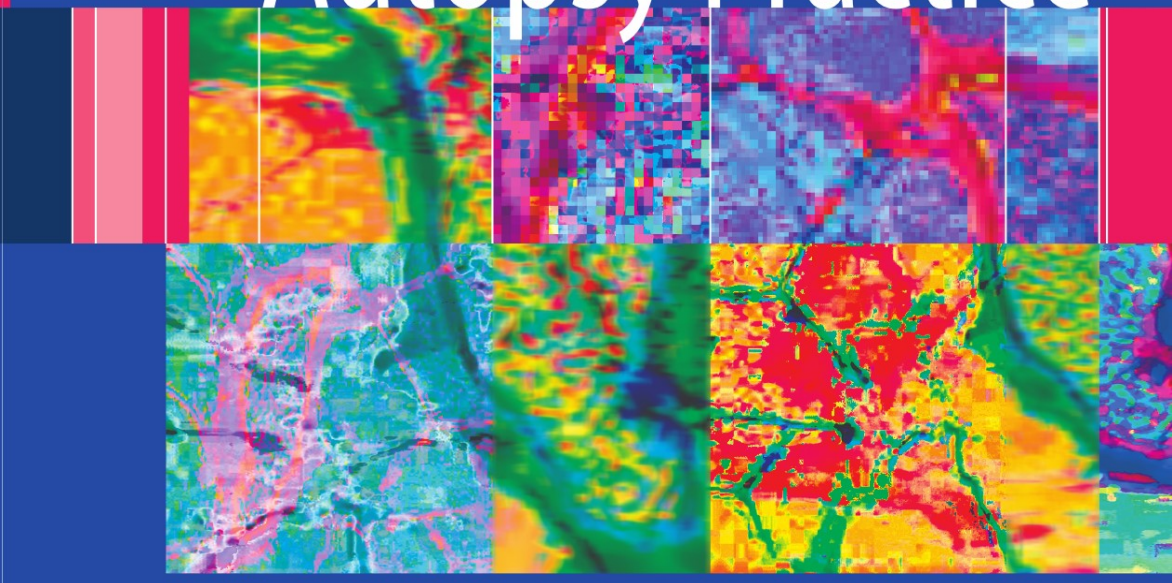
Guy N. Rutty

Essentials of Autopsy Practice



New Advances, Trends and Developments

Springer

Essentials of Autopsy Practice

Guy N. Rutty
Editor

Essentials of Autopsy Practice

Topical developments, trends and advances

Springer

Editor
Guy N. Rutty, MD, MBBS, FCRPath, DipRCPath(forensic), FFSSoc, FFFLM
East Midlands Forensic Pathology Unit
University of Leicester
Robert Kilpathic Building
Leicester Royal Infirmary
Leicester, UK

British Library Cataloguing in Publication Data
Essentials of autopsy practice: new advances, trends and development
1. Autopsy
I. Rutty, Guy N., 1963-
616'.0759

Library of Congress Control Number: 2007940380

ISBN: 978-1-84628-834-0 e-ISBN: 978-1-84628-835-7

Printed on acid-free paper.

9 8 7 6 5 4 3 2 1

Springer Science+Business Media
springer.com

Preface

Autopsy practice continues to change and evolve. In fact, since this series started the investigation of the dead has possibly changed more than in any part of its development through the centuries. For the first time we find ourselves in an era when not only the very necessity to undertake an autopsy is being questioned but that there may be a realistic alternative to invasive autopsies. The continued investigation into the role of MSCT and MRI as an adjunct if not, under certain circumstances, a replacement to traditional autopsy practice is moving at an accelerated pace. However at the time of writing of this edition of *Essentials*, we still have invasive autopsies and I am sure that we will still have them for years to come. Hence these areas are not addressed in this edition. Rather I have concentrated on providing again updates in a broad range of subjects to assist trainees and consultant, generalist and specialist alike. This edition is more medico-legally orientated although this in reality reflects on the type of autopsies that are predominantly undertaken throughout the world. Thus this volume remains relevant to all involved in autopsy practice and should not be viewed as a forensic text. The chapters also capture areas where there have been advances in autopsy processes or understanding of causation and yet they remain areas where pathologists may find themselves in need of updates or easy-to-find reference text. *Essentials* is designed to keep all involved in the investigation of death abreast of changes within this field and I hope that this, as with previous volumes, continues to fulfil this role.

Leicester, 2007 G.N. Rutty

Contents

Postmortem Toxicological Redistribution 1
Olaf H. Drummer

Examination of Fractures at Autopsy 23
Archie J. Malcolm

The Relationship of Body Weight and Sudden Death 45
James A.J.(Rex) Ferris

Fatal Sexual Abuse in Childhood 53
Roger W. Byard and Terence Donald

Elder Abuse and Gerontocide 77
Klaus Püschel

Air Crash Investigation for Histopathologists 113
Graeme Maidment

Bioterrorism 135
Sebastian Lucas

The Chronological Dating of Injury 167
Frank R.W. van de Goot

Index 183

Contributors

Roger W. Byard, MBBS, MD
Department of Pathology, Level 3 Medical School North Building, University of Adelaide, Adelaide, Australia

Terence Donald, FRACP
Child Protection Unit, Womans and Childrens Hospital, Adelaide, Australia

Olaf H. Drummer, PhD ARCPA
Victorian Institute of Forensic Medicine and Department of Forensic Medicine, Monash University, Melbourne, Australia

James A.J.(Rex) Ferris, MD., F.R.C.Path., F.F.Path.(I), D.M.J
Department of Forensic Pathology, LabPlus, Auckland Hospital, Auckland, New Zealand

Frank R.W. van de Goot, PhD
Dutch Forensic Institute, Laan van Ypenburg, The Hague, The Netherlands

Sebastian Brendan Lucas, BM BCh, FRCP, FRCPath
Department of Clinical Histopathology, KCL School of Medicine, St Thomas' Hospital, London, United Kingdom

Graeme Maidment, MA, PhD, BM, BCh, MRCPath, DMJ(Path), DipFHID, DAvMed, ARCM, MRAeS, RAF
RAF Centre of Aviation Medicine, RAF Henlow, Bedfordshire, United Kingdom

Archie J. Malcolm, FRCPath
Department of Histopathology, Royal Shrewsbury Hospital, Shrewsbury, United Kingdom

Klaus Püschel, MD, MPH
Institute of Forensic Medicine, University Medical Center Hamburg-Eppendorf, Hamburg, Germany

Postmortem Toxicological Redistribution

Olaf H. Drummer

Introduction

The interpretation of drugs in specimens taken postmortem is far more difficult than for toxicology results obtained in living persons due to the changes that occur to concentrations of drugs and other analytes after death. All too often conclusions have been drawn from toxicology results obtained from cadavers based on a simple extrapolation of the clinical pharmacokinetics in the living leading to potentially serious misinterpretations and miscarriages of justice.

Pivotal findings over 20 years ago observed that autopsy concentrations of digoxin in heart blood of patients with terminal illness were much higher than their antemortem vein blood concentrations. These increases were considered important due to the narrow therapeutic index of the drug and since they were in the potentially fatal concentration range (1). The threefold elevation of digoxin in postmortem blood compared to antemortem blood of 27 digitalised children who died in hospital suggested that excessive postmortem determinations cannot be interpreted as proof of toxic antemortem concentrations. This was reinforced by the absence of clinical or electrocardiographic digitalis toxicity (2). The issue of postmortem increases of digoxin has been raised many times since.

These postmortem changes for digoxin concentrations are not unique to this drug, but rather have been observed for a large number of drugs. These changes can be related both to the specimens sampled and to the unique changes that occur for many, if not all, biologically relevant substances. A number of useful reading materials are available (3–5).

For this reason it is imperative not only to understand these unique postmortem processes but also to appreciate what happens to exogenous substances during life, particularly the processes of absorption and distribution.

G.N. Rutty (ed.), *Essentials of Autopsy Practice*,

This chapter outlines the processes of drug distribution and presents the current knowledge of postmortem redistribution and how it affects drug concentrations. The drugs covered include References to the more common examples such as the amphetamines and other stimulants, cannabinoids, opioids, benzodiazepines and related drugs such as zolpidem, and the antidepressive and antipsychotic drugs. Other key drugs such as gamma-hydroxybutyrate (GHB) and ketamine are also briefly discussed.

Importantly, examples are provided through case examples of how interpretations can be made with redistribution and indeed other postmortem artefacts in mind.

Drug Absorption and Distribution

All drugs, no matter how they are introduced, will be distributed throughout the body into its various tissues. At some point these substances are excreted through one or more processes, often after some form of metabolic biotransformation. This process is termed disposition. Pharmacokinetics describes the time course of the blood and tissue concentration profiles for a particular drug and includes the absorption, distribution and elimination components of drug presence.

Orally administered drugs will need to be absorbed from the gastrointestinal tract before they can appear in the circulation and in body tissues. This absorption phase is relatively slow since most substances are absorbed in the small intestine and not in the stomach. Consequently, lag times exist from administration to the appearance of detectable drug in blood. Typically these may be 15 min, but gastric emptying can be dependent on a number of physiological variables including the presence of food (6).

Drugs introduced intravenously bypass the absorption stage and will be rapidly introduced to all tissues of the body within moments of the injection. Consequently, the rapid decline of blood concentration within minutes of a bolus injection represents this distribution phase. This is often designated the alpha-phase, whereas the often much slower elimination phase is termed the beta-phase. For anaesthetic drugs such as thiopentone and fentanyl the duration of action is terminated by the avid and rapid distribution of the drugs into tissues. This also occurs for Δ^9-tetrahydrocannabinol (THC) since the tissue concentrations are so much higher than for blood. The much slower elimination rates ultimately remove the drugs from the deep-tissue sites.

It should be of no surprise that the uptake of drugs into tissues is not uniform. The distribution of drugs into tissues depends on various factors, e.g. blood flow to and within the tissue, ionisation characteristics of the drug, affinity of the drug for the tissue and the degree of protein binding. For example, the distribution of warfarin is restricted to plasma and extracellular fluid, THC is avidly taken up into muscle and fat, whereas alcohol is almost equally distributed throughout the body.

While tissue concentrations depend on the relative affinity of the drug to blood, the time required to reach some form of steady state may be quite long (many hours to days). Consequently, it should be assumed that equilibrium is rarely achieved in a forensic case where the dynamics of recent drug use and drug-associated consequences occur.

Deep-tissue sites are usually the last to reach equilibrium and often include the non-parenchymal tissues such as skin, muscle and fat. These are also relatively poorly perfused by blood further retarding the rate at which equilibrium is reached. In single dose situations equilibrium will probably never be reached since the drug is being continually removed by the body through metabolism and excretion. However, under chronic dosing situations some form of dynamic equilibrium will be obtained in which daily increases and decreases occur in accord with the pharmacokinetics of the drug. For example, since THC has a terminal elimination half-life[1] of about 3 days, it will take approximately five half-lives for this equilibrium to occur, or about 2 weeks. On the other hand morphine with a terminal elimination half-life of about 2 h will take approximately 10 h to reach an equilibrium providing there has been a regular intake of drug.

