in cases of bilio-enteric anastomosis, in patients with gallstone ileus and during emphysematous cholecystisis. These findings have to be differentiated from air in the intestine and in the abdominal cavity.

**Table 112.7** Differential diagnosis of chalk and air on plain abdominal radiographs

| Findings | Differential diagnosis |
|---|---|
| **Chalk** | |
| Calcified gallstones, calcified empyema of the gallbladder, chalk milk bile, porcelain gall bladder | Kidney cysts, kidney stones<br>Tuberculosis of the kidneys<br>Cartilage of the ribs<br>Calcification in the liver, in the adrenal glands<br>Calcified blood vessels<br>Calcified lymphnodes<br>Neurofibroma, lipoma |
| **Gas, air** | |
| Gas clefts in gallstones | Air in the intestine |
| Status after sphincterotomy | Air in the abdominal cave |
| Bilio-enteric anastomosis, fistulas | Air in the pancreatic duct |
| Emphysematous cholecystitis | |
| Gallstone ileus | |

*Cholecystography* with orally or intravenously administered contrast medium is rarely performed nowadays, and has been abandoned in industrialised countries for decades. It is briefly described here for historical reasons.

The technique of *oral cholecystography* is old, safe, and insensitive. Contrast media are fat-soluble iodine containing compounds which after oral administration the evening before the investigation is mainly reabsorbed in the small intestine, bound to albumin and transported to the liver, where it is glucuronidated and secreted into bile. This is only possible when the passage from the liver to the intestine is unobstructed. An optimal concentration of the contrast medium is seen 12–15 h after its administration. At that time water is partly reabsorbed by the gallbladder mucosa which enhances the contrast. After administration of a test meal, of cholecystokinin or ceruletid, a synthetic octapeptide, gallbladder function can be investigated. The gallbladder function is said to be normal when it contracts at least by 30% of its surface on the X-ray film within 45 min. Partial inhibition of the bile flow caused by morphological alterations in the biliary tree, an insufficient stimulus of the gallbladder, diabetes mellitus, Billroth II resection of the stomach and liver cirrhosis can reduce contractility significantly, although there is no real dysfunction of gallbladder muscles.

The bile duct is visible in only 10% of patients undergoing oral cholecystography, but this figure increases to 20% after a test meal and to 80% after pharmacological stimulation. A large series of X-ray sections improves the result.

Two to 10% of all gallbladders are radiologically negative and in about 60% concentration of the contrast medium is rather faint and not sufficient for stone characterisation. A reduced reabsorption rate of the contrast medium, as well as vomiting, or diarrhea may be reasons for inadequate opacification. Other reasons are a reduced hepatic clearance of the contrast medium, intra- and extra-hepatic cholestasis or unfavorable anatomic

conditions of the biliary tree, e.g. post-sphincterotomy patients, in whom the contrast medium rapidly passes through the common bile duct into the intestine, or patients with a long and twisted "low-lying" cystic duct inserting near the papilla of Vater.

Side effects of oral contrast media are nausea, vomiting and occasionally diarrhea. These events are seen in about 50% of patients and their occurrence seems to be dose-dependent. If oral and intravenous contrast media are administered simultaneously, the risk of renal complications increases. Lethality rate of oral cholecystography is very low.

*Intravenous cholecystography* was described for the first time in 1953. With respect to gallbladder stones, it is not superior of cases to oral cholecystography, but bile duct stones can be demonstrated in 50–60% of cases, under optimal conditions even in 70–80%. Bile ducts remain invisible when serum bilirubin exceeds 3–4 mg%, alkaline phosphatase 300 U/L and the aminotransferases 100 U/L. Since in these cases excretion of the contrast medium occurs by the kidneys, kidney function must be investigated before performing intravenous cholecystography. To improve the procedure for bile duct stones the X-ray medium can be infused over a period of 6 h, but because the risk of side effects increases with the infusion time and is dose dependent other methods are instead recommended.

Side effects include nausea, vomiting, flushing, a metallic taste, and cardiac arrest. Minor complications are described in 0.5%, severe complications only in 0.007% and lethal events in approximately 0.002%.

*Computed tomography* is a second choice method for gallstone diagnosis. It is mainly used to investigate the surroundings of the gallbladder and the biliary tree. It is only indicated when the detection of slight calcifications which could not be clearly excluded by conventional radiology and when medicamentous litholysis or ESWL are planned. CT shows calcification in about 50% of the patients in whom stones on plain gallbladder pictures are radiolucent. In the case of air overlapping small concretions, they can be detected if the distance between the CT-sections is small enough. CT is also useful in the detection of chronic calculous cholecystitis, of abscesses which penetrate into the gallbladder bed or into the liver, as well as of fistulas into the pancreatic head.

*ERCP* plays a minor role in the diagnosis of gallbladder stones. ERCP may be indicated in patients with allergy to contrast medium, or when bile duct stones or malignancies are suspected.

*Percutaneous transhepatic cholangiography* (PTC) does not really play a role in the diagnosis of gallbladder stones. Only in a few selected patients PTC may be helpful. For instance, in the few cases with Mirizzi syndrome or with intrahepatic stones which could not be demonstrated by ERC.

*Magnetic resonance cholangiography* (MRCP) could be the future, although in 2008 most results are not yet satisfactory. MRCP is good at demonstrating fluids, e.g., bile. This means MRCP can be used without application of contrast media. It is not helpful in the diagnosis of intrahepatic stones of the peripheral bile ducts, of small pre-papillary concretions, or of stones located directly above duct stenoses and strictures.

Gallbladder stones can occasionally be demonstrated by *endoscopic ultrasound* (EUS). However, a systematic investigation of the gallbladder is not feasible. Therefore, EUS should not be performed in the diagnosis of gallbladder stones. The domain of EUS are common bile duct stones (see Chapter 113).

*Scintigraphy* plays no role in gallstone disease.

## Natural Course

80% of all gallstone carriers never experience pain (gallstone carriers), while about 20% will develop symptoms and complications (gallstone patients). The description of the natural course of gallstone disease is complicated by three different aspects. The first aspect is definition of typical pain. In former times noncharacteristic complaints such as pruritus, heartburn, nausea, vomiting, fat intolerance, pressure in the right upper abdomen, constipation or diarrhea were attributed to gallstone disease; nowadays, only colic is highly suggestive of gallbladder and bile duct stones.

Colic is defined as a severe continuing pain of 15–300 min, located in the epigastrium or the right upper abdomen, sometimes in the back, mainly observed during the night. This event may recur in irregular intervals. Not necessarily a colic is characterized by an increasing and decreasing undulating pain. A colic lasting for more than 5 h is suspicious for complications. These patients should be sent to a hospital and need to be carefully investigated.

Another problem which makes description of the natural course difficult is the observation that carriers of primarily silent gallstones describe symptoms, when they

learn from their physician that they have concrements. Nonspecific pain in the upper abdomen is now "typical for gallstone disease" and the stone carrier is mistaken for a stone patient. The third problem is of statistical nature. Reliable data on the natural course of a disease can only be established when a large population of a total region has been investigated and the follow-up time has been sufficiently long. Despite these caveats, the natural course of asymptomatic stone carriers and symptomatic gallstone patients is fairly well characterised.

## Asymptomatic Cholelithiasis

Asymptomatic cholelithiasis is defined as the presence of gallstones which have been detected incidentally in a patient who has never had biliary type pain. Today asymptomatic gallstones usually are detected by ultrasonography, though if the stones are calcified they can also be detected on a plane radiograph during investigation of other abdominal diseases.

Large epidemiological investigations have shown that 80% of all gallstone carriers remain asymptomatic during the entire life. Mainly this is correct for gallbladder stones. In about 10% of stone carriers, the concrements are located in the cystic duct. Since only 20% of these patients develop complaints, probably the majority of stones are not completely obstructing. Bile duct stones can be asymptomatic for a long period of time, and even a completely filled extrahepatic bile duct system may be clinically silent in some cases. Laboratory parameters are normal, sometimes the levels of alkaline phosphatase or gamma-glutamyl transpeptidase are increased. *In approximately 15% of patients undergoing cholecystectomy, synchronous bile duct stones are observed.*

Many aspects of the natural course of gallstone disease are unknown. So for instance, the time interval between the first secretion of a cholesterol supersaturated bile and the first appearance of gallstones, or whether the speed of growth or the stone type (cholesterol or pigment stone) influence the character of symptoms. According to investigations mainly from Italy during the years of 1982–1989 we can say that during an observation period of 30 years the annual incidence of colic is between 2% and 3%. Since the cumulative risk of developing pain probably depends on the interval free of symptoms, it is suggested that patients who are asymptomatic during a period of 10–15 years will remain asymptomatic for the rest of their life. After a period of 5 years without any complaints the risk of developing colic again is 10%, after 10 years only 5%. In some studies from Italy the risk of developing colic after 5 years was only 1% and later on only 0.5%. This means, that gallstones can be asymptomatic over long periods. The statement "once colic, always colic" obviously is wrong. In primarily asymptomatic patients, biliary complications will develop in 2.7% within 4–20 years, which corresponds to an annual incidence of 0.15% [12].

## Symptomatic Cholelithiasis

Patients who have suffered from typical colic are called symptomatic stone carriers or stone patients. But one has to consider that in older patients there may have been a long asymptomatic interval and the patient cannot remember any of his or her symptoms from a younger age, so that these symptomatic patients may be mistaken for asymptomatic stone carriers.

An analysis of 260 patients has shown that 70% of the patients had multiple and only 30% solitary gallbladder stones. Patients with multiple and smaller concrements more often suffered from biliary pain than patients with fewer or solitary concretions. This can be explained by stone migration or incarceration. Patients with a radiologically negative gallbladder three times more often had complaints than those with a functioning gallbladder. Women significantly more often complained of biliary pain than men. Stone characteristics and contractility of the gallbladder were identical in female and male patients, except during the short period of pregnancy (hypomotility). When gallstones were already present in other family members, complaints were observed more frequently than in patients with a stone-free family.

The natural course of symptomatic gallstone disease has been described only in older studies. According to these investigations, over a period of 30 years approximately 55–60% of 1,211 patients described recurrent symptoms and complications developed in 30%. The incidence of complications was much higher in symptomatic than in asymptomatic patients. Within 2 years complications developed in 69% compared with only 6.3% of patients with minimal pain. The annual incidence of complications is 1–3%. Prognosis in patients with early complaints is less favorable than in patients who where first called silent stone carriers,

and developed complaints only and many years later. But as already mentioned, primarily symptomatic patients may become asymptomatic again. In an Italian study, 4 years after the occurrence of colic 46% were asymptomatic again.

Since patients with gallbladder cancer have gallstones in about 80%, it was believed that gallstones themselves may cause malignancies. This hypothesis, however, has not been proven. It is also unknown whether the stone type or stone number influence the development of gallbladder cancer. There are only few reports on the association of pigment stones and cancer, but pigment stones in the gallbladder are rare. During an observation period of 20 years, gallbladder carcinoma developed in only 0.2–0.5% of the patients. Surgical statistics report on 1–2%, autopsy studies on 0.5%. Cholesterol stones, female sex and age older than 50 years are associated factors. Also stone size seems to be important. Patients with a concrement of 3 cm in diameter or more develop gallbladder carcinoma significantly more often than patients with smaller concrements. In these patients the large gallstone probably is responsible for the development of the tumor.

## Complications of Cholecystolithiasis

The experience of colic sometimes is called complicated gallstone disease, but according to the abovementioned prognosis this is an incorrect designation. A single episode of colic or even recurrent colic and discomfort may completely disappear and never recur; the gallbladder remains functioning, and an increased risk for the development of carcinoma does not exist.

Sequelae of gallstone disease include acute cholecystitis, chronic cholecystitis with additional complications like empyema or hydrops, phlegmon in the gallbladder wall, abscesses, biliary pancreatitis and other pathological alterations in the biliary tree originating from stone migration or developing during the course of chronic progressive inflammation.

### *Acute Bacterial Cholecystitis*

Acute cholecystitis is a disease of all age groups though there is predominance in older patients. In approximately 90%, gallbladder stones are the cause of the disease. During surgery, concrements of the cystic duct or stones in the infundibulum of the gallbladder are found in only 20%, though their presence in this location is the most frequent cause of acute cholecystitis.

Before acute cholecystitis develops the cystic duct is obstructed. Besides gallstones, torsion of the gallbladder, atypical blood vessels, parasites, polyps and gallbladder sludge have been discussed as other possible inciting factors. After sealing off of the cystic duct, a cascade of endotoxin meditated mechanisms is initiated which stimulate secretion of water and mucus by the gallbladder mucosa [15]. The gallbladder is distended, and the pressure of the incarcerated concrement on blood and lymph vessels in the gallbladder neck compromises blood and lymph flow and results in edema and hemorrhage of the gallbladder wall. At first, abacterial cholecystitis develops. Mucosal damage is induced by the enzymatic degradation of lecithin to lysolecithin by the enzyme phospholipase A. Probably later the bacterial degradation products of primary bile acids, such as deoxycholic acid and lithocholic acid, seem also to play a certain role. Cholecystitis therefore is a disease not only of the gallbladder but also of the cystic duct.

Only in 40–50% of the patients with acute cholecystitis are aerobic or anaerobic bacteria found in the gallbladder wall. Invasion of bacteria is a secondary event, and bile represents a perfect culture medium. The bacteria likely originate from the intestinal tract (Tables 112.8 and 112.9), and invade the gallbladder through the portal blood or ascend from the papilla of Vater. The second pathway seems to be improbable if the papilla has not been severed by sphincterotomy or surgery.

**Table 112.8** Spectrum of bacteria in acute cholecystitis and cholangitis

| | |
|---|---|
| Escherichia coli | 40–70% |
| Klebsiella spp | 10–20% |
| Enterococcus | 10–25% |
| Enterobacter | 5–15% |
| Streptoccocus | 5–15% |
| Pseudomonas aeruginosa | 1–5% |
| Bacteroides spp | 1–5% |
| Clostridia Welchii, perfringens | 1–5% |
| Salmonella spp | |
| Yersinia spp | |
| Mycobacterium tuberculosis | |
| Monomicrobial infections | 60–80% |
| Mixed spectra | 20–40% |
| Blood cultures positive | 40–90% |

**Table 112.9** Pathogenetic mechanisms in acute cholecystitis

| | |
|---|---|
| **Obstruction of the cystic duct** | Gallstone, sludge, torsion of the gallbladder, polyps, atypical blood vessels, arteriosclerosis, sclerosis of the sphincter of Oddi |
| **Initial alterations** | Increase of the pressure in the lumen, hemostasis, increased secretion of lymph and stasis, secretion of mucus and water, further increase of pressure |
| **Subsequent alterations** | Bacterial infection via the blood or the lymph (?), development of lysolecithin and toxic bile acids |
| **Tertiary alterations** | Damage of the gallbladder mucosa, atrophic and hyperplastic alterations, sedimentation of calcium, calcium deposits in the gallbladder wall |

The second pathway seems to be improbable if the papilla has not been severed by sphincterotomy or surgery

In 60–80% of all patients cholecystitis begins with a biliary colic. Often pain is mistaken for pain of a gastric or duodenal ulcer, but biliary colic typically starts around midnight, while pain of an ulcer usually starts during daytime. During cholecystitis a severe piercing pain develops in the right upper abdomen. Temperature may initially be normal or only slightly increased. Anorexia, nausea and vomiting subsequently develop. Laboratory investigations show a leukocytosis of 11–20,000/μL, ESR is variable. The levels of cholestatic liver enzymes (AP, γ-GT) can increase, and sometimes an accompanying rise of aminotransferases may be observed. In 20–30% of the patients hyperbilirubinemia develops.

If stone passage into the common bile duct occurs or the disease worsens, fever and chills develop. Leukocytosis increases and in the case of biliary pancreatitis hyperamylasemia develops. Percussing of the abdominal wall can augment pain and tension. Palpation during deep inspiration may elicit pain in by the gallbladder area ("Murphy's sign"). The so-called sign of Boas is characterised by hypersensibility under the right scapula, which, however, often is absent.

If at the time of ultrasound examination the culprit gallstone has migrated, the gallbladder will appear sonographically empty. The gallbladder wall appears thickened to more than 4 mm, the structure has loosened and shows three different layers, which especially can be demonstrated in the gallbladder bed. If phlegmon of the gallbladder wall or an abscess develops which extends to the liver, the whole region including the adjacent hepatic parenchyma becomes hypo– or even anechoic.

*Acalculous cholecystitis* by definition is not a complication of cholecystolithiasis but has to be considered in the differential diagnosis of patients with cholecystitis. For this reason, this variant of cholecystitis is discussed here. Acalculous cholecystitis is defined as an acute inflammation of the gallbladder without gallstones. Eighty percent of the patients are male. In more than 70%, classical findings and symptoms are absent, because in the mainly older patients signs and symptoms of other underlying diseases are superimposed. Fever of unknown origin and an increase of pancreatic enzymes in many patients are the only signs of stone-free cholecystitis. Nevertheless, the course of the disease very often is fulminant. In 50% of the patients complications like empyema, gallbladder perforation and cholangitis develop. Sensitivity and specificity of sonography is 70% and 90% and of computed tomography 90%.

Etiology and pathogenesis of stone-free cholecystitis are not well understood. Stone-free cholecystitis is seen in 1–10% of all cholecystectomies due to acute cholecystitis in up to 50% of patients after major trauma or burns. In 5–8% of the patients with acalculous cholecystitis bile duct stones are found.

In children stone-free cholecystitis is very often associated with simple bacterial infections. Further, immunosuppressive therapy, infections with salmonella spp., cytomegalovirus, the reflux of pancreatic juice, and gallbladder sludge during total parenteral nutrition are being discussed. In older patients blood supply of the gallbladder wall due to arteriosclerosis is reduced, which probably makes it more vulnerable to injury [39]. A reduced blood supply also exists in patients with stone-free cholecystitis and polyarteritis nodosa and vasculitis in lupus erythematosus. That reduced blood supply may be a cause of acalculous cholecystitis is also shown by the observation, that in 5% of patients after liver transplantation ischemic bile duct necrosis develops. In these cases the middle and proximal sections of the extrahepatic bile duct are inflamed, which is the area of an already physiologically inadequate blood supply. Etiologically and pathogenetically, prolonged periods of fasting, immobility, and vascular and hemorrhagic alterations may also play a role. This favors the development of a highly concentrated bile rich in toxic bile acids inducing epithelial damage. Histologically stone-free and calculous cholecystitis are identical. Since acalculous cholecystitis mainly develops in ill patients with multiple underlying acute and chronic comorbidities, early diagnosis is

difficult, and the mortality rate is higher than in calculous cholecystitis (7% vs 1–3%). In the American literature, mortality rates have been reported to be as high as 10–50%, likely reflecting the patient population (e.g. acutely ill patients in the intensive care unit) in whom this disease typically develops.

The treatment of acalculous cholecystitis includes the administration of broad-spectrum antibiotics (to cover Gram-negative aerobic and anaerobic bacteria), and immediate decompression of the gallbladder either by cholecystectomy, or by percutaneous cholecystostomy in severely ill patients. Alternatively, endoscopic drainage by a transpapillary approach (i.e. placing a plastic stent across the cystic duct into the gallbladder) or even by creation of a cholecysto-duodenostomy have both been described but are by no means the standard of care in 2008.

Besides acalculous cholecystitis, there exists also an acalculous biliary colic, which in 80% is seen in young women.

## Gallbladder Empyema and Hydrops

If suppurative cholecystitis develops sonographically, intraluminal echoes can be detected in the gallbladder. Hydrops and mucocele (mucin containing hydrops) develop in 95% of patients with incarcerated stones in the cystic duct. Occasionally a hydropic gallbladder can be palpated. Sonographically the gallbladder is enlarged, tightly filled and its contents anechoic. Laboratory investigations in these patients can be normal.

## Emphysematous Cholecystitis

Gas forming bacteria, such as *Escherichia coli* (50%). Clostridium welchii and perfringens (10%) and Klebsiella spp. can induce emphysematous cholecystitis which may lead to perforation in 20% of cases within 24–48 h. Male diabetics are especially at risk. The mortality rate is higher than in non-emphysematous inflammation. Sonographically and radiologically, the gallbladder wall contains gas and at surgery gallbladder crepitus can be appreciated. Emphysematous cholecystitis very often occurs in the context of acalculous cholecystitis.

## Chronic Cholecystitis

Recurrent acute cholecystitis can follow a chronic, slowly smouldering course. Compared to acute cholecystitis, symptoms diminish, laboratory tests are nonspecific and may even be normal. Sonographically the gallbladder wall is thickened, the stone-filled gallbladder shrinks, and in an extreme situation the gallbladder lumen completely disappears.

## Chalk Milk Bile, Porcelain Gallbladder

Recurring bouts of acute cholecystitis induce precipitation of calcium carbonate and other calcium compounds in bile. Usually the cystic duct is occluded by a concrement. However, it is not quite clear whether inflammatory bouts are really responsible for the development of chalk milk bile. Since, however, after acute inflammation calcium precipitates are being detected on gallstone surfaces these bouts could also be responsible for the development of chalk milk bile. The differential diagnosis includes muscular hypertrophy of the gallbladder neck and chronic fibrosing cholecystitis.

On plain radiographs one sees white, cloudy shadows which dissolve or disappear when the patient moves. On autopsy a thickened, crumbly or even pasty white mass can be seen, usually combined with complete shrinkage of the gallbladder.

It has been claimed that chalk milk bile can cause pain in the upper abdomen, sometimes even colic associated with an increase of aminotransferases, cholestatic enzymes as well as bilirubin.

In the case of a porcelain gallbladder, the gallbladder wall is partly or completely calcified. Usually porcelain gallbladder is clinically silent and laboratory tests are normal. Sonographically, it may be easily overlooked. Sometimes a half moon-shaped reflex with a dorsal shadow can be seen. Porcelain gallbladder is easily diagnosed by computed tomography.

Porcelain gallbladder is rare, seen in approximately 1–2% of patients with chronic gallbladder diseases. Because 7–20% of patients with porcelain gallbladder can go on to develop gallbladder cancer, it is considered a precancerous lesion. Diffuse calcification seems to be less dangerous than patchy alterations. Cholecystectomy is the treatment of choice.

### Mirizzi Syndrome

After incarceration of a gallstone in the cystic duct or gallbladder infundibulum, the resulting edema and inflammation can cause obstruction of the common bile and/or common hepatic duct. Cholestasis and biliary obstruction ensues. Diagnosis is suggested by transabdominal ultrasound, which in addition to findings of acute cholecystitis, typically shows a normal caliber of the distal common bile duct, with proximal common hepatic duct and intrahepatic duct dilatation. ERCP is both diagnostic as well as therapeutic (i.e., stent insertion with or without stone extraction), though cholecystectomy is the definitive treatment. Differential diagnosis of the Mirizzi syndrome includes gallbladder and bile duct cancer. In fact, for unclear reasons Mirrizi syndrome is associated with a higher incidence of gallbladder cancer.

### Stone Perforation and Stone Ileus

Erosions or ulcerations of the gallbladder wall can progress to gallbladder perforation. Suppurative peritonitis may develop. Especially endangered are patients with emphysematous or acalculous cholecystitis.

If gallbladder perforation develops slowly, at first pericholecystitis develops which initially may be "sealed off". Ninety percent of perforations are caused by gallstones, 4% by tumors or injuries and 6% by duodenal ulcer. Perforation may penetrate into neighbouring organs, such as the duodenum, the common bile duct, the stomach and the colon. Gallbladder perforation with penetration of gallstones into the kidneys, the bronchial system, the interpleural clefts, or into the aorta is extremely rare.

Eighty to 90% of all concrements which have passed into the intestine remain asymptomatic and are excreted in the stools. Stones with a diameter of 2.5 cm or more can be incarcerated either in the duodenum causing gastric outlet obstruction ("Bouveret syndrome"), or 20 cm above the ileocecal valve, which is the narrowest section of the terminal ileum ("gallstone ileus"). Since the concrement is difficult to be detected sonographically or radiologically, the mortality rate of gallstoneileus is still rather high (10–20%).

Gallstone ileus, which occurs in 1% of all gallstone perforations as well as of all cases with ileus, is rare. Most often it is diagnosed beyond the 60th–70th year of life in patients with other concomitant diseases and a less favorable prognosis. Patients usually complain of severe pain in the upper and lower abdomen accompanied by extreme meteorism. Tension of the abdominal wall can be severe. Radiologically, massive dilation of proximal small intestinal loops with air, pneumobilia, and a gallbladder filled with gas can be observed.

### Gallbladder Carcinoma

Four to 6% of all malignancies are gallbladder or bile duct carcinomas (see Chapter 116). Gallbladder cancer is associated with gallstones in 70–80% of cases. During an observation period of 20 years of symptomatic and asymptomatic patients with gallstones, 0.2–0.5% develop cancer. Since this figure is approximately the same as the mortality rate of conventional cholecystectomy, an operation is not indicated as prophylaxis of cancer. The annual incidence of gallbladder cancer in primarily asymptomatic stones is 0.01%. Annual incidence in symptomatic patients is 0.05%. The risk of developing gallbladder cancer is higher in patients with stones with a diameter of 3–3.5 cm, in patients with porcelain gallbladder and in American Indians. These patients are candidates for surgery.

### Biliary Pancreatitis

Biliary pancreatitis usually is seen in patients with bile duct stones (Chapter 113). Although not common, pancreatitis may be observed in patients presenting with acute cholecystitis if the gallbladder adheres to the pancreatic head, or after a perforating, penetrating duodenal or gastric ulcer. Whether inflammation in these cases reaches the pancreas by the lymphatic system or by contiguity is unclear.

## Gallstone Prophylaxis

Gallstone prophylaxis can be alimentary and pharmacological. Pharmacological prophylaxis is only indicated after successful oral litholysis or shock wave lithotripsy (ESWL) or in obese patients reducing their weight by

special diet or after weight loss surgery. These patients should be treated with UDCA plus CDCA, 6–8 mg/kg b.w. p.o. each, for at least 6 months.

Since it has been shown that a high calory diet rich in cholesterol especially in overweight women favors the development of cholesterol gallbladder stones, prophylactic measures aim at lipid metabolism and gallbladder motility. Slow reduction of bodyweight lowers the activity of hepatic HMG-CoA-reductase and therefore decreases cholesterol synthesis, but also cholesterol uptake by reduction of LDL-receptors on the cell surface. Avoiding fibrate-, progresterone- or estrogen-therapy increases bile acid synthesis, responsible for micellar solubility of cholesterol, increases esterification of cholesterol and reduces the number of LDL-receptors on hepatocyte membranes. Avoidance of fasting sustains regular contractions of the gallbladder excreting cholesterol-supersaturated bile and stimulates enterohepatic bile acid circulation. Breakfast plays a special role by kickstarting gallbladder function, and a glass of milk or chocolate in the evening before bedtime can also be helpful.

Drug prophylaxis of stone recurrence after oral litholysis could act on four different levels: first on cholesterol supersaturation, then on the production of mucus, nucleation factors and gallbladder motility. Only the first aspect has been investigated. After successful litholysis the administration of 300 mg ursodeoxycholic acid daily reduced recurrence rates by 30%, but not in all patients. Patients with primarily solitary concretions and patients with multiple stones and older than 50 years benefit less from prophylaxis than others. To reduce mucus production and secretion of nucleation factors after successful oral litholysis, patients were treated with 1,000 mg acetyl-salicylic acid or other non steroidal antiphlogistic drugs, for instance with 100 mg diclofenac daily. The results, however, were not convincing.

Prophylaxis for black and brown pigment stones is not available.

## Therapy

Silent, i.e. asymptomatic gallstones should not be treated [12]. The only exception to this rule is an increased risk of gallbladder carcinoma which occurs with an asymptomatic solitary stone ≥3 cm in size and with a so called porcelain gallbladder [2]. Since recent investigations have shown that the frequency of even severe colic can decrease and symptoms can disappear completely, in contrast to previous recommendations, colic is not an absolute indication for surgery, but rather an indication for patient surveillance and analgesic therapy (Table 112.10).

**Table 112.10** Definition of biliary colic and treatment

| |
|---|
| **Definition** |
| • Severe pain for at least 15 to maximal 300 min |
| • Location: epigastrium, right upper abdomen, back |
| • The patient asks for bed rest |
| • Most often in the evening and during night |
| • Tendency to recur |
| **Treatment** |
| Spasmolytics |
| N-butyl scopolamine: 1–4× 40 mg i.m. or i.v. |
| Combined with analgesics |
| Diclofenac: 75 mg i.m. |
| Indomethacin: 50 mg i.v. |
| Metamizol |
| Paracetamol |
| Opiates (in the case of severe pain) |
| Pethidine: 1–3× 25–150 mg i.v. |
| Morphine sulfate 2–4 mg i.v. or s.c. |
| Hydromorphone 1–2 mg i.v. |

The treatment plan depends on the intensity of symptoms. For patients with only minor symptoms, with nonspecific complaints in the upper abdomen, occasional pain in the gallbladder area or with single episodes of colic and in whom acute or chronic cholecystitis and other complications have been excluded, oral chemolitholysis or extracorporeal shock wave lithotripsy (ESWL) followed by oral litholysis may be discussed with the patient.

In a study of 260 newly diagnosed gallstone patients, 44% were suitable for oral litholysis and 16% for extracorporal shock wave lithotripsy, which corroborates an older study in which 59% of the patients were candidates for oral litholysis [19, 27]. However, in the era of laparoscopic cholecystectomy most patients nowadays will opt for this minimally invasive operative procedure.

### *Oral Litholysis*

Only cholesterol stones are suitable for oral litholysis; calcified cholesterol concrements, black and brown

pigment stones must be excluded. Ultrasonography is not helpful in this regard as it is only useful in establishing the diagnosis of gallstones. By means of plain radiographs, calcified stones or old concrements with central gas clefts can be excluded. Treatment is only successful in patients with non-calcified cholesterol stones of 1–1.5 cm in diameter and in whom the lumen of the gallbladder is not filled by more than 50%.

The two bile acids (chenodeoxycholic and ursodeoxycholic acid) have to be administered daily. Chenodeoxycholic acid (CDCA) inhibits the key enzyme of cholesterol synthesis in the liver, and ursodeoxycholic acid (UDCA) inhibits cholesterol reabsorption in the intestine. UDCA forms liquid crystals with stone cholesterol (multilamellar vesicles), while CDCA forms mixed micelles with cholesterol from gallstones. The result is complete stone dissolution within 12–24 months [41].

Oral litholysis is noninvasive, has no mortality rate and few side effects, and patients are treated as outpatients. The only side effects described are diarrhea, which occurs in approximately 2% of patients, and stone calcification in 10–15%.

With stones of 1 cm in diameter the mean dissolution rate after a treatment duration of 1.5–2 years is 60% (Table 112.11). When computed tomography is performed to exclude even the faintest calcifications, the dissolution rate increases to 90% [24]. Best results are obtained in so-called floating cholesterol stones. Floating stones are concrements with a diameter of 3–5 mm which float in a horizontal layer in a gallbladder filled with contrast medium with the patient in an upright position. Pigment stones of similar size would settle in the fundus of the gallbladder. It is recommended that the two bile acids be given in identical doses of 12–13 mg/kg b.w./day. After 6–12 months of therapy, more than 90% of floating and young cholesterol stones dissolve.

A major problem of oral litholysis is stone recurrence [38]. In patients with primarily solitary concrements, recurrence rate is 30% within the first 5 years after complete stone dissolution, and in patients with multiple stones as high as 50–70%. Continuing treatment after primary litholysis can reduce recurrence rates but not prevent recurrence completely and therefore it is not recommended. These observations have discouraged the use of oral litholysis. If stones have not recurred within the first 5 years after dissolution, however, studies using annual ultrasound have showed that future recurrence is extremely rare [25].

## *Extracorporeal Shock Wave Lithotripsy*

Since for oral litholysis only patients with concrements of 1–1.5 cm in diameter are suitable, larger stones have to be fragmented by extracorporeal shock wave lithotripsy (ESWL) before dissolution is started. ESWL enables chemical lysis of solitary concrements with a diameter of 2 cm or of a total of three concrements of 1 cm each. Usually multiple ESWL–sessions are necessary.

Exclusion criteria for ESWL therapy are the same as for oral litholysis (except for stone size and number) [9]. Because of the risk of bleeding, patients with gastric or duodenal ulcer, clotting disorders, anticoagulation treatment, aneurysm or a cyst in the way of the shock wave have also to be excluded. In most patients treatment results are very good. Ninety to 95% of the concrements can be fragmented to a diameter of less than 5 mm. The optimal size of a fragment for chemical dissolution is 3 mm. After a median duration of drug treatment of 18–24 months, 80–90% of the patients are stone-free (Table 112.11). Side effects of ESWL therapy are minor. Most important are biliary colic in 30%, migration of a stone fragment followed by biliary pancreatitis in 1–2%, and mild cholecystitis in 15% [30]. Cholecystectomy after ESWL is necessary in 2% of cases [13].

**Table 112.11** Results of conservative treatment regimes in patients with uncomplicated cholecytolithiasis (cholesterol stones)

| Method | Limitations | Stone free | Treatment time | Recurrence rate |
|---|---|---|---|---|
| **Oral litholysis** | Pigment, calcified stones, stones > 1.5 cm, gallbladder < 50% filled with stones, bile ducts occluded, acute cholecystitis,-angitis, susp. for bile duct carcinoma | 60–90% | 18–24 months | 30–60% |
| **Lithotripsy (ESWL)** | As above. solitary stones > 2 cm, 3 stones 1 cm, anatomic conditions | >80% | 12–24 months | 15–20% |
| **Contact litholysis** | As above, no limitation by stone number and size | 95% | 9 h | 30–60% |

As in oral litholysis, recurrence of stones after ESWL therapy is a major problem, although the rate is 10–15% lower than with oral treatment alone. Therefore, annual ultrasound examinations after ESWL therapy should be performed to monitor for stone recurrence. Bile acid therapy is recommended as follow-up treatment.

## Contact Litholysis

During this procedure a liquid solvent is injected into the gallbladder or the biliary tree [18, 37]. The gallbladder is punctured percutaneously through the gallbladder bed. After insertion of a thin catheter into the gallbladder, methyl-tertiary-butyl ether (MTBE) is instilled in small doses and immediately aspirated. Contact litholysis of bile duct stones can be performed after cholecystectomy through a T-tube, via a nasobiliary tube or after percutaneous transhepatic puncture of the bile ducts (PTC).

A European survey of more than 800 patients has shown that percutaneous gallbladder puncture is successful in 95% and that stones are completely dissolved in 96% of cases at a median time of 9 h (Table 112.11). For solitary stones the mean treatment time is 4 h, for multiple concrements 12 h [14, 21]. The most important complication is bile leakage after catheter removal, which occurs in 4% of the patients. As already mentioned, due to technical difficulties this procedure is no longer performed.

## Cholecystectomy

Cholecystectomy is the treatment of choice in patients with symptomatic cholecystolithiasis. In contrast to conservative treatment methods, the obvious benefit of cholecystectomy is that in addition to the removal of the offending stones, the gallbladder is also removed.

Contraindications for surgery today are rare and include significant comorbidities and untreated coagulopathies. Contraindications for laparoscopic cholecystectomy are severe cardiorespiratory diseases, portal hypertension, clotting disorders, ileus, diffuse peritonitis, infections of the abdominal wall, acute biliary pancreatitis, the Mirizzi syndrome and patients with suspected malignancy of the biliary system. Acute cholecystitis, bile duct stones, morbid obesity, gallbladder shrinkage and porcelain gallbladder, empyema of the gallbladder, and diaphragmatic hernia are all relative contraindications, mainly for less experienced surgeons.

Approximately 90% of all cholecystectomies are nowadays performed laparoscopically. Open cholecystectomy is only performed in 4–6% of cases due to the above mentioned complications. The results of the laparoscopic procedure are similar to that of the conventional method [3, 7, 29, 34]. Complications occur in 4.42% versus 5.57% during conventional operation. Disadvantages of laparoscopic operation are the time of the procedure, the more frequent occurrence of bile duct (0.6%) or vascular lesions (0.3%), and intestinal injuries (0.1%). In patients with acute cholecystitis the complication rate of laparoscopic operation is lower than that of the conventional procedure. The overall mortality rate is 0.08–0.4% (Table 112.12). Advantages of laparoscopic cholecystectomy are the lower doses

**Table 112.12** Results of conventional and laparoscopic cholecystectomy

| **Conventional cholecystectomy** | |
|---|---|
| *Mortality* | *Complications* |
| <40 years old: 0–0.1% | Total: 3–5.8% |
| ≥60 years old: 2–5% | In pts. with cholecystitis: 22.4% |
| | Residual stones: 1,2% |
| Plus revision of the common bile duct: 3% | Fistulas: 0.6% |
| Plus sphincterotomy: 9% | Bleeding: 0.5% |
| Chronic cholecystitis: 0.6% | Abscess, peritonitis: 0.4% |
| Acute cholecystitis: 2% | Bile duct injuries: 0.1–0.2% |
| Early-elective: 0.3% | |
| Interval operation: 1,5–5.3% | |
| Emergency operation: 10–15% | |
| With peritonitis: 10–20% | |
| **Laparoscopic cholecystectomy** | |
| *Overall mortality* | *Complications* |
| 0.1–0.5% | Total: 0.5–5.8% (mean 2.1%) |
| | In pts. with cholecystitis: 12.4% |
| | Residual stones: 15% |
| | Bile duct injuries: 0.6% |
| | Injuries of blood vessels: 0.3% |
| | Injuries of the gut: 0.1% |

of analgesics needed, a shorter recovery time, and a shorter hospital stay.