When drugs are administered by other routes, e.g. nasal insufflation, inhalation or dermal absorption, the rate of absorption influences the transfer of drug to the tissues of the body. Inhalation is a very effective and quick method to present drugs to the body and may approach the rate seen with intravenous injection. Absorption through the lungs occurs for substances that are smoked or inhaled, i.e. smoked cocaine, methamphetamine or heroin, THC from reefers or bongs, and even volatile substances. The use of "patches" is becoming increasingly popular for high-potency medicinal drugs that can be absorbed through the skin and include fentanyl (for pain control), oestrogens (as replacement hormones for women) and nicotine (treatment of nicotine dependency from smoking). Similarly, sub-lingual absorption is an effective way of introducing nitroglycerine and other related vasodilators, and for the opiate replacement buprenorphine. In these situations local absorption of drug will occur, i.e. skin (patches), mucous membranes (sub-lingual absorption or smoking) and lungs (smoking). This will clearly cause higher local drug concentrations than from other routes of administration and will therefore affect the overall distribution of drug into other tissues.

What is Redistribution?

Redistribution simply refers to a process or more likely a series of processes that occur after death and cause an alteration in the distribution of drug in tissues after death. Redistribution occurs because at some point after death cellular

[1] This term refers to the terminal phase of the elimination curve when metabolism and excretion are occurring, and absorption and distribution have been completed.

integrity has weakened (due to cell death and decompositional changes), or there is diffusion of drugs across membranes or movement through other cellular structures.

Typically these mechanisms manifest as an apparent change in drug concentration from what was expected. This is sometimes seen when antemortem specimens collected shortly before a death in hospital are compared to those taken at autopsy. These antemortem drug concentrations can be substantially lower than the postmortem concentration and cannot be attributed to changes in drug concentration due to natural elimination of the drug, but rather to postmortem processes.

The mechanisms operating are not fully understood but seem to be based on one or more of three postulated processes. These are:

(a) Diffusion of drug from neighbouring tissue
(b) Diffusion of drug from gastrointestinal tract to an adjacent tissue
(c) Movement of drug through blood vessels and other conduits to another site.

Tissue Diffusion

In its most simple explanation if a drug in muscle tissue surrounding a blood vessel is 10 times higher than the corresponding blood concentration at death, then after death the muscle and blood will eventually have the same concentration since the active processes causing the higher muscle concentration are no longer able to keep this concentration gradient. Unfortunately, this explanation is too simple since it does not take into account the natural affinity of the drug to various cellular structures.

For example, if a drug is poorly soluble in water at pH 7.4 and has a natural affinity for some protein or macromolecule in a tissue then the drug will never have the same concentration in the two tissues and will always favour the tissue to which it has a higher natural affinity.

Changes in the pH of the intra- and inter-cellular space that occur after death can affect tissue binding and has the potential to modify this equilibrium.

Contamination from the Gastrointestinal Tract

Pounder showed diffusion of ethanol from the stomach using a human cadaver model into pericardial fluid, left pulmonary vein, aorta, left heart, pulmonary artery, superior vena cava, inferior vena cava, right heart, right pulmonary vein and femoral vein in proportion to the amount of instilled alcohol and elapsed time (7). This was also demonstrated in a rat model (8).

In other experiments amitriptyline and paracetamol added to the stomach of drug-free cadavers was found to diffuse into the left lung, left lobe of the liver, the spleen and was also present in pericardial fluid. Diffusion into gall bladder bile, cardiac blood, aortic blood and blood of the inferior vena cava was less substantial. The left kidney contained more drug than the right kidney. Least affected was the right anterior lobe of the liver and the lung apexes (9). Similarly, volatile substances were also found to diffuse from the stomach to a number of tissues (10). Diffusion of diazepam and other drugs from the gastrointestinal tract into adjacent tissues has been observed, particularly the left posterior margin of the liver and the left basal lobe of the lung (11).

To reduce this problem of postmortem drug diffusion from the stomach and gastrointestinal tract, it has been recommended that blood be sampled from a peripheral vessel, liver from deep within the right lobe and lung from the apex rather than the base (11).

Postmortem femoral blood from eight cases were on average 3.3 times the concentration found in serum samples from the same subjects collected antemortem or perimortem. The drugs examined were amitriptyline, nortriptyline, imipramine, verapamil and chloroquine. Two additional cases with very early postmortem blood samples, as well as femoral blood samples from a later autopsy, involved amphetamine and THC (12).

Accordingly, the drug concentration found in femoral blood at autopsy cannot be regarded as being representative of the antemortem concentration.

Movement from Another Site

Another form of redistribution that may be quite common is movement of drug through blood vessels and other conduits such as lymph. This has been shown to occur for tissues adjacent to the gastrointestinal tract leading to elevation of concentrations in the liver (9, 13) and for ethanol concentrations in the left chambers of the heart from the lungs (14).

The permeation of morphine and morphine glucuronides has been demonstrated in vitro using the inferior vena cava; however, this was only particularly prominent once decompositional processes have occurred (15).

A further complication arises from diffusion of drugs in the bladder to blood causing elevation of femoral venous blood concentrations (16).

Table 1 summarises the main processes that affect drug redistribution (and distribution).

Figure 1 shows a stylised representation of drug redistribution. In this diagram only two major sites of drug diffusion or movement is represented; the lungs and pulmonary circulation to the heart, and the gastrointestinal system.

Table 1 Factors determining drug distribution and redistribution

Drug lipophilicity
Ionisation characteristics and p*K*a
Binding to macromolecules
Size of molecule
Volume of distribution
Solubility
Route of administration

Note some of these factors are interdependent.

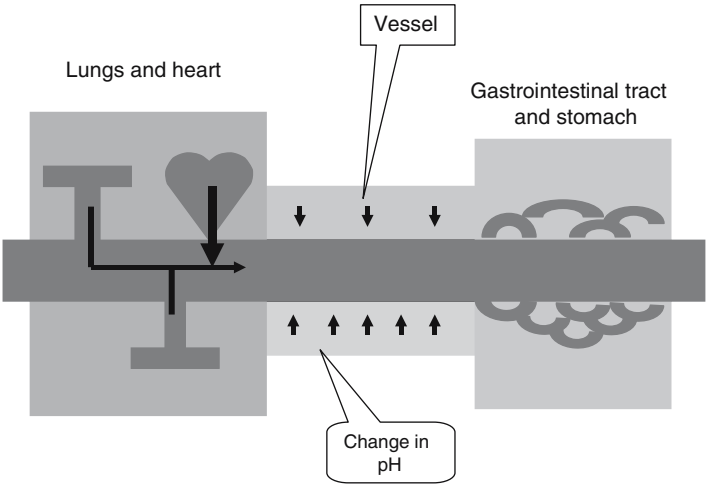


Fig. 1 Stylised representation of drug movement into a major blood vessel from the cardiopulmonary and gastrointestinal systems showing movement of drug through blood vessels and diffusion from surrounding tissues

Animal Studies

Animal studies have been used for many years to study the phenomenon of drug distribution and redistribution.

The earliest rat study showed 15-fold postmortem increases for blood concentrations of digoxin in rats (17). Morphine was also shown to undergo increases, but these were much less and averaged 68% over antemortem concentrations (18).

The diffusion of ethanol placed into the stomach of rats after death into the left lobe of the liver, left kidney, spleen occurred within 12 h at 5 °C and was quicker at higher ambient temperatures. At 30 °C ethanol was even detected in the brain (13).

Rises in blood taken from the heart and inferior vena cava occur within 2 h of death in rats given 20 mg amitriptyline subcutaneously. When the lungs of the rats were removed at the time of death smaller increases occurred suggesting movement of drug from the lungs (14). When rats were given much larger doses increases occurred up to 96 h post-dose. Tissues adjacent to the stomach had higher concentrations than those elsewhere, indicating movement of drug post-mortem (19). Rat studies on diazepam and triazolam suggest that the increases in liver and kidney concentrations that occurred in 24 h since death was largely due to diffusion of drug from the gastrointestinal tract (20). Diffusion from lung was seen as the reason for the two-fold increases in heart blood thioridazine and haloperidol concentrations in rats (21).

Amitriptyline placed in airways to simulate aspiration has been shown in rat models to increase drug concentrations within 5 h in heart blood and heart muscle. In rats where the trachea had been ligated, amitriptyline was found in the lungs after 96 h postmortem (22). Similarly, in rabbits given methamphetamine or amitriptyline ligation of large vessels around the heart prevented postmortem movement of drug from the lungs and pulmonary circulation (23). This was also confirmed by application of lidocaine into dead rabbits in which the drug was detected in heart blood 1 day later, but not in femoral muscle or cerebrum (24).

Rabbit studies have shown that basic drugs in the lungs diffuse rapidly into left cardiac chambers postmortem through the pulmonary venous supply (23). The authors therefore conclude that basic drugs in the myocardium do not contribute to increases in blood concentration that occur postmortem. Similar conclusions were reached with MDMA given intravenously to rabbits (25).

One of the drawbacks of these studies is the relevance these animals have to humans, particularly the smaller animals such as rats and rabbits. Studies on drug concentrations in pigs are an alternative to use of rats or rabbits and are particularly useful since these species have a much larger blood volume and may be less subject to volume changes (12). These studies using both oral and intravenous doses also show variable increases in the concentration of amitriptyline in heart blood. Consequently, reliance on only heart blood concentrations to determine the role of drugs subject to redistribution can lead to serious errors.

Rat studies have shown that chiral inversion does not occur for citalopram postmortem, although sixfold elevations have been reported in heart blood compared to the antemortem concentration (26). Table 2 summarises the results of animal models to study drug redistribution.

Specific Drug Examples

Particular common examples of drugs and their known postmortem redistribution are detailed below. A summary of average increases in blood concentration due to redistribution is shown in Table 3. These are based on an assessment of

Table 2 Literature studies on drug redistribution using animal models

Reference	Species	Drug	Extent of redistribution estimated from the perimortem period
Koren and MacLeod (17)	Rats	Digoxin (0.1 or 0.3 mg/kg for 5 days)	Showed 15-fold increase in blood concentration at lower dose, but not at higher dose
Sawyer and Forney (18)	Rats	Morphine (5 mg/kg i.v.)	Increases for free morphine occurred 24 and 96 h postmortem in cardiac blood and also particularly in liver
Koren and Klein, 1992 (49)	Rats	Morphine (4 mg/kg)	Blood concentrations increased almost threefold by 24 h
Kudo et al. (77)	Rats	Triazolam (5 mg/kg orally)	Triazolam diffuses into surrounding tissues, e.g. spleen, muscle, liver and kidney and blood
Hilberg et al. (19)	Rats	Amitriptyline 75 mg orally	Amitriptyline concentrations continued to increase from 2 to 96 h postmortem; left lobe of liver was higher than right lobe
Quatrehomme et al. (78, 79)	Rats	Secobarbital (250 mg/kg orally)	Increases in blood, liver, spleen, kidney concentrations over several days
Pounder et al. (27)	Rabbits	Dothiepin (20 mg i.v.)	Dothiepin concentrations in thoracic blood rose over antemortem concentration up to 12 h postmortem
Hilberg et al. (14)	Rats	Amitriptyline (20 mg s.c.)	Increases in heart blood and IVC blood 2 h postmortem
Gomez et al. (61)	Rabbits	Paracetamol (160 mg/kg orally)	Showed threefold increases in heart blood, but much smaller changes in femoral blood
Takayasu et al. (13)	Rats	Ethanol (oral, postmortem)	Diffusion of ethanol to neighbouring tissues occurred quickly
Moriya and Hashimoto (80)	Rats	Cocaine orally and i.m. to rats pre-treated with ethanol	Liver lost over 25% of the cocaine present at death after 1 h. Conversely, the hepatic cocaethylene concentrations doubled
Pohland and Bernhard (81)	Dogs	Fluoxetine 10 mg/kg orally for 5 days	Heart blood and vena cava blood concentrations were 6.0-and 2.2-fold higher than antemortem concentration at 2 h postmortem, and remained higher at 12 h. Decreases occurred in liver and lung
Moriya and Hashimoto (24)	Rabbits	Lidocaine (1 mg/kg) infused into trachea postmortem	Lidocaine detected in heart blood 1 day later, but not in femoral muscle or cerebrum

Table 2 (continued)

Reference	Species	Drug	Extent of redistribution estimated from the perimortem period
Hilberg et al. (82)	Pigs	Amitriptyline (20 mg/kg p.o. and 3.3 mg/kg i.v.)	Increases in heart blood 232%; and left lobe of liver after oral dosing
Rogde et al. (83)	Rats	Moclobemide (50 mg)	Very little increase in heart blood and IVC concentrations
Moriya and Hashimoto (23)	Rabbits	Methamphetamine (5 mg/kg iv) or amitriptyline (20 mg/kg s.c.)	Ligation of large vessels around heart prevented postmortem increases in blood concentration in left and right cardiac chambers
Hilberg et al. (60)	Rats	Various drugs given orally and distribution assessed at 2 h postmortem	Drug with V_d over 3–4 L/kg showed significant redistribution in heart blood, whilst vena cava blood resembled antemortem concentrations
De Letter et al. (25)	Rabbits	MDMA (1 mg/kg i.v.)	Increases in liver, decreases in lung, small changes in blood
Flanagan et al. (84)	Pigs	Clozapine (10 mg/kg, 7 days)	Increases in peripheral blood 155%, central blood 300%, also heart and muscle
Shiota et al. (20)	Rats	Diazepam (5 mg/kg) Triazolam (5 mg/kg)	Large increases in liver, kidney, smaller increases in heart blood
Kugelberg et al. (26)	Rats	Citalopram (100 mg/kg s.c.)	Heart blood showed threefold elevation postmortem, but no change occurred in proportions with desmethyl metabolite and in enantiomeric ratio
Castaing et al. (21)	Rats	Thioridazine or haloperidol (5 mg/kg i.p.)	Twofold increase over 48 h in cardiac blood with reduction in lung concentrations

i.m. = intramuscularly, i.p. = intraperitoneal, i.v. = intravenous, s.c. = subcutaneous, IVC = inferior vena cava, V_d = volume of distribution. References sorted by year of publication.

the changes based on the data available to the author. The references listed provide some data for readers to refer to when requiring more information on specific drugs.

Antidepressants

The tricyclic antidepressants such as amitriptyline, dothiepin, doxepin, imipramine and nortriptyline are archetypal examples of redistribution and all show substantial increases in blood concentration, particularly in blood collected

Table 3 Examples showing the approximate extent of redistribution of selected drugs in peripheral blood in humans

Drug	Extent of redistribution estimated from the perimortem period (% over AM)	Volume of distribution [a], V_d	Reference
Amitriptyline	600	15	(12)
Amylobarbital	200	1	(4, 5)
Chloroquine	4900	200	(12, 85)
Citalopram	<200	14	(30)
Cocaine	30	2	(4)
Diazepam	30	2	(4)
Digoxin	300	7	(86)
Fluoxetine	600	30	(29)
Flunitrazepam	<20	4	(36)
Gamma-hydroxybutyrate[b]	Endogenous production	0.5	(41, 44, 87)
Ketamine	350	4	(48)
MDMA	700	Unknown	(25, 56, 88)
Methamphetamine	300	4	(4, 89)
Methadone	250	4	(4, 53, 54)
Morphine	200	4	(52, 90, 91)
Nortriptyline	350	23	(12)
Paracetamol	100	1	(60)
Paroxetine	250	10	(29)
Pethidine	90	4	(4)
Propoxyphene	300	16	(4)
Quetiapine	300	10	(92, 93)
Risperidone	220	2	(29)
Sertraline	250	20	(29)
Temazepam	50	1	(4)
Venlafaxine	250	7	(29, 94)
Zopiclone	<50	1.5	(95)

AM = antemortem, PMA = para-methoxyamphetamine, MDMA = 3,4-methylenedioxymethamphetamine.
[a] taken from (96, 97).
[b] Possible endogenous production if blood concentration below 100 mg/L.

centrally (11, 14, 19, 27). As for other drugs increases are variable but can be 10-fold. While true peripheral blood reduces these changes it is still likely that two to threefold increases occur.

The serotonin reuptake inhibitors still tend to be redistributed. On average increases are threefold for centrally collected blood (28, 29). Fluoxetine tends to show larger increases than some of the other examples (29). Citalopram shows little difference between heart and femoral blood (30). Quetiapine and mirtazapine also show significantly higher concentrations in heart blood compared to femoral blood (31, 32).

Antipsychotic Drugs

As discussed previously for clozapine and haloperidol, animal models show increases in blood concentration postmortem (33). This has been confirmed in humans for olanzapine (34, 35). Since most, if not all, of these drugs are lipid soluble it is to be expected that they undergo redistribution after death. The use of peripheral blood concentrations will minimise these changes, but not eliminate changes.

Benzodiazepines

This class of drugs are less lipid soluble and on the whole show less redistribution postmortem than some of the other classes of drug. Redistribution studies on the nitrobenzodiazepines, nitrazepam, clonazepam and flunitrazepam showed that the concentrations of parent nitrobenzodiazepines were significantly higher in blood taken on admission to the mortuary; however, there was no difference in the respective 7-amino metabolites (36). There was also no significant difference in blood concentrations of these drugs when blood concentrations taken in hospital shortly prior to death were compared to postmortem blood.

The related drug zolpidem is also subject to small (twofold) increases in heart blood compared to peripheral blood (37, 38).

Cocaine

The interpretation of cocaine concentrations postmortem is even more problematic given its rapid degradation to benzoylecgonine and other degradants/metabolites (39). Analysis of cases involving paired specimens shows short-term increases in cocaine concentrations in aortic blood and decreases in peripheral blood (40).

The use of metabolites to assist in the interpretation is wise, however, these are formed in postmortem process, e.g. benzoylecgonine and ecgonine methyl ester. Care should be exercised in using concentrations of these species in any fluid or tissue. As for morphine, the true concentration in blood is not a good indicator of its likely response in the person concerned.

Gamma-hydroxybutyrate (GHB)

This date-rape drug is also an endogenous substance that is ordinarily present in low concentrations in living persons but can rapidly be produced in the dead.

Postmortem heart or femoral blood can approach 100 mg/L from endogenous productions under favourable conditions, well in the range capable of causing substantial clinical effects and even sudden death (41–43). Urine concentrations are more resistant to production with current research favouring 10 mg/L as a cut-off (44).

Ketamine

The prevalence of this drug is increasing and is often associated with MDMA (45, 46). This drug is also frequently seen in Asia (47). A case report showed three to fourfold higher concentration in heart blood compared to femoral blood suggesting redistribution or diffusion-related changes postmortem (48).

Morphine and Other Opiates

Morphine is one of the more common drugs detected at postmortem and clearly plays an important role in determining a likely toxic role in the death investigation. Rat studies outlined earlier show increases in blood morphine concentrations after death, perhaps as much as threefold (18, 49). These animal studies have not been confirmed in all human studies. Logan found little change in blood concentration of morphine in either centrally or peripherally collected blood (50). Similar conclusions were drawn in another review of 40 cases although there was a trend of higher concentrations in heart blood (51). More recently, site variations for morphine concentrations has been reported in 44 cases (52). In this study the left ventricular to femoral vein total morphine concentration ratio was 2.0, but with a range of 0.6–7. Centrally obtained morphine concentrations were on average twice as high compared with peripheral morphine concentrations.

It would seem that caution should be exercised in assessing postmortem concentrations for morphine (both free and total) since some increases are likely even in true peripheral blood. These possible postmortem changes in morphine concentration are further complicated by the deconjugation of morphine glucuronides in putrefying bodies or in blood specimens contaminated with bacteria. This issue is discussed later. Notwithstanding these changes there will still be enormous difficulty in interpreting morphine concentrations due to its ability to induce tolerance in individuals and the large variability in response seen between persons.

Similar considerations apply to the other opiates; however, the more lipid-soluble examples show larger postmortem changes. Methadone is thought to undergo significant redistribution since it has higher lipid solubility than morphine, although the changes are contradictory (53). Regression analysis of a number of cases suggested that peripheral postmortem concentrations were

twice those expected from the doses recorded for male subjects and three times those for female subjects (54). Cook et al. found that cases with amitriptyline, dothiepin, propoxyphene, methadone and propranolol increased about two to threefold, but not the hydrophilic paracetamol and salicylate (55).

MDMA and Other Amphetamines

MDMA has been shown to undergo redistribution. In five cases postmortem to antemortem ratios were between 1.1 and 6.6 for MDMA and 1.5 and 13.3 for MDA (56). Heart blood concentrations were also substantially higher than peripheral blood. Similar conclusions have been drawn from other studies (57).

Similar considerations apply to methamphetamine. Paired specimens show a twofold increase in peripheral blood compared to blood taken from the heart (23, 58). There is little data for other amphetamines, but it is likely that similar changes occur for the whole family of drugs.

THC

As for morphine the postmortem concentration changes observed for THC have been found inconsistent although they tend to demonstrate little increase after death (12, 59). In contrast rat studies show a small decrease in the THC blood concentration postmortem in the inferior vena cava (12). Two cases investigated by the author in which both antemortem and peripheral postmortem blood was collected showed increases of at least twofold after death (unpublished observations). Given the very high volume of distribution (10–20 L/kg) it is wise to assume that some increase in concentration in peripheral blood occurs after death.

Lipid Solubility and Volume of Distribution

Accordingly, the tissue distribution after death is determined by much of the same factors that determine distribution during life. However, these are rarely exactly the same and ultimately lead to alterations in the blood (and tissue) concentration after death. In practice much of the changes occurring postmortem that are attributed to redistribution can be related to the degree of lipid solubility. That is, most drugs that undergo redistribution do so because of their relative lipid solubility. Drugs with higher lipid solubility often have higher tissue concentrations and are more likely to be redistributed to blood after death (29, 60).

The volume of distribution (V_d) has a close relation with lipid solubility since it represents the average "virtual" volume (or concentration) to which drugs equilibrate. Values of V_d greater than 3–4 L/kg should be regarded as being redistributed by tissue diffusion unless otherwise proven (60). This does not take into account movement of drug through large blood vessels or diffusion of drug in areas of high concentration such as from gastric contents as that found for paracetamol (61) and other drugs of low lipid solubility (9, 49).