### Postcholecystectomy Syndrome

(See also Chapter 111).

Up to 40% of cholecystectomized patients complain postoperatively of pain, which collectively has been referred to as postcholecystectomy syndrome. In only 1.5% of these cases, however, is the pain attributed to a consequence of the operation; rather, 60% are of non-organic nature and 40% are organic. The frequency of the postcholecystectomy syndrome depends on the accuracy of preoperative diagnostic. When classic biliary colic is the indication for operation, only 5% of patients will suffer from postcholecystectomy syndrome. When colic and atypical complaints are the indications, however, 12% develop postoperative pain, and when further nonspecific gastrointestinal disorders are present, the incidence increases to 35–40% [28].

The most frequent cause of postcholecystectomy syndrome is idiopathic. Bile duct stones are found in 1–6% of patients, stenosis of the papilla of Vater in up to 30%, and a long cystic duct remnant in 20–50%. It is doubtful that this latter finding by itself is a cause of postcholecystectomy pain. Overall, the surgical reintervention rate for postcholecystectomy syndrome is only 1%. In one fourth of these patients a stone in the cystic duct, scar neuromas or granulomas of suture material are found.

The long-term effects of removing one's gallbladder are not completely understood. Some studies have shown, for example, an increased rate of right-sided colon and rectal cancers in patients who have undergone cholecystectomy. This has been shown mainly in older women between the 60th–80th years of life. The relative risk of developing colon carcinoma after cholecystectomy was 1.7–3.0%. As an explanation for this phenomenon, an increased circulation of the carcinogenic and co-carcinogenic bile acids, lithocholic acid and deoxycholic acid, in the enterohepatic circulation has been hypothesized. These two bile acids have not only been isolated from the stool of cholecystectomized patients but have also been found in patients of countries with a high incidence of benign and malignant tumors of the large intestine. In animal experiments these secondary bile acids favored the development of 1.2-dimethyl-hydracine-(DMH-) induced tumors.

On the other hand, the risk factors for the development of gallstones and for colon cancer may be the same, e.g., a diet rich in fat, cholesterol or calories. But it has to be mentioned that adenomas and carcinomas are significantly more frequent in cholecystectomized patients than in patients with gallbladder in situ and gallstones, and the prevalence of colorectal carcinoma is similar in men and women, whereas the prevalence of gallstones in women is two to three times that of men. A study from 1993 of 120,000 persons suggests that gallstone disease and colorectal cancer do not have the same origin, and that the development of tumors indeed seems to be the result of preceding cholecystectomy. But because the absolute risk of developing colon cancer after cholecystectomy is very small, the value of cholecystectomy is not reduced [10, 11, 22].

## *Treatment of Cholecystitis and Its Complications*

### Cholecystectomy

The standard treatment of acute cholecystitis is surgery. Cholecystectomy can be performed as an *immediate operation*, as an *early-elective operation* or as an *interval operation* after a period of conservative treatment.

Immediate, emergency operation is indicated in suppurative abscess-forming cholecystitis, in cases of gallbladder perforation, in patients with empyema and in associated peritonitis. In all other cases early-elective cholecystectomy is the method of choice, which means that the operation is performed within 24–72 h after diagnosis. As for antibiotics, usually ampicillin/sulbactam, or piperacillin/tazobactam is usually administered. Alternatively ciprofloxacin plus metronidazole, imipenem, or ampicillin plus gentamicin can be used. In 60–80% of conservatively treated patients, the disease initially improves; if surgery is not ultimately performed, however, up to 70% of patients develop recurrent cholecystitis within the next 6–8 weeks and approximately 10% need emergency surgery. Withholding surgery especially in older patients with accompanying comorbidities increases the risk significantly.

Early-elective cholecystectomy in contrast to interval operation (today not recommended any longer) has lower mortality and morbidity rates, is technically easier to do, and the number of emergency operations is markedly lower compared to interval operation with a longer waiting period. The mortality rate ofearly-elective operation in acute cholecystitis is 0.9%, whereas for interval operation it is 1.5–5.3%. If emergency operation is necessary, then the mortality rates are much higher: 20% in patients older than age 70, and10% for younger patients.

### Cholecystolithotomy, Cholecystostomy

Both procedures are not performed any more. If cholecystectomy is not possible, for example due to the risks of general anesthesia, than a rapid decompression of the acutely inflamed gallbladder by percutaneous cholecystostomy and drainage of the organ may be contemplated. Four to 6 weeks later, cholecystectomy should follow. Frequent complications include necrosis of the gallbladder wall, perforation of the gallbladder, bleeding and bile leakage.

## References

for this Chapters are listed at the end of Chapter 113 (page 1489)

# Bile Duct Stones

# 113

Ulrich Leuschner and Jason N. Rogart

## Chapter Outline



## Epidemiology

In patients with choledocholithiasis gallstones can be located in the extra- as well as in the intrahepatic bile ducts. Bile duct stones can be cholesterol concrements, and black and brown pigment stones. Cholesterol and black pigment stones exclusively originate from the gallbladder and therefore are called secondary bile duct stones. The brown calcium-bilirubinate stone is a primary bile duct stone, which originates from the bile ducts. In East Asia, brown bilirubinate stones can also be found in the gallbladder, but in Europeans it is extremely rare.

The relative frequency of bile duct stones is 23% and increases with age as it does for gallbladder stones. In 30 years old persons it amounts to 5%, in 60 years old persons to 15% and in the 80th year of life to 50%. In 70–80%, bile duct stones are secondary concrements. Since these stones originate from the gallbladder they all have the characteristic cubic, pyramid-like or polygonal shape and can easily be identified as secondary bile duct stones. *Fifteen percent of all patients with cholecystolithiasis simultaneously have bile duct stones and 80–95% of patients with bile duct stones have gallbladder stones.* Primary bile duct stones are round, cylindrical and layered due to their appositional growth and adapt their shape to the shape of the bile duct. Primary bile duct stones mostly develop after endoscopic or surgical manipulations of the biliary tree, and since bile duct stones proximal to stenoses, strictures or tumorous alterations as well as chronic inflammation are difficult to treat, primary gallstones tend to recur. Also, after endoscopic sphincterotomy bile duct stones can develop if the sphincterotomy was not large enough, when extra- and intrahepatic bile ducts are dilated, and the patient is of older age.

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Eighty percent of bile duct stones are located in the common bile duct, with the remaining 20% present in the common hepatic duct or intrahepatic ducts. Exclusive intrahepatic stones are rare. The relative prevalence of hepatolithiasis according to autopsy studies in Europe is 6.6–9.0%. In Russia it is said to be 17%, in China 38% and in Taiwan even 47% (Table 112.3). Usually intrahepatic stones are found in the left liver lobe. In most cases intrahepatic concrements are multiple, and in extreme cases the intrahepatic bile ducts can be completely filled with stones up to the periphery. Etiology and pathogenesis of gallstones have been discussed in Chapter 112.

## Clinics

Prevalence and incidence of asymptomatic bile duct stones are not known because bile duct stones in contrast to gallbladder stones are difficult to diagnose sonographically. It has been estimated that nearly one third of bile duct stones can remain asymptomatic over years. Two thirds develop early symptoms, and in 75% of this group jaundice develops. It is assumed that more or less all bile duct stones will become symptomatic sooner or later, but it is unknown when.

Gallbladder stones with a diameter upto 0.7 cm can pass through the cystic duct, larger concrements after gallbladder perforation directly into the common bile duct. Large concrements can induce ileus when they become incarcerated 20–25 cm proximal to the ileocecal valve. Smaller stones are able to migrate into the colon without difficulties and about 80–90% are excreted with the stool.

## Diagnosis

In asymptomatic gallstone patients laboratory data (leukocytes, bilirubin and aminotransferases) are normal or only slightly increased. The cholestatic enzymes AP and γGT may be elevated. When the common bile duct is completely occluded, cholestasis values increase drastically, jaundice develops, the stool turns a white (acholic) color and the urine is dark. When ascending cholangitis develops C-reactive protein increases as do the erythrocyte sedimentation rate and white blood cell count. In older patients, jaundice may be painless and therefore must be differentiated from malignancy.

### Ultrasound

*Conventional ultrasound* detects bile duct stones in only approximately 50% of cases (see Chapter 37). If stones are suspected, but cannot be shown sonographically, bile duct dilation is an indirect sign for the existence of stones which is superior to laboratory data alone. In distal extrahepatic occlusion the extrahepatic bile ducts, the gallbladder and the intrahepatic ducts become dilated; in patients with only proximal bile duct occlusion, however, only the intrahepatic bile ducts are dilated.

*Endosonography* is the most sensitive method for detecting common bile duct stones (especially small stones (< 5 mm) and those in the pre-papillary duct; see Chapter 42). Despite its high diagnostic accuracy of > 90%, this method is still underused in the diagnosis of choledocholithiasis. The most important disadvantage of endosonography is that it is a time-consuming procedure and requires gastroenterologists with specialized training. In the hand of a well-trained physician, however, endosonography is as valuable in stone diagnostics as endoscopic retrograde cholangiography and even superior to ERC with regard to the prepapillary and periductular region of the bile duct.

### Radiologic Techniques

Oral and intravenous cholangiography are only of historical interest. Scintigraphy also has no place in the diagnosis of bile duct stones. Computed tomography (CT) is less important for diagnosis of bile duct stones and shows concrements only incidentally.

*Magnetic resonance cholangio-pancreatography* (MRCP) is a non-invasive method which in optimal cases visualizes the biliary tree with the same quality as ERC. The specificity of bile duct alterations in some centers is 80–100%, and sensitivity 96%.

*Endoscopic retrograde cholangio-pancreaticography* (ERCP) is superior to all other techniques in demonstrating intra- and extrahepatic bile duct stones. Today, it is a routine procedure of which the risks are well-known. The importance of *diagnostic* ERCP has

been dramatically reduced over the last few years due primarily to MRCP and endoscopic ultrasound, and it has therefore become mainly a therapeutic procedure.

ERCP is indicated if choledocholithiasis is suspected, in biliary pancreatitis, acute cholangitis, in cases of stenosis of the papilla of Vater caused by gallstones and in cases after bilio-enteric anastamosis with a so-called sump-syndrome. Contraindications for ERCP are few and the reader is referred to Chapter 40 for further details. Worth mentioning here in particular are the presence of large communicating pancreatic pseudocysts and of large post-cholecystectomy bilomas resulting from bile leaks (risk of contamination and superinfection).

In patients with Billroth II gastric resection or Roux-en-Y gastric bypass, unassisted ERCP is only successful in about 50%. The proximal section of the bowel can be too long, so that it is impossible to advance the tip of the duodenoscope to the region of the papilla. A similar problem exists when the angle of the anastomosis of the gut is too acute. Additionally, the orientation of the bile duct in the afferent limb is rotated 180 degrees, making cannulation and therapy difficult for unexperienced operators. Recently, techniques such as double-balloon enteroscopy and transgastric laparascopic assisted approaches have made ERCP more successful in such patients with altered anatomy. Large juxtapapillary diverticula of the duodenum can be found in about 20% of the patients. If the papilla is sunken in the middle of the diverticulum or when it is located more to the right or the left side it may be nearly impossible to cannulate the bile duct and/or to perform an adequate sphincterotomy safely. In these patients it can be helpful for cannulation to support the papilla by injecting fluids in its surrounding (thus lifting the orifice) or to evert the diverticulum using endoclips. Additionally, papillary balloon dilation following a small sphincterotomy may provide a safer and more effective path for extracting stones.

The overall complication rate of ERCP is 2.5%, and the mortality rate of diagnostic ERCP is 0.07% (Table 113.1). Long time manipulations at the papilla of Vater increase the risk, as do repeated injections of contrast medium. The largest risk of ERCP is the uneducated and untrained endoscopist. In skilled hands, the success rate of ERCP is 90–95%.

If an endoscopic approach is not successful or possible, *percutaneous transhepatic cholangiography* (PTC) offers an alternative diagnostic and therapeutic route to the bile ducts. PTC helps to differentiate extra- and intrahepatic cholestasis, and is especially suited for investigations in the distal part of the common bile duct. An experienced interventional gastroenterologist or radiologist has a success rate of 95–98%. Puncture of the bile ducts is easier the more the ducts are dilated. The complication rate lies between 1–2%. The mean mortality rate is 0.5% and thus higher than for ERC (Table 113.1).

**Table 113.1** Results of diagnostic ERC and PTC in patients with choledocholithiasis

| Procedure successful | Complication rate | Mortality rate |
|---|---|---|
| ERC > 95% | 0.1–2.8%<br>Drugs: 0.2–0.6%<br>Extravasation: 0.1–0.5%<br>Pancreatitis: 1.0–1.6% | < 0.1% |
| PTC 94–98% | 1.0–2.0%<br>Sepsis: 1.4–3.1%<br>Leakage: 1.5–1.9%<br>Bleeding: 0.4% | 0.2–0.9% |

## Complications

Localisation and quality of pain in patients with uncomplicated choledocholithiasis is not different from that of cholecystolithiasis. But about 50% of patients develop colics, which is more than double of that of cholecystolithiasis. Five percent complain of a continuing pain in the right upper abdomen, radiating into the back or shoulder, 10% suffer from uncharacteristic complaints and only 25% are completely free of symptoms. The simultaneous occurrence of colics or pain, of fever, chills and of jaundice is a classical triad of symptoms caused by bile duct stones and is called Charcot-triad. In about 30% of the patients during physical examination the gallbladder is enlarged and sensible for pressure. Usually liver and spleen are not palpable.

Because bile duct stones can induce severe life-threatening complications when untreated, also asymptomatic concrements have to be removed. The most frequent complications are cholangitis and acute pancreatitis. The degree to which (if any) bile duct stones are responsible for the development of cancer of the biliary tree is unclear. The late complication of biliary cirrhosis has become a rarity in the time of ultrasonography and ERCP.

## Cholangitis

Cholangitis and sepsis are dangerous complications (see Chapter 114). The most frequent cause of cholangitis is bile duct stones, followed by iatrogenic causes, such as operations or endoscopic manipulations within the bile ducts, and then infestation with parasites. Bacterial infection of the bile ducts happens only when the pressure in the biliary tree increases, i.e. when the bile flow into the intestine is reduced or interrupted. Predisposing situations for a bacterial invasion are long-term obstruction by stones, foreign bodies and large peripapillary diverticula. Especially at risk are older patients, because in this group bacteria can be found in healthy bile ducts in 70% even in the absence of stones. Additionally, the absence of secretory IgA seems to favor infections. Bile cultures present with the same bacterial spectra as in acute cholecystitis: Escherichia coli, Klebsiella, Pseudomonas aeruginosa and enterobacteria. In suppurative cholangitis multiple organisms may be found. Blood cultures are positive in 40–90%. Besides the above-mentioned bacteria, salmonellas, yersinia and M.tuberculosis have been demonstrated. Cryptosporidia and cytomegaloviruses make the picture even more complicated in cases of AIDS.

Due to the occlusion of the bile ducts, jaundice develops, the stool becomes white and the urine dark. If the disease worsens sepsis, kidney insufficiency and septic shock may supervene. The fever increases to 40°C, bilirubin to 10 mg% (170 mmol) or even higher, and leukocytosis amounts to 20,000/mL or more. The risk of bleeding can be estimated by measuring platelet count and prothrombin time. The combination of *Charcot's triad* (fever, right upper quadrant pain, and jaundice) with mental status changes and hypotension (implying sepsis) is referred to as *Reynold's syndrome* or *pentad*.

The differential diagnosis of bacterial cholangitis includes acute alcoholic hepatitis, liver abscesses, perforation of an abscess into the abdominal cavity, and in rare cases the crises of sickle cell anemia. In patients with alcoholic hepatitis and sickle cell anemia the bile ducts are not dilated on ultrasonography. Liver abscesses can be diagnosed by ultrasound or by CT. Perforation of a visceral organ into the abdominal cavity is associated with signs and symptoms of acute peritonitis.

Especially endangered by suppurative cholangitis are patients older than 55 years of age, women, patients with other severe concomitant diseases (e.g. liver cirrhosis, liver abscess, renal insufficiency), and patients with cholangitis following PTC.

## Liver Abscess

A severe complication is liver abscess (see Chapter 65 and 66). If abscesses develop from diffuse cholangitis, the liver surface is speckled with multiple yellow dots. If these lesions coalesce, larger abscesses are formed. Large solitary abscesses can infiltrate the surrounding liver tissue, or when encapsulated can compress it.

A liver abscess has to be expected if sufficient drainage of the biliary system in combination with medicamentous therapy is unsuccessful, if the liver size increases, and if during palpation the patient has pain. Intrahepatic abscesses with kidney insufficiency are the most frequent complications of cholangitis.

## Biliary Pancreatitis

15% of all gallstone patients suffer from gallstone pancreatitis. Biliary pancreatitis is more frequently seen in patients with bile duct stones than with acute cholecystitis. The existence of a so-called common channel which drains both the pancreatic and the bile duct is seen in more than 90% of all patients. Surprisingly, bile duct stones are found in only 2–5% of patients with suspected biliary pancreatitis, however 80–90% of these patients have stones in the gallbladder. Therefore it is suspected that biliary pancreatitis is induced during stone passage. This is supported by the observation that more than 80% of the patients with pancreatits have gallstones in the stool, while in only 10% of patients without pancreatitis stones can be detected in the stool. The so-called microliths, which are very small concrements of less than 3 mm in diameter, induce pancreatitis four times more often than larger stones. The role of gallbladder sludge in the development of biliary pancreatitis is still unclear. But since 70% of all patients with a so-called idiopathic pancreatitis have gallbladder sludge, this finding could play a role similar to that of microliths and smaller concrements.

The clinical course of biliary pancreatitis is characterised by the combination of symptoms caused by

bile duct occlusion and those of acute pancreatitis. The patient complains of colicky pain due to the gallstones and of pain radiating into the back as seen in acute pancreatitis. In addition to the cholestatic enzymes alpha-amylase and lipase are increased in the serum. Cholestatic enzymes and bilirubin further increase when edema of the pancreatic head due to inflammation compresses and narrows the common bile duct. A correct diagnosis is possible in 90%. Differential diagnosis is papillary tumour, a tumour located in the pancreatic head, and parasite infestation.

Treatment includes the administration of analgesics, intravenous fluids, and antibiotics. Close observation for early signs of complications such as cardiac, renal or pulmonary insufficiency is absolutely necessary. In cases of sepsis, urgent ERCP within 24 h to decompress the bile duct significantly reduces mortality from 20% to 2%.

### *Bile Duct Carcinoma*

It is rather improbable that there is a connection between the rare bile duct carcinoma and the frequently observed bile duct stones. Only in 20% of patients with bile duct carcinoma are bile duct stones demonstrated. As with gallbladder carcinoma, bile duct carcinoma is a tumor of older age with the exception of patients with primary sclerosing cholangitis. Histologically in 95% it is adenocarcinoma. Gallbladder carcinoma is seen 2–3 times more often than bile duct carcinoma.

## Treatment of Choledocholithiasis and Cholangitis

### *Endoscopic Stone Extraction*

The treatment of choice for bile duct stones is endoscopic sphincterotomy (EST) followed by stone extraction [35]. The aim of this procedure is an efficient reduction of pressure in the biliary tree and subsequent drainage. In patients with severe symptoms and cholangitis, endoscopy is combined with antibiotics, and in cases with complicated concrements adjuvant procedures may be necessary. In patients with an in situ gallbladder, cholecystectomy should eventually be performed.

ERC with endoscopic sphincterotomy is an extremely successful approach to common bile duct stones and has reduced the need for surgical operations to 0.2%. Long-term post EST complications due to the severed papilla of Vater are exceedingly rare. Even when remnants of food or air can be demonstrated in the common bile duct and ascension of bacteria is theoretically possible, there is no convincing data to suggest an increased risk of future cholangitis or biliary obstruction. As long as bile flow is possible and the pressure in the common bile duct is low, these findings are harmless.

If sphincterotomy is considered to be high-risk (e.g., coagulopathic patients, ampulla located within a diverticulum), dilation of the papilla with or without a small sphincterotomy can be considered. Typically, dilation with balloon catheters with a diameter of 8–10 mm are used; it has been shown that these small balloons do not cause lacerations of muscle fibres and are safe [36]. In the case of large bile duct stones (>2 cm), dilation with larger balloons has been reported but may carry greater risks such as perforation and post-ERCP pancreatitis.

Medicinal dilation of the papilla can be induced by sublingual application of glycerol-trinitrate tablets. Approximately 1–2 min after application of the drug the muscles of the sphincter dilate. With this procedure only very small concrements have been shown to pass through the papilla and in a few cases even after preceding lithotripsy. Since the efficacy of this procedure is uncertain it is only indicated in special situations and is not routinely practiced.

In patients with simultaneous bile duct and gallbladder stones, ERCP is typically performed first, followed by elective cholecystectomy within the next 6 weeks. This has been clearly shown in two studies in which after EST "wait and see" was compared to EST followed by cholecystectomy within the above mentioned interval. In the "wait and see" procedure colic occurred in 20% vs. 5% of the surgical group, cholecystitis in 6%, cholangitis in 15% vs. 6%, emergency operations in 37% vs. 0%, and emergency ERC in 10% instead of 0%. Whether EST and stone extraction should precede or follow operation or should be performed during cholecystectomy is still under discussion.

In cases of acute cholecystitis, however, there is no good data on whether it is advantageous to perform ERCP or cholecystectomy first.

**Table 113.2** Surgical and endoscopic therapy of choledocholithiasis *plus* cholecystitis ("therapeutic splitting")

| |
|---|
| **Bile duct stones alone**: Endoscopic sphincterotomy, endoscopic stone extraction, no surgery |
| **Bile duct stones plus gallbladder stones**: "therapeutic splitting": |
| *First*: endoscopic sphincterotomy and stone extraction, especially in patients with cholangitis, biliary pancreatitis |
| *Second*: cholecystectomy later |
| With "therapeutic splitting" surgery should follow endoscopic procedures within 6 weeks, because: |
| In "wait and see" procedure colic develops in 20% instead of 5% after surgery |
| cholecystitis develops in 6% instead of 0% |
| cholangitis develops in 15% instead of 6% |
| emergency operation necessary in 37% instead of 0% |
| emergency ERCP necessary in 10% instead of 0% |

The mortality rate in patients with choledocholithiasis and cholangitis is 2–3%, though after emergency operation it increases to nearly 10–20% (Table 113.2).

The results of endoscopic sphincterotomy are excellent. EST is successful in 85% without any early or late complications. But in 3–5% restenosis can develop. After EST 85–95% are stone-free, but in 5–20% stone recurrence can occur, especially if the length of the incision of the sphincterotomy was too short [4].

The complication rate of ERC with sphincterotomy is low. The most important measure to prevent complications is drainage of the bile duct if stone extraction was not successful during the first session. The most frequent complication during EST is bleeding caused by injury to the arteria retroduodenalis or one of its branches, followed by cholangitis and pancreatitis (Tables 113.3 and 113.4). The most important early complication is retro-duodenal perforation. Frequency of bleeding is correlated with the length of the incision. Using a needle knife instead of the classic pull-type (e.g. Erlanger) sphincterotome seems to lower the complication rate. Drainage of the pancreatic duct diminishes the risk of pancreatitis in the case that the mouth of the duct is swollen due to manipulations at the biliary tree. Bleeding is treated endoscopically by balloon tamponade, thermocoagulation, or by injections of fluids such as dilute epinephrine. The indication for operation in cases of perforation depends on the amount of bile that leaks into the retroperitoneum and the degree of peritoneal infection. The mortality rate of EST amounts to 0.4–0.8%.

**Table 113.3** Complications of endoscopic sphincterotomy and stone extraction

| | Gallbladder removed or in situ | Gallbladder in situ |
|---|---|---|
| | (%) | (%) |
| Cholangitis | 1.6 | Cholecystitis 5–20 |
| Bleeding | 1.0–2.3 | Surgery necessary: 10–15 |
| Perforation | 0.3–0.8 | Mortality after operation: 0–26 |
| Pancreatitis | 1.4–5.0 | |
| Total | 6.9–10.0 | |
| Mortality due to ERCP: | 0.4–0.8 | |
| Mortality (total): | 2.3 | |

Source: Adapted from Freeman ML [9a], Endoscopy 1997

**Table 113.4** Results and early complications of endoscopic sphincterotomy in patients with bile duct stones

| Stone free | Complications | Finding | Mortality rate |
|---|---|---|---|
| 84–97% | 5–7% | Bleeding 2.3%<br>Cholangitis 1.6%<br>Pancreatitis 1.4%,<br>Perforation 0.8% | 0.5–1.0% |

Unsatisfactory results or complications which develop weeks or even months after sphincterotomy are called late complications. Late complications include stone recurrence, seen in 15–20%, stenosis of the papilla, and the recurrence or worsening of symptoms from which the patient suffered before surgery (Table 113.5).

## Adjuvant Treatment Procedures

If there is a prepapillary impacted stone, a pre-cut or a circumcision of the papilla with a needle knife can

**Table 113.5** Late results of endoscopic sphincterotomy in patients with bile duct stones and stenosis of the papilla

| | |
|---|---|
| Stone free | 73–94% |
| Recurrent stones | 3–21% |
| Stenosis after sphincterotomy | 2.3–6.1% |
| Restenosis | 8–33% |
| Symptom-free, improved | 87–91% |
| Stones: symptoms as before | 4–13% |
| Stones: symptoms deteriorated | 1–4% |
| Stenosis: symptoms unchanged | 9–19% |
| Stenosis: symptoms deteriorated | 1–8% |

**Table 113.6** Therapeutic results of endoscopically unextractable bile duct stones

| Method | Stone position | Stone free, comments |
|---|---|---|
| **Mechanical lithotripsy** | Common bile duct | 80–90%; stones ≥ 2.5 cm: 25% |
| **Litholysis** | Extra-, mainly intrahepatic | 50%, time consuming procedure |
| **Electrohydraulic lithotripsy** | Extra- and intrahepatic | 85%, expensive, costly |
| **Laser-lithotripsy** | Extra- and intrahepatic | 75–95%, expensive, costly, |
| **Ultrasound-lithotripsy** | ? | Under investigation |
| **Surgery** | Extra- and intrahepatic | 95–98%, increased morbidity and mortality |

be helpful. If the stone is too large to be extracted through the distal common bile duct or through the papilla, the intraduodenal segment of the bile duct can be dilated and/or the stone can be crushed using a mechanical lithotripter. Mechanical lithotripsy with a strengthened Dormia-type basket has a success rate of 80–95% for stones that could not be extracted with ordinary means (Table 113.6). The result depends on stone size and the degree of hardness [26]. The success rate with concrements of 2.5 cm in diameter, for example, is only 25%. Calcified or very old and hard stones can result in rupture of the lithotripter. The complication rate of mechanic lithotripsy is 1% and is mainly due to sphincterotomy. Stone gravel, small concretions or fragments which cannot be extracted with a Dormia basket can be removed by an extraction balloon.

Other procedures which have been developed to support endoscopic treatment are electrohydraulic lithotripsy (EHL), laser lithotripsy, ultrasound lithotripsy and extracorporeal shock wave lithotripsy (ESWL) [5]. EHL and laser lithotripsy are cumbersome procedures which typically require two skilled operators controlling a "mother" duodenoscope and "baby" cholangioscope which is inserted into the bile duct (cholangioscopy; see Chapter 41). The lithotripsy probe is passed through the accessory channel of the cholangioscope and applied to the stone under direct vision. Due to the expense of the equipment and the technical challenges of the procedure, it is only performed in specialized centers. In the hands of an expert, 80–90% of stones that have failed extraction with ordinary means and mechanical lithotripters can be removed. Laser lithotripters come in a variety of types. With a pulsed Neodyn-YAG-laser, for example, energy is carried forward through a 0.2 mm glass fibre to the stone surface. With the flash light pulsed colored laser (Rhodanium-6G-laser) the instrument differentiates between tissue and the concrement which diminishes the risk of tissue injury. Ultrasound lithotripsy is performed via a rigid nephroscope which leads the ultrasound probe percutaneously transhepatically through a 1.7 cm sinus-tract into the bile ducts. With this procedure intra- and extrahepatic concretions can be treated successfully.

Extracorporeal shock wave lithotripsy (ESWL) is approved in Europe for extra- and intrahepatic bile duct stones after endoscopic sphincterotomy and insertion of a nasobiliary drain. After fragmentation the stone remnants can be extracted. In patients with large stones complete removal is possible in 86%. The 30-day mortality rate amounts to 0.9%. ESWL-treatment is especially suitable for stones proximal to bile duct stenosis, after bends of the common bile duct and for concrements in the cystic duct after previous cholecystectomy and postcholecystectomy syndrome.

## *Treatment of Cholangitis*

Treatment of cholangitis includes stone extraction, drainage and the administration of antibiotics [16, 32]. All three measures have to be performed simultaneously. If immediate removal of the concrement is impossible a nasobiliary tube has to be placed into the bile duct allowing for sufficient drainage and decrease of pressure. From aspirated bile, cultures are performed. As in acute cholecystitis, Escherichia coli, Klebsiella and enterococcus predominate. After endoscopic or surgical manipulations Pseudomonas aeruginosa and other anaerobic bacteria are also found.

Medical treatment of cholangitis consists of infusion of intravenous fluid, administration of analgesics, and antibiotics (e.g. piperacillin and third generation cephalosporins with or without aminoglycosides or metronidazole). When the combination of endoscopic and drug therapy is successful, complaints and fever disappear within 24–48 h. Because endoscopic therapy of cholangitis has a mortality rate of only 5–8% it is superior to surgery with a mortality rate of 10–20%.

## Surgery for Bile Duct Stones

Surgical treatment of bile duct stones nowadays is only necessary in 0.2% of all patients, such as those with Billroth-II-resection of the stomach or Roux-en-Y gastric bypass in which the papilla cannot be approached by the tip of the endoscope [23]. In patients with stenosis of the papilla or incarceration of gallstones, transduodenal papillotomy is performed. In cases with a long stenosis of the common bile duct and with proximally located concretions latero-lateral choledochoduodenostomy or jejunostomy is performed. In the case of choledochojejunostomy a sufficiently long Roux-en-Y anastomosis prevents the disadvantage of previous procedures in which the development of a so-called sump-syndrome can occur. A *sump-syndrome* is defined as gallstones, sludge and bacteria in the eliminated portion of the common bile duct between papilla of Vater and the anastomosis. Here, new choledochal concrements can develop and lead to cholangitis and occlusion of the anastomosis. The risk of developing the so-called syndrome of Roux-en-Y anastomosis caused by an overgrowth of bacteria is a minor one. Which procedure is chosen by the surgeon depends on the general condition of the patient, on risk factors and on the patient's age. The mortality rate of surgical revision of the common bile duct lies between 0–4%. Stone clearance is achieved in 95–98% of patients; in 1–5%, residual stones remain. In 15% of patients, recurrent stones will develop.

## Therapy of Hepatolithiasis

The treatment of intrahepatic concrements is difficult and requires complex procedures. If the stone number is small one can try to extract the stones endoscopically after sphincterotomy, hoping that the more proximal concretions will follow spontaneously into the gut. Since extrahepatic and gallbladder stones often exist together with intrahepatic concrements, the usual procedure is cholecystectomy, revision of the common bile duct with or without bilio-enteric anastomosis and resection of a liver segment. Resection is always necessary when intrahepatic stenosis or prestenotic abscesses exist. The results of surgical treatment are good. The outcome depends on the age of the patient, the total risk, the mode of the procedure and especially the experience of the surgeon. Success rate amounts to 90%. While the recurrence for extrahepatic stones is rather low, intrahepatic stones recur in approximately 25% of patients.

## Cholelitholysis

Chemical dissolution of bile duct stones is only indicated when endoscopic procedures are unsuccessful and surgery is contraindicated. Whether the 10% of remaining stones after endoscopic procedures in an operable patient without contraindications should be treated surgically or chemically depends on the experience of the physicians involved. But the decision also depends on the wishes of the patient, the anatomical situation, on the stone type, the general condition of the patient, and whether the method of litholysis is available or not.

Chemical dissolution is always performed in the hospital because the litholytic solvents have to be instilled into the bile ducts via a catheter. This can be done transpapillary, via a nasobiliary tube, percutaneously transhepatically after puncture of the gallbladder, or through a T-tube after cholecystectomy. Only cholesterol concrements and brown calcium bilirubinate stones can be dissolved; calcified cholesterol stones, calcified brown pigment stones and black pigment stones of the gallbladder are insoluble. The advantages of litholysis are the low rate of complications and low costs. But the most important advantage of litholysis is the possibility also to dissolve intrahepatic stones, especially when they are located in the periphery. Disadvantages of litholysis are the long treatment time (up to 3 months and more) and the staffing situation. Since dissolution rate of bile duct stones does not exceed 50%, in some patients it makes sense only to reduce stone size and not completely to dissolve the concrements and then to extract the smaller stones endoscopically as early as possible. As mentioned previously, chemical dissolution of bile duct stones is rarely performed any longer.

For the treatment of cholesterol concrements methyl-tertiary-butyl-ether (MTBE) can be used. But MTBE should not enter the intestine and should not be injected into the biliary system with increased pressure. Treatment should start with small amounts of MTBE which have to be aspirated immediately after instillation. Dissolution of pure cholesterol stones is 100%, and treatment time is a couple of hours only. MTBE can induce mucosal damage in the bile duct,

and because it is an ether, somnolence after reabsorption in the intestine can occur.

Primary calcium bilirubinate concrements, the brown stones of the bile ducts usually seen after endoscopic or surgical manipulations, only dissolve in an EDTA-solvent at pH 9.4. The solvent is injected by means of a perfusor in a dosage of 15–20 mL/h. EDTA (ethylene-diamine-tetra-acetic acid) is a chelating solvent which chelates calcium bilirubinate in an alkaline milieu. The results are not convincing, but in some patients dissolution therapy may be the only option one has. The treatment of intrahepatic bile duct stones, especially when in a peripheral position of the biliary tree, is a great and life-long challenge, and dissolution therapy may be the only, although unsatisfactory, option one has.

In very few patients with complicated bile duct stones, only the combination of endoscopic procedures, extracorporeal shock wave lithotripsy and contact dissolution will be successful. Unfortunately, stone recurrence is frequent.

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# Biliary Infections

# 114

Ulrich Beuers

## Chapter Outline



## Bacterial Cholangitis

Acute bacterial cholangitis is a severe, potentially life-threatening disease which followed an always lethal course still a hundred years ago [46]. Bacterial cholangitis is characterized by the clinical signs and symptoms of jaundice, spiking fever (with chills), and right upper quadrant (RUQ) abdominal pain, also referred to as "Charcot's triad" [6, 20]. Introduction of medical and interventional treatment options has led to a reduction of mortality of acute bacterial cholangitis to < 10% at the end of the last century [36].

### *Pathogenesis*

Acute bacterial cholangitis develops in immunocompetent individuals when two pathological conditions are fulfilled: (1) Bacteria in bile ("bacterobilia") and (2) bile duct obstruction leading to an increased intraductal pressure.

#### Bacteria in Bile

Bile is sterile in healthy individuals [15, 25, 44]. Anatomical barriers protect bile from being invaded by bacteria both via the hematogenous route by hepatocellular and cholangiocellular gap junctions and via the enteric route by constant bile flow, the bacteriostatic action of bile acids and immunoglobulins, particularly IgA in bile, and a well functioning sphincter of Oddi [52].

Risk factors for invasion of bacteria into the biliary tree include common bile duct (CBD) stones, benign, and to a markedly lesser degree, malignant biliary

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strictures, endoscopic sphincterotomy, and biliary endoprosthesis. Obstructive jaundice, recent clinical infections, age > 70 years, diabetes mellitus, and previous biliary interventions may further enhance the risk for bacterobilia [52]. In the early phase of acute cholangitis, bacteria are detected in at least 75% of bile cultures and often in blood cultures taken during episodes of fever. Mixed infections of bile are found in 30–80% of bacterial cholangitis and in > 80% of cases with severe acute suppurative cholangitis [49, 57]. The most commonly detected bacteria are Gram-negative E. coli and Klebsiella spp. as well as Gram-positive Enterococcus faecalis (Table 114.1) [37, 52, 57]. Enterobacter spp., Citrobacter spp., Proteus spp. and anaerobes like Bacteroides spp. or Clostridium spp. may be found in a minority of cases. After biliary endoscopic or surgical interventions, a mixed flora of the above mentioned Gram-negative bacteria together with Gram-positive bacteria, anaerobes or with Pseudomonas spp. can be expected [45]. Insertion of biliary stents is universally associated with bacterobilia, and stent occlusion frequently leads to bacterial cholangitis. It is generally assumed, but not proven, that bacteria invade the biliary tree under pathological conditions via the duodenum. The pattern of germs found in bile mirrors the predominant bacteria isolated from the upper GI tract [2].