Table 3 lists the extent of redistribution against volume of distribution. Linear regression analysis shows an r^2 of 0.41 without using chloroquine and 0.98 with chloroquine. Chloroquine has such a high degree of redistribution that it tends to bias any relationship. This shows that V_d does provide a guide to predict redistribution although it is by no means the only factor that determines postmortem changes in concentration.

Other Artefacts Associated with Redistribution

While not strictly redistribution, stability or, rather, instability of substances can lead to changes in drug concentration that can often be confused with redistributive changes.

One of the more important examples is the deconjugation of morphine glucuronides back to the parent drug after prolonged postmortem periods. This was first suggested by Moriya and Carroll (62, 63). It has now been confirmed by Skopp et al. (64). The Shipman deaths also illustrate this phenomenon very well (65). Glucuronides are known to be unstable for other drug metabolites; hence their hydrolysis back to morphine in cases of decomposition is not surprising. The net effect of this is to overestimate the amount of morphine (or heroin) consumed, and may, if not recognised, lead to serious interpretation errors.

Other examples include the nitrobenzodiazepines that are actively converted by bacteria in the postmortem interval, to their respective metabolites (66). Consequently flunitrazepam is rarely observed in blood specimens taken at autopsy. Other drugs such as cocaine have also been subject to conversion postmortem to its metabolite, benzoylecgonine being formed as a result of hydrolysis of the parent drug (67).

Co-administration of drugs can lead to redistribution. For example, 1000 units of heparin intravenously led to increases in concentrations of N-desmethyldiazepam (nordiazepam) (68).

Dothiepin, a tricyclic antidepressant, not only shows increases in blood concentration postmortem but also is subject to bacterial degradation due to putrefaction (27, 69).

Variations in drug concentration due to poor quality of postmortem blood has been shown for morphine in cases of heroin overdose (70). Similarly, changes in the quality of postmortem blood affect the THC concentration (59). A review of these pre-analytic issues has been conducted (71).

Other Tissues

Adipose Tissue

Adipose tissue is in general poorly vascularised and has about 2% of total blood flow. Studies on this tissue in cadavers before and after livor have shown little change in concentration of methamphetamine, morphine, temazepam and diazepam in single cases and cocaine/benzoylecgonine in three cases (72). Earlier studies had concluded that there was no redistribution of antipsychotics and antidepressants into adipose tissue (73, 74). Uptake into this tissue is further complicated by the effect of binding competition between lean and adipose tissue (75). THC has an avid affinity for adipose tissue with concentrations about 200-fold higher than circulating blood (59).

Muscle

Muscle may be an alternative to liver because of the high perfusion of this tissue and relative protection from postmortem redistribution (21).

Liver

This tissue is most commonly used to supplement blood data; however, drugs can be absorbed by diffusion from the gastrointestinal tract. Hence sampling from the right anterior lobe or deep within the tissue is preferred to reduce such changes (9).

Redistribution of Endogenous Substances

These substances have been reviewed elsewhere (76). Electrolytes are notoriously unreliable postmortem. Sodium concentrations slowly decline while potassium increases due to loss of cellular integrity causing release and diffusion of intracellular contents. Postmortem glycolysis results in utilisation of most of the blood glucose leading to glucose concentrations under the detection limit.

Vitreous humour is often used to provide some indication of antemortem biochemistries. Typically concentrations are well below 1 mmol/L but can be over 10 mmol/L in cases of hyperglycaemia. Acetone and other acetone bodies are often also present to suggest a pathological state. Urea in vitreous humour can be useful to assess dehydration and kidney function.

Case Studies

A. The sudden and unexpected death of a 40-year-old woman with no significant pathology (after a full autopsy) showed the presence of sertraline in heart blood at 3.2 mg/L. While this concentration is much higher than expected following therapeutic use (usually less than 0.5) it may have been elevated postmortem, particularly in heart blood. No useful conclusion can be made of the toxicology results. It would have been useful to also collect (and analyse) peripheral blood and or right lobe of liver and gastric contents. If the peripheral blood is also elevated and this is confirmed by a high right-lobe liver concentration (over 20 mg/kg) then an overdose is most likely. The presence of substantial drug in the gastric contents will assist in coming to this conclusion, but the absence does not necessarily exclude an overdose if the death is delayed.

B. The sudden and unexpected death of a 36-year-old heroin user with moderate atherosclerotic changes showed the presence of morphine in femoral blood at 0.5 mg/L, 6-acetylmorphine in urine as well as morphine (total) of 15 mg/L. The toxicology confirms the use of heroin in the recent past. The peripheral blood concentration of morphine may be elevated post-death due to redistribution; however, given the variable nature of morphine responses due largely to the development of tolerance the concentration itself cannot be usefully interpreted as necessarily being potentially fatal. The determination of the cause and manner of death will be largely dictated by the circumstances and the elimination of other relevant factors.

C. A man was found deceased in his apartment with the presence of ethanol in femoral blood and vitreous humour of 0.26 and 0.28%, respectively, and temazepam in femoral blood of 1.4 mg/L. This strongly suggests death from the toxic effects of drugs, in the absence of an alternative cause. The vitreous humour result is consistent with the blood ethanol result. It is slightly higher due to higher water content of vitreous humour. The temazepam concentration is elevated and since it undergoes little redistribution it probably reflects the perimortem concentration. The addition of a second blood specimen from another site or the analysis of a liver specimen would confirm the temazepam result.

D. An elderly woman with substantial coronary artery disease and an enlarged heart was found deceased in her home. A femoral blood digoxin concentration was 5.2 ng/mL suggesting possible digoxin toxicity since the upper therapeutic concentration is about 2.5 ng/mL and death has been reported from 3.5 ng/mL. The vitreous humour was 3.2 ng/mL suggesting therapeutic use and postmortem elevation of the blood concentration. Police investigations showed that her usage of digoxin based on her remaining tablets was consistent with her prescribed dose. The vitreous humour concentration is often closer to the perimortem blood concentration than the postmortem peripheral blood concentration. This information as well as an evaluation of the circumstances suggests that her death is probably natural.

Summary

In special cases where the diagnosis of overdose is to be used as coronial or judicial evidence, a result from a single sample of blood may prove insufficient. In such cases, analyses of two samples of blood, or blood and another specimen will increase the possibility of reaching a correct conclusion. Blood taken from the femoral region is still regarded as being the best source of blood for toxicological purposes; however, even this specimen can be subject to redistribution. Care should therefore be exercised in the interpretation of drug concentrations in a postmortem context and wherever possible toxicology data should be compared to the available drug history, circumstances of death and pathology findings.

References

1. Aderjan R, Mattern R. Validity of digoxin concentrations in blood determined post mortem (author's transl). Z Rechtsmed 1980; 86(1): 13–20.
2. Koren G, Parker R. Interpretation of excessive serum concentrations of digoxin in children. Am J Cardiol 1985; 55(9): 1210–1214.
3. Barnhart FE, Bonnell HJ, Rossum KM. Post-mortem drug redistribution. Forensic Sci Rev 2001; 13: 101–128.
4. Prouty RW, Anderson WH. The forensic science implications of site and temporal influences on postmortem blood–drug concentrations. J Forensic Sci 1990; 35(2): 243–270.
5. Pounder DJ, Jones GR. Postmortem drug redistribution—a toxicological nightmare. Forensic Sci Int 1990; 45: 253–263.
6. Basic Clinical Pharmacokinetics 4th edn. by Michael E. Winter. Lippincott, Williams & Wilkins, 2003.
7. Pounder DJ, Smith DR. Postmortem diffusion of alcohol from the stomach. Am J Forensic Med Pathol 1995; 16(2): 89–96.
8. Takayasu T, Ohshima T, Tanaka N, et al. Experimental studies on postmortem diffusion of ethanol-d6 using rats. Forensic Sci Int 1995.
9. Pounder DJ, Fuke C, Cox DE, Smith D, Kuroda N. Postmortem diffusion of drugs from gastric residue: an experimental study. Am J Forensic Med Pathol 1996; 17(1): 1–7.
10. Fuke C, Berry CL, Pounder DJ. Postmortem diffusion of ingested and aspirated paint thinner. Forensic Sci Int 1996; 78(3): 199–207.
11. Pounder DJ, Adams E, Fuke C, Langford AM. Site to site variability of postmortem drug concentrations in liver and lung. J Forensic Sci 1996; 41(6): 927–932.
12. Hilberg T, Rogde S, Morland J. Postmortem drug redistribution—human cases related to results in experimental animals. J Forensic Sci 1999; 44(1): 3–9.
13. Takayasu T, Ohshima T, Tanaka N, et al. Experimental studies on postmortem diffusion of ethanol-d6 using rats. Forensic Sci Int 1995; 76(3): 179–188.
14. Hilberg T, Morland J, Bjorneboe A. Postmortem release of amitriptyline from the lungs; a mechanism of postmortem drug redistribution. Forensic Sci Int 1994; 64(1): 47–55.
15. Skopp G, Lutz R, Potsch L, et al. An in vitro experiment for postmortem vascular permeation. The passage of morphine and morphine glucuronides across a vascular wall. J Forensic Sci 1997; 42(3): 486–491.
16. Moriya F, Hashimoto Y. Postmortem diffusion of drugs from the bladder into femoral venous blood. Forensic Sci Int 2001; 123(2–3): 248–253.

17. Koren G, MacLeod SM. Postmortem redistribution of digoxin in rats. J Forensic Sci 1985; 30(1): 92–96.
18. Sawyer WR, Forney RB. Postmortem disposition of morphine in rats. Forensic Sci Int 1988; 38: 259–273.
19. Hilberg T, Bugge A, Beylich KM, Ingum J, Bjorneboe A, Morland J. An animal model of postmortem amitriptyline redistribution. J Forensic Sci 1993; 38(1): 81–90.
20. Shiota H, Nakashima M, Terazono H, et al. Postmortem changes in tissue concentrations of triazolam and diazepam in rats. Legal Med 2004; 6(4): 224.
21. Castaing N, Titier K, Canal-Raffin M, Moore N, Molimard M. Postmortem redistribution of two antipsychotic drugs, haloperidol and thioridazine, in the rat. J Anal Toxicol 2006; 30: 419–425.
22. Hilberg T, Bugge A, Beylich KM, Mørland J, Bjorneboe A. Diffusion as a mechanism of postmortem drug redistribution: an experimental study in rats. Int J Legal Med 1992; 105: 87–91.
23. Moriya F, Hashimoto Y. Redistribution of basic drugs into cardiac blood from surrounding tissues during early-stages postmortem. J Forensic Sci 1999; 44(1): 10–16.
24. Moriya F, Hashimoto Y. Postmortem diffusion of tracheal lidocaine into heart blood following intubation for cardiopulmonary resuscitation. J Forensic Sci 1997; 42: 296–299.
25. De Letter EA, Belpaire FM, Clauwaert KM, Lambert WE, Van Bocxlaer JF, Piette MH. Post-mortem redistribution of 3,4-methylenedioxymethamphetamine (MDMA, "ecstasy") in the rabbit. Part II: post-mortem infusion in trachea or stomach. Int J Legal Med 2002; 116(4): 225–232.
26. Kugelberg FC, Kingback M, Carlsson B, Druid H. Early-phase postmortem redistribution of the enantiomers of citalopram and its demethylated metabolites in rats. J Anal Toxicol 2005; 29(4): 223–228.
27. Pounder DJ, Hartley AK, Watmough PJ. Postmortem redistribution and degradation of dothiepin. Human case studies and an animal model. Am J Forensic Med Pathol 1994; 15: 231–235.
28. Kunsman GW, Rodriguez R, Rodriguez P. Fluvoxamine distribution in postmortem cases. Am J Forensic Med Pathol 1999; 20(1): 78–83.
29. Rodda KE, Drummer OH. The redistribution of selected psychiatric drugs in postmortem cases. Forensic Sci Int 2006; 164: 235–239.
30. Jenkins AJ, Gubanich K. Disposition of citalopram in biological specimens from postmortem cases. J Forensic Sci 2002; 47: 159–164.
31. Anderson DT, Fritz KL, Muto JJ. Distribution of mirtazapine (Remeron) in thirteen postmortem cases. J Anal Toxicol 1999; 23(6): 544–548.
32. Anderson DT, Fritz KL. Quetiapine (Seroquel) concentrations in seven postmortem cases. J Anal Toxicol 2000; 24(4): 300–304.
33. Flanagan RJ, Amin A, Seinen W. Effect of post-mortem changes on peripheral and central whole blood and tissue clozapine and norclozapine concentrations in the domestic pig (*Sus scrofa*). Forensic Sci Int 2003; 132(1): 9.
34. Horak EL, Jenkins AJ. Postmortem tissue distribution of olanzapine and citalopram in a drug intoxication. J Forensic Sci 2005; 50(3): 679–681.
35. Merrick TC, Felo JA, Jenkins AJ. Tissue distribution of olanzapine in a postmortem case. Am J Forensic Med Pathol 2001; 22(3): 270–274.
36. Robertson MR, Drummer OH. Postmortem distribution and redistribution of nitrobenzodiazepines in man. J Forensic Sci 1998; 43(1): 9–13.
37. Gock SB, Wong SH, Nuwayhid N, et al. Acute zolpidem overdose—report of two cases. J Anal Toxicol 1999; 23: 559–562.
38. Meeker JE, Som CW, Macapagal EC, Benson PA. Zolpidem tissue concentrations in a multiple drug related death involving Ambien. J Anal Toxicol 1995; 19: 531–534.

39. Logan BK, Smirnow D, Gullberg RG. Lack of predictable site-dependent differences and time-dependent changes in postmortem concentrations of cocaine, benzoylecgonine, and cocaethylene in humans. J Anal Toxicol 1997; 21(1): 23–31.
40. Hearn WL, Keran EE, Wei HA, Hime G. Site-dependent postmortem changes in blood cocaine concentrations. J Forensic Sci 1991; 36(3): 673–684.
41. Elliott S, Lowe P, Symonds A. The possible influence of micro-organisms and putrefaction in the production of GHB in post-mortem biological fluid. Forensic Sci Int 2004; 139(2–3): 183–190.
42. Moriya F, Hashimoto Y. Endogenous gamma-hydroxybutyric acid levels in postmortem blood. Legal Med (Tokyo) 2004; 6(1): 47–51.
43. Berankova K, Mutnanska K, Balikova M. Gamma-hydroxybutyric acid stability and formation in blood and urine. Forensic Sci Int 2006; 161(2–3): 158–162.
44. Elliott SP. Gamma hydroxybutyric acid (GHB) concentrations in humans and factors affecting endogenous production. Forensic Sci Int 2003; 133(1–2): 9–16.
45. Degenhardt L, Copeland J, Dillon P. Recent trends in the use of "club drugs": an Australian review. Subst Use Misuse 2005; 40(9–10): 1241–1256.
46. Kelly BC, Parsons JT, Wells BE. Prevalence and predictors of club drug use among club-going young adults in New York city. J Urban Health 2006; 83(5): 884–895.
47. Fang YX, Wang YB, Shi J, Liu ZM, Lu L. Recent trends in drug abuse in China. Acta Pharmacol Sin 2006; 27(2): 140–144.
48. Lalonde BR, Wallage HR. Postmortem blood ketamine distribution in two fatalities. J Anal Toxicol 2004; 28(1): 71–74.
49. Koren G, Klein J. Postmortem redistribution of morphine in rats. Ther Drug Monit 1992; 14(6): 461–463.
50. Logan BK, Snirnow D. Postmortem distribution and redistribution of morphine in man. J Forensic Sci 1996; 41: 37–46.
51. Gerostamoulos J, Drummer OH. Postmortem redistribution of morphine and its metabolites. J Forensic Sci 2000; 45(4): 843–845.
52. Crandall CS, Kerrigan S, Blau RL, Lavalley J, Zumwalt R, McKinney PE. The influence of site of collection on postmortem morphine concentrations in heroin overdose victims. J Forensic Sci 2006; 51(2): 413–420.
53. Levine B, Wu SC, Dixon A, Smialek JE. Site dependence of postmortem blood methadone concentrations. Am J Forensic Med Pathol 1995; 16(2): 97–100.
54. Caplehorn JR, Drummer OH. Methadone dose and post-mortem blood concentration. Drug Alcohol Rev 2002; 21(4): 329–333.
55. Cook DS, Braithwaite RA, Hale KA. Estimating antemortem drug concentrations from postmortem blood samples: the influence of postmortem redistribution. J Clin Pathol 2000; 53(4): 282–285.
56. Elliott SP. MDMA and MDA concentrations in antemortem and postmortem specimens in fatalities following hospital admission. J Anal Toxicol 2005; 29(5): 296–300.
57. De Letter EA, Bouche MP, Van Bocxlaer JF, Lambert WE, Piette MH. Interpretation of a 3,4-methylenedioxymethamphetamine (MDMA) blood level: discussion by means of a distribution study in two fatalities. Forensic Sci Int 2004; 141(2–3): 85–90.
58. Moriya F, Hashimoto Y. Redistribution of methamphetamine in the early postmortem period. J Anal Toxicol 2000; 24(2): 153–155.
59. Chu M. Studies on the Toxicology of Cananbis [PhD(med)]. Melbourne: Monash University, 2002.
60. Hilberg T, Ripel A, Slordal L, Bjorneboe A, Morland J. The extent of postmortem drug redistribution in a rat model. J Forensic Sci 1999; 44(5): 956–962.
61. Gomez HF, Mcinney P, Phillips S, Roberts DV, Brent J, Watson WA. Postmortem acetaminophen pharmacokinetics: an experimental study of site and time-dependent changes. J Forensic Sci 1995; 40: 980–982.

62. Moriya F, Hashimoto Y. Distribution of free and conjugated morphine in body fluids and tissues in a fatal heroin overdose: is conjugated morphine stable in postmortem specimens? J Forensic Sci 1997; 42(4): 736–740.
63. Carroll FT, Marraccini JV, Lewis S, Wright W. Morphine-3-D glucuronide stability in postmortem specimens exposed to bacterial enzymatic hydrolysis. Am J Forensic Med Pathol 2000; 21: 323–329.
64. Skopp G, Potsch L, Klingmann A, Mattern R. Stability of morphine, morphine-3-glucuronide, and morphine-6-glucuronide in fresh blood and plasma and postmortem blood samples. J Anal Toxicol 2001; 25(1): 2–7.
65. Pounder DJ. The case of Dr. Shipman. Am J Forensic Med Pathol 2003; 24: 219–226.
66. Robertson MD, Drummer OH. Stability of nitrobenzodiazepines in postmortem blood. J Forensic Sci 1998; 43(1): 5–8.
67. Moriya F, Hashimoto Y. Postmortem stability of cocaine and cocaethylene in blood and tissues of humans and rabbits. J Forensic Sci 1996; 41(4): 612–616.
68. Routledge PA, Kitchell BB, Bjornsson TD, Skinner T, Linnoila M, Shand DG. Diazepam and N-desmethyldiazepam redistribution after heparin. Clin Pharmacol Ther 1980; 28: 528–532.
69. Batziris HP, McIntyre IM, Drummer OH. The effect of sulfur-metabolising bacteria on sulfur-containing psychotropic drugs. Int Biodeterior Biodegradation 1999; 44(2–3): 111–116.
70. Skopp G, Lutz R, Ganssmann B, Mattern R, Aderjan R. Postmortem distribution pattern of morphine and morphine glucuronides in heroin overdose. Int J Legal Med 1996; 109(3): 118–124.
71. Skopp G. Preanalytic aspects in postmortem toxicology. Forensic Sci Int 2004; 142(2–3): 75–100.
72. Levisky JA, Bowerman DL, Jenkins WW, Johnson DG, Karch SB. Drugs in postmortem adipose tissues: evidence of antemortem deposition. Forensic Sci Int 2001; 121(3): 157–160.
73. Moor MJ, Steiner SH, Jachertz G, Bickel MH. Adipose tissue distribution and chemical structure of basic lipophilic drugs: desipamine, N-acetyldesipramine, and haloperidol. Pharmacol Toxicol 1992; 70: 121–124.
74. Bickel MH, Graber BE, Moor M. Distribution of chlorpromazine and imipramine in adipose and other tissues of rats. Life Sci 1983; 33(20): 2025–2031.
75. Minder S, Daniel WA, Clausen J, Bickel MH. Adipose tissue storage of drugs as a function of binding competition. In-vitro studies with distribution dialysis. J Pharm Pharmacol 1994; 46(4): 313–315.
76. Coe JI. Postmortem chemistry update. Emphasis on forensic application. Am J Forensic Med Pathol 1993; 14(2): 91–117.
77. Kudo K, Nagata T, Kimura K, Imamura T, Urakawa N. Postmortem changes of triazolam concentrations in body tissues. Nihon Hoigaku Zasshi 1992; 46(5): 293–296.
78. Quatrehomme G, Bourret F, Xhioua M, Lapalus P, Ollier A. Post mortem kinetics of secobarbital. Forensic Sci Int 1990; 44: 117–123.
79. Quatrehomme G, Bourret F, Liao Z, Ollier A. An experimental methodology for the study of postmortem changes in toxic concentrations of drug using secobarbital as an example. J Forensic Sci 1994; 39: 1300–1304.
80. Moriya F, Hashimoto Y. The effect of postmortem interval on the concentrations of cocaine and cocaethylene in blood and tissues: an experiment using rats. J Forensic Sci 1996; 41(1): 129–133.
81. Pohland RC, Bernhard NR. Postmortem serum and tissue redistribution of fluoxetine and norfluoxetine in dogs following oral administration of fluoxetine hydrochloride (Prozac). J Forensic Sci 1997; 42: 812–816.
82. Hilberg T, Ripel A, Smith AJ, Slordal L, Morland J, Bjorneboe A. Postmortem amitriptyline pharmacokinetics in pigs after oral and intravenous routes of administration. J Forensic Sci 1998; 43(2): 380–387.