Bacteria in a normal bile duct with low pressure (8–12 cm $H_2O$) do not cause clinical cholangitis, as can be observed after routine endoscopic retrograde cholangiography when cholangitis is only observed in a very low percentage of patients, although bacterial contamination of the biliary tree can barely be avoided [7]. Experimental studies indicate that transfer of bacteria from bile to blood mainly via a transcellular route requires an elevation of biliary pressure to at least 15–20 cm $H_2O$ [26].

**Table 114.1** Bacteria cultured in bile during bacterial cholangitis [37, 52, 57]

| | |
|---|---|
| E. coli | 44–58% |
| Klebsiella spp. | 14–34% |
| Streptococcus faecalis (Enterococcus) | 0–53%. |
| Enterobacter spp. | 5–13% |
| Citrobacter spp. | 0–6% |
| Proteus spp. | 3–10% |
| Bacteroides spp. | 1–18% |
| Clostridium spp. | 4–16% |

### Bile Duct Obstruction

The most frequent cause of bacterial cholangitis is choledocholithiasis. In approximately 10–15% of patients with cholecystolithiasis, gallbladder stones pass into the common bile duct (CBD), and this rate may rise to 25% in the elderly. Thus, all patients with symptomatic cholecystolithiasis should be screened for the presence of bile duct stones [21]. CBD stones remain undetected in 1–5% of patients after cholecystectomy for symptomatic cholelithiasis. Bile duct stones deriving from the gallbladder are most commonly cholesterol stones. In contrast, those developing in the biliary tree after cholecystectomy, during chronic recurrent cholangitis, during parasitic infections of the biliary tree, due to congenital anatomical anomalies such as Caroli's disease or due to secondary stricturing, sclerosis, and dilation of the bile ducts, are mainly pigment stones. Choledocholithiasis leads to bacterial cholangitis in about 10% whereas malignant strictures of the biliary tree only rarely induce bacterial cholangitis as long as interventions have not been performed.

## *Diagnosis*

The diagnosis of bacterial cholangitis is based on clinical findings as well as laboratory tests and imaging studies (Table 114.2) [6]. Typical *clinical signs* and *symptoms* such as the "Charcot's triad" (fever, jaundice, right upper quadrant pain) are observed today less frequently than 25 years ago: fever is observed in 90% of patients with bacterial cholangitis, but jaundice is only observed in two of three patients, and right upper quadrant abdominal pain in less than half of patients [8, 32]. However, severe cases with sepsis and

**Table 114.2** Diagnostic workup of patients with suspected bacterial cholangitis

| | |
|---|---|
| **Signs and symptoms** | Fever, jaundice, RUQ pain, confusion, hypotension (Charcot's trias, Reynold's pentad) |
| **Biochemical tests** | Leukocytes, CRP, bilirubin, alkaline phosphatase, lipase |
| **Microbiological tests** | Blood culture |
| **Imaging** | Abdominal ultrasound<br>EUS **or** MRC |
| **Imaging and treatment** | ERCP |

shock who fulfill "Reynold's pentad" (fever, jaundice, right upper quadrant pain, confusion, hypotension) are still found in 5% of the mostly elderly patients with acute bacterial cholangitis.

*Laboratory tests* may help to confirm the diagnosis of bacterial cholangitis: Leukocytosis (> 10 G/L) is common (70%), but leukopenia may be found in severe cases [32]. CRP is frequently elevated at least 10- to 20-fold. Hyperbilirubinemia is greater than 2 mg/dL (>34 µmol/L) in 80% of cases, and there is a two- to fivefold increase of alkaline phosphatase in up to 75% of patients. Serum transaminases are markedly elevated only in a minority of patients [32].

Blood cultures are positive in 60% of cases. Bile cultures are not routinely recommended before beginning antibiotic treatment, but may be positive in 75–100%.

A meta-analysis of 22 studies, including more than 9,000 patients with symptomatic cholecystolithiasis, analyzed ten clinical, biochemical and ultrasound findings for the prediction of choledocholithiasis as the most frequent cause of bacterial cholangitis [1]. The positive likelihood ratio (LR + : sensitivity/1-specificity) indicating choledocholithiasis was highest for Charcot's triad (18.3), suspicion of bile duct stones on ultrasound (13.6), jaundice (10.1) and a dilated common bile duct > 6–7 mm (LR + 6.9). Hyperbilirubinemia (4.8), elevated alkaline phosphatase (2.6), pancreatitis (2.1), cholecystitis (1.6), and elevated amylase (1.5) had a limited predictive value for choledocholithiasis. None of the criteria alone reached a sufficient sensitivity for the detection of bile duct stones although seven of ten criteria reached a specificity above 90% [1].

*Abdominal ultrasound* has a sensitivity of up to 50% to detect bile duct stones. A common bile duct > 6–7 mm enhances its sensitivity to 75% in non-cholecystectomized patients [40]. Conventional abdominal *computed tomography* (CT) has a sensitivity similar to ultrasound, but is associated with considerable exposure to irradiation and requires more sophisticated technical equipment. Conventional CT has, therefore, no place in routine diagnostic workup when choledocholithiasis is suspected. In contrast, *endoscopic ultrasound* (EUS) has a high sensitivity (95–100%) and specificity (97–98%) for detection of extrahepatic bile duct stones, but also can detect other causes of biliary obstruction and has therefore become the diagnostic procedure of choice in centers with highly skilled endoscopists [55]. *Magnetic resonance cholangiography* (MRC) has recently developed into a highly sensitive and specific (> 85–100%) technique equivalent to EUS for the detection of causes of biliary obstruction [16, 22, 23, 33, 54, 55]. MRC is not invasive and not accompanied by exposure to irradiation. Thus, MRC may be helpful in the diagnostic work-up of a patient with bacterial cholangitis in centers equipped with up-to-date technology. Accuracy of *helical computed-tomographic cholangiography* (HCT-C) for detection of choledocholithiasis appears comparable to MRC, but HCT-C is associated with exposure to irradiation and a risk of adverse reactions to the contrast agent administered intravenously [33].

*Endoscopic retrograde cholangiography* (ERC) remains the gold standard for concomitant detection and treatment of choledocholithiasis and other causes of bile duct obstruction. In experienced hands, a sensitivity of 90–100% and a specificity > 90% for the detection of bile duct stones are reached. Although accompanied by complications such as pancreatitis, cholangitis, or bleeding after endoscopic sphincterotomy (EST), this procedure combines high diagnostic accuracy with therapeutic options for efficient biliary drainage such as basket or balloon extraction of stones, dilatation of strictures, and biliary decompression via stenting or temporary nasobiliary drainage.

## Treatment

Therapeutic measures are directed against the two key pathogenetic factors of cholangitis: bacteria in bile and obstruction of bile flow. Thus, adequate antibiotic treatment and decompression and drainage of the biliary tree are equally warranted [6].

### Antibiotic Treatment

The antibiotic is chosen empirically according to (i) the most common pathogens expected to cause cholangitis (see above), (ii) biliary penetration and activity of the antibiotic, and (iii) the likelihood of resistance of pathogens in the clinical environment [56, 57]. Acylaminopenicillins like mezlocillin and piperacillin cover Gram-negative bacteria as well as enterococcus spp. more efficiently than aminopenicillins, are effectively secreted into bile not only in healthy persons, but also in patients with cholangitis, and may reach biliary levels 200- to 300-fold higher than the mean inhibitory

concentration of common biliary pathogens [19, 20]. In addition, effective biliary bactericidia in vivo has been shown [47]. Addition of a beta lactamase inhibitor broadens the spectrum of amino- and acylaminopenicillins and diminishes bacterial resistance. Cephalosporins (group 2 and 3) also cover Gram-negative bacteria and are adequately excreted into bile, but are less effective against Enterococcus spp. and Pseudomonas aeruginosa. Ciprofloxacin is excreted into bile effectively, can be administered orally, and covers Gram-negative bacteria and Pseudomonas aeruginosa [57]. The new generation fluoroquinolone (group 4), moxifloxacin, is also effectively secreted into bile not only in health, but also in obstructive cholangitis, reaching biliary concentrations sufficiently above the minimal inhibitory concentrations for most of the expected bacteria including Enterococcus spp. and anaerobes [47]. Aminoglycosides (e.g., gentamicin, tobramycin), active against Gram-negative bacteria and Pseudomonas spp., and vancomycin, active against Enterococcus spp., are inadequately excreted into bile. Nevertheless, aminoglycosides in combination with amoxicillin are used for the initial systemic treatment of severe bacterial cholangitis in some centers in order to counteract the consequences of systemic bacteremia. It needs to be kept in mind, however, that the risk of oto- and nephrotoxicity of aminoglycosides is aggravated in cholestasis. Imipenem/meropenem represent potent parenteral antibiotics which cover the whole spectrum of bacteria causing ascending cholangitis including Gram-negative strains, Pseudomonas aeruginosa, Enterococcus spp., and anaerobes [57]. They should, however, be reserved for most severe, life-threatening infections when other regimens have failed (Table 114.3).

## Decompression of Bile Ducts

Severe bacterial cholangitis requires immediate mechanical decompression of the biliary tree to prevent sepsis and septic shock. ERC is the therapeutic procedure of choice which allows, after endoscopic sphincterotomy (EST) (or endoscopic balloon dilation [EBD] of the papilla in patients with coagulopathy and small calculi), extraction of calculi, stenting of stenoses or biliary drainage via a nasobiliary tube [3, 5, 14, 31, 35, 51]. Stenting has been shown to be equivalent to nasobiliary drainage in the acute management of severe obstructive cholangitis [48]. Pregnancy or advanced

**Table 114.3** Antibiotics recommended for treatment of acute bacterial cholangitis

Gram-negative enterobacteriaceae (E. coli, Klebsiella spp., Enterobacter spp., Citrobacter spp., and Proteus spp.) and Enterococcus spp., (as well as Pseudomonas spp. when endoscopic or surgical interventions at the biliary tree have previously been performed) should be adequately covered when empirical antibiotic treatment of bacterial cholangitis is considered [57]. Thus, aminopenicillins + beta lactamase inhibitors, ureidopenicillins (+ beta lactamase inhibitors), group 2 or 3 cephalosporins (+ metronidazol), or group 2 fluorchinolones (+ metronidazol) appear useful as first-line treatment considering the spectrum of bacteria covered, potential side effects, and costs. Aminoglycosides (e.g., gentamycin, tobramycin, gernebcin; cave: oto-, nephrotoxicity, aggravated in cholestasis), active against Gram-negative bacteria and Pseudomonas spp., and vancomycin, active against Enterococcus spp., are inadequately excreted into bile. Aminoglycosides in combination with amoxicillin are used for the initial systemic treatment of severe bacterial cholangitis in some centers in order to counteract the consequences of systemic bacteremia. Group 4 fluorchinolones and carbapenemes should be reserved for severe cases unresponsive to other treatment options.

| Antibiotic | Gram-neg. | Enterococcus spp. | Pseudomonas spp. | Anaerobes | High daily i.v. dose for adults with normal kidney and liver function |
|---|---|---|---|---|---|
| Amoxicillin + Clavulanic acid * | + | ± | | ± | 3 × 2.2 g |
| Ampicillin + Sulbactam * | + | ± | | ± | 3 × 3 g |
| Mezlocillin | + | + | | ± | 3 × 5 g |
| Mezlocillin + Combactam | + | + | | + | M:3 × 5 g C:3 × 1 g |
| Piperacillin | + | + | + | ± | 3 × 4 g |
| Piperacillin + Tazobactam | + | + | + | + | 3 × 4.5 g |
| Ceftazidime (group 3b ceph.) | + | | + | | 3 × 2 g |
| Cefotaxime (group 3a ceph.) | + | | | | 3 × 2 g |
| Cefuroxime (group 2 ceph.) | + | | | | 4 × 1.5 g |
| Ciprofloxacin (group 2 fluo.) * | + | ± | + | | 3 × 0.4 g |
| Moxifloxacin (group 4 fluo.) * | + | + | + | + | 1 × 0.4 g |
| Imipenem/Meropenem | + | + | + | + | 3 × 1 g |
| Metronidazole * | | | | + | 3 × 0.5 g |

*Oral administration possible

age are not contraindications for ERC [27, 30]. Only when ERC cannot be performed for technical reasons (e.g., after Billroth II and other gastrointestinal surgery, or tumor growth impairing endoscopic access to the biliary tree), the percutaneous transhepatic route should be chosen to achieve biliary decompression.

## Prophylaxis

After *biliary tract surgery*, the rate of wound infections was reported to be 15% (3–47%) as documented in a meta-analysis encompassing 42 randomized controlled trials with 4,125 patients [42]. Risk factors included age > 60 years, common bile duct stones, previous biliary tract interventions, jaundice, morbid obesity and diabetes mellitus. Antibiotic prophylaxis with β-lactam antibacterials (e.g., ceftriaxone, cefuroxime, cefotaxime, piperacillin) effectively diminished the rate of wound infections in these cohorts by 9%. A single dose before surgery was as effective as multiple doses. Fluoroquinolones have recently been shown to be as effective as common β-lactam antibacterials [57].

After *percutaneous transhepatic cholangiography* (PTC), the effect of antibiotic prophylaxis has not been studied in trials comparable to those after surgery or ERCP. However, the frequency of infectious complications which reaches up to 20% without prophylaxis, may justify regular use of antibiotic prophylaxis until appropriate clinical trials are available. A protocol like the one mentioned below for patients at risk for post-ERCP cholangitis may be considered.

After *endoscopic retrograde cholangiopancreaticography (*ERCP), cholangitic sepsis was observed in 1.4% of 10,881 patients studied for post-ERCP complications in 10 large cohorts and in 2% of a large unselected population of 10,000 patients assembled by postal questionnaires [7, 52]. Mortality of post-ERC cholangitis reached 10 to 16% in severe cases, but was only 2.9% in a series of prospective, placebo-controlled trials on use of antibiotic prophylaxis in which 6.5% of 539 placebo-treated patients developed cholangitis [4, 7, 52]. A meta-analysis revealed that antibiotic prophylaxis diminished rates of bacteremia, but not cholangitis and sepsis after ERCP and could, therefore, not be recommended for routine use during ERCP [24].

The most important risk factor for septicemia following endoscopic biliary stenting was incomplete biliary drainage (91%!) in a prospective study of 347 patients with obstructive cholestasis [43]. Additional risk factors were prior cholangitis, elevated white blood cell count, bile duct strictures, and number of ERCPs.

These data suggest that antibiotic prophylaxis before/after ERCP should be restricted to

(i) Patients with unrelieved biliary obstructions–antibiotic treatment should be continued in these patients until complete biliary drainage is achieved
(ii) Patients with primary sclerosing cholangitis, as they are at particular risk for at least focal incomplete biliary drainage and chronic bacterobilia after ERCP and
(iii) Immunocompromised individuals at high risk for infectious complications (e.g., post liver transplant; HIV) (Table 114.4)

A regimen of ciprofloxacin, 750 mg p.o. 2 h before ERCP and 6 h after ERCP has been proposed as first line antibiotic prophylaxis in these patients, as it appropriately

**Table 114.4** Antibiotic prophylaxis prior to surgical or endoscopic (ERC) interventions of the bile ducts [6]. No adequate data are available for prophylactic antibiotic treatment prior to percutaneous transhepatic cholangiography (PTC)

| Intervention | Rate of infectious complications | Recommended antibiotic regimen | Ref. |
|---|---|---|---|
| **Biliary tract surgery** | Wound infection 15% (3–47%) | β-lactam antibiotic (e.g., cefuroxime, ceftriaxone, cefotaxime, piperacillin) or fluoroquinolone 1 h before surgery | 9, 39 |
| **ERC** | Cholangitis 1–2% | | 9, 14, 42 |
| (a) No biliary obstruction | | (a) None | |
| (b) Biliary obstruction expected or unrelieved | | (b) β-lactam antibacterial (cephalosporin or ureidopenicillin ± beta lactamase inhibitor) or fluoroquinolone, until obstruction relieved (see Table 114.3) | |
| (c) Sclerosing cholangitis | | | |
| (d) Immunodeficiency (e.g., HIV, immunosuppressive treatment after liver transplantation) | | (c + d) ciprofloxacin 750 mg p.o. 2 hrs before and 6 hrs after ERC or β-lactam antibacterial i.v. (optimal dose and duration of treatment unknown) | |

considers activity against the bacterial spectrum expected, adequate biliary drug enrichment even in cholestasis, drug tolerability, and costs [52]. As an alternative, amoxicillin plus beta-lactamase inhibitors have been considered as suitable for prophylactic treatment in patients undergoing endoscopic interventions in the biliary tree [38]. Optimal duration of prophylactic treatment after endoscopic interventions remains to be determined.

After surgical construction of a *bilio-enteric anastomosis*, some patients may develop recurrent ascending cholangitis and may need long-term antibiotic prophylaxis to avoid development of biliary fibrosis/cirrhosis. As an alternative to oral fluoroquinolones, cotrimoxazole (trimethoprim/ sulfamethoxazole) has been proposed for long-term use in these patients at risk (Table 114.4, see also above) [57].

## Fungal Cholangitis

Fungal cholangitis remains anecdotal in otherwise healthy individuals. "Fungobilia" has only recently attracted more clinical attention when biliary Candida infections were reported in cohorts of patients with primary sclerosing cholangitis (PSC) and dominant biliary strictures or immunocompromised patients, e.g. after liver transplantation [18, 34]. In these patients, repeated endoscopic interventions, frequent antibiotic treatment in the recent past, and immunosuppressive therapy may have contributed synergistically to fungal infection of the biliary tree (see Chapter 68).

### *Diagnosis*

In the case series reported, clinical signs and symptoms of cholangitis were only described in a portion of the patients affected. Hyperbilirubinemia and CRP elevation were more pronounced in PSC patients with Candida infection than in those without Candida infection [34]. Different Candida species (C. albicans, C. glabrata, C. tropicalis) were identified in bile of these patients.

### *Treatment*

The antifungal drugs fluconazole and caspofungin are secreted into bile and may be expected to reach adequate levels to eradicate Candida from bile [10]. Nevertheless, antifungal treatment of biliary candidiasis did not always lead to fungal clearance, and spontaneous clearance without antifungal treatment was also reported in single cases [34]. Thus, the effectiveness of antifungal treatment for biliary candidiasis remains to be proven.

## Parasitic Cholangitis

Biliary parasites are responsible for cholangitic features at considerable rates mainly in tropical and subtropical areas of the world [6]. The nematode Ascaris lumbricoides and the trematodes ("liver flukes") Clonorchis sinensis, Opisthorchis viverrini, Opisthorchis felineus, Dicrocoelium dendriticum, Fasciola hepatica and Fasciola gigantica are the most common biliary parasites. The cestodes Echinococcus granulosus and Echinococcus multilocularis usually are diagnosed as focal lesions of the liver or other organs and are, therefore, not discussed here although they may invade the biliary tree in rare cases and, thereby, cause biliary complications (see Chapters 66 and 67).

### *Pathogenesis*

Parasite-induced bile duct damage and inflammation with bile duct strictures, focal bile duct dilatations, and biliary fibrosis may be induced by chemical irritation of biliary endothelia by parasitic secretion and parasite eggs, mechanical bile duct obstruction, and initiation of pigment stone formation [11]. In addition, parasite-induced bacterial superinfection together with mechanical obstruction, may be responsible for episodes of acute cholangitis.

### *Diagnosis and Treatment*

#### Nematode Cholangitis

Ascaris lumbricoides is found worldwide, but is reported as a major cause of biliary pain and cholangitis often in young patients from tropic and subtropic areas, e.g. India or South America. Nematode eggs are mostly

ingested with contaminated vegetables via the oral route. Cholangitis is usually induced by migration of larvae or young adult worms into the biliary tree where they may cause – together with comigrating intestinal bacteria – suppurative cholangitis, biliary obstruction, abscesses, cholangiectases, sometimes papillary hyperplasia, gallstone formation, biliary fibrosis and even cirrhosis. The diagnosis of ascaris lumbricoides cholangitis is made by blood eosinophilia and stool examination, ultrasound, CT, and ERCP.

The treatment includes antibiotics for bacterial superinfection, spasmolytic agents for pain relief, and anthelmintic drugs (mebendazole 100 mg p.o. bid for 3 days; albendazole 400 mg p.o. daily for 3 days; pyrantel 10 mg/kg b.w. p.o. once). Worms can then be removed via the endoscopic route.

### Trematode Cholangitis

The "liver flukes" Clonorchis sinensis, Opisthorchis felineus, Opisthorchis viverri and Dicrocoelium dendriticum are found mainly in Asia and Eastern Europe. They undergo a biphasic life cycle in an intermediate host (a freshwater snail and subsequently freshwater fish) and a definitive host, a mammal. Trematode eggs are passed in the feces of the definitive host, and develop to ciliated microcidia in freshwater which are ingested by or penetrate into snails and multiply into tailed cercariae. Cercariae are taken up by or penetrate freshwater fish. Humans and other mammals develop liver fluke cholangitis after ingestion of raw infected fish. After infection, patients are often asymptomatic, but may develop recurrent cholangitis, secondary gallstone disease, and fibrosing bile duct alterations. Long-term biliary infection is a serious risk factor for development of cholangiocarcinoma. The diagnosis is made by detection of ova in the stool or in bile.

The treatment of choice is praziquantel; alternatively, albendazole can be administered (Table 114.5).

"Liver flukes" like *Clonorchis sinensis* have been held responsible for the development of *recurrent pyogenic cholangitis* (RPC; synonymous: oriental cholangiohepatitis, hepatolithiasis) [29, 39]. RPC is mostly restricted to Southeast Asian countries and emigrants from this part of the world, and is mainly found in rural areas with a low socioeconomic status. Patients mostly present with initial symptoms before the age of 40. Clinical symptoms include Charcot's triad with jaundice, spiking fever, and right upper quadrant pain. Hepatomegaly is a frequent finding. Endoscopic retrograde cholangiography (ERC) reveals dilation of extra- and large intrahepatic bile ducts, biliary strictures at the papilla and at intrahepatic sites and typical involvement specifically of the lateral part of the left hepatic duct. Bile ducts are filled with debris and small stones in almost all patients. Complications of RPC include Gram-negative septicemia and septic shock, empyema of the gallbladder, rupture or fistulization of an obstructed intrahepatic bile duct into neighbouring structures, hemobilia, portal vein thrombosis, abscesses in liver, lungs, and brain, biliary cirrhosis, and acute pancreatitis. Cholangiocarcinoma mainly in the left atrophic hepatic lobe was observed in 3%, and overall mortality was 10% in 427 patients undergoing surgical or nonsurgical transcutaneous treatment during follow-up for 4–10 years [28].

*Fasciola hepatica* occurs worldwide [17]. Hosts are cattle and sheep, but humans may also be infected after ingestion of contaminated watercress or other waterplants. Signs and symptoms of cholangitis and blood eosinophilia develop in the early migratory phase of infection. Eosinophilic infiltrates, liver abscesses, and fibrosing cholangitis are observed later in the course. The diagnosis is made by detection of ova in stool or bile, fasciola antigen in stool, and a serologic test.

Treatment of choice is triclabendazole, 10 mg/kg b.w. p.o. on one or two subsequent days after a meal.

## HIV Cholangiopathy

Cholestasis and liver injury are common in HIV-infected patients [6]. HIV cholangiopathy represents only one of numerous causes (see also Chapters 52 and 69). Elevation of serum biochemical markers of cholestasis such as alkaline phosphatase and γ-glutamyl transpeptidase levels have been reported in up to 55% of patients [53]. These alterations can in part be explained by concomitant infections with hepatitis B or C or alcohol abuse. Drugs represent a major culprit of liver damage in HIV-infected patients and may cause relevant liver injury in up to 10% of patients under highly active antiretroviral therapy (HAART) with nucleoside and non-nucleoside reverse transcriptase inhibitors or protease inhibitors [50]. Antimicrobial agents for the prophylaxis of respiratory infections and Mycobacterium avium complex disease (e.g., clarithromycin, azithromycin), for the prophylaxis and treatment of pneumocystis jiroveci pneumonia

(e.g., trimethoprim-sulfamethoxazole), antifungal agents for the treatment of esophageal candidiasis and systemic mycoses (e.g., ketoconazole, itraconazole, to a lesser extent fluconazole), and antituberculosis agents (e.g., isoniazid, rifampin, pyrazinamide) are associated with considerable rates of hepatotoxicity.

Systemic infections with involvement of the hepatobiliary system accompanying HIV disease include bacillary angiomatosis caused by Rochalimaea henselae and R. quintana, mycobacterial infections including M. avium complex (MAC) and M. tuberculosis, as well as fungal infections with Cryptococcus neoformans, Coccidioides imitis, Sporothrix schenckii, or histoplasmosis.

Beside these numerous causes of hepatobiliary injury in HIV disease, a specific type of cholangitis first reported in 1986 has been described mainly in advanced stages of HIV infection which resembles primary sclerosing cholangitis and has been named HIV cholangiopathy [41].

## Pathogenesis

The cause of HIV cholangiopathy most likely is infectious. No single causative agent has been identified so far. In biopsy specimens, cytomegalovirus, cryptosporidium, microsporidia, and mycobacterium avium complex have all been isolated, albeit at variable rates up to 75% [53].

## Diagnosis

Clinical symptoms include epigastric or right upper quadrant pain (73–90%) and fever (50–65%), whereas jaundice and pruritus are uncommon. Alkaline phosphatase is markedly (mean five- to sevenfold the upper limit of normal) elevated in almost all patients whereas serum transaminases are only mildly elevated. Abdominal ultrasound and CT scan are of limited diagnostic help. Endoscopic retrograde cholangiography (ERC) or magnetic resonance cholangiography (MRC) disclose the typical features of bile duct damage. Four patterns of HIV cholangiopathy have been described:

(i) Sclerosing cholangitis and papillary stenosis (50%)
(ii) Papillary stenosis alone (15%)
(iii) Intrahepatic sclerosing cholangitis (20%) and
(iv) Extrahepatic bile duct strictures with or without intrahepatic sclerosing cholangitis (15%) [12]

Liver histology reveals features of cholangitis with portal edema, neutrophilic infiltrations, bile ductular proliferation, and cholestasis.

## Treatment

Treatment of HIV cholangiopathy includes endoscopic sphincterotomy in symptomatic patients with papillary stenosis which may relieve RUQ pain, but does not regularly affect biochemical abnormalities [9, 13, 53, 58].

**Table 114.5** Parasitic cholangitis

| | Parasite | Source of infection (distribution) | Anthelminthic medical treatment options[a] |
|---|---|---|---|
| **Nematodes** | Ascaris lumbricoides | Vegetables (worldwide, but mainly Asia and South America) | Mebendazole (100 mg bid, 3 days)<br>Albendazole (400 mg daily, 3 days)<br>Pyrantel (10 mg/kg b.w. once) |
| **Trematodes** | (a) Clonorchis sinensis<br>Opisthorchis felineus<br>Opisthorchis viverrini | (a) Raw freshwater fish (Asia and Eastern Europe) | (a) Praziquantel (25 mg/kg b.w. tid, 1 day)<br>Albendazole (400 mg bid, 7 days) |
| | (b) Fasciola hepatica | (b) Watercress and other waterplants | (b) Triclabendazole (10 mg/kg b.w. once, 1 or 2 days) |
| **Cestodes** | Echinococcus granulosus<br>Echinococcus multilocularis | Vegetables or wild fruits contaminated with fox (or dog) feces (worldwide) | Albendazole (12–15 mg/kg b.w. daily in 2 doses, for adults 400 mg bid, 3 months)<br>Mebendazole (50–60 mg/kg b.w. daily in 3 doses during meals, 3–12 months) |

[a]All drugs are given p.o.

Medical treatment with ursodoexycholic acid improves cholestasis, but long-term effects are unknown. In contrast, treatment of opportunistic pathogens does not seem to have an effect on HIV cholangiopathy. In studies in the 1990s, survival of patients suffering from HIV cholangiopathy was poor (14–41% after 1 year), mainly due to the progression of the underlying HIV disease [9, 13]. This indicates that HIV cholangiopathy is a late manifestation of HIV infection.

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# Benign Tumors

# 115

Marcus Wiedmann, Christian Wittekind, Michael Tröltzsch, and Joachim Mössner

## Chapter Outline



## Benign Tumors of the Gallbladder

### *Definition*

Benign tumors of the gallbladder consist of neoplasms that are derived from the gallbladder's wall.

A *classification* of benign gallbladder tumors was first proposed in 1970: they are primarily divided into neoplastic and non-neoplastic lesions [16]. Currently the WHO classification of tumors is most commonly used [32].

The most common neoplastic lesion is an adenoma. Benign mesodermal tumors such as leiomyomas and lipomas are rare. The most common non-neoplasic lesions (pseudotumors) are cholesterol polyps (the presence of which is referred to as "cholesterolosis"), followed by adenomyomas (the presence of which is referred to as "adenomyomatosis"), and inflammatory polyps.

### *Epidemiology*

Benign gallbladder tumors (polyps) can be found in 0.004% to 14% of resected gallbladders and between 1% to 7% of gallbladders assessed by ultrasonography [15, 35, 37, 51, 57, 81]. A solitary polyp is found in approximately half of the cases, two polyps in about 18%, and three or more polyps in approximately 31%. Most of the polyps (about 71%) are located in the corpus of the gallbladder, about one quarter in the infundibulum, and the remaining in the fundus. Average size of the polyps is between 3 and 5 mm. No clear association has been observed between the presence of polyps and the patient's age (although they have only

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rarely been described in children), sex, weight, number of pregnancies, use of estrogens/gestagens in women, or any other risk factors [37].

The prevalence of adenomas of the gallbladder is below 5% [57, 59]. They are rarely associated with Peutz-Jeghers-syndrome and sometimes with Gardner's syndrome [25, 74]. Granular cell tumors of the gallbladder represent only 4% of all granular cell tumors that occur in the biliary tract [80].

Tumor-like lesions are more common within the gallbladder than within the extrahepatic bile ducts. Adenomyomatosis of the gallbladder can be found in up to 5% of cholecystectomy specimens. It occurs more commonly in females and in patients with a mean age of 53 years.

## Etiology and Pathogenesis

The majority of gallbladder tumors (polyps) are not neoplastic but hyperplastic (adenomyomatosis) or represent lipid deposits (cholesterolosis). Both entities are mucosal abnormalities of the gallbladder wall.

Cholesterolosis in association with gallstones is by far the most common pathologic finding in the gallbladder [37]. Its etiology is unknown and unrelated to atherosclerosis. Abnormal deposits of triglycerides, cholesterol precursers, and cholesterol esters can be found in the gallbladder wall.

Adenomyomatosis is a benign condition of the gallbladder characterized by overgrowth of the mucosa, thickening of the muscular wall, and formation of intramural diverticuli or sinus tracts termed Rokitansky-Aschoff sinuses, likely caused by incorporation of cholesterol. According to localisation one can distinguish the focal form ("polypoid") from the segmental form (mostly in the fundus) and from the diffuse adenomyomatosis.

Heterotopia (aberrant tissue) is defined as ectopic, non-neoplastic, regularly composed tissue found in the gallbladder. It is derived from dysontogenetic dislocation of cells from the former foregut, mostly stomach or pancreatic tissue [16, 78]. Potential complications include mucosal ulceration, obstruction, hemorrhage, and pancreatitis. Very rarely, liver, thyroid gland, or adrenal gland tissue can be found [9, 12, 20, 34].

Malacoplakia is a rare entity, usually found in the urinary tract but described in a wide range of tissues. It is a rare and special form of inflammation and usually incidentally discovered in cholecystectomy specimens. The pathogenesis of malacoplakia is not proven, but it is thought to arise from chronic infection and a deficient lytic response by macrophages. The preponderance of malacoplakia in the urinary tract and the colon, and its rarity in the gallbladder, may be explained by the fact that exposure to coliform organisms in the former sites is much more frequent. Immunosuppression is thought to be another important pathogenetic factor in the development of malacoplakia.

## Pathology

The histologic types of benign gallbladder tumors include the following:

- Epithelial Tumors
  - Adenoma (tubulary, papillary, tubulo-papillary)
  - Cystadenoma
  - Papillomatosis (Adenomatosis)
- Non-epithelial Tumors
  - Granular cell tumor
  - Ganglioneurofibromatosis
  - Leiomyoma
  - Lipoma
  - Hemangioma
  - Lymphangioma
  - Neurofibroma
- Tumor-like Lesions
  - Cholesterol polyps
  - Adenomyomas
  - Inflammatory polyps
  - Regenerative epithelial atypia
  - Papillary hyperplasia
  - Intestinal metaplasia
  - Pylorus-gland-metaplasia
  - Squamous metaplasia
  - Heterotopia
  - Xanthogranulomatous cholecystitis
  - Cholecystitis with lymphoid hyperplasia
  - Malacoplakia
  - Stump neuroma after amputation

Adenomas are benign neoplasias of the gland epithelium, which are typically polypoid and distinct. They are divided according to their growth pattern into tubulary, papillary, and tubulo-papillary adenomas.

Cholesterol polyps are tumor-like lesions that are less than 1 mm in diameter in about two third of cases, which gives the mucosa a coarse and granular appearance that is characteristic of the diffuse or planar type of cholesterolosis. The remaining one third of cases are referred to as the polypoid form in which nodules are larger and polypoid in appearance. In the polypoid form the deposits give rise to solitary or multiple cholesterol polyps that are attached to the underlying mucosa with a fragile epithelial pedicle, the core of which is composed of lipid filled macrophages (Fig. 115.1). The macroscopic aspect of cholesterol polyps are yellow deposits, which on the background of hyperemic mucosa led to the description of this finding as "strawberry gallbladder".

Pathologically, the gross appearance of malacoplakia is of a soft yellowish mass. Microscopically, malacoplakia is characterized by a mononuclear cell infiltration. The so-called Michaelis Gutman inclusion bodies seen in some of these cells are pathognomonic. Michaelis Gutman bodies are discrete, lamellated structures 5–10 μm in diameter, which are thought to represent remnants of phagosomes mineralized by calcium and iron deposits. A case of malacoplakia of the gallbladder is described by Charpentier et al. [14]. The cytoplasm of histiocytes in the gallbladder wall was filled with granules positive for periodic acid-Schiff, von Kossa's, and Perls' stains, which is highly suggestive of malacoplakia. Both local inflammation and recent neoplasia could have played a role in the histogenesis of the malacoplakia.

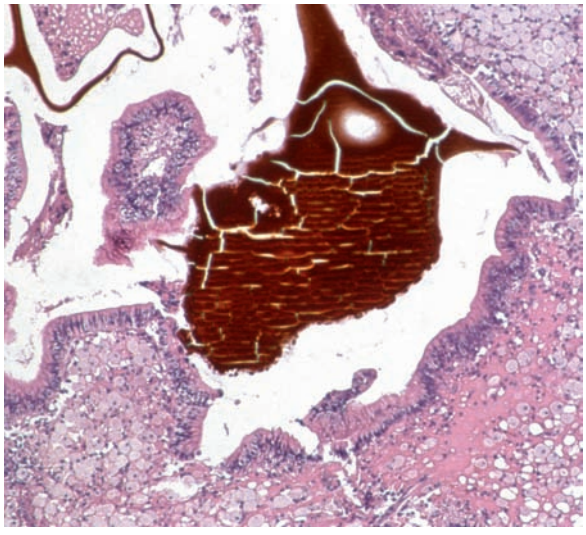

**Fig. 115.1** Histological image of cholesterolosis resulting from abnormal deposits of triglycerides, cholesterol precursers, and cholesterol esters

## Clinical Manifestations

Clinical symptoms of patients are not characteristic. Abdominal discomfort or upper abdominal pain is commonly reported, similar to complaints for cholelithiasis [52, 63]. Since sometimes both entities occur together, a precise allocation of symptoms is difficult.