83. Rogde S, Hilberg T, Teige B. Fatal combined intoxication with new antidepressants. Human cases and an experimental study of postmortem moclobemide redistribution. Forensic Sci Int 1999; 100(1–2): 109–116.
84. Flanagan RJ, Amin A, Seinen W. Effect of post-mortem changes on peripheral and central whole blood and tissue clozapine and norclozapine concentrations in the domestic pig (*Sus scrofa*). Forensic Sci Int 2003; 132(1): 9–17.
85. Kuhlman JJ, Jr., Mayes RW, Levine B, Jones R, Wagner GN, Smith ML. Chloroquine distribution in postmortem cases. J Forensic Sci 1991; 36(5): 1572–1579.
86. Koren G, Beatie D, Soldin S, Einerson TR, MacLeod S. Interpretation of elevated postmortem serum concentrations of digoxin in infants and children. Arch Pathol Lab Med 1989; 113(7): 758–761.
87. Kintz P, Villain M, Pelissier AL, Cirimele V, Leonetti G. Unusually high concentrations in a fatal GHB case. J Anal Toxicol 2005; 29(6): 582–585.
88. Garcia-Repetto R, Moreno E, Soriano T, Jurado C, Gimenez MP, Menendez M. Tissue concentrations of MDMA and its metabolite MDA in three fatal cases of overdose. Forensic Sci Int 2003; 135(2): 110.
89. Barnhart FE, Fogacci JR, Reed DW. Methamphetamine—a study of postmortem redistribution. J Anal Toxicol 1999; 23: 69–70.
90. Logan BK, Smirnow D. Postmortem distribution and redistribution of morphine in man. J Forensic Sci 1996; 41(2): 221–229.
91. Gerostamoulos J, Drummer OH. Postmortem redistribution of morphine and its metabolites. J Forensic Sci 2000; 45(4): 843–845.
92. Wise S, Jenkins AJ. Disposition of quetiapine in biological specimens from postmortem cases. J Forensic Sci 2005; 50: 209–214.
93. Flammia DD, Valouch T, Venuti S. Tissue distribution of quetiapine in 20 cases in Virginia. J Anal Toxicol 2006; 30(4): 287–292.
94. Jaffe P. The Distribution and Redistribution of Four Serotonin Reuptake Inhibitors in Postmortem Specimens [B Sc (Honours)]. Melbourne: Monash University, 1997.
95. Pounder DJ, Davies JI. Zopiclone poisoning: tissue distribution and potential for postmortem diffusion. Forensic Sci Int 1994; 65(3): 177–183.
96. Drummer OH. Clarke's Isolation and Identification of Drugs, 3rd edn. London: The Pharmaceutical Press, 2004.
97. Drummer OH, Odell M. The Forensic Pharmacology of Drugs of Abuse. London: Arnold, 2001.

Examination of Fractures at Autopsy

Archie J. Malcolm

Introduction

Most pathologists undertaking autopsies will be familiar with the appearances of fresh fractures. Fresh or very recent fractures are often seen following fatal road traffic collisions and there is rarely any need to examine these by histology. Equally, osteoporotic femoral neck fractures, whether untreated or treated, do not usually warrant histology. All pathologists are familiar with resuscitation fractures of the anterior parts of the ribs, particularly in the elderly patient, and again these require no further investigation. However, there are other circumstances where histology may be vital to determine:

(a) Is it a fracture?
(b) What age is the fracture?
(c) Is the fracture through an otherwise normal bone?

There are normal variations within bone that can be misinterpreted as fractures (1). In these circumstances, it may be necessary to examine the area by histology to determine whether the abnormality seen is indeed a fracture or some other condition. If the age of the fracture, or fractures, is an important component of the examination, then histology provides a more accurate assessment than radiology. Where the presence of fractures is an important component of a case, or indeed has contributed to death, it may be necessary to ascertain whether those fractures occurred through otherwise normal bones or whether there was a pre-existing condition that had caused or contributed to the fracturing of those bones. This may mean not only examining the fractures but perhaps taking some bone from the iliac crest to determine the presence or absence of a metabolic bone disease.

Fractures (2) may be simple (closed) when there is no communication between the fractured bone and the body surface. An open or compound

G.N. Rutty (ed.), *Essentials of Autopsy Practice*,

fracture is when the fracture site is exposed to the body surface. In certain situations, the type or pattern of fracture may also be of importance. Transverse fractures are usually caused by traction (pulling forces) or a direct blow to the bone. Oblique fractures are more often caused by compression. Helical or spiral fractures are most often caused by torsion or twisting of the affected bone. A comminuted fracture is when the bone at the fracture site is broken into several pieces. An avulsion fracture is where a ligament or tendon inserts into a bone and the stress placed upon the ligament or tendon results in avulsion of the tendon from the underlying bone, usually with some bone still attached to the tendon. These are often seen around the knee in infants who have been swung by their legs. A greenstick fracture is occasionally seen in the resilient bones of infants and young children. This is an incomplete fracture of the bone as the bone can bend more in infants without breaking than can a fully calcified adult bone.

Is It a Fracture?

There are some circumstances when there may be radiological or macroscopic doubt about whether changes seen are truly fractures. Cranial fissures in infants are well recognised in the field of radiology, but these are less well understood by pathologists. A fissure in the skull is an area of fibrous tissue, which usually runs off from the saggital sinus (Fig. 1 and Fig. 2). After death, red blood cells leak into the fibrous tissue imparting a red line very similar to a linear fracture of the skull in a neonate or infant. The histology of such a fissure shows poorly cellular collagenous

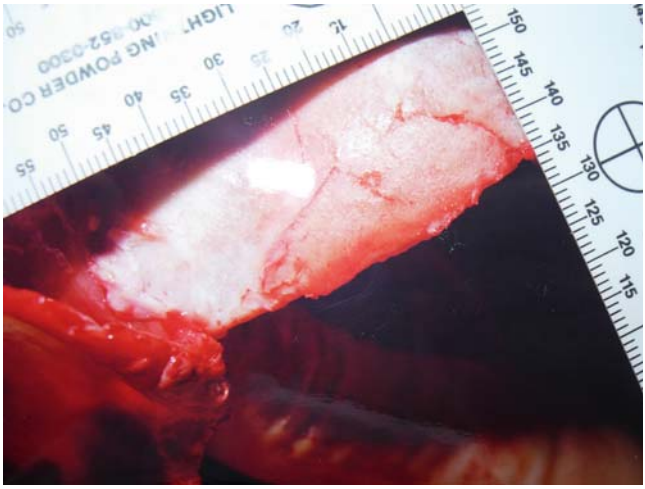


Fig. 1 Two fissures running right to left from the saggital suture across the left parietal bone

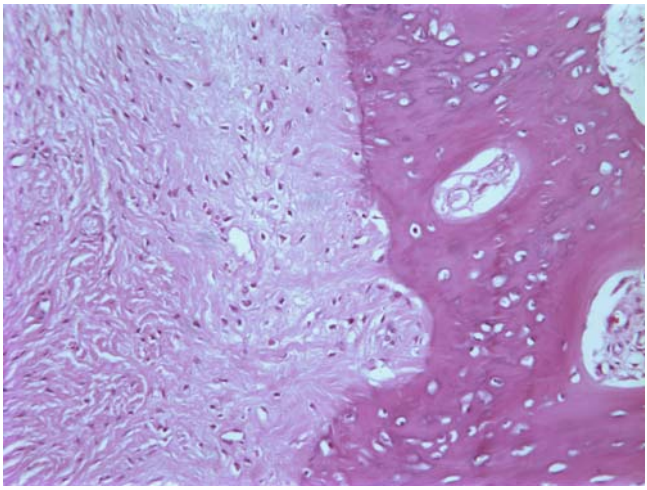


Fig. 2 Collagenous fibrous tissue of a fissure with fibres continuous with the adjacent bone. Haemotoxylin and eosin (H&E)

fibrous tissue, with some of the collagen bands being continuous with the lamellae of the adjacent skull bones. This is unlike the repair reaction seen in a fracture.

Example

A mother was in bed with her 2-month-old girl. When the mother awoke, the baby appeared to be lifeless and there was a small amount of blood-stained fluid coming from its nose. The mother claimed that she must have overlaid the baby while asleep. The post-mortem showed some pulmonary congestion with a small amount of intra-alveolar blood and there was diffuse hypoxic brain damage. The prosecution and defence pathologists both agreed that there was at least one fracture running from the saggital sinus across the parietal bone. The initial court case accepted that a skull fracture could not have occurred due to overlying and the mother was found guilty. However, at appeal, the histology of the so-called skull fracture was reviewed and this was a skull fissure, a variation of normal, and that there was no evidence of a fracture. The mother was released.

The presence of a periosteal reaction in the tibias of neonates and infants is often caused by a gripping or twisting injury to the ankles. However, periosteal new bone in the long bones of neonates and infants can be a variation of normal physiology (3, 4). This is particularly true in the tibias. Therefore, histology of such an area may help to separate physiological periosteal new bone, which has a symmetrical arrangement, and periosteal new bone caused by trauma, where the bone formation is irregular and there will be evidence of recent or old

haemorrhage. There are also variations in the shapes of the epiphysis and metaphysis in neonates and infants that can be misinterpreted as previous trauma.

In both infants and adults, there may be primary bone pathology that can give rise to misinterpretation that the changes seen on an X-ray may be due to trauma. The presence of osteomyelitis often stimulates periosteal new bone. If this is at the distal end of the femur or within the tibia, the changes may be interpreted as trauma, and histology should be used to verify or refute this. Similarly, Paget's disease of bone may cause bone deformity, which may be misinterpreted as a healing fracture.

What Age Is the Fracture?

A fracture is a disruption in the continuity of a bone. It is usually caused by a single violent injury, but it can occasionally be caused by repeated injuries such as a fatigue or stress fracture. A pathological fracture may occur after trivial injury and occasionally even spontaneously. The contribution of a bone disease to a fracture is discussed later in the chapter.

The healing of a fracture goes through several stages from the initial haematoma to an inflammatory reaction, removal of necrotic tissue, ingrowth of granulation tissue, early matrix formation, removal of necrotic bone, maturing matrix with its mineralisation, restoration of the fractured cortex and finally remodelling with eventual removal of the callus and reconstitution of the cortex (5–7). Although these are separate activities, there are overlaps as the healing of a fracture is a continuum. Thus, the ageing of a fracture is not an exact science. As the fracture reaction matures, there is less accuracy in its ageing. When ageing a fracture, account must be taken of the circumstances of the fracture and the health of the fractured individual.

Perimortem Fractures

A perimortem fracture is one that occurs 12 h, or less, prior to death. The first changes following a fracture are that the blood vessels in the medulla, cortex and periosteum of bone are severed and there is a haematoma between and around the fractured bone ends. If the fracture damages adjacent soft tissue, this haemorrhage will be more extensive. There will be evidence of fibrin at the fracture site.

12 Hours to 3 Days

At some 6–12 h following a fracture, there is an inflammatory reaction with polymorphs around the margins of the fracture. With increasing time, there will be an increasing number of polymorphs. Around 48 h after the fracture, there

will be a gradual accumulation of macrophages. The haemopoietic marrow and marrow fat cells will show signs of necrosis 24–48 h following the fracture, but osteocytes do not tend to show necrosis (empty lacunae) until about day 7.