## Diagnosis

Tumors (polyps) are usually found incidentally on ultrasonography (US) or after cholecystectomy. They appear as little echogenic immobile or pedunculated tumors (the patient needs to change position during the US examination for the differentiation from gallstones!) of the gallbladder's wall without dorsal echo extinction (Fig. 115.2a). However, ultrasound characteristics alone are insufficient to exclude the possibility of gallbladder carcinoma or premalignant adenomas (Fig. 115.2b). When detected on ultrasonography, their clinical significance relates largely to their malignant potential. Gallbladder polyps are well-visualized, the detection of a vessel at the polyps' base by color duplex ultrasound is helpful for the assumption of adenoma (Fig. 115.2c). Intramural cystic formation (anechoic diverticula) with echogenic foci and/or reverberation artifacts (so-called comet's tail artifacts) together with full or partial thickening of the gallbladder wall are considered to be the diagnostic findings for adenomyomatosis on US examination (Fig. 115.2d). If intramural diverticula are not identified, differentiating adenomyomatosis from other causes of gallbladder wall thickening, such as inflammation or carcinoma, is difficult. However, in comparison to malignancies of the gallbladder vascular architecture remains intact.

Endoscopic ultrasound (EUS) may be helpful for distinguishing benign polyps and adenomyomatosis from adenomas and adenocarcinomas [27, 65]. Precision of endoscopic US is higher than that of transabdominal US (87–97% vs. 52–76%) [2, 72]. Computed tomography (CT) is less helpful in distinguishing benign from malignant polyps [28]. In contrast, dynamic magnetic

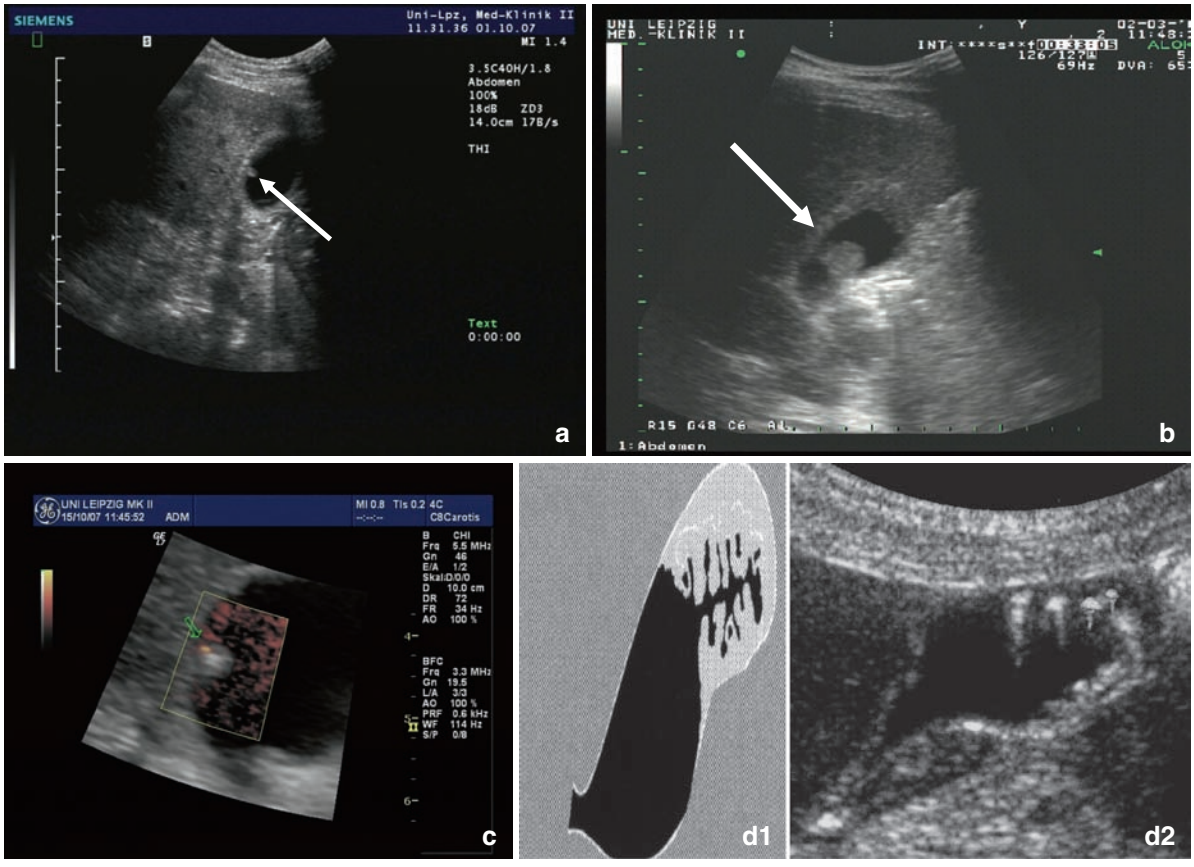


**Fig. 115.2** (**a–d**) Ultrasound imaging of (**a**) a small polyp, appearing as little echogenic immobile tumor of the gallbladder's wall without dorsal echo extinction, (**b**) adenoma of the gallbladder (size above 6 mm with a broad basis, more roundish than a cholesterol polyp), (**c**) adenoma of the gallbladder with a vessel at the polyps' base, and (**d**) adenomyomatosis of the gallbladder (intramural cystic formation with echogenic foci and reverberation artefacts together with full or partial thickening of the gallbladder wall)

resonance imaging (MRI) and MR cholangiography (MRC) can help differentiate benign from malignant lesions in equivocal cases, and provide information as to disease extent [67]. Small polypoid lesions of strawberry gallbladder can be differentiated successfully from gallbladder carcinoma using positron emission tomography (PET) scanning with 18-fluorodeoxyglucose (FDG). PET reveals a focus of FDG uptake at the site of gallbladder carcinoma. No focal uptake is noted in cholesterol polyps.

## *Differential Diagnosis*

The most important differential diagnoses for benign tumors of the gallbladder are: gallbladder carcinoma (see Chapter 116), hepatocellular carcinoma, metastatic disease to the gallbladder fossa, empyema, hematoma, sludge, acute/chronic cholecystitis, carcinoid tumor, metastatic melanoma, gallbladder wall thickening caused by acute hepatitis, ascites, hypoalbuminemia, liver cirrhosis, congestive heart failure, pancreatitis, and gallbladder wall-varices.

## *Therapy and Prognosis*

Gallbladder polyps are mostly benign, representing cholesterol or inflammatory polyps most of the time. Whereas malignant polyps usually occur as single polyps within the gallbladder, cholesterol or inflammatory polyps are usually multiple. According to an analysis

by Shinkai et al., 37% of the patients develop gallbladder neoplasia if there are less than three polyps within the gallbladder with a size of 5–10 mm [69]. In contrast, only 6% of patients develop neoplasia if the size of the polyps is below 5 mm.

*Cholecystectomy is recommended if polyp size exceeds 10 mm.*

In several large studies, polyps with a size > 10 mm were likely to show neoplastic transformation (adenoma) and the risk for carcinoma was almost 50% [44, 47, 48, 51, 57, 76, 81]. For a polyp size of 5–10 mm cancer risk is lower, but cholecystectomy should be considered if there are further risk factors, such as patient's age > 50 years, detection of a solitary polyp, and/or concurrent gallstones [19, 40, 50, 57, 76]. For polyps > 18–20 mm, open cholecystectomy is the method of choice because of a high risk of malignancy [40, 41, 44, 59]. If cholecystectomy is not performed, a close monitoring of the polyp size is recommended (first every 6 months, then, if polyp size is stable, every year) [44, 51]. Polyps < 5 mm do not require surgery, but rather a frequent follow-up period.

Surgical excision and antibiotic therapy form the mainstay of therapy against malacoplakia, although a variety of other treatments have been tried. Cholecystectomy, with or without peri-operative antibiotics, is likely to be adequate treatment for malakoplakia of the gallbladder, although the small number of cases do not allow for a detailed assessment of therapeutic efficacy at this stage.

## Benign Tumors of the Extrahepatic Bile Ducts

### Definition

Benign tumors of the extrahepatic bile ducts consist of neoplasms that are derived from the bile ducts's wall. Currently WHO classification of tumors is most commonly used [32].

### Epidemiology

The most common neoplastic lesions are epithelial tumors, which are mostly adenomas.

Cystadenomas are more common in the extrahepatic bile ducts than in the gallbladder. After incomplete resection recurrence rate is high.

Papillomatosis (adenomatosis) is a rare disease characterized by multiple papillary and recurrent adenomas, which cover extended sections of the extrahepatic bile ducts and even can reach into the gallbladder and intrahepatic bile ducts [18]. It is a tumor of middle-age to elderly people, with a male: female ratio of 2:1. Associated conditions are Clonorchis sinensis infection, primary sclerosing cholangitis (PSC), choledochal cyst, Caroli's syndrome eventually confined to one liver lobe, and dysplasia of the gallbladder.

Non-epithelial benign tumors, such as leiomyomas and lipomas are very rare. Less than 1% of granular cell tumors are located in the extrahepatic bile ducts, although this is the most common non-epithelial benign tumor of the extraheptic bile ducts [80]. The majority of the patients are black and female [23]. The common bile duct (50%) and cystic duct (37%) are most commonly involved. Additional biliary pathologic disorders are present in more than half of the patients, e.g. papillary "pseudocarcinomatous" hyperplasia of epithelium [79].

### Etiology and Pathogenesis

Adenomas of the extrahepatic bile ducts are sometimes associated with familial adenomatosis. The risk of malignancy of extrahepatic cystadenoma is unknown, but some cases are reported. Adenomas of papillomatosis often contain areas with high-grade dysplasia, which are sometimes difficult to distinguish from cholangiocarcinoma. Risk of malignancy is much higher for papillomatosis than for single adenomas. Granular cell tumor is a benign mesenchymal tumor, which can occur in the whole body, but is mostly located in the mouth-jaw-area. According to current opinion, the tumor is derived from Schwann-cells (localization of the nervous-system-specific protein (S-100 protein) in the granular component cells) and therefore classified as a neurogenic tumor. Ganglioneurofibromatosis is very rare and can be part of multiple endocrine neoplasia (MEN) type 2b. It occurs predominantly in children and young adults. Papillary hyperplasia is usually associated with chronic cholecystitis, ulcerative colitis and primary sclerosing cholangitis. In these cases it occurs

focally or segmentally, and intestinal metaplasia is common. Primary papillary hyperplasia is not associated with inflammation and is much rarer.

## Pathology

The histologic types of benign bile duct tumors include the following:

- Epithelial Tumors
  - Adenoma (tubulary, papillary, tubulo-papillary)
  - Cystadenoma
  - Papillomatosis (Adenomatosis)
- Non-epithelial Tumors
  - Granular cell tumor
  - Ganglioneurofibromatosis
  - Leiomyoma
  - Lipoma
  - Hemangioma
  - Lymphangioma
  - Neurofibroma
- Tumor-like Lesions
  - Regenerative epithelial atypia
  - Papillary hyperplasia
  - Intestinal metaplasia
  - Pylorus-gland-metaplasia
  - Squamous metaplasia
  - Malacoplakia
  - Congenital cysts
  - Primary sclerosing cholangitis (PSC)

Papillary adenomas consist of tissue papillae, which are covered by cubic and cylindrical mucinous epithelium. Metaplasia and enterochromaffin cells were described in this adenoma type. Kushima et al. reported this phenomenon as "pyloric gland type adenoma with squamoid spindle cell metaplasia" [42]. According to the literature, epithelial dysplasia is more commonly found in papillary than in tubulary adenomas [25, 39, 52, 55, 66]. Tubulo-papillary adenomas are diagnosed if at least 20% of the lesions consist of tubular, papillary structures.

Grossly, papillomatosis presents as friable, papillary masses. At microscopy it appears as multiple papillary adenomata with complex gland formation and variable degrees of atypia (Fig. 115.3b and c). Laminin staining reveals frequent invasion which cannot be appreciated on hematoxylin and eosin staining. Aspiration cytology shows distinctive features permitting differentiation from cholangiocarcinoma, namely (1) hypercellular smear, (2) very broad and often double-cell layered sheets of ductal columnar epithelium, (3) papillary configuration, (4) preserved honeycomb pattern with even nuclear spacing, and (5) dysplastic but not frankly malignant nuclear features. Atypia and dysplastic changes are frequently found and transition to adenocarcinoma frequently reported.

Ganglioneurofibromatosis consists of Schwann-cells, proliferation of ganglia cells in the lamina propria, and enlarged nerve bundles in the lamina muscularis and lamina subserosa.

Regenerative epithelial atypia as a tumor-like lesion represents non-dysplastic atypia, which occurs in regenerative or defective epithelium. It consists of a mixture of cylindric epithelial cells with or without mucus, atrophic cubic epithelium, and thin rod-like cells.

Primary papillary hyperplasia involves the total mucosa and shows no metaplasia.

## Clinical Manifestations

Clinical symptoms of patients are not characteristic; very often, abdominal discomfort or upper abdominal pain is reported. Sometimes patients present with jaundice or biliary colic due to bile duct obstruction by the tumor.

## Diagnosis

Tumors are usually found incidentally on ultrasonography. Ultrasound characteristics alone are insufficient to exclude the possibility of cholangiocarcinoma or premalignant adenomas. When detected on ultrasonography, their clinical significance relates largely to their malignant potential.

In papillomatosis, non-shadowing biliary masses are found on ultrasound. In addition to the usual features of obstruction, there is frequently sludge and even choledocholithiasis. On CT, intrahepatic masses and duct dilatations can be seen, but the findings are nonspecific and variable. Extent of the lesions can be better outlined by MRI than by CT. On endoscopic retrograde cholangiopancreaticography (ERCP), mucinous discharge from a dilated ampulla is the typical finding; in addition there are multiple filling defects

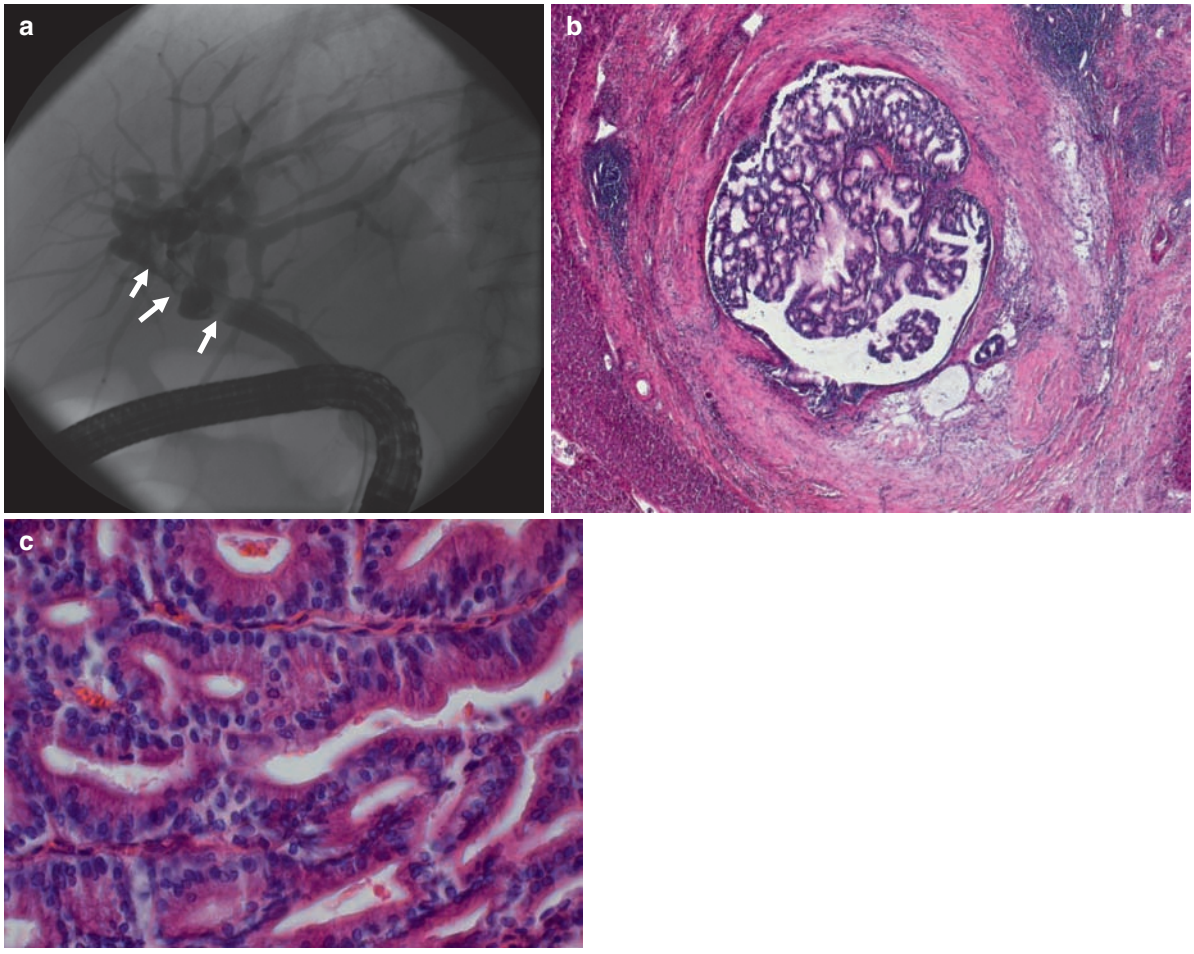


**Fig. 115.3** (**a**–**c**) Papillomatosis (adenomatosis) of the common bile and right hepatic duct in a 76-year-old female patient. (**a**) Tumor lesions are shown by ERC (*white arrows*). (**b**, **c**) bile duct histology after right-sided hemihepatectomy confirmed diagnosis

and supple stenoses (Fig. 115.3a). ERCP is not sufficient to delineate extent; one author with experience of nine cases found additional tumor in 4 patients with transhepatic cholangiography/cholangioscopy where ERCP suggested only extrahepatic involvement [38].

## *Differential Diagnosis*

Cholangiocarcinoma of the extrahepatic bile ducts (see Chapter 116) is the most important differential diagnosis. Benign, segmental, non-traumatic inflammatory strictures of the biliary tract were infrequently reported with the exception of primary sclerosing cholangitis [30, 31, 53, 70]. Many benign nontraumatic inflammatory strictures of the common bile duct have been generally considered to be a variant of primary sclerosing cholangitis. However, Standfield et al. described 12 cases of benign strictures of unknown etiology, and differentiated them from the localized form of sclerosing cholangitis [71]. Inflammatory conditions of the common bile duct, which are potential etiological factors, included bacteria or virus infection, parasite infestation, abdominal trauma, congenital abnormality, inflammatory pseudotumors, and complication of chemotherapy.

## *Therapy and Prognosis*

There are no reliable diagnostic methods to distinguish benign lesions from cholangiocarcinoma in patients without histologically confirmed adenocarcinoma.

*Therefore, in the presence of bile duct obstruction of unknown cause, potentially resectable lesions should always be explored and resected to offer patients with a malignancy the chance for cure.*

Treatment of papillomatosis with hepatectomy can be curative; however, recurrence after 'curative' hemihepatectomy has been described. Stenting and brachytherapy with $^{192}$ Iridium after electrocoagulation have been used for palliation. Liver transplantation has been advocated.

## Benign Tumors of the Ampulla of Vater

### Definition

Benign tumors of the Ampulla of Vater are most commonly villous and tubulovillous adenomas; although classified as benign, ampullary adenomas have the potential to undergo malignant transformation to ampullary carcinomas, similar to the adenoma-carcinoma sequence of other gastrointestinal tumors [79]. Currently WHO classification of tumors is most commonly used [32].

### Epidemiology

Benign neoplasms of the ampulla of Vater are rare, representing less than 10% of periampullary neoplasms. Adenomas are considered precancerous and usually occur within the fifth or sixth decade (tumors may occur much earlier in patients with familial polyposis syndromes). The prevalence of ampullary adenoma has been estimated to be 0.04–0.12% in autopsy series. However, they are being increasingly recognized with widespread screening programs for high-risk patients such as those with familial adenomatous polyposis (FAP).

Brunner's gland hyperplasia (BGH) usually presents in middle age with no sex predominance; however, cases have been described from early infancy to 80 years of age. Hyperplasia of these glands is a rare condition, with only 144 cases being described in the literature. BGH around the ampulla of Vater is particularly rare, having been described only twice in the literature [36, 49].

### Etiology and Pathogenesis

Ampullary adenomas can occur sporadically, or in the setting of familial polyposis syndromes such as FAP [46]. For these patients, there is a 100- to 200-fold increased risk of adenocarcinoma [58].

The pathogenesis of BGH remains poorly understood. Gland stimulation by gastric hyperacidity was originally thought to induce hyperplasia; however, only 45% of patients demonstrate hyperacidity and 20% have low gastric acidity. Other suggested mechanisms include proliferation in response to local irritation or excessive parasympathetic activity.

### Pathology

The histologic types of benign tumors of the Ampulla of Vater include the following:

- Villous adenoma
- Tubulovillous adenoma
- Fibroadenoma
- Hemangioma
- Leiomyoma
- Leiofibroma
- Lipoma
- Lymphangioma
- Hamartoma
- Gangliocytic paraganglioma
- Neuroendocrine tumors
- Brunner's gland hyperplasia (BGH)

Gangliocytic paraganglioma (GP) is a rare, typically benign tumor that shows neuroectodermal (neurosustentacular or Schwannian and neuronal) and neuroendocrine differentiation. Once thought to arise exclusively from the periampullary region as a solitary lesion, recent reports have documented both origins of GP in a variety of extra-duodenal sites as well as synchronous multifocal presentation of the tumor. The epithelioid tumor cells show diffuse immunohistochemical expression of keratin (CAM 5.2), chromogranin, and synaptophysin, supporting true neuroendocrine differentiation; ganglion cells express S-100 protein and neurofilament protein; and the spindled elements express S-100 protein, neurofilament protein, and glial fibrillary acidic protein indicating Schwannian differentiation. The finding of another GP

occurring outside the periampullary region bolsters the argument for a stem cell origin of this unusual tumor.

Brunner's glands are mucus-secreting acinar glands located in the deep mucosa and submucosa of the duodenum, emptying into the crypts of Lieberkuehn. The glands secrete mucus, pepsinogen, and urogastrone in response to acid stimulation. In 1934, Feyrter classified the abnormal glandular proliferation into three types–type 1, type 2, and type 3 [24]. Type 1 has diffuse nodular hyperplasia, in which multiple sessile projections are found throughout the duodenum. Type 2 has circumscribed nodular hyperplasia limited to the duodenal bulb. Type 3 has glandular adenoma with polypoid lesions. It is unclear whether these three histological types are different manifestations of a single disease. The histological similarity between diffuse hyperplasia, hamartoma, and adenoma may indicate a common pathological origin. However, Feyrter's classification is controversial, and some authors suggest that all forms should be considered [45]. Furthermore, the nomenclature used in the literature is inconsistent. The terms adenoma, brunneroma, and hamartoma have been used interchangeably with BGH. Consequently, there is no consensus regarding the classification of benign pathology of Brunner's glands. Histological features favoring hamartoma include lack of encapsulation; admixture of muscular, glandular, and adipose tissues; presence of continuous sheets of Brunner's glands from the submucosa through the body of the tumor; and lack of any cellular atypia [24]. The presence of both ductal and glandular components is a further evidence of a hamartomatous origin, features which are unusual in hyperplasia or neoplasia. Dysplasia is not seen in Brunner's gland tumors, suggesting that the term adenoma is a misnomer [45]. A case, presented by Janes et al., does not support these concepts as there was no evidence of hamartoma [36]. It rather suggested that hyperplasia can occur independently from hamartomatous change in Brunner's glands.

## Clinical Manifestations

The occurrence of biliary obstruction presenting as jaundice in 50–75% of patients with benign ampullary tumors is the most common clinical finding [75]. Nonspecific symptoms, such as weight loss, vague abdominal pain (rarely biliary colic), nausea, vomiting, fever, and anorexia have all been described. Benign ampullary tumors can cause heme-positive stools and may lead in some patients to iron-deficiency anemia. Up to 25% of patients have associated common bile duct stones secondary to cholestasis. Some patients infrequently develop cholangitis or acute pancreatitis [56].

More than half of the patients with BGH present with abdominal pain, 43% have melena, and 12% experience hematemesis.

## Diagnosis

Initial evaluation of patients with clinical features suggesting an ampullary neoplasm should include radiologic imaging, typically beginning with a transabdominal ultrasound. A CT scan or MRI is frequently obtained to exclude pancreatic mass in patients with evidence of bile duct obstruction on ultrasound. Patients with biliary obstruction should undergo cholangiography, which is typically accomplished by MRCP and/or ERCP. Whereas MRCP is less invasive, ERCP with the use of a side-viewing endoscope allows direct visualization of the tumor and the acquisition of biopsies, preferably after sphincterotomy. However, biopsies cannot reliably exclude the presence of malignant foci within an ampullary adenoma which are reported in 30–60% [17, 62, 73, 77, 79]. Where local expertise is available, EUS and intraductal ultrasound (IDUS) can provide valuable tumor staging information.

Small-bowel radiological studies have a sensitivity of 92% for detecting BGH. A smooth-walled filling defect in the duodenum is the commonest finding. Localized tumors can present as pedunculated or sessile filling defects with a sharp border, typical of submucosal lesions. However, adenocarcinoma arising from Brunner's gland can also appear as a sessile polypoid lesion; so further investigation is indicated when these features are present. CT scanning may demonstrate fat within the lesion or enhancement after intravenous contrast. Only two reports describe EUS features of BGH, showing a submucosal mass in the fourth echolayer [8, 36].

## Differential Diagnosis

Differential diagnoses include ampullary carcinoma (see Chapter 116), duodenal adenocarcinoma, leiomyosarcoma, gastrointestinal stromal tumor, lymphoma,

pancreatic carcinoma and distal bile duct carcinoma invading the ampulla of Vater, intraductal papillary mucinous neoplasm (IPMN), and ectopic pancreatic tissue.

## Therapy and Prognosis

Whereas it is generally agreed upon that all ampullary tumors should be removed or resected, patient selection for the various treatments, including pancreaticoduodenectomy, local resection and endoscopic treatment, remains controversial. Stage of disease, patient characteristics (i.e. age and comorbid conditions), and local availability of expertise determine treatment options. The observation that histologic progression of papillary adenomas to carcinomas is very low in FAP patients (11% in one study of Burke and colleagues) prompted some experts to advocate surveillance endoscopy with biopsies, rather than excision, for ampullary adenomas with normal endoscopic appearance and without dysplasia on biopsy [11]. However, large prospective studies on this issue have not been performed.

*Pancreaticoduodenectomy (Whipple procedure)* is still considered to be the definitive approach for achieving curative resection of ampullary adenomas as this therapy has been shown to have a low tumor recurrence rate.

However, although mortality rates have fallen to remarkably low levels for such an extensive operation (0–10%), morbidity (25–65%), length of hospital stay, and possibly quality-of life issues continue to fuel interest in less invasive alternatives.

*Surgical ampullectomy*, first reported in 1899, has experienced resurgent interest as a possible less morbid option [4].

A number of technical variations of the procedure have evolved. Most entail mobilization of the duodenum and longitudinal duodenotomy, followed either by simple excision of the ampullary neoplasm or an extended excision, involving the ampulla and adjacent duodenal and ductal tissue [10, 22]. Hospital mortality after ampullectomy is less than 0.4%, and surgical morbidity, e.g. cholangitis, below 10% [3]. However, tumor recurrence rate ranges from 0% to approximately 50% in several reports [1, 10, 13, 17, 22, 29, 62, 64, 77]. Therefore, patients treated with local resection should undergo surveillance endoscopy.

*Endoscopic snare excision (papillectomy)* for ampullary adenomas (Fig. 115.4a) was first described in the late 1980s, followed by further reports in the 1990s, and early 2000s [5, 6, 54, 68]. Its role remains controversial and it is currently performed in only a small number of referral centers with expertise in interventional endoscopy. Of critical importance is retrieval of the entire resected specimen to ensure the absence of a focus of adenocarcinoma. Temporary drainage of biliary and pancreatic ducts (Fig. 115.4b) is advisable to prevent severe obstruction and pancreatitis. A systematic review estimated that success rates range from 46% to 92%, while recurrence rates range from 0% to 33% [33]. Complication rates are approximately 10–15%, consisting mainly of acute pancreatitis, bleeding, and late papillary stenosis.

Endoscopic ablation with the Nd: YAK laser, mono-/bipolar thermal ablation, photodynamic therapy

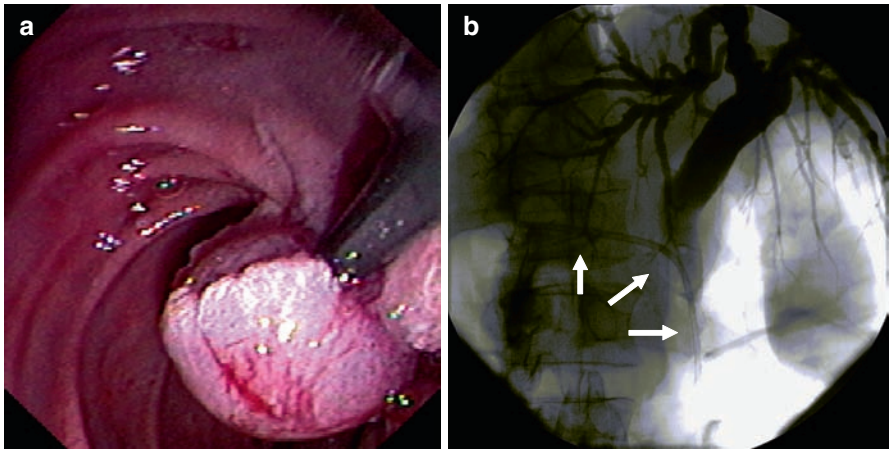


**Fig. 115.4** (**a**, **b**) Ampullary adenoma. (**a**) Endoscopic snare excision (papillectomy) and (**b**) plastic stent insertion into the pancreatic duct after papillectomy to protect against post-ERCP pancreatitis (*white arrows*)

(PDT), and argon plasma coagulation (APC) have been described, too [7, 21, 26, 43, 61, 68].

Finally, in patients who refuse surgical or endoscopical tumor resection, or are not candidates due to comorbidities, but who have symptoms attributable to biliary or pancreatic duct obstruction, endoscopic drainage alone may provide effective symptomatic relief.

The efficacy of COX-2 inhibitors (which reduce colonic polyps inpatients with FAP) in reducing ampullary adenomas in FAP patients has not been well studied, although at least one study suggested a possible benefit [60]. In this randomized, double blind, placebo controlled study of celecoxib (100 mg twice daily (n = 34) or 400 mg twice daily (n = 32) ) versus placebo (n = 17), given orally twice daily for six months to patients with FAP, efficacy was assessed qualitatively by blinded review of shuffled endoscopy videotapes comparing the extent of duodenal polyposis at entry and at six months, and quantitatively by measurement of the percentage change in duodenal area covered by discrete and plaque-like adenomas from photographs of high and low density polyposis. As a result, shuffled and blinded video review showed a statistically significant effect of 400 mg twice daily celecoxib compared with placebo treatment (p = 0.033) with all five independent observers scoring a beneficial effect. Overall, patients taking celecoxib 400 mg twice daily showed a 14.5% reduction in involved areas compared with a 1.4% for placebo (p = 0.436). However, patients with clinically significant disease at baseline (greater than 5% covered by polyps) showed a 31% reduction in involved areas with celecoxib 400 mg twice daily compared with 8% on placebo (p = 0.049).

Whether managed surgically or endoscopically, the outcome of BGH is uniformly good. Endoscopic removal offers a safe and cost-effective alternative to open surgery.

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# Malignant Tumors

# 116

Marcus Wiedmann, Helmut Witzigmann,
and Joachim Mössner

## Chapter Outline



## Gallbladder Cancer

### *Definition*

Gallbladder carcinoma is a malignant tumor originating from the epithelium of the gallbladder. According to de Groen, approximately 67% of the biliary tract tumors are gallbladder carcinomas (Fig. 116.1a) [69]. The American Joint Committee on Cancer (AJCC) and UICC (Union Internationale Contre le Cancer) have designated staging by the TNM classification of 2002 (Table 116.1) [354].

### *Epidemiology*

Gallbladder cancer affects women two to six times more often then men and its incidence steadily increases with age (the peak incidence is 70–75 years). In 2007, 9,250 new cases and 3,250 deaths from gallbladder (and other biliary) cancer were estimated in the United States. The highest frequency of gallbladder cancer is reported for Japan, Korea, Poland, Israel, Northern India, Pakistan, and South American countries like Chile, Ecuador, Mexico, and Bolivia [319, 361]. Low incidence is seen in Singapore, Nigeria, and USA. Incidence also varies within a country and shows large racial and ethnic variations (thus, in the USA, there is a 50% greater incidence in white people of both sexes than in black populations; Native, Japanese, and Hispanic Americans also show a high incidence).

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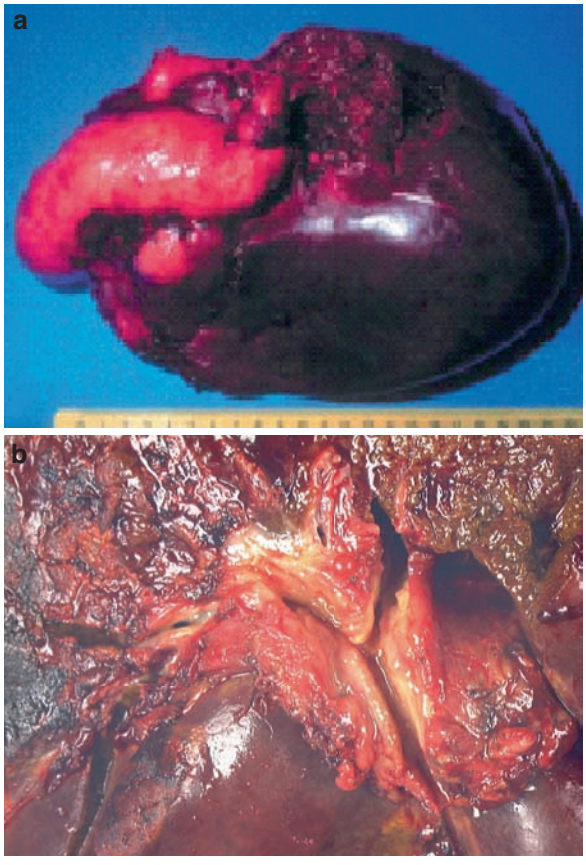


**Fig. 116.1** Resection specimens of **(a)** gallbladder cancer and **(b)** hilar cholangiocarcinoma

**Table 116.1** TNM- and UICC-Classification of gallbladder cancer

| | | | |
|---|---|---|---|
| TX | Primary tumor cannot be assessed | | |
| T0 | No evidence of primary tumor | | |
| Tis | Carcinoma *in situ* | | |
| T1 | Tumor invades lamina propria or muscle layer | | |
| T1a | Tumor invades lamina propria | | |
| T1b | Tumor invades the muscle layer | | |
| T2 | Tumor invades the perimuscular connective tissue; no extension beyond the serosa or into the liver | | |
| T3 | Tumor perforates the serosa (visceral peritoneum) and/or directly invades the liver and/or one other adjacent organ or structure, such as the stomach, duodenum, colon, or pancreas, omentum or extrahepatic bile ducts | | |
| T4 | Tumor invades main portal vein or hepatic artery or invades multiple extrahepatic organs or structures | | |
| NX | Regional lymph nodes cannot be assessed | | |
| N0 | No regional lymph node metastasis | | |
| N1 | Regional lymph node metastasis | | |
| MX | Distant metastasis cannot be assessed | | |
| M0 | No distant metastasis | | |
| M1 | Distant metastasis | | |
| Stage 0 | Tis | N0 | M0 |
| Stage IA | T1 | N0 | M0 |
| Stage IB | T2 | N0 | M0 |
| Stage IIA | T3 | N0 | M0 |
| Stage IIB | T1-3 | N1 | M0 |
| Stage III | T4 | any N | M0 |
| Stage IV | any T | any N | M1 |

Regional lymph nodes are: cystic duct node and the pericholedochal, hilar, peripancreatic (head only), periduodenal, periportal, celiac, and superior mesenteric nodes

## *Etiology and Pathogenesis*

Cholelithiasis (65–90%) is an associated finding in the majority of gallbladder cancer cases, but less fewer than 1% of patients with cholelithiasis develop this cancer. Additional cholecystitis was found in approximately 10% of the patients [303]. Cancer risk is higher in patients with symptomatic cholelithiasis than in patients with asymtomatic cholelithiasis [428]. Asymtomatic gallstones > 3 cm or "porcellain gallbladder" (wall thickening) significantly increases the cancer risk. Additional risk factors are gallbladder polyps (see Chapter 115), Mirizzi's syndrome, anomalous pancreaticobiliary duct junction (APBDJ), carcinogens (methylcholanthrene, o-aminoazotoluene, nitrosamines, oral contraceptives, methyldopa, and isoniazide), segmental adenomyomatosis of the gallbladder (see Chapter 115), chronic inflammatory bowel disease (IBD), polyposis coli, obesity, multiparity and chronic infections like Salmonella typhi, Salmonella paratyphi, Helicobacter bilis, and Helicobacter pylori [12, 38, 67, 88, 203, 233, 259, 274, 319, 322].

Studies investigating genetic abnormalities during carcinogenesis of gallbladder cancer are still limited. It is hypothesized that chronic irritation of the gallbladder mucosa over a period of years, by pancreaticobiliary reflux in patients with APBDJ or cholecystitis in patients with cholelithiasis, may predispose to malignant transformation, or act as promoter for carcinogenic exposure or genetic predisposition. *K-RAS* mutations were discovered in 39–59% and abnormalities of tumor suppressor gene *P53* (mutations especially in exons 5 and 8, loss of heterozygosity (LOH) in chromosome

area 17p13.1, and *P53*-overexpression) in 35–92% [218, 250]. Whereas *K-RAS* mutations are more common in APBDJ patients, abnormalities of tumor suppressor gene *P53* are more common in patients with cholelithiasis and chronic cholecystitis. In addition, LOH in the APC (3%)-, DCC (45%)-, RB (13%)- and NM23-H1 (7%)- genes were reported besides microsatellite instability (17%) [426]. TNF (tumor necrosis factor) mRNA and TNF protein expression is increased during the development of gallbladder mucosa from hyperplasia, dysplasia to carcinoma, and is increased with tumor stage. This finding suggests that TNF is involved in the pathogenesis of gallbladder carcinoma induced by gallstones and the TNF expression in cancer cells may serve as a marker for tumor stage [341].

The occasional presence of hTERT protein in normal and regenerative gallbladder mucosa reflects their regenerative capacity. Nevertheless, significantly higher hTERT indices in low and high grade dysplastic epithelia and in gallbladder adenocarcinomas are probably a consequence of hTERT re-expression, an early event in the multistep process of gallbladder carcinogenesis [236].

## Pathology

The histologic types of gallbladder cancer include the following:

- Carcinoma *in situ*
- Adenocarcinoma, not otherwise specified (NOS)
- Papillary carcinoma
- Adenocarcinoma, intestinal type
- Mucinous carcinoma
- Clear cell adenocarcinoma
- Signet-ring cell carcinoma
- Adenosquamous carcinoma
- Squamous cell carcinoma
- Small cell (oat cell) carcinoma*
- Undifferentiated carcinoma*
- Carcinoma, NOS
- Carcinosarcoma

The most common type (80–90%) is adenocarcinoma. Some histologic types have a better prognosis than others; papillary carcinomas have the best prognosis. Grossly, gallbladder cancer can appear as infiltrative, nodular, papillary, or as a combination of these morphologies. The lack of a well-defined muscularis layer permits early vascular, lymphatic, and neural invasion. Tumors frequently extend outside of the gallbladder, invading adjacent organs, particularly the liver, as they grow.

*Grade 4 by definition.

## Clinical Manifestations

The most common symptoms caused by gallbladder cancer are right-sided upper abdominal pain (75%), nausea and vomiting (30%), anorexia, and jaundice in an advanced stage with liver infiltration. However, patients with early invasive gallbladder cancer are most often asymtomatic, or they have nonspecific symptoms. In patients who present with symptoms, the tumor is rarely diagnosed preoperatively. Very often, patients report a long history of cholelithiasis and chronic cholecystitis.

## Diagnosis

Laboratory studies are generally nondiagnostic; an elevated alkaline phosphatase or serum bilirubin may be related to bile duct obstruction. Serum tumor markers such as carcinoembryonic antigen (CEA) or CA 19-9 are often elevated but not diagnostically useful because they lack specificity. Ultrasound is often the first test performed in the evaluation of gallbladder cancer. On ultrasound, gallbladder cancer appears as thickening of the gallbladder wall or as a mass lesion compressing or growing into the bile duct. Inflammatory changes can be difficult to distinguish from gallbladder cancer, however ultrasound contrast agents have proven to be useful for clarification of biliary tumors. Endoscopic ultrasound may be helpful in staging tumor extent (also for defining lymph node involvement in the porta hepatis or peripancreatic regions) and to distinguish benign polyps and adenomyomatosis from adenomas and adenocarcinomas [106, 329]. Computed tomography (CT) is less helpful in distinguishing benign from malignant polyps [110]. In contrast, dynamic magnetic resonance imaging (MRI) and MR cholangiography (MRC) can help differentiate benign from malignant lesions in equivocal cases, and provide information as to disease extent [339].

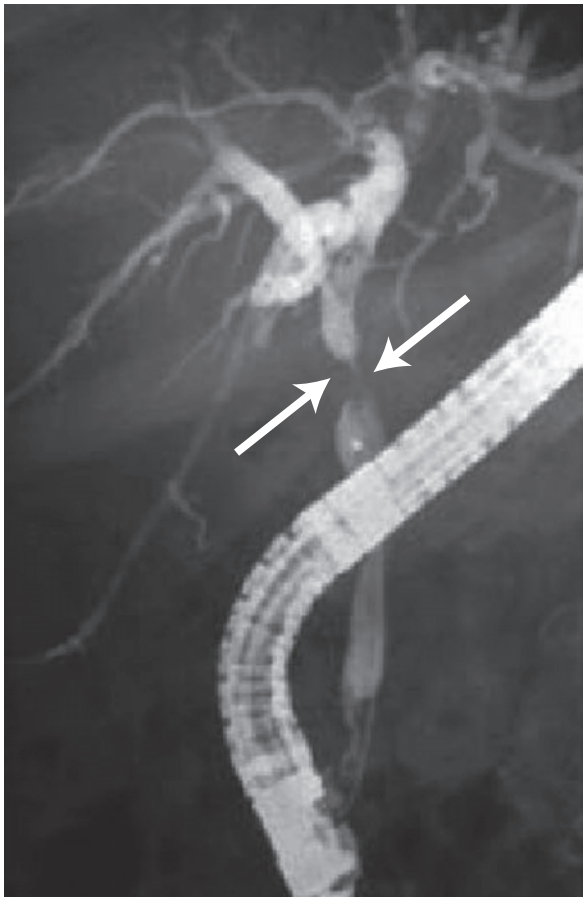

**Fig. 116.2** ERC from a patient with gallbladder cancer. Stenosis in the middle of the common hepatic bile duct (*white arrows*)

MRI is particularly useful for visualizing invasion into hepatoduodenal ligament, portal vein encasement, and lymph node involvement [427]. Endoscopic retrograde cholangiography (ERC) and percutaneous transhepatic cholangiography (PTC) are of little use for patients suspected of having gallbladder cancer since the gallbladder is not displayed in most cases (Fig. 116.2). These procedures may be helpful in planning the surgical procedure (as they may indicate tumor growth into intrahepatic bile ducts or into the common bile duct) and in decompressing the common bile duct in case of cholestasis with or without cholangitis. Fine-needle aspiration cytology (FNAC), guided by (endoscopic) ultrasound or CT, is becoming more frequently used for preoperative cytodiagnosis; the reported sensitivity is 88% [8]. This technique has particular relevance for confirming diagnosis in advanced stages in which non-surgical treatment is being pursued. Brushings of obstructed bile ducts or bile duct cytology studies have a low diagnostic yield. However, laparoscopy and biopsy are extremely useful for assessment of peritoneal metastasis, extent of disease, and suitability of surgery in patients with locally advanced disease.

## *Differential Diagnosis*

Most important differential diagnoses for gallbladder carcinoma are: empyema, hematoma, sludge, acute/chronic cholecystitis, gallbladder wall thickening caused by acute hepatitis, ascites, hypoalbuminemia, liver cirrhosis, congestive heart failure, pancreatitis, gallbladder wall-varices, benign tumors (such as polyps, hemangioma, fibroma, myoma, adenoma), and adenomyomatosis.

## *Therapy and Prognosis*

### Curative Treatment

When gallbladder cancer is previously unsuspected and is discovered in the mucosa of the gallbladder at pathologic examination, it is curable in more than 80% of cases. Gallbladder cancer suspected before surgery because of symptoms, however, usually penetrates the muscularis and serosa and is curable in fewer than 5% of patients.

In patients with superficial cancer (T1 or confined to the mucosa) the disease is often cured after cholecystectomy without further therapy with a 5-year survival rate of nearly 100%, according to a study by Shirai et al [347]. Therefore, follow-up without a second operation is very often recommended for pT1 cancer without a positive margin. However, many surgeons favor simple cholecystectomy for patients with T1a cancers only, while more radical resections are considered for patients with T1b tumors – derived from the fact that T1b tumors are associated with a higher incidence of lymph node metastases than are T1a tumors (15% vs. 2.5%) – although there is no general consensus about this issue [187, 252, 279, 363, 401, 420].

In a Japanese study, 5-year survival for patients with pT2 and pT3 tumors was 40% and 0% after cholecystectomy alone [347]. Results of a radical second

operation showed that patients with pT2 cancer had a 5-year survival of 90%, which was significantly better than cholecystectomy alone ($p < 0.05$). In addition, there was a prolongation of survival in patients with pT3 or pT4 [347]. These results were confirmed by other studies [252, 347, 364, 400, 419].

Another study reported on patterns of lymph node spread from gallbladder cancer and outcomes of patients with metastases to lymph nodes in 111 consecutive surgical patients in a single institution from 1981 to 1995 [389]. There was no neurovascular invasion or lymph node involvement in 15 patients with pT1 tumors, however 60 of 96 patients with pT2-4 tumors had lymph node metastases. The pericholedochal lymph node was the most common metastatic lymph node, followed by the cystic lymph node. The frequency of metastases in retroportal, posterosuperior pancreaticoduodenal, and interaorticocaval lymph nodes was > 15% in all cases. pT3-4 tumors had significantly more lymph node involvement (79%) and significantly higher N2:N1 ratios (2.5) than pT2 tumors (46% and 0.6, respectively). There was no difference in 5-year survival between N0 and N1 groups in pT2-4 tumors (66% in N0 and 53% in N1). Patients with N2 disease had a significantly worse prognosis, but four patients survived > 5 years. Therefore, the pericholedochal and cystic lymph nodes are the initial site of spread from gallbladder cancer and the frequency of lymph node involvement is strongly influenced by the depth of invasion of the primary tumor.

*It is therefore concluded that a radical (second) operation, including extended tumor resection ± partial hepatectomy (commonly segments IVb and V) and removal of regional lymphatics and lymph nodes (including the porta hepatis and superior pancreatic nodes), should be carried out for pT2 or more advanced carcinoma* [206, 210, 400].

The role of extended lymphadenectomy, including the retropancreatic and aortocaval basins, is unclear and should be attempted only in selected cases [348]. Implantation of the carcinoma at all port sites (including the camera site) after laparoscopic removal of an unsuspected cancer is a problem. Even for stage I cancers, the port sites must be excised completely [235, 282, 349, 408]. In jaundiced patients, there should be consideration of preoperative percutaneous transhepatic biliary drainage for relief of biliary obstruction. Among the absolute contraindications to surgery are liver or peritoneal metastases, ascites, extensive involvement of hepatoduodenal ligament, and encasement or occlusion of major vessels. Morbidity and mortality rates from resection for gallbladder cancer vary widely, with major morbidity rates ranging from 5% to 54%, and perioperative mortality rates from 0% to 21%.

Beneficial adjuvant chemotherapy has been demonstrated in only a single multicenter prospective randomized study, especially for patients after incomplete (R1-) resection [366]. The five-year survival rate in patients treated with mitomycin C and 5-Fluorouracil was 26% in comparison to 14% for the untreated group ($p < 0.05$). Adjuvant external radiotherapy or chemoradiation have shown some benefit in small studies only [147, 211, 381].

*Therefore, adjuvant treatment for gallbladder cancer is currently not standard procedure and patients should be included in clinical studies.*

## Palliative Treatment

With the exception of some patients with focal UICC stage IIA disease, stage IIB-IV cancer cannot be completely resected, although it represents the majority of cases of gallbladder cancer. Often the cancer invades directly into adjacent liver or biliary lymph nodes or has disseminated throughout the peritoneal cavity. Spread to distant parts of the body is not uncommon. At this stage, standard therapy is directed at palliation. The goal of palliation is relief of pain, jaundice, and bowel obstruction, along with prolongation of life.

*The preferred approach to biliary obstruction is percutaneous transhepatic radiologic catheter bypass or endoscopically placed stents* [19].

Palliative surgery, such as segment-III-choledochojejunostomy, may relieve bile duct obstruction and is warranted when symptoms produced by biliary obstruction (pruritus, hepatic dysfunction, and cholangitis) outweigh other symptoms from the cancer. A few patients have very slow-growing tumors and may live several years [56, 180]. However, nowadays this procedure has only a small relevance, because of the high technical success rate of endoscopic and percutaneous transhepatic stenting.

The use of external-beam radiation therapy (EBRT) with or without chemotherapy as a primary treatment has been reported in small groups of patients to produce short-term control. Similar benefits have been reported for radiation therapy with or without chemotherapy administered following resection [138, 353].

*To date, external-beam radiation therapy is not standard procedure, however, it can, on occasion, alleviate biliary obstruction in some patients and may supplement bypass procedures.*

*Standard chemotherapy has not been established for gallbladder cancer, though occasional patients may be palliated* [138].

Most clinical studies have been small and uncontrolled; a combination of 5-FU/doxorubicine/ mitomycine (FAM), gemcitabine-monotherapy, and 5-FU in combination with interferon alpha-2b yielded response rates up to 30% [50, 136, 295]. Adding cisplatin and doxorubicin to the last protocol (PIAF) did not significantly increase response rate (35%), but did worsen toxicity [294]. Combining infusional 5-FU with cisplatin alone or with cisplatin and epirubicine (ECF) resulted in an overall response rate of 24% and 19–40% in studies that included also bile duct cancer patients [81, 89, 320]. Capecitabine, an orally active fluoropyrimidine derivative, appears to be an active agent for gallbladder cancer, both as a single agent, and in combination with cisplatin and oxaliplatin [189, 266, 293]. Whether 5-FU and leucovorin adds benefit to gemcitabine alone remains an open question. On the other hand, the combination of gemcitabine and the oral 5-FU prodrug capecitabine seems to be active for gallbladder cancer. In a phase II study, one third of 24 gallbladder cancer patients had a partial response, and the median survival was 16 months [64]. Also promising seems to be a combination of gemcitabine und cisplatin, which yielded a response rate of 64% and a median survival of 42 weeks in a small phase II study (8 patients with advanced gallbladder cancer) [240]. A second study, including a higher number of gallbladder cancer patients (n = 30), showed a response rate of only 37% and a median survival of 20 weeks [77]. Others have reported good benefit and good tolerability for GemOx (gemcitabine plus oxaliplatin) [14, 133]. Early results of a multicenter phase II trial of 35 patients with chemotherapy-naïve advanced or metastatic gallbladder carcinoma using gemcitabine plus oxaliplatin and 5-FU are promising [398]. Median survival was 10 months, with an 1-year survival rate of 30%. Finally, patients treated with docetaxel ± gemcitabine showed only a minor response between 0% and 20% [213, 283, 297].

*Generally, clinical trials should be considered as a first option for most patients. At present, they are exploring ways of improving local control with radiation therapy combined with radiosensitizer drugs and new chemotherapy regimens.*

### Recurrent Gallbladder Cancer

The prognosis for any treated cancer patient with progressing or recurrent gallbladder cancer is poor. The question and selection of further treatment depends on many factors: tumor burden, prior treatment, site of recurrence, and individual patient considerations. Patients may have portal hypertension caused by portal vein compression by the tumor. Transperitoneal and intrahepatic metastases are not uncommon.

*For recurrent gallbladder cancer, clinical trials are appropriate and should be considered when possible.*

## Extrahepatic Cholangiocarcinoma

### Definition

According to de Groen approximately 22% of biliary tract tumors are hilar carcinomas (Klatskin tumors) (Fig. 116.1b). Eight percent occur in the distal bile duct [69]. The American Joint Committee on Cancer (AJCC) and UICC (Union Internationale Contre le Cancer) have designated staging by the TNM classification of 2002 (Tables 116.2 and 116.4) [354]. Hilar cholangiocarcinomas are further sorted according to the Bismuth-Corlette classification (Table 116.3, Fig. 116.3) [35].

### Epidemiology

Overall, cholangiocarcinoma is a rare neoplasm. Worldwide, it accounts for approximately 3% of all gastrointestinal cancers. The incidence rates of cholangiocarcinoma vary greatly among different areas of the world, and this variation is related to distribution of risk factors. Intrahepatic and extrahepatic cholangiocarcinoma have different epidemiologic features. Whereas the incidence of intrahepatic carcinoma is increasing in North America and Western Europe since 1970, as has been reported by Wetzel et al and West et al, the

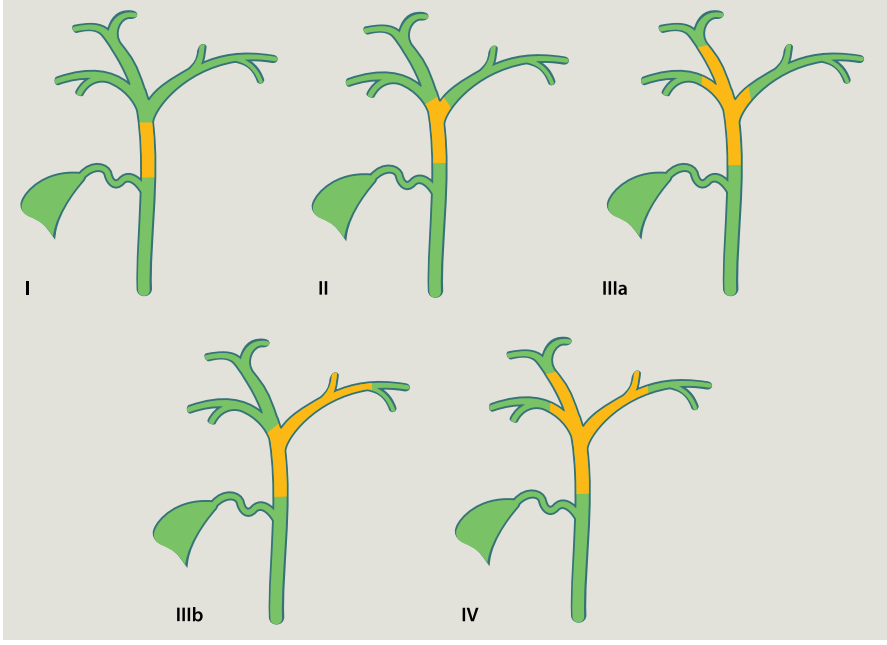


**Fig. 116.3** Bismuth-Corlette-classification of hilar cholangiocarcinoma (From [30]. With permission)

**Table 116.2** TNM- and UICC-Classification of extrahepatic cholangiocarcinoma

| | | | |
|---|---|---|---|
| TX | Primary tumor cannot be assessed | | |
| T0 | No evidence of primary tumor | | |
| Tis | Carcinoma in situ | | |
| T1 | Tumor confined to bile duct | | |
| T2 | Tumor invades perifibromuscular connective tissue | | |
| T3 | Tumor invades liver, gallbladder, pancreas, and/ or unilateral branches of portal vein (right or left) or of hepatic artery (right or left) | | |
| T4 | Tumor invades adjacent structures: main trunk of portal vein or its bilateral branches, hepatic artery, and or neighbor organs/structures like colon, stomach, duodenum, abdominal wall | | |
| NX | Regional lymph nodes cannot be assessed | | |
| N0 | No regional lymph node metastasis | | |
| N1 | Regional lymph node metastasis | | |
| MX | Presence of distant metastasis cannot be assessed | | |
| M0 | No distant metastasis | | |
| M1 | Distant metastasis | | |
| Stage 0 | Tis | N0 | M0 |
| Stage IA | T1 | N0 | M0 |
| Stage IB | T2 | N0 | M0 |
| Stage IIA | T3 | N0 | M0 |
| Stage IIB | T1-3 | N1 | M0 |
| Stage III | T4 | any N | M0 |
| Stage IV | any T | any N | M1 |

**Table 116.3** Bismuth-Corlette-classification of hilar cholangiocarcinoma

| | |
|---|---|
| I | Tumor below the confluence |
| II | Tumor confined to confluence |
| IIIA | Tumor extention into right hepatic duct |
| IIIB | Tumor extention into left hepatic duct |
| IV | Tumor extention into right and left hepatic ducts |

incidence of extrahepatic cholangiocarcinoma is decreasing [406, 407]. The cause of this rise is not exactly known–some authors postulate the rising incidence due to the increasing prevalence of chronic infection with hepatitis B and C–and does not appear to be explained simply by improvements in diagnosis or changes in coding practice. In the United States, the reported age-adjusted incidence of extrahepatic cholangiocarcinoma is 1.2 in 100,000 for men and 0.8 in 100,000 for women [49]. As a general rule, the incidence increases with age, with the typical patient between 50 and 70 years old. However, patients with primary sclerosing cholangitis (PSC) and those with choledochal cysts present nearly two decades earlier [39]. Moreover, the age-adjusted mortality rate of extrahepatic cholangiocarcinoma is also decreasing in Western countries, with the exception of Italy and Japan [186]. In the United states, the age-adjusted mortality rates declined from 0.6 in 100,000 in 1979 to 0.3 in 100,000 in 1998 [292]. To this end, current evidence indicates minor improvements in 5-year survival rates of extrahepatic cholangiocarcinoma from 11.7% in 1973–1977 to 15.1% in 1983–1987 [49].

**Table 116.4** TNM- and UICC-classification of ampullary carcinoma

| | | | |
|---|---|---|---|
| TX | Primary tumor cannot be assessed | | |
| T0 | No evidence of primary tumor | | |
| Tis | Carcinoma in situ | | |
| T1 | Tumor confined to Ampulla of Vater or sphincter of Oddi | | |
| T2 | Tumor invades duodenal wall | | |
| T3 | Tumor invades pancreas | | |
| T4 | Tumor invades peripancreatic soft tissue and/or other neighboring organs/structures | | |
| NX | Regional lymph nodes cannot be assessed | | |
| N0 | No regional lymph node metastasis | | |
| N1 | Regional lymph node metastasis | | |
| MX | Presence of distant metastasis cannot be assessed | | |
| M0 | No distant metastasis | | |
| M1 | Distant metastasis | | |
| Stage 0 | Tis | N0 | M0 |
| Stage IA | T1 | N0 | M0 |
| Stage IB | T2 | N0 | M0 |
| Stage IIA | T3 | N0 | M0 |
| Stage IIB | T1-3 | N1 | M0 |
| Stage III | T4 | any N | M0 |
| Stage IV | any T | any N | M1 |

## *Etiology and Pathogenesis*

Approximately 10% of patients with cholangiocarcinoma are found to have an established risk factor [26]. Risk factors for cholangiocarcinoma are PSC (perhaps smoking as additional enhancing risk factor) and/or ulcerative colitis, chronic empyematous cholangitis, chronic intrahepatic lithiasis (Oriental cholangiohepatitis), chronic salmonella infection, choledochal cysts, Caroli's disease, liver cirrhosis, and biliodigestive anastomosis. The annual incidence of cholangiocarcinoma in patients with PSC has been estimated to be between 0.6% and 1.5% per year, with a life time risk of 8–20% [27, 39, 208]. Rare factors in the Western world are carcinogens (thorium dioxide (in the past), asbestos, radon, nitrosamines, methyldopa, isoniazide, dioxin, polychlorinated biphenyls, and cigarette smoke), chronic hepatitis B or C, and biliary parasites (Opistorchis viverrini and less common Clonorchis sinensis) [27, 31, 53, 62, 75, 201, 228, 289, 344, 357, 373, 380, 384, 402]. Solitary adenoma, Lynch syndrome II, and biliary papillomatosis are regarded as precancerous conditions (see Chapter 115). An association between diabetes mellitus, obesity (BMI > 30), thyrotoxicosis, alcoholic liver disease, chronic pancreatitis and cholangiocarcinoma has been suggested [7, 405, 409].

During the last years, molecular biology enabled fundamental insight into tumor pathogenesis. Thus, transformation from normal into malignant bile duct tissue requires a sequence of different gene mutations, similar to the adenoma-dysplasia-carcinoma-sequence in colon carcinoma, although our knowledge is limited in comparison to this much more frequent tumor type [126]. These mutations can be grouped into several gene classes: proto-oncogenes, tumor suppressor genes, and mismatch-repair genes. In general, all tumors develop genetic and functional mechanisms to induce self-sufficiency in growth signals, insensitivity to anti-growth signals, tissue invasion and metastasis, limitless replicative potential, sustained angiogenesis, and evading apoptosis [125, 130].

Mutated oncogenes, such as *K-RAS, C-MYC, C-ERB-B1* (epidermal derived growth factor-1, *EGF-1), C-ERB-B2* (epidermal derived growth factor-2, *EGF-2),* and *C-MET* stimulate tumor cell proliferation, in addition to *COX-2* (cyclooxygenase-2)-overexpression, which induces *C-ERB-B1*- and *AKT*-gene-activation (anti-apoptotic protein) [6, 54, 66, 109, 129, 314, 350, 362]. Proinflammatory cytokines, such as interleukin-6 (IL-6), induce cell proliferation via MAPK (mitogen activated protein kinase)- and JAK/STAT (Janus kinase/signal transducer and activator of transcription)-signalling-transduction-pathways and upregulation of inducible nitric oxide synthase (iNOS), which induces increased production of nitric oxide (NO) [137]. NO itself or peroxynitrite, generated by the reaction of NO with superoxide anions, may modify or alter DNA bases, resulting in direct DNA damage, inhibit apoptosis by caspase 9-nitrosylation and MCl-1-induction (anti-apoptotic protein), or inactivate DNA-repair-proteins and regulate the activity of DNA methyltransferases [157, 158, 164, 165, 202, 385, 404]. TNF (tumor necrosis factor)-alpha substantially activates NFkappaB, MAPK and Akt signalling which in turn activates matrix metalloproteinase-9 (MMP-9) secretion and tumor cell invasiveness [371]. Therefore, inflammation processes and bile salts generate growth- and anti-apoptotic signals and promote carcinogenesis. Detectable tumor suppressor gene mutations involve *P53* and *P16* [28, 45, 71, 97, 135, 372, 390]. These mutations result in impaired cell aging, supported by telomerase-overexpression (> 70% of tumors) [161, 334]. In addition, there are modifications in cell cycle- and apoptosis-regulation [5, 146, 160, 313, 343,

372, 423]. These modifications cause a reduced rate of apoptosis and increase cell cycle progression. Invasiveness and ability to induce metastases is influenced by *VEGF (*vascular endothelial growth factor)-*, MMP* (matrix-metallo-proteinase)- and *HAAH* (humane aspartyl-asparaginyl-beta-hydroxylase)-overexpression [24, 153, 181, 217, 237, 238, 271, 374]. This phenomenon is accompanied by increased rate of angiogenesis and inhibition of cell adhesion by deactivation of *E-CADHERIN* [90, 181]. *WISP1v* (Wnt-inducible secreted protein 1)-overexpression in 49% of tumors induces lymphatic and perineural invasion [370]. In addition, human cholangiocarcinoma cells escape immune surveillance either by disabling FasR signalling and/or increasing FasL expression to induce apoptosis of invading T cells [313]. Recent experimental results reported activities of the Fas and FasL promoters being regulated by NF-kappaB [280]. The exact meaning of further phenomenons, such as *CCK-BR* (CCK-B/gastrin receptor)-, *ETS-1*-, *REG-1* alpha (regenerating gene-1)-, *PKR* (RNA activated protein kinase)- expression, *APC*-gene mutations, and aberrant methylation of death-associated protein kinase (DAPK) still has to be explored [47, 132, 159, 179, 231, 375].

## Pathology

Adenocarcinomas are the most common type of extrahepatic bile duct cancers. The histologic types are listed below:

- Carcinoma *in situ*
- Adenocarcinoma
- Papillary adenocarcinoma
- Adenocarcinoma, intestinal type
- Mucinous adenocarcinoma
- Clear cell adenocarcinoma
- Signet-ring cell carcinoma
- Adenosquamous carcinoma
- Squamous cell carcinoma
- Small cell (oat cell) carcinoma
- Undifferentiated carcinoma
- Carcinoma, not otherwise specified (NOS)

Malignant mesenchymal tumors, although rare, include the following:

- Embryonal rhabdomyosarcoma
- Leiomyosarcoma
- Malignant fibrous histiocytoma

Lesions can be described as mass-forming, periductal or intraductal, or as mixed mass-forming and periductal [291].

## Clinical Manifestations

Cholangiocarcinoma usually becomes symptomatic when the tumor obstructs the biliary drainage system, causing painless jaundice. Common symptoms include pruritus (66%), abdominal pain (30–50%), weight loss (30–50%), and fever (up to 20%). The pain is generally described as a constant dull ache in the right upper quadrant.

Physical signs include jaundice (90%), hepatomegaly (25–40%), or right upper quadrant mass (10%). A palpable gallbladder, caused by obstruction distal to the takeoff of the cystic duct (Courvoisier's sign), occurs rarely.

## Diagnosis

The first suspicion of extrahepatic cholangiocarcinoma generally relies on clinical examination and increased cholestasis parameters (γ-GT, alkaline phosphatase, and bilirubin), whereas transaminases are either normal or only slightly elevated. Since sensitivity and specificity of tumor makers are low, they should be used only as adjunct after diagnosis has been made. Tumor diagnosis should never rely on tumor markers only. CA 19-9, CEA, and CA-125 are currently the most commonly used markers. They are elevated in approximately 85%, 30%, and 40–50%, respectively, of patients with cholangiocarcinoma [69, 150, 290, 317]. It is important to know that cholestasis causes false increase of tumor markers. For CA 19-9, a borderline value of > 100 U/mL has been proposed for diagnosis of cholangiocarcinoma, if there is no cholestasis [51, 269, 290]. In a study, accuracy for the diagnosis of cholangiocarcinoma in patients with PSC was 86% for a combination of tumor markers using the formula CA 19-9 + (CEA × 40) [317]. Recent studies indicate that detection of circulating hTERT (human telomerase reverse transcriptase) and serum trypsinogen-2 may be used as novel tumor markers [223, 224].

It is the aim of imaging methods to evaluate bile duct irregularities in terms of their dignity, to determine local tumor extent and resectability, and to diagnose/exclude

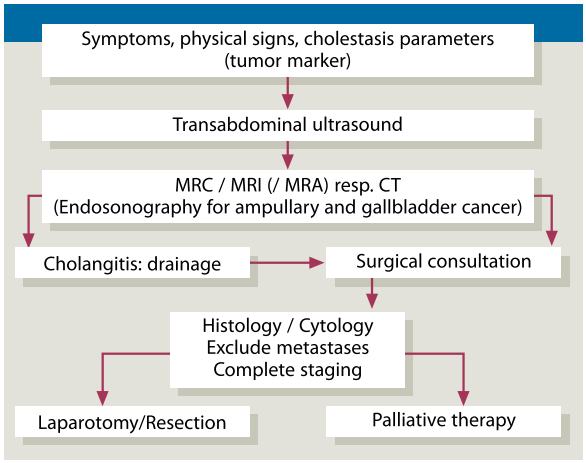


**Fig. 116.4** Diagnostic flowsheet for extrahepatic cholangiocarcinoma and gallbladder cancer. MRC, magnetic resonance cholangiography; MRI, magnetic resonance imaging; MRA, magnetic resonance angiography; CT, computed tomography

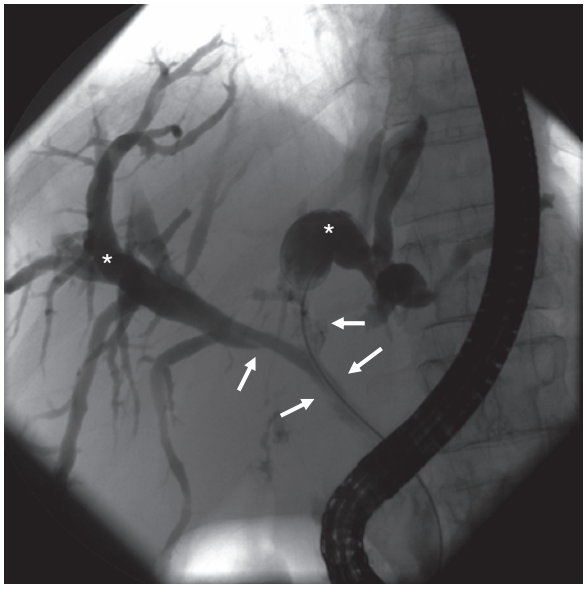

**Fig. 116.5** ERC from a patient with hilar cholangiocarcinoma Bismuth type IV: high-grade irregular bile duct stenosis which extends 11 mm subhilar, 9 mm into the right and 11 mm into the left hepatic duct (*white arrows*). Bile ducts are dilated intrahepatically (*white asterices*)

distant metastases in case of malignant tumors, in order to enable stage related therapy [351]. Usually, transabdominal ultrasound with color duplex is performed first, because it is cheap and widely available (Fig. 116.4). During recent years, new technological developments, such as THI (tissue harmonic imaging), and use of contrast-enhanced ultrasound, such as Sonovue™, allow improved imaging of hilar cholangiocarcinoma [21]. If bile ducts are sonographically dilated, non-invasive combined dynamic magnetic resonance imaging (MRI) and MR cholangiography (MRC) should follow [131]. Several studies affirm a sensitivity between 70% and 100% for this combination [3, 15, 108, 232, 241, 421, 432]. Magnetic resonance angiography (MRA) can be added to this procedure, if blood vessel invasion is suspected. Cholangiocarcinoma appears as a hypointense lesion on T1-weighted images, that is hyperintense on T2-weighted images (Fig. 116.6a). Two recently introduced technical improvements have contributed to further increasing the diagnostic value of MRI, including MRCP. The first is parallel imaging (iPAT), and the second is respiratory independent sequences navigator triggering, which have substantially increased the spatial resolution as a critical parameter in biliary imaging [429]. If MRC and MRI are not available, contrast enhanced spiral computed tomography (CT) should be performed, which is less sensitive according to a study by Nebit et al [267]. However, there is a lack of larger studies comparing sensitivity of MRI and CT for the diagnosis of cholangiocarcinoma. In addition, recently multidetector row CT (MDCT) has been developed with a tumor detection rate of up to 90%, thus leading to an important improvement in CT technology.

Following initial imaging, surgical consultation is recommended at an experienced hepatobiliary center to decide about tumor resectability [187]. Endoscopic retrograde cholangiography (ERC) and, to a lesser degree, percutaneous transhepatic cholangiography (PTC), are important for tissue sampling and therapy planning despite of the high sensitivity of MRI/MRC (Fig. 116.5) [40]. In addition, endoscopy should be reserved for treatment of cholangitis and preoperative and palliative stenting. Combined brush cytology and forceps biopsy should be performed, because this combination yields a positive histology for extrahepatic cholangiocarcinoma in 40–70% [185]. Transpapillary cholangioscopy significantly increases the ability to distinguish between malignant and benign dominant bile duct stenoses in patients with PSC [379]. Procedures, such as digital imaging analysis for evaluation of cell aneuploidia and fluorescence in situ hybridization (FISH) for chromosomal analysis, may increase sensitivity of histology [327]. Sensitivity may be even further increased by methylation analysis of tumor suppressor genes p16INK4a and p14ARF and real-

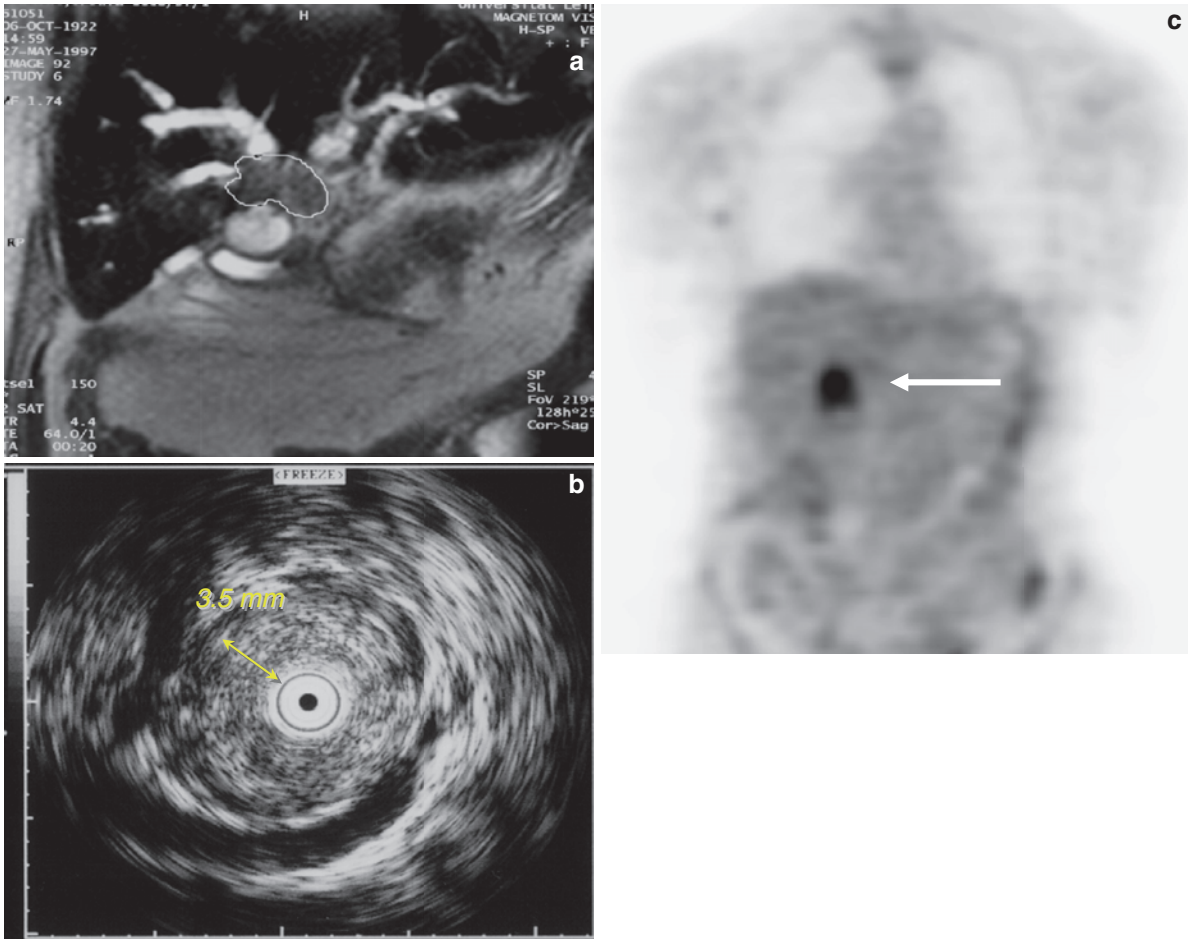


**Fig. 116.6** Hilar cholangiocarcinoma. (**a**) MRI with magnetic contrast medium of Bismuth-Corlette type IV tumor (encircled), (**b**) tumor imaging with intraductal ultrasound (IDUS; 15 MHz, 6 French probe), and (**c**) imaging of the 2.4 × 1.2 ×2.7 cm sized tumor with F-18-deoxy-glucose-positron emission tomography (FDG-PET)

time reverse transcription polymerase chain reaction (RT-PCR) assays for the detection of human aspartyl (asparaginyl) beta-hydroxylase (HAAH) and homeobox B7 (HoxB7) mRNA from intraductal bile and brush cytology specimens [96, 195]. Whether endoscopic ultrasound (EUS)-guided fine-needle aspiration of suspected hilar cholangiocarcinoma in potentially operable patients with negative brush cytology will become a standard procedure is unknown, although an initial study showed a sensitivity of 89% and specificity of 100% [104].