3–7 Days

From about the third day, there will be evidence of ingrowth of granulation tissue in the form of delicate blood vessels and occasional fibroblasts (Fig. 3). This process will continue and mature and is usually very obvious by about the seventh day. From approximately the third day onwards, there is proliferation of cells in the periosteum, particularly where the torn periosteum joins the cortex. These primitive mesenchymal cells will develop into osteoblasts to produce matrix (Fig. 4). Between days 3 and 7, osteoclasts will appear at the edge of the fracture and start removing bone at the fracture site. Osteoblasts will be seen in the adjacent marrow, and by day 7, there should be delicate new woven unmineralised bone formation in both the periosteum and within the medullary canal. Within the medulla, some of this new bone formation may be on top of pre-existing fragments of bone, the new bone being appositional on the old bone. There is continuing removal of necrotic tissue such as necrotic fat cells, fibrin and blood. Around day 7, the bone at the fracture site will show empty osteocyte lacunae due to necrosis (Fig. 5). There may be some cartilage formation in the periosteum at the fracture gap if there is movement at the fracture site. Cartilage is frequently seen in the healing of rib fractures.

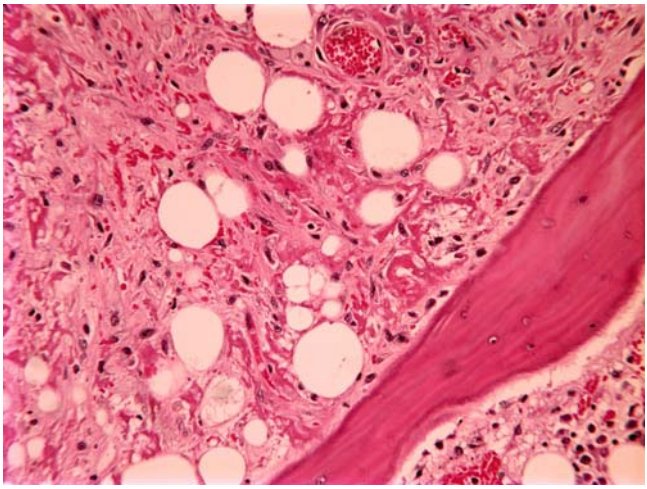


Fig. 3 Fat necrosis with fibrin and early granulation tissue, day 3 with persisting osteocytes. H&E

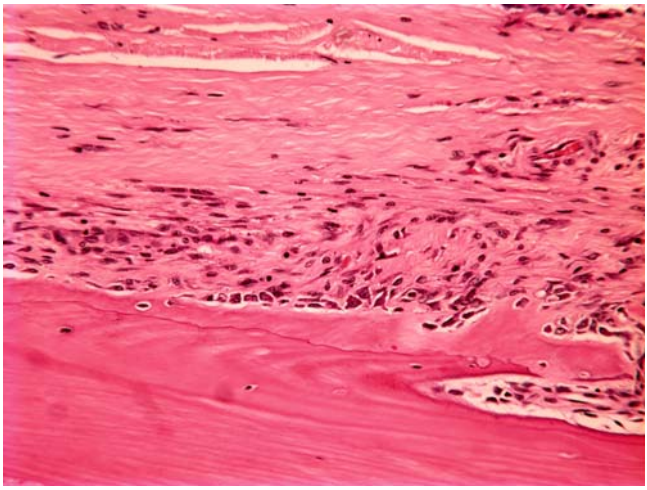


Fig. 4 Early proliferation of periosteal osteoblasts adjacent to cortex about 15 mm from the fracture site. H&E

7–14 Days

During this period, there will be definite primary callus formation. The internal callus forms within the medullary canal, while the external callus forms a sleeve

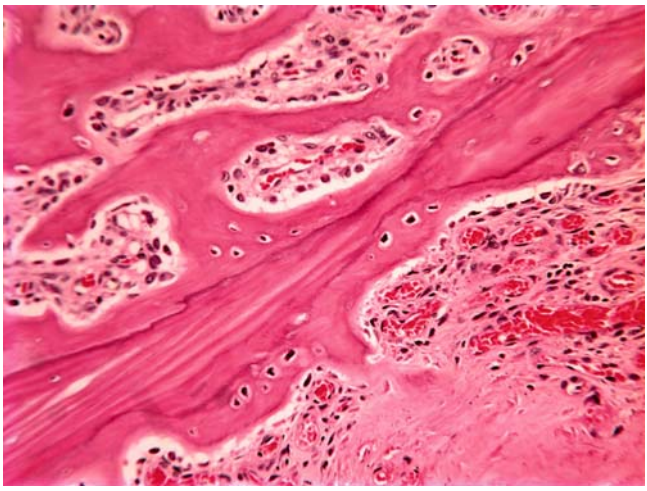


Fig. 5 Central pre-existing necrotic bone trabeculum, with empty osteocyte lacunae, encased in reactive new bone (appositional bone formation) at about 7 days. H&E

Fig. 6 Swellings due to external callus on the pleural side of ribs following a fracture 2 weeks previously

of reparative tissue around the fracture site. The periosteal reaction is often much more pronounced than the internal callus, and the stability of a fracture relies mainly on the external callus (Fig. 6). Histologically, there will be much woven bone being formed by numerous plump osteoblasts, some of the bone within the medulla being formed de novo and some being formed on a scaffolding of necrotic pre-existing bone. During the 7- to 14-day period, there will be significant osteoclastic resorption of the fractured cortical bone (dying back) (Fig. 7). The external callus at this stage will show arcades of reactive new woven bone with or without islands of cartilage (Fig. 8). During

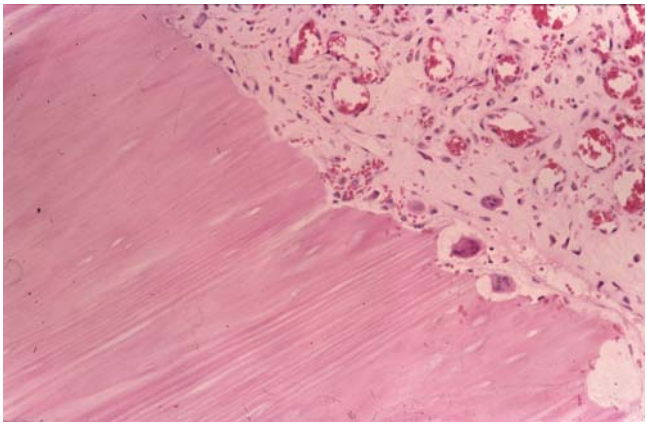


Fig. 7 Osteoclastic resorption of necrotic cortical bone at the fracture site with granulation tissue in the fracture gap and empty osteocyte lacunae. H&E

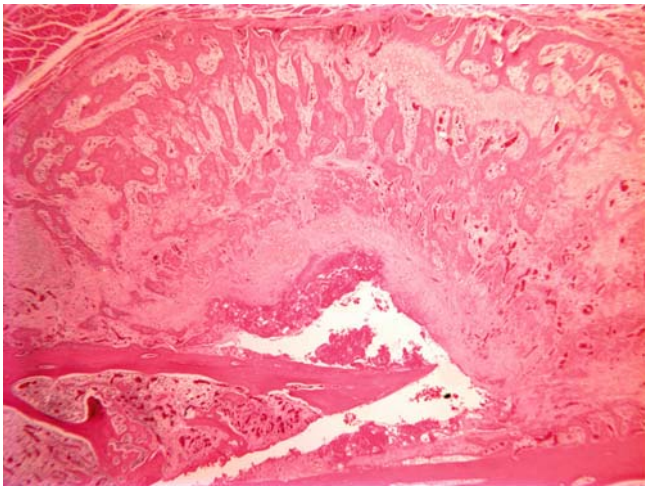


Fig. 8 Two-week-old external fracture callus with a persisting fracture gap (*below*). The outer fracture callus is parallel to the fractured cortex while most of the reactive bone is forming arcades at right angles to the cortex. H&E

the 7- to 14-day period, there is continuing removal of fibrin and necrotic debris by macrophages.

14–21 Days

The internal and external callus reaches its maximum size during this period (Fig. 9). The new matrix starts to calcify and new bone will form between the fractured cortical ends (intermediate callus). In this period, most of the necrotic debris will have been removed, and necrotic medullary bone will either have been removed or will be encased in new bone. By the end of the third week, the callus is calcified and so the fracture is likely to be stabilised and therefore painless. The mass in and around the fracture site is called the provisional or primary callus.

3–6 Weeks

From about 3 weeks onwards, the woven bone and any cartilage in the callus mineralise, and the space between the bone within the callus starts to lose its cellularity and osteoblastic activity becomes less. Over the 3- to 6-week period, any cartilage undergoes enchondral ossification, and the arcades of newly formed bone have a more mature appearance and are mineralised. From the fourth week onwards, there may be osteoclastic remodelling of both internal and external callus (Fig. 10). The fracture gap, including the gap between the

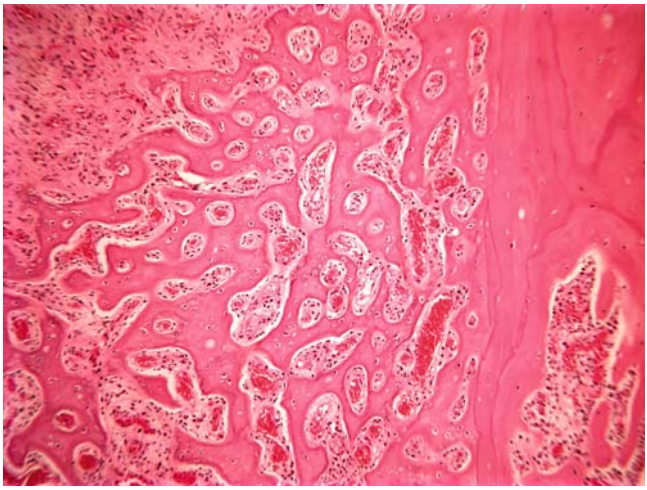


Fig. 9 Well-formed medullary callus at 2–3 weeks using the endosteal cortex as a scaffold (appositional new bone). H&E

fractured cortices, becomes increasingly filled with new bone formation. By the sixth week, the fracture gap should have closed. Once the fractured bone ends have been united by a mineralised primary callus, lamellar bone will form

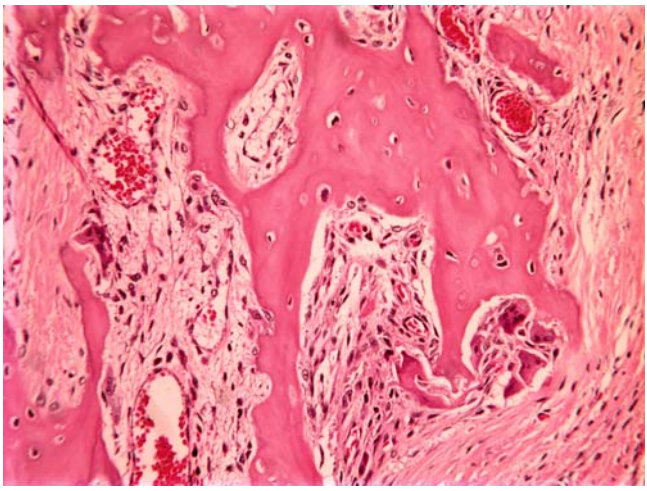


Fig. 10 An arcade of new bone in the external callus undergoing osteoclastic remodelling. H&E

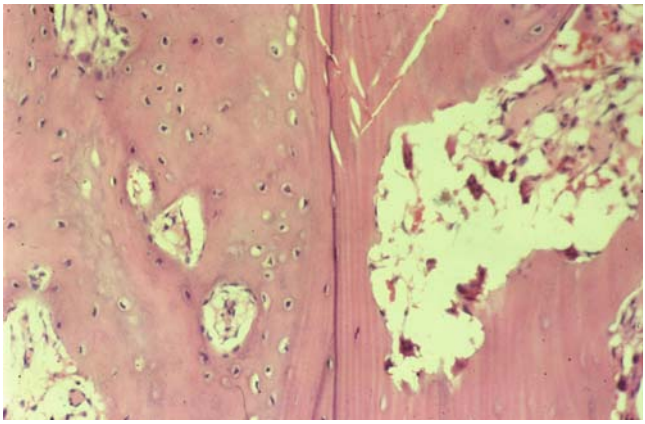


Fig. 11 Cortex at the fracture site being removed by osteoclasts (*right*) after callus formation has bridged the fracture gap with weight-bearing new bone (*left*). H&E

between the fractured bone ends and eventually the callus will be remodelled (Fig. 11).