Recently introduced imaging techniques, such as intraductal endoscopic ultrasound (IDUS), positron emission tomography (PET), and PET/CT, are not yet routine procedures (Fig. 116.6b and c) [13, 104, 107, 183, 194, 300, 311, 324].

Laparoscopy before laparotomy to exclude peritoneal metastases is recommended in some cases [168].

## Differential Diagnosis

It is important for the clinician to realize that there is a remarkably high number of patients with presumed Klatskin tumors, which turn out to be benign fibrosing cholangitis after resection (Fig. 116.7). It has been agreed, however, that *any localized extrahepatic bile duct obstruction coexisting with intrahepatic bile duct dilatation should be considered to be malignant until proven otherwise* [118, 143, 196, 312]. This agreement arose because of the difficulty in obtaining a

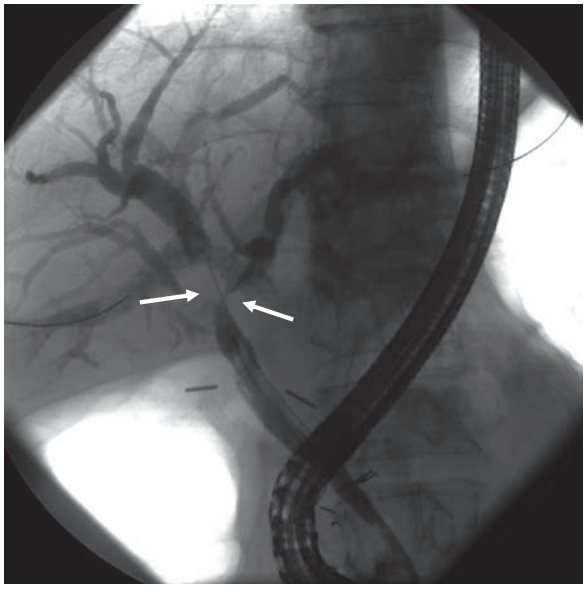

**Fig. 116.7** ERC from a patient with fibrosing cholangitis. Hilar stenosis mimics hilar cholangiocarcinoma (*white arrows*)

histopathologic diagnosis in patients with obstructive jaundice caused by a bile duct stricture. The lack of histopathological evidence might result in some patients being inappropriately treated for malignant disease when a benign stricture was present and vice versa. In the study of Gerhards et al, a false-positive preoperative diagnosis of malignancy resulted in a 15% resection rate of benign lesions in patients with suspicious hilar strictures [118].

The lack of clinical constitutional symptoms such as body weight loss and elevated tumor markers may implicate the possible benign entity of the disorder. Benign, segmental, non-traumatic inflammatory strictures of the biliary tract were infrequently reported with the exception of primary sclerosing cholangitis [118, 123, 168, 352]. Many benign nontraumatic inflammatory strictures of the common bile duct have been generally considered to be a variant of primary sclerosing cholangitis. However, Standfield et al described 12 cases of benign strictures of unknown etiology, and differentiated them from the localized form of sclerosing cholangitis [360]. Inflammatory conditions of the common bile duct, which are potential etiological factors, included bacteria or virus infection, parasite infestation, abdominal trauma, congenital abnormality, inflammatory pseudotumors, and complication of chemotherapy.

The current state of diagnostic imaging fails to deliver a reliable discrimination between benign and malignant hilar lesions. All clinical and imaging features are quite compatible with a malignant tumor in the liver hilum. Application of improved diagnostic methods, such as thin-section spiral CT, MRI/MRC and PET, can potentially increase the diagnostic accuracy, but neither could reliably differentiate malignant from benign lesions [288]. A recent study using FDG-PET for diagnosing hilar cholangiocarcinoma could not demonstrate any significant benefit in the differentiation between cholangiocarcinoma and Klatskin mimicking tumors [104]. However, only two patients with Klatskin-mimicking lesions were included into this study.

The decision to undertake resection of the strictures in these patients should not be considered as an error of judgment. In analogy to lesions of the pancreatic head, resection of the lesion is still the most reliable way to rule out malignancy. Therefore, resection of a benign stricture mimicking a malignant lesion in the extrahepatic bile duct cannot be avoided completely. Most benign segmental strictures of the extrahepatic bile duct reported in the literature are located at the hilum or the distal common bile duct.

*In conclusion, there are no reliable diagnostic methods to distinguish Klatskin tumors from benign lesions in patients without histologically confirmed adenocarcinoma. Therefore, in the presence of hilar obstruction, potentially resectable lesions should always be explored and resected to offer patients with a potential Klatskin tumor the chance for cure.*

## *Therapy and Prognosis*

### Curative Treatment for Hilar Cholangiocarcinoma

It has been recognized that only curative resection, which requires hepatic resection, offers patients with hilar cholangiocarcinoma a chance of cure and long-term survival [127, 166, 168, 182, 205, 268, 302, 340]. Recent results from the American SEER (Surveillance, Epidemiology, and End Results) database suggest a cumulative 53.7% improvement in survival for extrahepatic cholangiocarcinoma after surgical resection from 1973 through 2002. This trend may largely be caused by developments in imaging technology, improvements in patient selection and advances in surgical techniques [264].

The clear superiority of curative (R0) resection over palliative endoprosthesis placement is confirmed in several studies [168, 192, 193, 302, 417]. Even resection with a histologically positive margin offers a survival benefit as compared to palliative treatment in most reports except for the study by Jarnagin et al [139, 168, 182, 209, 302, 417]. In older studies, fewer than 30% of patients are suitable for formally curative resection (R0 resection) [168]. However, in the last 5–10 years, resectability rates could be improved to 50–60% by increased surgical radicality [139, 182, 268, 340]. Clarification of operability (pre- and intraoperatively) as well as the surgical procedure itself requires a high level of experience [32, 33].

Typical criteria for non-resectability are: (1) patients unfit or otherwise unable to tolerate a major operation, (2) hepatic cirrhosis, (3) hepatic duct involvement up to secondary biliary radicals bilaterally, (4) encasement or occlusion of the main portal vein proximal to its bifurcation (relative criterion; portal vein resection and reconstruction may be possible), (5) atrophy of one hepatic lobe with encasement of contralateral portal vein branch, (6) atrophy of one hepatic lobe with contralateral involvement of secondary biliary radicals,(7) unilateral tumor extension to secondary biliary radicals with contralateral portal vein branch encasement or occlusion, and (8) distant metastases (peritoneum, liver, lung, N2 lymph nodes) (Jarnagin and Shoup 2004) [169].

The close contiguity of the tumor to important structures in the hepatic hilum such as the hepatic artery and portal vein and the longitudinal spread mostly along and within the intrahepatic bile ducts are the most limiting factors to yield tumor-free margins. Despite extended resection, only short longitudinal and lateral tumor-free margins can be obtained.

## Preoperative Biliary Drainage

Liver dysfunction caused by obstructive jaundice is a relevant risk factor in major hepatic resections. There are no prospective randomized trials investigating the effect of preoperative biliary drainage on morbidity and mortality in jaundized patients before liver resection. In non-randomized studies, no difference in mortality was observed between patients with and without biliary drainage [61, 116, 144, 268, 302]. No difference with respect to morbidity was seen in four studies [116, 268, 302, 417]. On the other hand, higher complication rates after biliary stenting were reported by other authors [61, 144, 168]. Despite these non-conclusive data, the authors of the majority of recent studies dealing with hilar cholangiocarcinoma recommend biliary drainage in order to decrease the serum bilirubin level below 2–5 mg/dL before resection [85, 139, 182, 205, 209, 254, 340, 382]. Recovery of hepatic function after biliary decompression takes about 4 weeks, so that surgery should be done not too early [340].

## Resection of Hilar Cholangiocarcinoma

Basic principles of oncological surgery are R0 resection with wide longitudinal and lateral safety margins, monobloc-resection of the tumor and no touch technique, i.e. no manipulation at the tumor side. Obedience to these rules is limited by the anatomical setting. Hilar resection alone may result in formal R0 resection in patients with Bismuth-Corlette type I/II tumors, however safety margins are only several millimeters. Therefore, limited resection should only be performed in patients with poor general and functional operability. Hilar resection with left-sided liver resection requires dissection in close vicinity of the tumor for preparation of the right liver artery and right main trunk of the portal vein, therefore it can not be completely complied with no touch technique. Moreover, risk of complication is increased for extended left-sided resection by the high number of right-sided bile duct anastomoses to be sewn together. With right trisegmentectomy (Brisbane classification 2000), i.e. resection of liver segments 4–8 including segment 1 and portal vein segment resection de-principe, highest radicality can be achieved [23]. Therefore this technique is the procedure of first choice, if permitted by tumor extent, technical conditions of operation and functional conditions [268]. By cutting the left hepatic bile duct at segmental bifurcation 2/3, i.e. 3–5 cm distant to the hilar region, and principal portal vein segment resection between portal vein main stem and left main trunk with end-to-end anastomosis, preparations levels are not in close vicinity to the tumor (no touch technique) and extended safety margins can be achieved (Fig. 116.8).

The rates of hilar resection in series of resected hilar cholangiocarcinomas range between 0% and 24% [48, 139, 151, 167, 168, 182, 190, 192, 205, 209, 254, 268, 302, 340, 417]. Except for two reports in which 42%

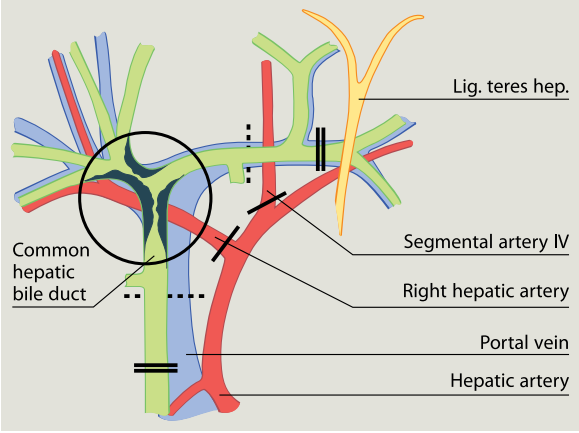


**Fig. 116.8** Right-sided trisegmentectomy. I: right hepatic artery and liver segment IV artery are cut through. II: choledochal duct is resected suprapancreatically and left hepatic duct is resected at the umbilical fissure at the area of segment 2/3 bifurcation. By resecting the portal vein, tumor free margin can be extended

and 71% of patients respectively underwent hilar resection alone [116, 382]. Concomitant liver resection revealed higher rates of R0 resection as compared to hilar resection alone in all studies but one [167, 168, 192, 254, 268]. Surprisingly, survival time did not differ between patients with and without additional liver resection in four reports [193, 254, 382, 417]. In three studies, hepatic resection proved to be a significant prognostic factor [167, 168, 205]. These controversial data with respect to survival may be caused by the small number of patients with hilar resection alone in these studies. In two series in which 75% of Bismuth-Corlette type I/II tumors were treated with hilar resection alone, survival of these patients was worse than for those with type III/IV tumors with liver resection [205, 268]. Capusotti et al reported longer survival of patients with Bismuth-Corlette type I/II tumors treated with liver resection as compared to hilar resection alone [48]. On the basis of these results, concomitant hepatic resection should be routinely performed even in patients with Bismuth-Corlette type I/II tumors.

Concomitant vascular resection had no impact on survival, using multivariate analysis, in six studies [139, 167, 192, 205, 340, 417]. Whenever curative resection of tumor-infiltrated vascular segments is possible, the patients have a similar long-term prognosis to those without vascular tumor involvement. Neuhaus et al recommend right trisegmentectomy with portal vein resection as procedure of choice, combining “no touch technique” and increased lateral radicality [268].

There is no relevant evidence that portal vein embolization, which is recommended before extended hepatic resections by some groups, is important to reduce operative mortality [139, 182, 205, 340].

Caudate lobectomy is recommended as an obligatory procedure for curative resection by the majority of authors, although the data did not show any impact on outcome [48, 167, 192, 302].

High complication rates ranging from 14% to 85% were observed in the literature and were dominated by infective complications and bile leaks [48, 116, 190]. In-hospital mortality rates range from 7.5% to 18% in studies which included at least 40 patients and used an aggressive resectional approach. However, there are two recent series with 0% and one report with 1.3% in-hospital mortality [34, 182, 205].

In our own study, neither the UICC 2002 system nor the Bismuth-Corlette classification system providing information only about longitudinal tumor extent, correlated preoperatively with the likelihood of R0 resection [34, 354, 417]. This finding was confirmed by Hemming et al for the Bismuth-Corlette classification and by Jarnagin et al for the AJCC system, which is comparable to the UICC system from 1997 [98, 139, 168, 355]. Understaging was the main problem in our series, as was also seen by Gerhards et al [116]. This can be partly explained by the periductal and intraductal type of tumor growth with minimal mass lesion and the difficulty to assess lymph node involvement. Therefore, because of limitations of current diagnostic tools laparotomy is indicated in any patients except for those with distant metastases, poor general condition or obviously locally advanced tumor. In patients with locally advanced tumors preliminary laparoscopy should be performed to preclude peritoneal and liver metastases in order to avoid unnecessary laparotomy. New preoperative staging systems, such as the proposed T–staging of Jarnagin et al, for prediction of resectability based on new imaging techniques should be considered [168].

Five-year survival rates vary in 11 relevant publications, with a minimal study length of 10 years between 12.5% and 32.8% (Table 116.5). These publications include many patients that were not operated according to the current radicality principles. In contrast, recent publications including only radically and/or R0-resected patients report 5-year survival rates of 35–44%, respectively 72% (Table 116.6). Despite variability in patient populations and adjuvant chemoradiation in some patients, these new data display an

**Table 116.5** Hilar cholangiocarcinoma: 5-year overall survival after resection in studies with at least 10 years duration[a]

| Author and year | (n) | (%) |
|---|---|---|
| (Pichlmayr et al. 1996) [302] | 125 | 27.1 |
| (Miyazaki et al. 1998) [254] | 76 | 26 |
| (Neuhaus et al. 1999) [268] | 95 | 22[b] |
| (Kosuge et al. 1999) [209] | 65 | 32.8 |
| (Launois et al. 1999) [216] | 40 | 12.5[c] |
| (Todoroki et al. 2000) [382] | 101 | 28[d] |
| (Jarnagin et al. 2001) [168] | 80 | 27 |
| (IJitsma et al. 2004) [151] | 42 | 22 |
| (Rea et al. 2005) [321] | 46 | 26 |
| (Witzigmann et al. 2006) [417] | 60 | 22[e] |
| (Baton et al. 2007) [20] | 59 | 20[f] |

[a]Including perioperative mortality and R0-, R1-, and R2-resections
[b]n = 15 liver transplantations with Kausch-Whipple-operation
[c]n = 4 liver transplantations
[d]Postoperative radiation in 28 patients
[e]n = 1 liver transplantation
[f]n = 25 adjuvant therapy

**Table 116.6** Hilar cholangiocarcinoma: 5-year overall survival after liver resection and/or R0-resection

| Author and year | (n) | (%) |
|---|---|---|
| (Neuhaus et al. 1999) [268] | 34 | 72[a] |
| (Kawasaki et al. 2003) [182] | 79 | 40[b] |
| (Seyama et al. 2003) [340] | 58 | 40[c] |
| (Hemming et al. 2005) [139] | 53 | 35[d] |
| (Sano et al. 2006) [331] | 102 | 44[e] |
| (Liu et al. 2006) [229] | 27 | 41 |

[a]Right trisegmentectomy with portal vein and R0-resection in all + patients, no perioperative mortality
[b]Only patients with R0-resection
[c]Extended right or left hemihepatectomy in all patients; only one surgeon
[e]Postoperative chemoradiation after R1-, R2-resection and for positive lymph nodes
[f]n=39 intrahepatic cholangiocarcinoma with hilar infiltration

improvement of long-term survival after resection of Klatskin-tumors.

## Liver Transplantation for Hilar Cholangiocarcinoma

Oncological goals are best met by liver transplantation, including complete removal of the bile duct system, no touch technique, and wide tumor-free margins. However, 5-year survival rates in small studies are only between 17% and 36% and therefore not better than after tumor resection [162, 302, 326]. Even extended so-called "cluster-transplantations" and the combination of pancreaticoduodenectomy (Whipple procedure) with liver transplantation did not improve survival rate [162, 268]. Evaluation of relevance of liver transplantation as an oncological concept was neglected in the past decade due to the inadequate supply of donor organs. Perhaps adult-to-adult living donor liver transplantation and excellent results of neoadjuvant chemoradiation at Mayo Clinic in Rochester/USA may revitalize the indication of liver transplantation for cholangiocarcinoma [172, 321].

*Nowadays, a general exclusion of patients with Klatskin-tumors from liver transplantation is no longer justified.*

## Resection of Distal Cholangiocarcinoma

*Patients with cancer of the distal end of the bile duct and no distant metastases require pancreaticoduodenectomy (Whipple procedure), which is the only curative treatment.*

Pancreaticoduodenectomy is usually performed in locally resectable cases including biliodigestive anatomosis with a Roux-en-loop. A pylorus-preserving operation is preferable, and is feasible in most patients. Of all patients with distal cholangiocarcinoma, approximately 50% present with resectable tumors at time of diagnosis. However, resectability rate varies between 22% and 91% in published studies [100, 261, 262, 397].

Perioperative mortality after radical resection of distal cholangiocarcinoma varies between 1.4% und 11%, with the rate of clinically relevant and severe morbidity between 22% and 41% [100, 397, 422, 425]. Whereas 5-year survival rates were below 20% at the beginning of the 1990s, 5-year survival rates in later reports are between 16% and 44% (Table 116.7). Long-term survival depends on R0 status in most of the studies. Lymph node status and tumor differentiation are

**Table 116.7** Distal cholangiocarcinoma: 5-year overall and median survival after resection

| Author and year | (n) | (months) | (%) |
|---|---|---|---|
| (Nagorney et al. 1993) [260] | 50 | 24 | 44 |
| (Fong et al. 1996) [100] | 45 | 33 | 27 |
| (Yeo et al. 1997) [422] | 65 | 20 | 16 |
| (Yoshida et al. 2002) [425] | 26 | 20.5 | 37 |
| (Jang et al. 2005) [166] | 151 | 25 | 32.5 |

independent prognostic factors in some, but not all of the studies [100, 166, 422, 425].

## Neoadjuvant and Adjuvant Therapy

Neoadjuvant chemoradiation prior to resection has been investigated so far only in small case series [245]. Chemoradiation is followed by inflammation and necrosis at the liver hilum, increasing complication risk of subsequent resection [178, 321]. Therefore, neoadjuvant chemoradiation in combination with liver resection is not established. The approach of chemoradiation and subsequent liver transplantation has recently been investigated in a small study with highly selected patients [321]. In this study, patients with hilar cholangiocarcinoma at UICC stages I and II and PSC as mainly underlying disease were treated. The neoadjuvant concept comprised chemoradiation (total dose of 45 Gy) with 5-Fluorouracil (5-FU) as bolus during the first 3 days of radiation, followed by intraluminal brachytherapy with 20–30 Gy and staging-laparotomy.

Patients without distant and lymph node metastases were treated with 5-FU or capecitabine until transplantation. Those patients that were transplanted after this selective neoadjuvant treatment reached 5-year survival rates of 82%. However, there was no histological or cytological proof of malignancy in 8 of 38 finally transplanted patients (21%), neither at time of diagnosis nor at time of operation.

Adjuvant strategies for hilar cholangiocarcinoma were tested in only two prospective comparative studies, so far (Table 116.8). In the first study, including patients with hilar cholangiocarcinoma, postoperative external radiation neither prolonged survival nor improved quality of life in comparison to patients who

**Table 116.8** Hilar cholangiocarcinoma: Adjuvant therapy

| Author and year | Therapy | Mode dose (Gy) | (n) | Median survival (months) | 5-YSR (%) |
|---|---|---|---|---|---|
| | | **Radiation** | | | |
| (Reding et al. 1991) [323] | RT | NA | 27 | 13 | NA |
| | no RT | | 98 | 8 | NA |
| (Pitt et al. 1995) [304] (prosp) | RT | EBRT 46 Gy + BT 13 Gy | 23 | 14 | NA |
| | no RT | | 27 | 15 | NA |
| (Gonzalez Gonzalez et al. 1999) [124] | RT | EBRT 40–45 Gy (+BT 10 Gy) | 52 | 24 | 24 |
| (Todoroki et al. 2000) [382] | RT | IORT 21 Gy (+ EBRT 44 Gy) | 28 | 32* | 33.9* |
| | no RT | | 19 | 10 | 13.5 |
| (Gerhards et al. 2003) [117] | RT | EBRT 42 Gy + BT 10 Gy | 41 | 21 | 17 |
| | RT | EBRT 46 Gy | 30 | 30* | 24 |
| | no RT | | 20 | 8 | 11 |
| (Kelley et al. 2004) [184] | CRT | EBRT 39–54 Gy (5FU) | 41 | 40.5* | NA |
| | no RT | | 53 | 24 | NA |
| (Sagawa et al. 2005) [330] | RT | EBRT 37 Gy (+ BT 37 Gy) | 39 | 23 | 24 |
| | no RT | | 30 | 20 | 30 |
| | | **Chemotherapy** | | | |
| (Takada et al. 2002) [366] (prosp) | CT | MMC + 5-FU | 58 | NA | 26.7 |
| | no CT | | 60 | NA | 24.1 |
| (Takeda et al. 2004) [369] | CT (i.a.) | 5-FU c.i. | 18 | | 47.6*[1] |
| | no CT | | 19 | | 39.5[a] |

Prosp: prospective study; RT: radiation therapy; CRT: chemoradiation; CT: chemotherapy; i.a.: intraarterial; c.i.: continuous infusion (24 h); EBRT: external radiation therapy; IORT: intraoperative radiation therapy; BT: brachytherapy; MMC: mitomycin C; NA: non-applicable

*$p < 0{,}05$

[a]3-year survival rate

had only operation alone [304]. In a second published study, adjuvant chemotherapy, consisting of single dose mitomycin C at the day of operation and two 5-day cycles of 5-FU, followed by orally administered 5-FU until tumor progression, was compared to postoperative surveillance of patients, only. The 5-year survival rate was 26.7% in the adjuvant arm compared to 24.1% in the control arm ($p > 0.05$) [366]. In both studies, however, inhomogeneous populations with both curatively and palliatively resected tumors were included. Formal randomization was not part of the Pitt-study. Also, the types of therapy, radiation without chemotherapy and orally administered 5-FU can not be regarded as standard therapy. In contrast, some retrospective surgical studies reported about long time survival and improved tumor control after adjuvant radiation therapy. In a study by Todoroki et al. patients with locally advanced tumors received intra- and/or postoperative radiation after R1 resection or no further treatment. The five-year survival rate was 34% in the adjuvant therapy group in comparison to 13.5% in the untreated group [382].

Data was confirmed by another study, where some of the patients received postoperative adjuvant external radiation and/or intraluminal brachytherapy. The five-year survival rate was 19% in the adjuvant therapy group in comparison to 11% in the untreated group, and median survival was 24 months in comparison to 8 months [117].

Adjuvant chemoradiation reduced recurrence rate of biliary carcinoma in non-randomized studies, yielding a median survival time of up to 41 months and a 5-year survival rate of up to 53.5%. In these studies, 5-FU was used as radiosensitizer [184, 392].

In our own pilot-study, it was the aim to determine the toxicity and efficacy of a postoperative regimen combining radiotherapy and chemotherapy with gemcitabine and capecitabine in extrahepatic bile duct cancer. Patients were eligible after surgery for extrahepatic bile duct adenocarcinoma. Surgery included resection of lymph node positive cancer, incomplete resections and diagnostic laparotomy in unresectable tumors. Patients received a fractionated radiotherapy of 49.6 Gy accompanied by gemcitabine once a week. After a 2-week rest, patients were treated with gemcitabine and capecitabine on a 3-week cycle. The treatment continued for six cycles in nonmeasurable disease or until disease progression or intolerable toxicity. There were 18 patients (resection/laparotomy 7/11) enrolled between August 2003 and April 2005. Radiotherapy was completed in all patients and a total of 66 cycles of chemotherapy was administered. Fatigue and nausea were the most common mild adverse events. Grade 3 and 4 toxicity was rare after resection but frequent in unresectable disease and consisted of fatigue, nausea, duodenal ulcer, cachexia, and cholangitis in 1, 2, 2, 4, and 4 patients, respectively. We observed a 50% disease stabilization rate in patients with measurable disease. Median overall survival was 7.9 months in patients with unresectable tumors. Median overall survival in patients after resection has not been reached at a median follow-up of 19.5 months. Thus, radiochemotherapy using gemcitabine followed by gemcitabine and capecitabine is an active regimen with manageable toxicity after resection of extrahepatic bile duct cancer but has significant toxicity in unresectable disease [337].

*In summary, neoadjuvant and adjuvant chemotherapy/chemoradiation have shown some benefit for patients with hilar cholangiocarcinoma, but they do not represent an established therapy. Further prospective, randomized studies are required.*

A new approach might be the use of photodynamic therapy (PDT) for the treatment of hilar cholangiocarcinoma in a neoadjuvant setting, because of the high tumor recurrence rate of up to 76% after curative (R0) resection. Having accomplished a successful treatment in a single case, we investigated the use of PDT prior to tumor resection in six patients and in one patient prior to liver transplantation in a small pilot study [29, 30, 411]. In all patients, R0 resection was achieved. Four patients developed minor surgical complications, even though the bilioenteric anastomoses were sewn to PDT-treated bile ducts. No viable tumor cells were found in the inner 4 mm layer of the surgical specimens. The PDT-pretreated epithelium of the tumor-free proximal resection margins exhibited only minimal inflammatory infiltration. The 1-year recurrence-free survival rate was 83% and 5-year overall survival 42%. Similar results have also been reported by Nanashima et al. who treated eight patients with porfimer sodium PDT in an adjuvant setting [263]. Five patients had extrahepatic bile duct cancer, two had intrahepatic cholangiocarcinoma, and one had ampullary carcinoma. Cancer cells were microscopically detected in the stump of the hepatic duct in six patients, and biliary stenosis caused by remnant tumor was observed in one patient. One patient had tumor recurrence with occlusion of the bile duct. At 48 h prior to PDT, porfimer

sodium was injected intravenously. A pulse laser by an eximer dye laser (50–100 J/cm$^2$) with a wavelength of 630 nm was applied through an endoscope to the hepatic stump or tumor lesion. Marked destruction of the tumor and ductal epithelium was observed on day 1 after PDT. After PDT, four patients developed mild dermatitis, but no severe morbidity or mortality was noted. In patients who underwent PDT for the stump, one patient showed distant metastasis at 31 months, and four patients did not show tumor recurrence at 17, 12, 12, and 6 months, respectively. However, one of the eight patients died at 2 months of an unrelated cause. In two patients with occlusion caused by tumor growth, resolution of bile duct stenosis was noted on day 7. These patients showed re-occlusion by tumor at 20 and 8 months.

*In summary, neoadjuvant and adjuvant PDT in these two small studies was a safe and a useful option for a better survival benefit in patients with bile duct cancer undergoing surgical resection, however further studies are required.*

## Palliative Management

The goal of palliative treatment is to achieve stable long-term biliary drainage in order to improve quality of life and prolong survival. Refractory obstructive cholestasis leads to death owing to severe bacterial cholangitis or liver failure [94].

### Stenting for Extrahepatic Cholangiocarcinoma

The median survival time of patients with nonresectable hilar cholangiocarcinoma is approximately 3 months without intervention and 4–10 months with biliary drainage [73, 94, 230, 305]. Successful endoscopic drainage rates with plastic stents have been reported to range from approximately 40–80% [80, 230, 305, 306]. In contrast, a randomized study by Ortner et al, which contained 79% Bismuth type IV tumors, showed a decrease of cholestasis by at least 50% within the first week in only 21% with stenting alone [278]. An endoscopic approach for biliary stent insertion is preferred over an ultrasound-guided percutaneous approach. Cholestasis was more efficiently reduced and 30-day and overall mortality rates were lower in a randomized study [359]. Percutaneous transhepatic cholangiodrainage (PTCD) should be used after failure of endoscopy and for anatomically difficult situations, i.e. after partial gastrectomies, especially with Roux-en-y anastomosis [74]. In these cases the papilla Vater can often not be reached endoscopically. In selected cases both methods can be combined as“rendezvous-approach”. Recently, a new technique of endoscopic biliary-enteric bypass was introduced which comprises endosonographically guided bile duct puncture followed by biliary drainage into the duodenum [43]. The major complication of endoprosthesis implantation is stent occlusion with consecutive cholangitis [258]. Light and electron microscopy studies of blocked stents revealed that the material blocking the lumina was composed of a matrix of bacterial cells and their fibrillar anionic extracellular products. Crystals of calcium bilirubinate, calcium palmitate, and cholesterol were embedded within this matrix. Bacterial cells were attached to the stent surface by a fibrillar matrix, suggesting that the initial event in stent clogging is the development of an adherent bacterial biofilm. Bacterial enzyme activity (beta-glucuronidase and phospholipase) leads to the deposition of crystals. Therefore, it was assumed that the use of antibiotics and/or ursodeoxycholic acid may reduce bacterial adhesion and stent clogging. However, prophylactic long-term antibiosis and/or ursodeoxycholic acid have not turned out to be useful in several studies [128].

*Therefore, a close monitoring of patients for early detection of latent biliary sepsis is important for long-term success of stent therapy. As part of this monitoring, plastic endoprostheses should be routinely exchanged every 3 months [310].*

Technical questions regarding stent material, number and location of stents have not been completely answered. Polyurethane and polyethylene seemed to be slightly better in comparison to Teflon according to a preclinical study [215]. However in a prospective randomized trial of Teflon versus polyethylene stents for distal malignant biliary obstruction Teflon material did not improve patency in biliary stents with an Amsterdam-type design [395]. Early complication rates were similar in both groups (10%). The median follow-up was 142 days. Stent dysfunction occurred in 28 Teflon and 29 polyethylene stents. The thirty-day mortality was 14% in both groups. Patient survival did not differ significantly between the groups (median survival: Teflon 165 days, polyethylene 140 days). The median stent patency was 83 days for Teflon and 80 days for polyethylene stents, and was not significantly different either. To

assess whether the use of large stents is justified, Speer et al. retrospectively reviewed the results of 8F stents with pigtails and 10F straight stents in the palliation of biliary obstruction due to malignancy [358]. The incidence of cholangitis following stent insertion was significantly lower with 10F stents compared with 8F stents (5% vs. 34%, $p < 0.001$). Stent survival until blockage was significantly longer for 10F compared with 8F (median 32 weeks vs. median 12 weeks, $p < 0.001$). In addition, Kadakia and Starnes retrospectively compared the efficacy and complications of 10F biliary stents with 11.5F stents in the management of malignant and benign biliary tract diseases [175]. They concluded that 10F stents have the same success rate and complication rate as 11.5F stents in the management of biliary tract diseases, thus a stent size bigger than 10F offers no significant advantage. A new approach may be the use of an Olympus DoubleLayer stent (DLS; perfluoro alkoxy, without sideholes). In a prospective randomized comparison of this stent with the standard polyethylene stent (with sideholes), including a total of 120 patients with jaundice due to malignant strictures of the middle to distal third of the common bile duct, 28 DLS patients (47%) and 17 polyethylene stent patients (29%) died without clinical evidence of stent occlusion after a mean of 114 and 105 days, respectively ($p < 0.05$). Twenty-six DLS patients (43%) and 38 polyethylene stent patients (63%) had symptoms of stent clogging after a mean of 144 and 99 days, respectively ($p < 0.05$). Stent dysfunction (stent orifice impacted on the bile duct or duodenal wall, stent migration) was recorded in 6 DLS patients (10%) and 5 polyethylene patients (8%) (n.s.). Kaplan-Meier analysis of DLS and PE stent clogging-free survival showed a significantly longer patency period with the DLS stents ($p = 0.0005$). Tringali et al. concluded that these results showed that DoubleLayer stents have a longer patency period than polyethylene stents [386]. Another new development, as described by Raju et al., is a 10F "winged" stent without a lumen to prevent stent occlusion [316].

A prospective, randomized study revealed a lower cholangitis rate after unilateral stent insertion in comparison to bilateral stent insertion [70].

*However, so far general consensus regarding unilateral versus bilateral bile duct drainage has not been obtained.*

In general, at least 25% of liver tissue should be drained and contrast medium should be injected only in those bile duct segments that will be finally drained. There is no need to drain a certain liver lobe, it is better to drain the easiest accessible one. In case of concurrent single-sided liver atrophy as a result of tumor induced blood vessel occlusion the primarily intact liver lobe has to be drained. Recent studies showed that MRCP-guided drainage of the widest dilated bile ducts can improve results of stent therapy [102, 142].

Cost analyses in patients with middle and distal bile duct or ampullary cancer showed that metallic stents were advantageous in patients surviving more than 6 months, whereas a plastic stent was advantageous in patients surviving 6 months or less according to several studies [174, 199, 310, 399].

Self-expanding metal stents (SEMS), which can be inserted endoscopically and percutaneously, have the advantage of a higher patency rate with consecutive lower rates of reinterventions and rehospitalizations in comparison to less costly plastic stents [73, 399]. The higher patency rate of metallic stents is related to the wider lumen and better drainage of segmental bile duct branches. These data were confirmed by a recent single-center prospective randomized controlled trial comparing the patency of 10F polyethylene stents and covered 30F (10mm)steel Wallstents™ in patients with nonresectable malignant common bile duct strictures [356]. As a result, median patency times were 1.8 and 3.6 months in the polyethylene stents and Wallstents™ groups, respectively ($p = 0.002$). Interestingly, if overall costs of therapy, determined by adding the cost of hospitalization to the costs of the stent, were compared, there was no difference between both groups. The authors recommended the more-effective SEMS in unresectable patients with malignant common bile duct strictures, who survive a median of 4.5 months and less costly plastic stents for patients who have distant metastases. A newly designed Y stent for advanced hilar cholangiocarcinoma may improve technical and clinical efficacy of endoscopic stent placement [222]. Despite the higher patency rate of SEMS, stent dysfunction can be induced by tumor ingrowth, overgrowth, clogging with sludge or stones, or stent dislocation. Stent dysfunction can be managed by insertion of additional plastic or metal stents, laser treatment or balloon dilatation [376]. Whether stent occlusion may be prevented by covered SEMS or brachytherapy is currently unknown. A prospective randomized study evaluating patients with distal malignant biliary duct obstruction showed that a polyurethane covered Diamond™-stent is superior to a non-covered metallic stent in

terms of patency rate [155]. However, cholecystitis and pancreatitis rates were higher in the covered metallic stent group. Overall survival was equivalent in both groups. In contrast, a recent study comparing polyurethane-covered Wallstents™ that were used in 36 patients with uncovered Wallstents™ that were used in 41 patients found no significant difference between stent patencies [424]. Cholecystitis occurred in one patient of the covered Wallstent™ group but in none of the uncovered Wallstent™ group. Stent occlusion occurred after a mean of 398 days in the covered group and after 319 days in the uncovered Wallstent group ($p > 0.05$). Stent patency rates were 83%, 78%, 67%, and 54% at 100, 200, 300, and 400 days, respectively, in the covered group and 83%, 66%, 54%, and 36% in the uncovered group, which was not significantly different. However it has to be mentioned that this study was not a prospective, randomized study and sample size was not large. However, another non-randomized study by Park et al. supported these results [285].

### Photodynamic Therapy (PDT) for Hilar Cholangiocarcinoma

PDT is based on the administration of a photosensitizer which localizes selectively within the target, mostly tumor tissue. The mechanisms by which this localization occurs are complex and not fully understood. High vascular permeability of the agents, as well as their affinity for proliferating endothelium and the lack of lymphatic drainage in tumors may contribute to an accumulation in tumors [76]. Moreover, tumors might have increased lipid content, elevated numbers of low-density lipoprotein receptors, abnormal vasculature, and decreased pH. In a second step, non-thermal laser light of a specific wavelength is applied, adapted to the absorption spectrum of the sensitizer to be excited. Following the absorption of light the compound is transformed into a relatively long-lived electronically excited state via a short-lived excited singlet state [140]. This so-called triplet state can undergo two kinds of reactions. In a type I reaction, it can react directly with a substrate, such as the cell membrane or a molecule, and transfer a hydrogen atom or electron to form radicals. The radicals interact with oxygen to produce oxygenated products. Alternatively, in a type II reaction, which is more common, the triplet can transfer its energy directly to oxygen to generate singlet oxygen. Therefore, the effects of both PDT reaction types are oxygen dependent. Tumor destruction, mediated by oxygenated products and singlet oxygen, can be explained by several mechanisms causing necrosis and apoptosis. First, PDT kills tumor cells directly, secondly it damages the tumor-associated vasculature, leading to tumor infarction, and finally can activate an immune response against tumor cells.

*In detail, PDT has been demonstrated to induce (1) direct cytotoxicity by degeneration of lipids in cell membranes, (2) microvascular damage from platelet activation and thromboxane-induced vessel constriction and thrombus formation, probably mediated by nitric oxide, (3) cytosolic and mitochondrial membrane damage with cytochrome C release, bcl-2 depletion and induction of apoptosis, and (4) release of inflammatory mediators that induce T-lymphocyte mediated cellular immune response.*

The extent of photodamage and cytotoxicity is multifactorial and depends on the type of photosensitizer used, its localization and administered dose, the light source used, generating the light exposure dose and light fluence rate, the oxygen availability in the target, and finally the time interval between the administration of the photosensitizer and the light treatment. Because PDT is a cold photochemical process, there is no tissue heating, and connective tissues such as collagen and elastin are largely unaffected. PDT should not be performed in patients with acute porphyria, poor kidney or liver function (creatinine > 3 mg/dL, international normalized ratio of prothrombin time [INR] > 2.2), encasement or thrombosis of the main blood vessels, leukopenia (leukocytes < 2,000/cmm), thrombocytopenia (< 50,000/ $mm^3$), and terminal tumor stage.

Interest in using PDT for palliative treatment of advanced non-resectable CC has been given rise to by a case report, documenting the success of PDT performed via percutaneous cholangioscopy in a single patient with incompletely resected bile duct carcinoma (Table 116.9) [244]. This type of treatment caused a prolonged survival time of more than 4 years in a tumor entity with a median survival of only 4–6 months. Ortner et al performed the first prospective, non-randomized single-arm study including nine patients with advanced Bismuth type III and IV hilar CC, who showed no sufficient drainage after endoscopic stent insertion [277]. Two days after intravenous administration of porfimer sodium (Photofrin™) at 2 mg/kg body weight, intraluminal photoactivation at 630 nm was performed

**Table 116.9** Palliative photodynamic therapy in hilar cholangiocarcinoma

| Protocol | n | Photosensitizer | Drainage | Survival# | Reference |
|---|---|---|---|---|---|
| Phase I | 1 | Dihemato-porphyrin 2 | T-Drain | 48 | (McCaughan et al. 1991) [244] |
| Phase II | 9 | Photofrin II | Plastic stent | 14.6 (3–19.2) | (Ortner et al. 1998) [277] |
| Phase II | 21 | Photofrin II | Plastic stent | 15 | (Ortner 2000) [276] |
| Phase II | 23 | Photofrin II | Plastic stent | 12.0 (10.4–13.6) | (Berr et al. 2000b; Wiedmann et al. 2004) [30, 410] |
| Phase II | 8 | Photosan-3 | Plastic stent and PTCD | 4.0 (1.7–14.8) | (Zoepf et al. 2001a) [434] |
| Phase II | 4 | 5-ALA | Plastic stent | NA | (Zoepf et al. 2001b) [435] |
| Phase II | 6 | Photofrin II | Plastic stent | NA | (Rumalla et al. 2001b) [328] |
| Phase II | 24 | Photofrin II | Metallic stent | 9.9 (6.4–13.4)$^{+}$ | (Dumoulin et al. 2003) [83] |
| Phase III | 20 | Photofrin II | Plastic stent | 16.4$^{**}$ | (Ortner et al. 2003) [278] |
| | 19 | | Plastic stent | 3.3 | |
| Phase II | 8 | Photofrin II | Plastic stent | 9.2$^{+}$ | (Harewood et al. 2005) [134] |
| Phase II | 36 | Photofrin II | Plastic stent | 11.2 (2.5–17.3) | (Pereira et al. 2005) [299] |
| Phase II | 24 | Hematoporphy-rine derivative | PTCD | 18.6 (2.1–27) | (Shim et al. 2005) [342] |
| Phase II | 25 | Photofrin II | Plastic stent | 8.1 | (Prasad et al. 2005) [308] |
| Phase III | 16 | Photosan-3 | Plastic stent | 21$^{*}$ (3–30) | (Zoepf et al. 2005) [433] |
| | 16 | | Plastic stent | 7 (1–24) | |
| Phase II | 68 | Photofrin II | Plastic stent | 12$^{**}$ (8.8–15.3) | (Witzigmann et al. 2006) [417] |
| | 56 | | Plastic stent | 6.4 (4.2–8.5) | |

NA: non-applicable; #median (range) in months from diagnosis
$^{*}p < 0.05$; $^{**}p < 0.01$; $^{+}$median (range) in months from therapy

cholangioscopically with laser diffusing fibers. After PDT, bilirubin serum levels declined significantly ($p = 0.004$) with no increase during the two monthly follow-ups. Quality of life indices improved dramatically and remained stable. Thirty-day mortality was 0%, and median survival 439 days. Side effects were limited to skin hyperpigmentation, but no acute phototoxicity, in all patients. One patient also reported fever and pain, self-limited, for 2 weeks after PDT. Ortner's work was subsequently expanded to a larger study of 21 patients [276]. We performed our own study, including 23 patients with hilar CC (Bismuth type III, n = 2; type IV; n = 21) who were treated with a combination of bile duct stenting and endocopically performed Photofrin™-PDT [30]. In detail, we intubated the tumor stenosis with a translucent ERC-cannula and inserted a 400 µm thin quartz fiber with either a 2, 3, or 4 cm long cylindrical light diffuser and radioopaque markers (Lambda Plaus PDL-2, Coherent, Dieburg, Germany). The power emmitted by the diffuser tip through the ERC cannula had been calibrated to 0.400 W/cm before PDT and checked thereafter using an integrating sphere power meter. The tumor stenoses were exposed to laser light for 10 min with a light dose of 242 J/cm$^2$. The patients showed a 6 months survival rate of 91% after diagnosis and 74% after start of PDT with a median local tumor response of 74%, 54%, 29%, and 67% after the first, second, third, and fourth PDT session. Cholestasis, performance, and quality of life of the patients improved clearly. Cholangitis rate with PDT was not higher than in historical control patients with Bismuth type III tumors and bile duct drainage, only. After a five year follow-up, median survival time after diagnosis was 18 months for patients without peritoneal carcinosis (n = 19) and 12 months for all patients (n = 23) [415]. Survival was 63%, 26%, 16%, and 5% after 1, 2, 3, and 4 years, respectively. Of the patients who died, 73.9% (n = 17) were because of tumor progression; 26.1% died as a result of cholangitis (n = 4), septic shock (n = 1), or appendicitis/peritonitis (n = 1). For all patients, except one with diffuse liver metastases, there was improvement in cholestasis, performance, and quality of life, which was maintained for an extended period of time. These data were confirmed by a similar recent phase II study including 36 patients [299]. In 2001, Zopf et al. described the use of a novel laser diode PDT system in the treatment of eight patients with Bismuth II-IV non-resectable CC using Photosan-3 (2 mg/kg; dihematoporphin ether) and a light dose of 200 J/cm$^2$ at a wavelength of 633 nm [434]. Four weeks after initial

PDT, which was performed transpapillary (n = 4) or percutaneously (n = 4), all patients showed a marked reduction of bile duct stenosis. The median serum bilirubin value declined from 5.8 to 1.0 mg/dL. The median survival time was 119 days (52–443 days). The therapeutic value of percutaneous transhepatic PDT in patients with advanced bile duct cancer was subject of another prospective trial [342]. The utility of intraductal ultrasonography (IDUS) for the assessment of responses and for regular follow-up after PDT was also examined. PTCD was initiated before PDT. Following dilation and maturation of the PTCD tract, percutaneous PDT was performed. Intraluminal photoactivation was carried out using percutaneous cholangioscopy 2 days after intravenous application of a hematoporphyrin derivative. All patients were additionally provided with percutaneous bile duct drainage catheters after PDT. IDUS was conducted monthly to measure the thickness of the tumor mass before and after PDT. Twenty-four patients with advanced cholangiocarcinomas (Bismuth IIIa, n = 4; IIIb, n = 10; IV, n = 10) were treated with PDT. At 3 months after PDT, the mean thickness of the tumor mass had decreased from 8.7 ± 3.7 mm to 5.8 ± 2.0 mm ($p < 0.01$). At 4 months after PDT, the thickness of the mass had increased to 7.0 ± 3.7 mm. Quality of life indices improved dramatically and remained stable 1 month after PDT; the Karnofsky index increased from 39 to 58 points ($p = 0.003$). The 30-day mortality rate was 0%, and the median survival time was 558 days (range 62–810 days). Another single-arm study described a new method of applying photodynamic therapy in the biliary tract by using accessories available in the United States [328]. Patients were injected with 2 mg/kg of sodium porfimer and 48 h later a commercially available cylindrical diffusing laser fiber was inserted into an 8 F biliary catheter equipped with a 0.038 inch side-hole at its distal tip. After positioning of a 0.035 inch guidewire proximal to the biliary stricture, the preloaded catheter was advanced over the guidewire by using the monorail technique. Laser light was applied at a power of 4 mW/cm fiber for a total energy of 180 J/cm$^2$. By using the preloaded biliary catheter, adequate positioning of the laser fiber was achieved in all patients. A fracture of the diffuser tip occurred during one of the treatments. Two patients developed acute cholangitis and two patients experienced skin phototoxicity. The applicability of this technique was confirmed in a recent study by Harewood et al. [134] It was concluded that (1) PDT treatment every 3 months is applicable, (2) patient selection for PDT need not to be based on response to primary biliary stenting, and (3) the average cost of treatment per patient would be US $ 10,337, which is based on direct medical costs estimated from USA Medicare ambulatory patient classification (ABC 2003) plus professional fees for hospital-based outpatient procedures and can be compared with the costs of management with biliary stenting alone.

Another study examined factors associated with increased survival after PDT [309]. Twenty-five patients with cholangiocarcinoma who were treated with PDT at the Mayo Clinic in Rochester/USA from 1991 to 2004 were studied. Patients received PDT treatments every 3 months. Plastic biliary stents (10–11.5 F) were inserted to decompress the biliary system after PDT. Survival analysis was performed using Kaplan-Meier curves and Cox proportional hazards models. The median overall survival period was 344 days. The median survival period after PDT was 214 days. The 1-year survival rate was 30%. On multivariate analysis, the presence of a visible mass on imaging studies (hazard ratio, 3.55; 95% confidence interval, 1.21–10.38), and increasing time between diagnosis and PDT (hazard ratio, 1.13; 95% confidence interval, 1.02–1.25) predicted a poorer survival rate after PDT. A higher serum albumin level (hazard ratio, 0.16; 95% confidence interval, 0.04–0.59) predicted a lower mortality rate after PDT. The conclusion from this study was that patients with unresectable cholangiocarcinoma without a visible mass may benefit from earlier treatment with PDT.

Another prospective phase II study investigated PDT and consecutive metal stent insertion for palliation of hilar CC [83]. It was feasible but there was only a modest benefit in overall survival in comparison to a historical control group. In addition, a small study investigated the use of 5-aminolevulinic acid (5-ALA) for the palliative treatment of hilar CC [434]. Light activation was performed 5–7 h after oral administration of 5-ALA. All patients had an endoprosthesis placed in the bile duct after PDT. However, 4 weeks after PDT, 5-ALA had failed to significantly reduce malignant bile duct obstruction and thus can not be recommended for the palliative treatment of bile duct cancer. Finally, we reported the first prospective, uncontrolled trial in patients with hilar cholangiocarcinoma which compared outcome after palliative PDT and resectional therapy [417].

As expected, survival after palliative PDT was inferior to R0 resection. However, patients treated with PDT showed a similar survival time to that of patients with incomplete R1/R2 resection, although the former were more ill.

Despite these improvements in palliative treatment by PDT, *an aggressive resectional approach is justified in view of the facts that even during surgery completeness of tumor resection can hardly be defined and long-term survival is observed after complete resection of advanced tumor stages and even after R1 resection*.

The first prospective, randomized multicenter trial, including our hospital, confirmed preliminary data from the above mentioned non-randomized studies [278]. Patients with hilar CC fulfilling inclusion criteria (non-resectable tumor, tumor diameter > 3 cm, tumor clearly visible on CT and ERCP, unequivocal positive histology, no evidence of cancer of another organ) were randomized to group A (stenting and subsequent PDT) and group B (stenting alone). For PDT, Photofrin™ at 2 mg/kg body weight was injected intravenously 2 days before intraluminal photoactivation (wavelength, 630 nm; light dose, 180 J/cm$^2$). Further treatments were performed in cases of residual tumor in the bile duct. Oral ciprofloxacin therapy 250 mg twice daily was started before ERCP and continued for 14 days. The primary outcome parameter was survival time. Secondary outcome parameters were cholestasis and quality of life. PDT resulted in prolongation of survival (group A: n = 20, median 493 days; group B: n = 19, median 98 days; $p < 0.0001$). It also improved biliary drainage and quality of life. The study was terminated prematurely because PDT proved to be so superior to simple stenting treatment that further randomization was deemed unethical. It was remarkable that an additional group of 31 patients who were excluded from randomization because of a statistically significant lower Karnovsky performance status, but received PDT treatment voluntarily, performed as well as the randomized PDT group.

The second prospective randomized trial investigated the effect of Photosan-3 based PDT plus stenting on survival time in 32 patients with advanced hilar CC [433]. In the control group, patients were treated with endoprostheses but no PDT. PDT group and the control group were comparable due to age, gender, performance status, bilirubin level, and Bismuth stage. The median survival time after randomization was 7 months for the control group and 21 months for the PDT group ($p = 0.01$). In half of the initially percutaneously treated patients, a change from percutaneous to transpapillary drainage after PDT was possible. Four patients showed infectious complications after PDT versus one patient in the control group.

*In summary, in 2 prospective randomized studies palliative PDT for non-resectable hilar CC was effective in restoring biliary drainage and improving quality of life and prolonging survival; thus, PDT should become a standard treatment*.

Finally, Cheon et al. suggest that serum Il-6 concentration may serve as a marker for monitoring the response to treatment of cholangiocarcinoma with PDT [60].

## Surgical Palliation

Because surgical drainage procedures do not offer any advantage to nonsurgical palliation, biliary stenting is regarded as the palliative method of choice.

## Palliative Chemotherapy

In general, cytostatic therapy has to be evaluated critically according to the goal of palliative treatment to improve quality of life and prolong survival. Time for treatment and hospitalization for complications of chemotherapy have to be substrated from the limited total survival time. In addition, toxicity of therapy may negatively influence quality of life. Reserve of liver function is normally limited for these tumors, which may result in an increased rate of complications of cytostatics as a consequence of decreased drug metabolization. There is an additional risk of latent cholangitis and sepsis for patients with extrahepatic cholangiocarcinoma with the typical feature of bile duct obstruction. This risk is increased with cytostatic therapy.

In the nineties, in a randomized phase III study including biliary tract and pancreatic cancer patients, 5-FU-based chemotherapy was shown to be better than best supportive care (Table 116.10) [121]. While the prolongation of overall survival failed to reach significance for biliary tract cancer patients (2.5 versus 6.5 months, $p = 0.1$) – possibly due to the low number of patients (n = 37) – quality of life was improved and the average quality-adjusted survival was prolonged in the group of patients randomized to the chemotherapy. However, the FELV-protocol (5-FU, etoposid and

**Table 116.10** Randomized studies of palliative chemotherapy for biliary cancer

| Author and year | (n) | Drugs | Dose (mg/m$^2$), Protocol | Response rate (%) | Median Survival (months) | 1-YSR (%) |
|---|---|---|---|---|---|---|
| **Phase III** | | | | | | |
| (Glimelius et al. 1996) [121]** | 90 | F(E)LV | d 1–3 / 3 week | (8) | 6* | NA |
| | | BSC | | (2) | 2.5 | |
| (Rao et al. 2005) [320] | 54 | FELV | d 1–3 / 3 week | 15 | 12 | 50 |
| | | ECF | d 1(EC), d 1–21 (F) / 3 week | 19 | 9 | 21 |
| **Phase II** | | | | | | |
| (Falkson et al. 1984) [93]*** | 87 | 5-FU | 600 p.o. d 1–5 / 6 week | 10 | 5.3 / 6.5 | NA |
| | | 5-FU/Strepto | 600 (F)+500 (S) d 1–5 / 6 (F)/12 (S) week | 8 | 3.5 / 3 | NA |
| | | 5-FU/ mCCNU | 500 d 1–5/6 week (F) + 150 d 1 (C) | 10 | 2.5 / 2 | NA |
| (Takada et al. 1994) [367]a | 81 | 5-FU | 310 (F) d 1–5 / 6 week | 0 | 6.0 | 25 |
| | | FAM | 310 (F), d 1–5, 12 (A) d 8, 6 (M) d 1 / 6 week | 4 | 6.2 | 14 |
| (Kornek et al. 2004) [207] | 51 | MMC/Gem | d 1 (M), d 1,15 (G) / 4 week | 20 | 6.7 | 23 |
| | | MMC/Cap | d 1 (M), d 1–14 (C) / 4 week | 31 | 9.3 | 41 |
| (Ducreux et al. 2005) [82] | 58 | HD 5-FU | d 1,8,15,22,29,36 / 8 week | 7 | 5 | 29 |
| | | 5-FU /LV/ CDDP | d 1,(8),15,(22),29,(36) / 8 week | 19 | 8 | 30 |

*$p < 0.05$ (pancreatic and biliary cancer; $p = 0.1$ for biliary cancer only [n = 37])
**Response rate was not formally investigated
***Survival was separately investigated for gallbladder cancer and bile duct cancer
[a]Study included pancreatic cancer patients
*FELV*: 5-Fluorouracil + Etoposide + Leucovorin; *BSC*: best supportive care; *ECF*: Epirubicine + Cisplatin + 5-Fluorouracil; *Strepto*: Streptozotocin; *mCCNU*: methyl-*CCNU*; *FAM*: 5-FU + Adriamycin + Mitomycin C; *MMC*: Mitomycin C; *Gem*: Gemcitabine; *Cap*: Capecitabine; *HD*: high dose; *LV*: Leucovorin; *CDDP*: Cisplatin; *NA*: non-applicable

leucovorin) that was followed in the study is used only seldomly because of its toxicity, although epirubicin, cisplatin and 5-FU (ECF) combination was not more effective in a second phase III study [320].

Ten years ago a randomized trial demonstrated the superiority of gemcitabine over 5-FU in pancreatic cancer considering clinical benefit response, progression-free and overall survival [44]. Based on the analogy of carcinogenesis of biliary tract cancer and pancreatic cancer, gemcitabine was adopted for palliative chemotherapy for biliary tract cancer. Nevertheless, a randomized controlled trial of gemcitabine compared to 5-FU has not been performed up to date and there is no evidence based on non-randomized trials supporting superiority of gemcitabine alone compared to 5-FU alone in biliary tract cancer [86].

Favourable results have been published from a GERCOR (Groupe Coopérateur Multidisciplinaire en Oncologie) trial evaluating the GemOx regimen in biliary tract cancer [14]. GemOx comprises gemcitabine at a dose of 1,000 mg/m$^2$ infused over 100 min (fixed dose rate of 10 mg/m$^2$/min) on day 1, followed by oxaliplatin 100 mg/m$^2$ on day 2, repeated every 14 days. Remarkably, untreated patients with good performance status (WHO 0–2) and adequate renal and liver functions (serum creatinine < 1.5 × upper limit of normal [ULN] and bilirubin < 2.5 × ULN) were enrolled (group A) as well as patients with performance status > 2 or bilirubin > 2.5 × ULN, or prior chemotherapy for advanced disease (group B). Such an enrollment policy is unusual for trials but represents well the reality of the patients with advanced biliary tract cancer. Noteworthy, patients with performance status of > 2 are capable of only limited selfcare, confined to bed or chair more than 50% of waking hours. According to the NCCN guidelines these patients would have received best supportive care only. Of course, results of group A (n = 33) were better than of group B (objective response 36% vs 22%, stable disease 26% vs 30%, median progression-free survival

5.7 vs 3.9 months and overall survival 15.4 vs 7.6 months, respectively). However, results from group B indicate that GemOx has some activity even in patients with a poor prognosis who have received previous chemotherapy. Tolerability of GemOx in group B did not differ significantly from that in group A patients, indicating that this combination is safe in patients with a poor prognosis or pretreated by chemotherapy [14].

A meta-analysis by Eckel et al. failed to demonstrate an increase of the activity of gemcitabine combined with fluoropyrimidenes compared with gemcitabine alone [86]. Nevertheless, results of some trials were remarkable. The combination of gemcitabine with capecitabine (response rate (RR) 31%, tumor control rate (TCR) 73%, progression free survival (PFS) 7 months, overall survival (OS) 14 months) did better than the active combination with continuous infusional 5-FU (RR 33%, TCR 63%, PFS 3,7 months, OS 5,3 months) [197, 198]. Even if the differences in survival times may be partly due to selection bias, the combination of gemcitabine and capecitabine is an interesting one, as this combination demonstrated significant prolongation of survival in patients with pancreatic cancer in a large randomised controlled trial compared to gemcitabine alone [68].

Guidelines for the treatment of cholangiocarcinoma have been published 2002 by the BASL (British Association for the Study of the Liver) [185]. Consensus conclusion from predominately phase II trials suggest: (1) RRs of 5-FU-based and (older) single agents are 10–20%, (2) RRs of newer single agents, such as gemcitabine, vary from 20% to 30%, (3) RRs of recent phase II combinations vary from 20% to 40%, and (4) gemcitabine in combination with cisplatin shows 30–50% RRs. The results of the above mentioned recent pooled analysis of clinical trials are somewhat different but agree in principle concerning the combination of gemcitabine with cisplatin: (1) more than half of fluoropyrimidine based trials reported RRs of more than 20%, (2) more than a half of single agent gemcitabine trials and nearly all trials of newer single agents reported RRs of less than 20%, (3) about 40% of combination trials reported RRs of 20% or less, and (4) the middle half of gemcitabine plus platinum combinations results in RRs between 26% and 50%, i.e. one quarter of this combination trials reported RRs of 50% or greater [86]. Furthermore, significant correlations of RRs and tumor control rates with progression-free and overall survival times were found in this analysis. Nevertheless, there is no evidence based on large randomized controlled trials supporting the combination of gemcitabine and platinum compounds as accepted standard treatment. Large, randomized controlled trials, such as the UK National Cancer Research Institute ABC-02 trial, evaluating the definite role of platinum compounds in combination with gemcitabine compared to gemcitabine alone are needed for a higher level of evidence.

The guidelines of the US National Comprehensive Cancer Network (NCCN v.1.2007) recommend, depending on the patient's general condition, best supportive care, a clinical trial, 5-fluorouracil (5-FU), or gemcitabine.

*In summary, owing to the lack of randomized phase III studies (Tables 116.11–116.17), there is no standard regimen for palliative chemotherapy of cholangiocarcinoma.*

## Palliative Radiotherapy

Regarding palliative radiation therapy, there are even fewer studies and there is no proof that external or intraluminal radiation improves survival or quality of life (Tables 116.18 and 116.19) [186, 252]. Single case reports demonstrate improvement of cholestasis and pain [204, 256, 273]. Median survival of patients varies widely in different studies between 4 and 23 months [105, 176, 346, 368, 388]. Low patient numbers, an uncontrolled and mostly retrospective study design, as well as a high variation in radiation dose and method (external, intraluminal, percutaneously or endoscopically, intraoperatively or combined) permit no final conclusion.

Attention may be paid to complications after radiation therapy. In studies of intraluminal brachytherapy the cholangitis rate was as high as 44% and the rates of gastroduodenal ulcer and stenoses were as high as 25% after combined treatment [41, 105, 124, 176, 204, 368].

## Palliative Chemoradiation

The status of combined chemoradiation in comparison to chemotherapy, radiation or stenting is unclear (Table 116.20) [191].

*In summary, it has to be concluded that currently there is no standard protocol for palliative radiation therapy or chemoradiation of cholangiocarcinoma. Therefore, patients should be included in randomized phase III studies.*

**Table 116.11** Monotherapy with fluoropyrimidines

| Author, Year | Entity | (n) | Drugs | Dose (mg/m²), Protocol | Response rate (%) | Median survival (mo) | 1-YSR (%) |
|---|---|---|---|---|---|---|---|
| (Chen et al. 1998) [57] | BC | 19 | 5-FU/LV | 2600 c.i./ 150 d 1,8,15,22,29,36 / 8 week | 33 | 7 | NA |
| (Choi et al. 2000) [65] | BC | 28 | 5-FU/LV | 375b/25 d 1–5 / 4 week | 32 | 6 | 39 |
| (Malik and Aziz 2003) [239] | GBC | 30 | 5-FU/LV | 425b/20 d 1–5 / 4 week | 7 | 14.8 | NA |
| (Patt et al. 2004) [293] | BC | 18 | Cap | 2000 d 1–14 / 3 week | 6 | 8.1 | 44 |
| | GBC | 8 | | | 50 | 9.9 | 38 |
| (Mani et al. 1999) [242] | BC | 13 | UFT/LV | 300/90 d1–28 / 5 week | 0 | 7 | NA |
| (Chen et al. 2003) [58] | BC | 16 | UFT/LV | 300/60 d1–28 / 5 week | 0 | 5 | 19 |
| (Ikeda et al. 2005) [152] | BC | 19 | UFT | 360 daily | 5 | 8.8 | 21 |

BC: biliary carcinoma; GBC: gallbladder cancer; b: bolus; c.i.: continuous infusion (24 h); LV: folic acid (Leukovorin); UFT: Uracil/Tegafur; Cap: Capecitabine; NA: non-applicable

**Table 116.12** Monotherapy with gemcitabine

| Author and year | Entity | (n) | Drugs | Dose (mg/m²), Protocol | Response Rate (%) | Median Survival (mo) | 1-YSR (%) |
|---|---|---|---|---|---|---|---|
| (Mezger et al.. 1998) [248] | BC | 13 | Gem | 1000 d 1,8,15 / 4 week | 8 | 6 | NA |
| (Raderer et al. 1999) [315] | BC | 19 | Gem | 1200 d 1,8,15 / 4 week | 16 | 6.5 | NA |
| (Gebbia et al. 2001) [114] | BDC/ GBC | 18 | Gem | 1000 d 1,8,15 / 4 week | 22 | 8 | 22 |
| (Arroyo et al. 2001) [17] | BDC/ GBC | 42 | Gem | 1000 d 1,8,15 / 4 week | 33 | 6.5 | 26 |
| (Kubicka et al. 2001) [212] | ICC/ECC | 23 | Gem | 1000 d 1,8,15 / 4 week | 30 | 9.3 | NA |
| (Gallardo et al. 2001) [112] | GBC | 26 | Gem | 1000 d 1,8,15 / 4 week | 36 | 7.5 | 33 |
| (Lin et al. 2003) [227] | BDC | 24 | Gem | 1000 d 1,8,15 / 4 week | 13 | 7.2 | 17 |
| (Tsavaris et al. 2004) [387] | BDC/ GBC | 30 | Gem | 800 every week | 30 | 14 | 57 |
| (Park et al. 2005) [286] | BDC/ GBC | 23 | Gem | 1000 d 1,8 / 3 week | 26 | 13.1 | 50 |
| (Okusaka et al. 2006)[275] | BC | 40 | Gem | 1000 d 1,8,15 / 4 week | 18 | 7.6 | 25 |
| (von Delius et al. 2005) [396] | BDC/ GBC | 18 | Gem | 100 c.i. d 1,8,15 / 4 week | 6 | 7.5 | 34 |
| (Eng et al. 2004) [91] | BDC/ GBC | 15 | Gem | 1500 FDR d 1,8,15 / 4 week | 0 | 5 | 0 |
| (Gelibter et al. 2005) [115] | BC | 40 | Gem | 1000 FDR d 1 / 1 week | 15 | 10 | 26 |
| (Penz et al. 2001) [298] | BC | 32 | Gem | 2200 d 1 / 2 week | 22 | 11.5 | 44 |

BC: biliary carcinoma; BDC: bile duct carcinoma; GBC: gallbladder carcinoma; ICC: intrahepatic cholangiocarcinoma; ECC: extrahepatic cholangiocarcinoma; c.i.: continuous infusion (24 h); Gem: Gemcitabine; FDR: fixed dose rate; NA: non-applicable

**Table 116.13** Monotherapy with topoisomerase-inhibitors

| Author and year | Entity | (n) | Drugs | Dose (mg/m²), Protocol | Response Rate (%) | Median Survival (mo) | 1-YSR (%) |
|---|---|---|---|---|---|---|---|
| (Sanz-Altamira et al. 2001) [333] | BC | 25 | CPT-11 | 125 d 1,8,15,22 / 6 week | 8 | 10 | NA |
| (Alberts et al. 2002) [10] | BDC/GBC | 36 | CPT-11 | 125/100 d 1,8,15,22 / 6 week | 8 | | |
| (Abou-Alfa et al. 2005) [2] | BDC/GBC | 42 | Exatecan | 0.5 d 1–5 / 3 week | 5 | 7 | 32 |

BC: biliary carcinoma; BDC: bile duct carcinoma; GBC: gallbladder carcinoma; CPT-11: Irinotecan; NA: non-applicable

**Table 116.14** Combination therapy with fluoropyrimidines and gemcitabine

| Author and year | Entity | (n) | Drugs | Dose (mg/m²), Protocol | Response rate (%) | Median survival (mo) | 1-YSR (%) |
|---|---|---|---|---|---|---|---|
| (Iyer et al. 2007) [163] | BDC/GBC | 12 | Gem | 1000 d 1,8 / 3 week | 17 | 14 | 58 |
| | | | Cap | 1300 d 1–14 / 3 week | | | |
| (Knox et al. 2005) [197] | BDC/GBC | 45 | Gem | 1000 d 1,8 / 3 week | 31 | 14 | 49 |
| | | | Cap | 1300 d 1–14 / 3 week | | | |
| (Iqbal et al. 2006) [154] | BDC/GBC | | Gem | 1000 d 1,8 / 3 week | 18 | 7 | NA |
| | | | Cap | 1300 d 1–14 / 3 week | | | |
| (Cho et al. 2005) [64] | BDC/GBC | 44 | Gem | 1000 d 1,8 / 3 week | 32 | 14 | 58 |
| | | | Cap | 1300 d 1–14 / 3 week | | | |
| (Chang et al. 2005) [52] | BDC | 20 | Gem | 1000 d 1, 8, 15 / 4 week | 15 | 7.8 | NA |
| | GBC | 11 | Cap | 1660 d 1–21 / 4 week | 9 | | |
| (Knox et al. 2004) [198] | BDC/GBC | 27 | Gem | 900 d 1,8,15 / 4 week | 33 | 5.3 | 26 |
| | | | 5-FU | 200 c.i. d 1–21 / 4 week | | | |
| (Alberts et al. 2005) [9] | BDC/GBC | 42 | Gem | 1000 d 1,8,15 / 4 week | 10 | 9.7 | 36 |
| | | | 5-FU | 600b d 1,8,15 / 4 week | | | |
| | | | LV | 25 d 1,8,15 / 4 week | | | |
| (Gebbia et al. 2001) [114] | BC | 22 | Gem | 1000 d 1,8 / 3 week | 36 | 11 | 36 |
| | | | 5-FU | 400b d 1,8 / 3 week | | | |
| | | | 5-FU | 600 c.i. d1,8 / 3 week | | | |
| | | | LV | 100 | | | |
| (Hsu et al. 2004) [148] | BDC/GBC | 30 | Gem | 800 d 1,8,15 / 4 week | 21 | 4.7 | NA |
| | | | 5-FU | 200 c.i. d 1,8,15 / 4 week | | | |
| | | | LV | 300 d 1,8,15 / 4 week | | | |

BC: biliary carcinoma; BDC: bile duct carcinoma; GBC: gallbladder carcinoma; b: bolus; c.i.: continuous infusion (24 h); Gem: Gemcitabine; Cap: Capecitabine; LV: Leucocorin; NA: non-applicable

**Table 116.15** Combination therapy with fluoropyrimidines and platinum-derivatives

| Author, Year | Entity | [n] | Drugs | Dose (mg/m²), Protocol | Response Rate [%] | Median Survival [mo] |
|---|---|---|---|---|---|---|
| (Ducreux et al. 1998) [81] | BC | 25 | 5-FU | 1000 c.i. d 1–5 / 4 week | 24 | 10 |
| | | | CDDP | 100 d 2 / 4 week | | |
| (Taieb et al. 2002) [365] | BC | 29 | CDDP | 50 d 2 / 2 week | 34 | 9.5 |
| | | | LV | 200 d 1 / 2 week | | |
| | | | 5-FU | 400b d 1 / 2 week | | |
| | | | 5-FU | 600 c.i. d 1–2 / 2 week | | |
| (Kobayashi et al. 2006) [200] | BDC/GBC | 42 | 5-FU | 160 c.i. d 1–7 / 1 week | 43 | 7.5 |
| | | | CDDP | 60 d 1,4 / 1 week | | |
| (Kim et al. 2003) [189] | BDC/GBC | 42 | Cap | 2500 d 1–14 / 3 week | 21 | 9.1 |
| | | | CDDP | 60 d 1 / 3 week | | |
| (Glover et al. 2005) [122] | BC | 15 | Cap | 1500 d 1–14 / 3 week | 27 | NA |
| | | | Oxali | 130 d 1 / 3 week | | |
| (Hong et al. 2007) [145] | BDC/GBC | 32 | Cap | 2500 d 1–14 / 3 week | 41 | 12.4 |
| | | | CDDP | 60 d 1 / 3 week | | |
| (Nehls et al. 2006) [266] | BDC/GBC | 65 | Cap | 2000 d 1–14 / 3 week | 20 | NA |
| | | | Oxali | 130 d 1 / 3 week | | |
| (Nehls et al. 2002) [265] | BDC/GBC | 16 | Oxali | 85 d 1 / 2 week | 19 | 9.5 |
| | | | LV | 500 d 1 / 2 week | | |
| | | | 5-FU | 1500–2000 d 1–2/2 week | | |
| (Sanz-Altamira et al. 1998) [332] | BC | 14 | Carbo | 300 d 1 / 4 week | | |
| | | | LV | 25 d 1–4 / 4 week | 21 | 5.0 |
| | | | 5-FU | 400 d 1–4 / 4 week | | |

BC: biliary carcinoma; BDC: bile duct carcinoma; GBC: gallbladder carcinoma; b: bolus; c.i.: continuous infusion (24 h); CDDP: Cisplatin; Oxali: Oxaliplatin; Carbo: Carboplatin; Gem: Gemcitabine; Cap: Capecitabine; LV: Leucocorin; NA: non-applicable

**Table 116.16** Combination therapy with fluoropyrimidines and topoisomerase-inhibitors

| Author, Year | Entity | [n] | Drugs | Dose (mg/m²), Protocol | Response Rate [%] | Median Survival [mo] | 1-YSR [%] |
|---|---|---|---|---|---|---|---|
| (Feisthammel et al. 2007) [95] | ICC/ GBC | 30 | CPT-11 5-FU/ LV | 80 d 1,8,15,22,39,46 / 8 week | 10 | 5.5/9.1 | 25 |
| | | | | 2000 c.i. d 1,8,15, 22,39,46 / 8 week | | | |

ICC: intrahepatic cholangiocarcinoma; GBC: gallbladder carcinoma; CPT-11: Irinotecan; LV: Leucovorin; c.i.: continuous infusion (24 h)

**Table 116.17** Combination Therapy with Gemcitabine and Platinum-Derivatives

| Author, Year | Entity | [n] | Drugs | Dose (mg/m²), Protocol | Response Rate [%] | Median Survival [mo] | 1-YSR [%] |
|---|---|---|---|---|---|---|---|
| (Malik et al. 2003) [240] | GBC | 8 | Gem | 1000 d 1, 8 / 3 week | 64 | 10 | NA |
| | | | CDDP | 70 d 1 / 3 week | | | |
| (Thongprasert et al. 2005) [378] | BDC/GBC | 43 | Gem | 1250 d 1,8 / 3 week | 28 | 9 | NA |
| | | | CDDP | 75 d 1 / 3 week | | | |
| (Misra et al. 2005) [251] | GBC | 40 | Gem | 1000 d 1, 8, 15 / 4 week | 55 | 8 | NA |
| | | | CDDP | 80 d 16,17 | | | |
| (Gebbia et al. 2005) [113] | BDC/GBC | 24 | Gem | 1000 d 1, 8 / 3 week | 50 | 10 | NA |
| | | | Oxali | 100 d 1 / 3 week | | | |
| (Giuliani et al. 2006) [120] | BDC/GBC | 38 | Gem | 1000 d 1,8 / 3 week | 32 | 8+ | |
| | | | CDDP | 75–80 d 1 / 3 week | | | |
| (Doval et al. 2004) [77] | GBC | 30 | Gem | 1000 d 1,8 / 3 week | 37 | 5 | 18 |
| | | | CDDP | 70 d 1 / 3 week | | | |
| (Lee et al. 2006) [220] | BDC | 24 | Gem | 1000 d 1,8 / 3 week | 21 | 9.3 | NA |
| | | | CDDP | 70 d 1 / 3 week | | | |
| (Kim et al. 2006) [188] | BC | 29 | Gem | 1250 d 1,8 / 3 week | 35 | 11 | NA |
| | | | CDDP | 60 d 1 / 3 week | | | |
| (Park et al. 2006) [284] | BC | 27 | Gem | 1000 d 1,8,15 / 4 week | 33 | 10 | 36 |
| | | | CDDP | 75 d 1 / 4 week | | | |
| (Abid et al. 2003) [1] | GBC | 36 | Gem | 1250 d 1,8 / 3 week | 25 | 5.7 | NA |
| | | | CDDP | 70 d 1 / 3 week | | | |
| (Reyes-Vidal et al. 2003) [325] | GBC | 44 | Gem | 1250 d 1,8 / 3 week | 46 | 7 | NA |
| | | | CDDP | 35 d 1,8 / 3 week | | | |
| (Julka et al. 2006) [173] | GBC | 20 | Gem | 1000 d 1,8 / 3 week | 35 | 7.9 | 43 |
| | | | Carbo | AUC 5 d 1 / 3 week | | | |
| (Andre et al. 2004) [14]* | BDC/GBC | 33 | Gem | 1000 FDR d 1 / 2 week | 33/22 | 15.4/7.6 | 57/31 |
| | | | Oxali | 100 d 2 / 2 week | | | |
| (Harder et al. 2006) [133] | BC | 31 | Gem | 1000 d 1,8,15 / 4 week | 26 | 11 | NA |
| | | | Oxali | 100 d 1,15 / 4 week | | | |
| (Charoentum et al. 2007) [55] | BC | 42 | Gem | 1250 d 1,8 / 3 week | 21 | 10.8 | 40 |
| | | | CDDP | 75 d 1 / 3 week | | | |
| (Lee et al. 2007a) [221] | BDC/GBC | 39 | Gem | 1250 d 1,8 / 3 week | 17 | 8.6 | 30 |
| | | | CDDP | 70 d 1 / 3 week | | | |
| (Wagner et al. 2006) [398] | GBC | 35 | Gem | 900 d 1,8 / 3 week | NA | 9.9 | 30 |
| | | | Oxali | 65 d 1,8 / 3 week | | | |
| | | | 5-FU | 1500 d 1,8 / 3 week | | | |

BC: biliary carcinoma; BDC: bile duct carcinoma; GBC: gallbladder carcinoma; AUC: area under the curve; FDR: fixed dose rate; CDDP: Cisplatin; Oxali: Oxaliplatin; Carbo: Carboplatin; Gem: Gemcitabine; NA: non-applicable

*Separate analysis for patients in poor general condition with bilirubin > 3x upper limit of normal or second line chemotherapy

**Table 116.18** Radiation therapy for biliary carcinoma

| Author, Year | [n] | Entity | EBRT Dose [Gy] | ILBT Dose [Gy] | Median Survival [mo] |
|---|---|---|---|---|---|
| (Shinchi et al. 2000) [346] | 30 | HC | Yes | – | 10.6 |
| (Zheng et al. 2006) [430] | 38 | ICC | 50 | – | |
| (Ohnishi et al. 1995) [273] | 14 | HC | 50–60 | – | |
| (Lee et al. 1997) [219] | 15 | ECC | + | + | 8 |
| (Shin et al. 2003) [345] | 17 | ECC | 50.4 (36–55) | – | |
| | 14 | ECC | 50.4 (36–55) | 15 | |
| (Schleicher et al. 2002) [336] | 30 | HC | 30 | – | 3.9 |
| | | HC | 30 | 40 | 9.1 |
| (Milella et al. 1998) [249] | 10 | ECC | + | – | 11.5 |
| | 12 | ECC | + | + | 14.3 |
| (Bowling et al. 1996) [37] | 28 | HC | 30 | 60 | 10 |
| | 28 | HC | – | – | 7 |
| (Meyers and Jones 1988) [247] | 27 | ECC | 30–45 | 30–50 | 14.3 |
| | | ECC | – | 30–50 | 3.6 |
| (Ishii et al. 2004) [156] | 25 | ECC | 30–50 | 24–40 | 9.3 |
| (Kuvshinoff et al. 1995) [214] | 12 | HC | 50.4 | 20 | 14.5 |
| (Takamura et al. 2003) [368] | 93 | ECC | 50 | 39.2 (27–50) | 12 |
| (Eschelman et al. 1996) [92] | 11 | ECC | 45 (25–56) | 25 (15–31) | 22.6 |
| (Foo et al. 1997) [101] | 24 | ECC | 50.4 | 20 | 12.8 |
| (Vallis et al. 1996) [394] | 38 | BDC | 23.8 (22.5–55) | 40 | 15 |
| (Genzalez Gonzalez et al. 1999) [124] | 38 | HC | 60–68 | 22–25 (n=19) | 10.4 |
| (Kamada et al. 1996) [176] | 54 | ECC | 7.5–80 | 20–95 | 12.4 |
| (Tsujino et al. 1995) [388] | 27 | ECC | 45 (12–50) | 7–95 | 13 |
| (Fritz et al. 1994) [105] | 30 | ECC | 30–45 | 20–45 | 10 |
| (Lu et al. 2002) [234] | 18 | ECC | 45 | 7–21 | 12.2 |

HC: Hilar cholangiocarcinoma; BDC: bile duct carcinoma; ICC: intrahepatic cholangiocarcinoma; ECC: extrahepatic cholangiocarcinoma; NA: non-applicable

**Table 116.19** Intraluminal brachytherapy for biliary carcinoma

| Author, Year | [n] | Entity | ILBT [Gy] | Stent Patency [Months] | Median Survival [mo] |
|---|---|---|---|---|---|
| (Bruha et al. 2001) [41] | 17 | HC | 30 | SEMS (13.9) | 11.9 |
| | 11 | GBC | 30 | SEMS (7.3) | 7 |
| (Chen et al. 2004) [59] | 14 | BDC | 12–28 | SEMS (12.6) | 9.4 |
| | 20 | BDC | – | SEMS (8.3) | 6 |
| (Dvorak et al. 2002) [84] | 13 | BDC/GBC | 36–42 | NA | 9.2 |
| (Leung et al. 1996) [225] | 16 | BDC | NA | NA | 23 |
| (Kocak et al. 2005) [204] | 8 | ECC | 20 | SEMS (5) | 5.5 |
| (Fletcher et al. 1983) [99] | 19 | HC/GBC | 44.7 | Drainage (4.2) | 11 |

HC: Hilar cholangiocarcinoma; BDC: bile duct carcinoma; GBC: gallbladder carcinoma; ICC: intrahepatic cholangiocarcinoma; ECC: extrahepatic cholangiocarcinoma; NA: non-applicable

## Future Directions of Palliation

### Progress in PDT

In the future, further progress may be made by using photosensitizers with a higher penetration depth for PDT than the currently used Photofrin™ and Photosan™, whose effect is limited to is 4 – 4.5 mm. Temoporfin (Foscan™), with a strong absorption at 652 nm, or tetrahydroporphyrin tetratosylat (THPTS) with a strong absorption at 760 nm may be good candidates. Further modifications of Foscan™ like a liposomal formulation (Foslip™) or pegylation (Fospeg™) aim to reduce photosensitizer dose, side effects and costs. In addition to the development of new photosensitizers, other innovative ideas are currently under investigation. For

**Table 116.20** Chemoradiation for biliary carcinoma

| Author, Year | [n] | Entity | EBRT [Gy] | ILBT [Gy] | Chemotherapy | Median Survival [mo] |
|---|---|---|---|---|---|---|
| (Urego et al. 1999) [11] | 24 | ECC | 46 | 25 | 5-FU (+ FAM/MMC) | 12 |
| [392] | 34 | BDC | 42–54.6 | – | 5-FU/LV, IFN, (Taxol) | 14 |
| (Morganti et al. 2000) [257] | 20 | BDC | 39.6–50.4 | 30–50 | 5-FU | 21.2 |
| (Deodato et al. 2006) [72] | 22 | ECC | 39.6–50.4 | 30–50 | 5-FU | 23 |
| (Park et al. 2006) [287] | 19 | ECC | 45 | – | Doxifluridin + Paclitaxel | 14 |
| (Schoppmeyer et al. 2006) [338] | 11 | ECC | 49.6 | – | Gemcitabine | 8,2 |
| (Matsumoto et al. 2004) [243] | 23 | HC | 41.4 | – | Epirubicin, MMC, 5-FU i.a. | 19,5 |
| (Ben-Josef et al. 2005) [25] | 46 | ICC | 60,75 | – | Floxuridin i.a. | 13,3 |
| (Nomura et al. 2002) [270] | 5 | ECC | – | 10–30 | – | 4,6 |
| | 12 | ECC | < 40–59.4 | 10–30 | 5-FU, CDDP, Adriamycin | 16,2 |

HC: Hilar cholangiocarcinoma; BDC: bile duct carcinoma; GBC: gallbladder carcinoma; ICC: intrahepatic cholangiocarcinoma; ECC: extrahepatic cholangiocarcinoma; FAM: 5-FU/Adriamycin/Mitomycin C; LV: Leukovorin; IFNα: Interferon α; MMC: Mitomycin C; CDDP: Cisplatin; NA: non-applicable

instance, fractionated drug-dose PDT regimens were reported to result in a superior therapeutic effect, compared to single-dose regimens, and were able to induce long-term tumor growth control. Moreover, the use of specific targeting carriers, such as conjugated antibodies directed to tumor-associated antigens or vascular antigens are supposed to direct the photosensitizer to a certain cell type or compartment. Others suggest the use of different advanced delivery systems, such as ligand-based targeting with insulin, epidermal growth factor or adenoviral proteins, protease-mediated drug delivery, photosensitizing adenoviruses, water-soluble polymer carriers, and pH-responsive polymeric micelles. A completely different approach is the protection of normal tissue with drugs like WR-2721 and WR-77913 from possible PDT side effects if the photosensitizer is used in a high dose [415].

## Progress in Chemotherapy

S-1 is a novel orally administered drug that is a combination of tegafur, 5-chloro-2,4-dihydroxypyridine (CDHP), and oteracil potassium in a 1:0.4:1 molar concentration ratio. CDHP is a competitive inhibitor of DPD, which is involved in the degradation of 5-FU, and acts to maintain efficacious concentrations of 5-FU in plasma and tumour tissues. Oteracil potassium, a competitive inhibitor of orotate phosphoribosyltransferase, inhibits the phosphorylation of 5-FU in the gastrointestinal tract, reducing the serious gastrointestinal toxicity associated with 5-FU. A phase II study investigated the efficacy and safety of S-1 in patients with biliary tract cancer. Four patients achieved a partial response, giving an overall response rate of 21%. The median time-to-progression and median overall survival period were 3.7 and 8.3 months, respectively. S-1 was well tolerated in patients with this disease [391].

Uracil-tegafur (UFT) is an orally administered drug that is a combination of uracil and tegafur in a 4:1 molar concentration ratio. Uracil prevents degradation of 5-FU by inhibiting dihydropyrimidine dehydrogenase (DPD), which leads to an increased level of 5-FU in plasma and tumor tissues. A phase II study of UFT in patients with advanced biliary tract cancer was performed. Only one patient (5%) achieved a partial response with a duration of 2.0 months and six further patients (32%) showed stable disease. The authors concluded, that UFT appeared to have little activity as a single agent in treating patients with advanced biliary tract cancer [152]. A subsequent early phase II trial of UFT plus doxorubicin demonstrated moderate activity against advanced biliary tract cancer with an objective response rate of 12.5% and 54% stable disease [111].

Becatecarin (XL119, rebeccamycin analog) is a novel anti-tumor antibiotic with both topoisomerase I and II activity, as well as DNA intercalating properties. In a phase I trial the presence of a second peak during the elimination phase as well as a high concentration of becatecarin in biliary fluid compared with the corresponding plasma measurement was suggestive of enterohepatic circulation [78]. Encouraging responses and prolonged stable disease were observed in patients

with biliary cancers and prompted a phase II trial. Response rate was 7% (11% including unconfirmed responses) and tumor control rate 44%. Preliminary observations were suggestive of enhanced clinical benefit with near doubling of survival as compared to historical controls [79]. However, the phase III trial was terminated early after enrollment of the first 225 of up to 600 planned patients due to no clear indication of efficacy benefit in the investigational arm. In addition, differences regarding early deaths, survival, treatment duration, and the number of serious adverse events in favor of the comparator arm (5-FU) were noted.

The multitargeted antifolate pemetrexed is a novel antifolate that inhibits at least three enzymes involved in folate metabolism and DNA synthesis. These enzymes are thymidylate synthase, dihydrofolate reductase, and glycinamide ribonucleotide formyl transferase. The implication of these enzyme targets in the cytotoxicity of pemetrexed is the inhibition of both pyrimidine and purine pathways and is supported by the fact that both thymidine and hypoxanthine are required to circumvent cellular death caused by pemetrexed. Four patients with biliary tract cancer have been enrolled in a phase I trial of pemetrexed plus gemcitabine. Objective response was observed in two patients with cholangiocarcinoma [4]. Recently phase II results of the NCCTG phase I/II trial (N9943) of gemcitabine and pemetrexed in patients with biliary tract cancer were presented at ASCO 2007 in Chicago. Median survival was 6.3 months and median time-to-progression was 3.8 months. In conclusion, results of this trial were similar to what would be expected with gemcitabine alone [246].

Davanat™ is a carbohydrate polysaccharide polymer composed of mannose and galactose. Its mechanism of action is based upon binding to lectins on the cell surface. It is theorized that davanat targets specific lectin receptors (galectins) that are over-expressed on cancer cells. Current research indicates that galectins affect cell development and play important roles in cancer, including tumor cell survival, angiogenesis and tumor metastasis. In pre-clinical studies co-administration of davanat with 5-FU increased efficacy and decreased toxicity compared to 5-FU alone. Two international phase II trials, both designed as first-line therapies in colorectal and biliary cancers, administering Davanat™ in combination with 5-FU are currently recruiting patients.

Bendamustine is a bifunctional alkylating agent combining a purine antagonist with an alkylating nitrogen mustard group. Its antineoplastic and cytotoxic properties are attributable mainly to crosslinking of the DNA single and double strands by alkylation. This leads to a disturbance of the matrix function of DNA and to hampered DNA synthesis. Bendamustine has activity as single-agent therapy or in combination with other cytotoxic drugs in the treatment of plasmocytoma, chronic lymphocytic leukemia, non-Hodgkin's lymphoma, and breast cancer. We performed a pilot study to evaluate the safety and tolerability of bendamustine in patients with advanced hilar bile duct cancer and impaired liver function [337]. Six patients with histologically proven, unresectable adenocarcinoma of the hilar bile duct were treated with bendamustine 140 mg/m$^2$ intravenously on day 1 of the first cycle and with bendamustine 100 mg/m$^2$ on days 1 and 2 of the second to fourth cycle. Treatment cycles were repeated every 21 days. Primary endpoint was the safety and tolerability of the treatment; secondary endpoints were response rate, time to progression and overall survival. Transient lymphopenia grade 3 occurred in all six patients. No other grade 3 or 4 toxicities were present. The most common nonhematologic toxicity was mouth dryness grade 2 in six patients. Three patients had stable disease. No partial or complete responses were observed. Median time to progression was 3.3 months; median overall survival was 6 months. Our study demonstrated that bendamustine can be safely administered in patients with hilar bile duct cancer and impaired liver function. A potential role of bendamustine in combination therapies for bile duct cancer will be a subject of further trials.

## Molecular Targeted Therapy

Receptor tyrosine kinases (TKs) have emerged as clinically useful drug target molecules for treating biliary tract cancer [416].

Imatinib mesilate (STI-571, Gleevec™), an inhibitor of c-kit receptor, which was primarily designed to treat chronic myeloid leukemia, exerts marked effects on tumor growth inhibition in vitro and in vivo [63, 177, 413].

The epidermal growth factor receptor (EGFR) family, which is involved in cell proliferation, metastasis and angiogenesis, is another important target [16, 253]. In 2006, Philip and colleagues published a manuscript describing a phase II study of EGFR-1 (ErbB-1, HER- 1) inhibitor erlotinib in patients with advanced

biliary cancer [301]. The primary objective of this study was to determine the proportion of patients with advanced biliary cancer who were progression-free at 6 months. Patients with either unresectable or metastatic disease were studied. Only one prior systemic or locoregional therapy was allowed. Erlotinib was administered continuously at a dose of 150 mg per day orally. Forty-two patients with advanced biliary cancers were enrolled and 57% of patients had received prior chemotherapy. EGFR-1 expression by immunohistochemistry in tumor cells was detected in 29 (81%) of the 36 assessable patients. Seven of the patients (17%) were progression free at 6 months. Three patients had partial response with a duration of 4, 4, and 14 months, respectively. All responding patients had mild (grade 1/2) skin rash and two patients had positive tumoral EGFR-1 expression. Three patients (7%) had toxicity-related dose reductions of erlotinib due to grade 2/3 skin rash. These results suggest a therapeutic benefit for EGFR blockade with erlotinib in patients with biliary cancer.

In addition, the dual EGFR-1/EGFR-2 (ErbB-2, HER-2) inhibitor NVP-AEE788 demonstrated significant in vitro and in vivo effects on tumor growth inhibition [412]. In the same year, Safran et al presented a two-stage, phase I evaluation of GW572016 (lapatinib), an orally active small molecule that reversibly inhibits EGFR-1 and EGFR-2 tyrosine kinases at GI ASCO. Patients with advanced adenocarcinoma of the pancreas or bile ducts were treated with GW572016 and either weekly gemcitabine (1 g/m$^2$/week, 3 weeks on, 1 week off) or GemOx (gemcitabine 1 g/m$^2$ over 100 minutes and oxaliplatin 100 mg/m$^2$, every 14 days). Cohort 1: weekly gemcitabine + GW572016, 1,000 mg/day. Cohort 2: weekly gemcitabine + GW572016, 1,500 mg/day. Cohort 3: GemOx + GW572016 1,000 mg/day. Cohort 4: GemOx + GW572016 1,500 mg/day. Twenty-five patients had been treated [pancreatic cancer (n = 18), biliary cancer (n = 7)]. One of 11 patients in cohort 2 had grade 3 diarrhea. Dose limiting grade 3 nausea occurred in 2 of 5 patients in cohort 4. Two patients had a temporary decrease in cardiac ejection fraction. Five of 22 evaluable patients (23%) responded and median survival (n = 24) was 10 months. It was concluded that GW572016 at 1,500 mg/day can be administered will full dosage gemcitabine. The MTD of GW572016 was 1,000 mg/day with GemOx. Dramatic responses had been demonstrated in patients with diffuse liver and peritoneal metastases suggesting that EGFR-1/ EGFR-2 signaling may be important in pancreaticobiliary cancers. Further evaluation of GW572016 in pancreaticobiliary cancer was recommended. In contrast, Ramanathan et al did not detect any activity of GW572016 monotherapy (1,500 mg daily orally) in patients with biliary tract cancer, showing a median survival of 5.2 months [318].

The expression level of EGFR-1 and EGFR-2 may have important implications for the treatment of different types of biliary tract cancer with EGFR inhibitors. According to Thomas et al, biliary tract cancer expresses EGFR-1 and EGFR-2 receptors in only 8% and 30% and EGFR-2 gene amplification was only present in 22% [377]. Intrahepatic cholangiocarcinoma, which was the main tumor in the study by Ramanathan et al, had no EGFR-1 expression and EGFR-2 gene amplification at all [318]. In contrast, studies examining only extrahepatic cholangiocarcinoma detected EGFR-1 and EGFR-2 receptor expression in 32–43% and 65–84% [271, 412, 431].

A relatively new experimental approach may be the combination of different targeting strategies, such as EGFR-1 and MAPK inhibition or dual EGFR-1/ EGFR-2 and Hedgehog inhibition [170, 171]. In addition, in vitro EGFR-1 inhibition with ZD1839 (gefitinib, Iressa) enhances the effect of radiotherapy [253].

Phase II studies examining the combination of gemcitabine, oxaliplatin and angiogenesis inhibitor bevazicumab (GemOx-B) in unresectable or metastatic biliary tract cancer/gallbladder cancer are in progress, but as yet a final conclusion regarding the efficacy of this regime cannot be drawn.

Other promising new drugs, currently under preclinical and clinical evaluation, are cyclooxygenase-2 (COX-2) inhibitors, mammalian target of rapamycin (m-TOR) inhibitors, MAPK inhibitors, proteasome inhibitors, multikinase inhibitor sorafenib, histondeacetylase (HDAC) inhibitors, anti-interleukin-6 receptor antibodies, hepatocyte growth factor (HGF) antagonists, somatostatin analogues, peroxisome proliferator-activated receptor-γ (PPARγ) ligands, γ-aminobutyric acid (GABA), antisense oligonucleotides against aspartyl (asparaginyl) β-hydroxylase (AAH), adoptive immunotherapy against MUC-1-positive target tumor cells, interferon-γ, tumor necrosis factor-related apoptosis-inducing ligand (TRAIL)/Apo2L ± triptolide as sensitizer, adenovirus vector expressing p27$^{kip1}$, RGD-modified COX-2 promoter driven herpes simplex virus thymidine kinase (HSV-TK) adenoviral expression vectors, tauroursodeoxycholate, and tannic acid [18, 36, 87, 149, 170, 281, 393, 414].

## Recurrent Extrahepatic Cholangiocarcinoma

The prognosis for any treated cancer patient with progressing, recurring, or relapsing extrahepatic bile duct cancer is poor. Deciding on further treatment depends on many factors, including prior treatment and site of recurrence, as well as individual patient considerations. Relief of recurrent jaundice will usually improve quality of life.

*Clinical trials are appropriate and should be considered when possible in patients with recurrent extrahepatic cholangiocarcinoma.*

## Management of Tumor Complications

Malignant gastric outlet obstruction or duodenal obstruction is a complication of advanced hepatobiliary carcinomas with an incidence of about 25% [335]. It usually represents a terminal event, but also grossly impairs patients' quality of life. Relief of obstruction to improve symptoms as well as to allow enteral feeding, thereby improving weight and possibly increasing life expectancy, is the primary aim in these patients. Surgical bypass is the standard treatment with a reported success rate up to 90%. The procedure, however, carries a high complication rate of 25–35% and a perioperative mortality of approximately 2% [226]. A procedure that prolongs hospital stay and increases costs dramatically may not always be appropriate, especially in terminally ill patients with a poor clinical condition. Previous endoscopic interventions like balloon dilatation or laser recanalization provide only transient success, resulting in high reocclusion rates. Based on the experiences in palliation of obstructive esophageal and biliary tract cancer, enteral SEMS have been also used for malignant gastric outlet and duodenal obstructions since the early 1990s. In contrast to surgical bypass surgery, endoscopic placement of duodenal SEMS is a minimally invasive procedure that may result in shorter hospitalization time and lower costs. However, some studies reported complications that may increase length of hospital stay, especially tumor ingrowth, tumor overgrowth and stent dislocation. In our own prospective study, comprising 20 patients with biliary-tract-cancer (7 gallbladder, 13 Klatskin tumors), successful stent placement was achieved in all patients [335]. An additional stent was required in six cases (4 occlusions, 2 dislocations). Median survival was 20.5 weeks, no treatment related deaths occurred. Twenty-eight biliary stent exchanges were performed in 13/20 (65%) patients. Erosive reflux esophagitis improved in 11/12 (92%) cases. After 4 weeks, all 17 surviving patients tolerated soft-solid or solid food, while 13/17 (77%) tolerated a more solid diet ($p < 0.001$, gastric-outlet-obstruction-scoring-system). Twelve of 17 (71%) patients gained a median of 1.5 kg weight ($p = 0.001$). The median Karnofsky-Index increased from 50% to 60% in 13/17 (77%) patients.

*In conclusion, SEMS are a safe, efficacious and minimally invasive treatment option for palliation of duodenal obstruction in biliary tract cancer. Technical complications can be managed endoscopically and the bile duct remains accessible for endoluminal treatment.*

## Prognostic Indicators in Patients with Hilar Cholangiocarcinoma

Two recent studies identified surgical resection, C-reactive protein (CRP) and bilirubin at time of diagnosis as important prognostic factors for survival in patients with hilar cholangiocarcinoma [119, 403].

In contrast, age, tumor stage according to Bismuth-Corlette classification, and types of intervention were not significant parameters for survival.

In surgical patients with hilar cholangiocarcinoma, residual tumor classification and grading seem to be the most important prognostic factors. There are controversial data on the prognostic significance of involved lymph nodes. Out of 10 surgical studies, tumor stage (UICC or AJCC staging system) was not shown to be an independent prognostic parameter in eight series [139, 167, 192, 209, 268, 340, 417]. It proved to be of prognostic significance in two reports [205, 302]. Vascular tumor invasion in resected specimens, a main component of the pT category and UICC classification from 2002, did not prove to be a significant factor for survival in multivariate analysis in recent studies [168, 182, 192, 205, 209, 302]. In two series, vascular invasion influenced survival on the basis of univariate analysis, and in the investigation by Ebata et al macroscopic but not microscopic portal vein invasion proved to be an independent prognostic variable [85, 168, 302].

Finally, microvessel density in tumors may be an important prognostic parameter for survival [255].

## Malignant Tumors of the Ampulla of Vater

### Definition

Periampullary carcinomas arise within 2 cm of the major duodenal papilla. Primary ampullary neoplasms can arise from epithelium of the common bile duct, the pancreatic duct, or the duodenal mucosa. The American Joint Committee on Cancer (AJCC) and UICC (Union Internationale Contre le Cancer) have designated staging by the TNM classification of 2002 (Table 116.4) [354].

### Epidemiology

Malignant primary tumors of the papilla of Vater are very rare with an incidence of approximately six cases per million. They account for only 6% of lesions that arise in the periampullary region. Since the 1980s, largely through increased use of endoscopy, malignant ampullary tumors are more frequently recognized. The average age at diagnosis of sporadic ampullary carcinoma is 60–70 years.

### Etiology and Pathogenesis

Strong evidence supports the adenoma-to-carcinoma sequence, which is similar to the progression in colon cancer [126]. Adenomas are considered precancerous and usually occur within the 5th or 6th decade (see Chapter 115). Patients with familial adenomatous polyposis (FAP), an autosomal dominant disease, are predisposed to duodenal adenomas, especially near the ampulla. There is a 100- to 200-fold increased risk of adenocarcinoma [296]. This is also true for patients with hereditary non-polyposis colorectal cancer (HNPCC).

*K-RAS* mutations are an early event in ampullary carcinogenesis, with an incidence of 37%. High expression of *COX-2* has been detected in 78% of ampullary carcinomas.

### Pathology

Ninety percent of ampullary malignancies are adenocarcinomas. Five percent of gastrointestinal malignancies are ampullary or periampullary adenocarcinomas. They may arise from villous adenomas or villoglandular polyps; there is usually a co-existing adenoma present.

### Clinical Manifestations

Ampullary tumors are relatively rare, but the occurrence of biliary obstruction early in the disease course facilitates diagnosis. Nonspecific symptoms, such as weight loss, vague abdominal pain, dyspepsia, malaise, fever, and anorexia have all been described. Ampullary tumors can cause heme-positive stools and may lead in some patients to iron-deficiency anemia.

### Diagnosis

With technological advances, methods of diagnosis, treatment, and management of ampullary tumors are constantly evolving. However, despite rapid improvements in these areas, preoperative differentiation between adenomas and adenocarcinomas remains difficult (Fig. 116.9a). Transabdominal ultrasound is usually the first-line imaging test performed when obstructive jaundice is suspected. CT and MRI are useful for determining metastatic disease, although these imaging modalities are poor for detecting small ampullary tumors. Endoscopic ultrasound (Fig. 116.9c) is the test of choice for local staging [46]. MRCP may take over the diagnostic role of ERCP. Nevertheless, the value of ERCP lies in its ability to visualize the ampulla directly, to rule out other causes of obstruction, to obtain tissue samples, and to decompress the biliary system (Fig. 116.9b). Forceps biopsy specimens can accurately

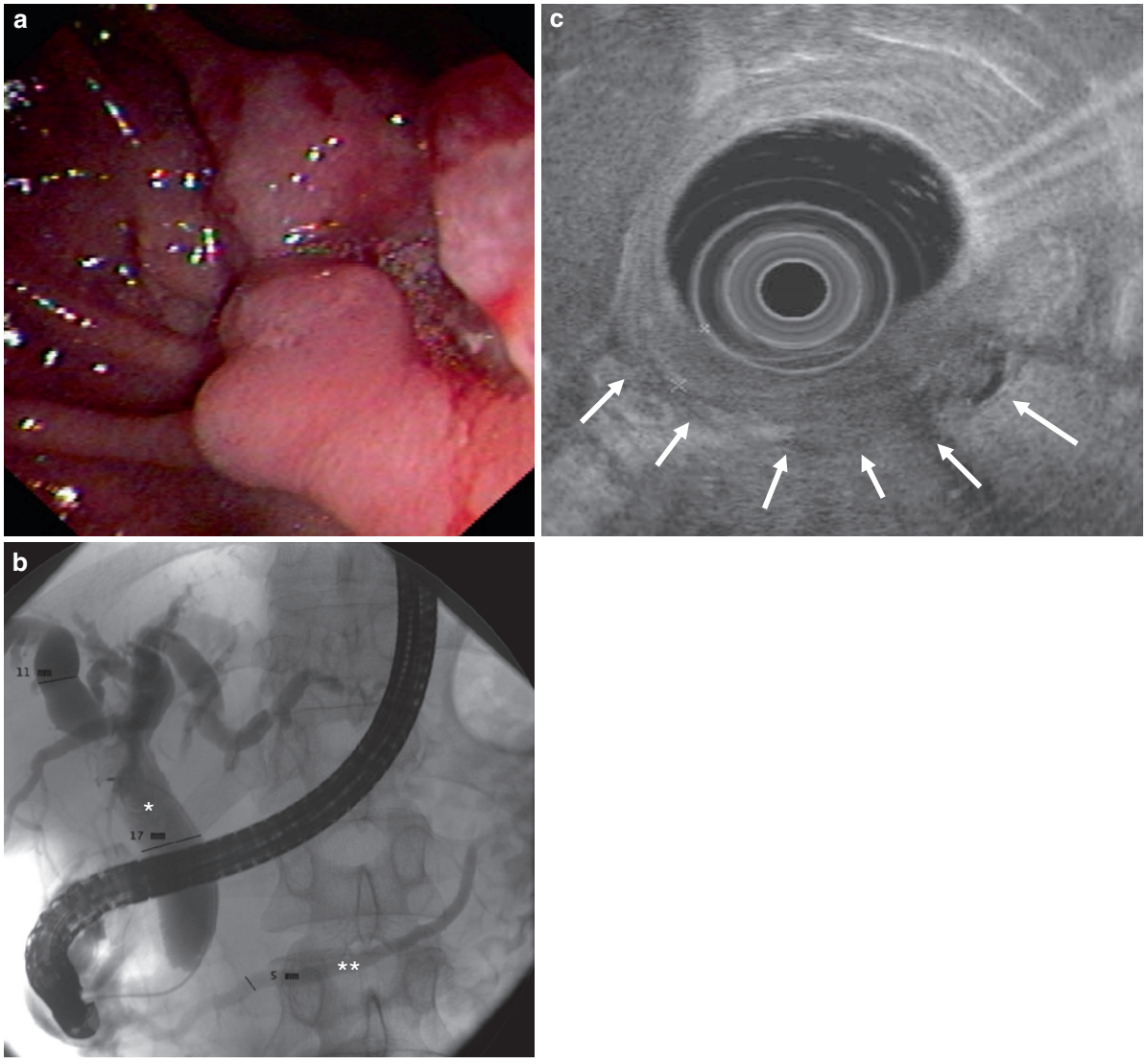


**Fig. 116.9** Patient with ampullary carcinoma. (**a**) Endoscopic view, (**b**) ERCP, depicting a dilated bile (single asterix) and pancreatic duct (double asterix), and (**c**) endoscopic ultrasound (EUS) staging (uT3uN+) (white arrows depict tumor, lymph node not shown)

detect the presence or absence of adenomatous changes, but they have a high false negative rate for adenocarcinoma, ranging from 25% to 60%. Therefore, some authors advocate a two-stage approach for lesions that appear to arise from within the ampulla. Sphincterotomy is initially performed during the first endoscopic procedure. A second procedure is done 7–10 days later to obtain forceps biopsy and cytology specimens. Further improvements with flow cytometry and immunocytochemistry may improve diagnostic capabilities in the future. Serum tumor markers are not specific for ampullary carcinomas and have limited diagnostic application. Intraductal ultrasonography (IDUS) and magnification endoscopy with narrow-band imaging (NBI) may become useful supplemental diagnostic tools.

## *Differential Diagnosis*

Differential diagnoses include duodenal adenocarcinoma, leiomyosarcoma, gastrointestinal stromal tumor, lymphoma, pancreatic carcinoma and distal bile duct carcinoma invading the ampulla of Vater, intraductal

papillary mucinous neoplasm (IPMN), ampullary hamartoma, gangliocytic paraganglioma, neuroendocrine tumors, ectopic pancreatic tissue, fibroadenoma, and Brunner's gland hyperplasia (BGH).

## *Therapy and Prognosis*

*An oncological resection of cancer of the ampulla by means of a pylorus-preserving partial pancreaticoduodenectomy or the Kausch-Whipple resection is the surgical procedure of choice*.

In a large study, the 3- and 5-year survival rates were 72% and 52%, respectively, in patients with R0 resections [22]. Two recent studies display even a better overall 5-year survival rate of 68% [42, 418].

In patients with low-risk cancer in stages pTis and pT1 N0 M0, G1 or G2, local resection with ampullectomy including local lymph node dissection is debated [307]. In these cases, annual or semiannual upper endoscopic follow-up surveillance is recommended for early detection or recurrence.

*With regard to other treatments, chemotherapy and radiation should only be used in the context of controlled trials*.

Poor prognostic factors are: high stage, tumor size 2.5 cm or more, perineural invasion, angiolymphatic invasion, invasion of muscle of sphincter of Oddi, nodal metastases, signet-ring histology, poor differentiation, and positive margins.

Papillary histology is regarded a favorable prognostic factor.

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