6–12 Weeks

This is the period when a secondary callus will gradually emerge and the primary callus will be replaced with lamellar bone, which will be laid down in apposition on the surface of some of the woven bone of the primary callus. This lamellar bone is formed along the lines of stress, according to Wolff's law (8), and over 6–12 weeks, the cortex will reform with lamellar bone, which will increase the strength of the cortex and thus reduce the reliance on callus for stability at the fracture site. The fracture site will be remodelled by osteoclasts, and the internal and the external callus will gradually become smaller. Increasingly, the external callus will disappear and any woven bone in the internal callus will be removed. From approximately 12 weeks onwards, the fracture should have fully united and only minor cortical remodelling will be continuing.

The above is a description of the healing of a simple fracture where the bone ends are not significantly displaced and there is no soft tissue interposition between the fractured bone ends. The timing of the healing process is quicker in infants and very young children. Equally, the sequence of events is slower in elderly or old individuals.

The skull, some of the flat bones and the femoral neck all heal slightly differently as there is no true periosteum. Indeed, a femoral neck fracture relies exclusively on medullary callus for repair as the femoral neck has no periosteum (Fig. 12), a source rich in blood vessels and progenitor osteoblasts. Similarly, skull fractures tend to rely on medullary callus with limited external callus formation.

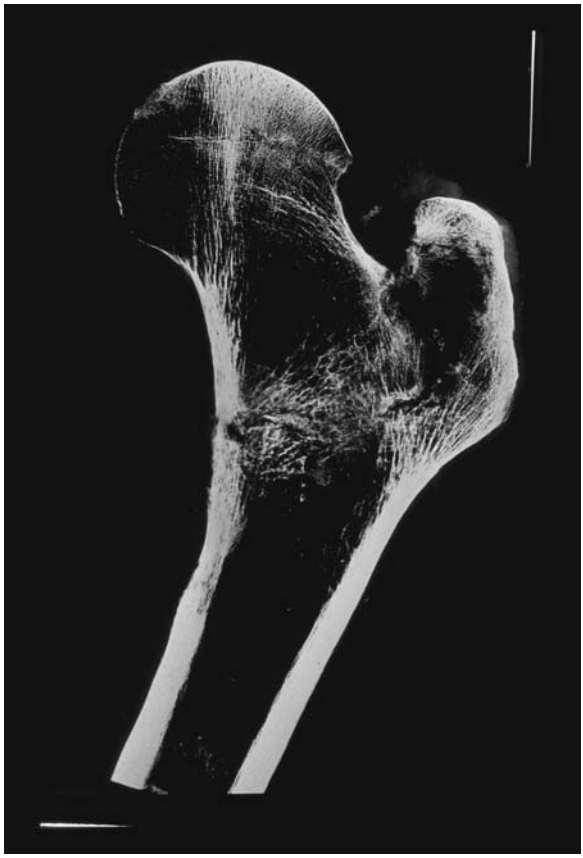


Fig. 12 Inter-trochanteric fracture of femur at 3 weeks showing calcified medullary callus with no external callus as there is no periosteum and limited soft tissue at this site

There are a number of local and systemic factors that may alter the healing process of a fracture. Local factors that interfere with fracture healing are infection and foreign bodies around the fracture site and interposition of muscle or soft tissues at the fracture site. Poor immobilisation results in a delay in fracture healing, and this would manifest itself by the presence of large amounts of cartilage and limited amounts of bone at, and around, the fracture site (Fig. 13). Significant displacement of the fracture will also delay fracture healing. Avascularity such as in the femoral head following a fracture or poor blood supply will all delay the fracture healing process. Clearly, if the fracture is through a tumour, then healing may not take place.

Systemic factors include all generalised metabolic bone diseases or generalised disorders of skeletal development. The nutrition and metabolic state of the patient (e.g. abnormal calcium or phosphate metabolism or serum levels or vitamin D or C deficiency) may also delay fracture healing. Certain drugs such as steroids and cytotoxic therapy may delay fracture healing. Therefore, when ageing a fracture, all these other factors may need to be taken into

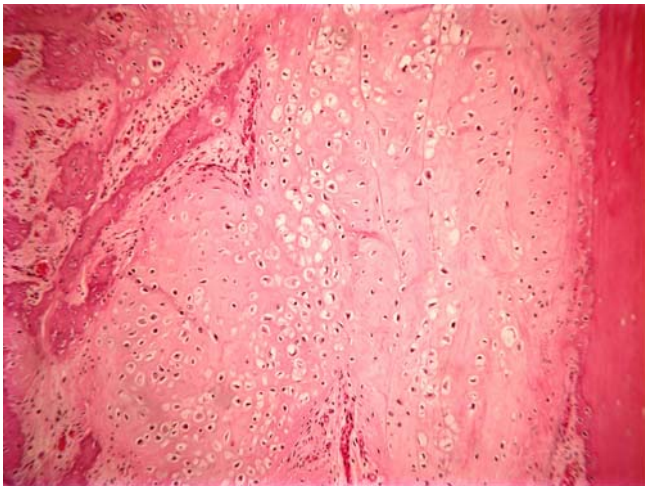


Fig. 13 Extensive cartilage callus formation (cortex at the bottom) in a healing 3-week-old rib fracture. H&E

account. Thus, the ageing of a fracture may be more imprecise if the fracture is complicated or the patient is otherwise unwell.

Comparing Radiological with Pathological Features of Fracture Healing

Apart from the fracture defect, which may be difficult to identify in some cases, the earliest radiological change to be seen is loss of mineralisation of the bone on either side of the fracture. This is usually visible 1–2 weeks after the fracture and corresponds to the removal of necrotic bone at the fracture site (dying back). The next feature at 2–3 weeks is the presence of a soft tissue mass around the fracture site with faint mineralisation. As the callus matures, it becomes more mineralised, and by weeks 4–6, reparative changes within the medullary bone lead to the fracture line becoming hazy and indistinct due to the calcified callus (Fig. 14). Eventually, the fracture line will disappear completely and then over the ensuing weeks to months the internal and external callus will be removed and the bone will essentially be restored to normal (9). These changes may occur a little quicker in infants and children (10). There is considerable agreement by experienced paediatric radiologists that early periosteal new bone can be seen from 7 to 21 days following a fracture with an average of 10–14 days. The loss of the fracture line definition is 14–21 days, with a calcified callus appearing from 14–42 days depending on the type of fracture. The remodelling takes from 2 months to up to 2 years in those fractures that showed gross displacement.

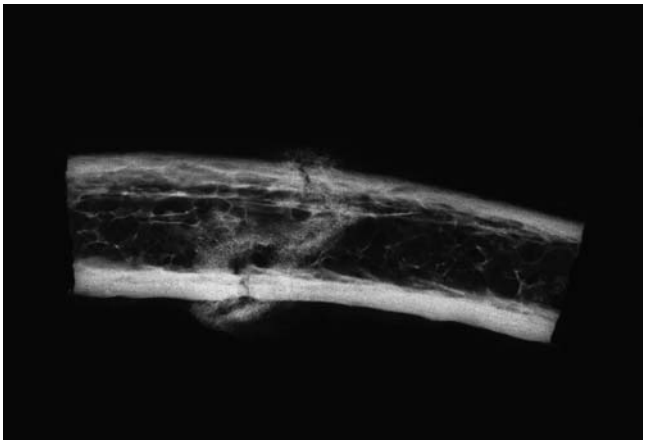


Fig. 14 Specimen radiograph of a 3-week-old fracture with calcified external callus and bridging of the medullary fracture gap and persistence of the cortical gap

Examples

A 3-month-old male infant was found in his cot dead at 7.0 a.m., with mother and father being present in the house. A post-mortem examination was undertaken and there was evidence of multiple bilateral lateral rib fractures and pneumonia. The cause of death was thought to be a pneumonia precipitated or exacerbated by the rib fractures. Histology of the rib fractures showed that these were between 3 and 7 days old. The father had only returned from 2 weeks on the oil rig the day before the baby was found dead.

A 54-year-old man was involved in a pub fracas when he was knocked to the floor and hit his head. He managed to make his own way home. Five days later, he was admitted to hospital because he felt generally unwell, but before he could be investigated, he slipped in the ward toilet and banged his head on the edge of the toilet rim. An X-ray showed a fractured skull and a CT scan showed a subdural haematoma, and he died 2 days later. Histology of the skull fracture showed a healing reaction consistent with a fracture of about 7 days old and inconsistent with a fracture of 2 days duration.

Fractures in Infancy

Rib Fractures in Infancy

Birth Injury

It is recognised that fractures to the ribs can occur during birth, although exceptionally rare. However, posterior fractures at the costovertebral junction

are not described in the literature. This may be because these fractures often cannot be detected on X-ray or because they do not occur.

Resuscitation

Anterior, and even more rarely lateral, rib fractures very occasionally result from resuscitation in infants, especially inexpert resuscitation. The elasticity of a child's chest during resuscitation enables it to tolerate considerable compression without causing rib fractures. In one study where 94 infants and children had received resuscitation, there were rib fractures in the anterior part of the chest in only two (11).

Non-accidental Rib Fractures

Non-accidental rib fractures are the commonest cause of rib fractures in immobile infants. The fractures are often of different ages and are more common in the posterior part of the chest. Anterior and lateral rib fractures can occur due to blows or considerable compression of the chest, front to back (12, 13). The production of rib fractures in infancy requires the application of significant force. The infant ribcage is very elastic and pliable and so fracturing requires a force way beyond rough handling.

Fractures to the rib neck, at the back of the rib, result from backward and inward bowing of the rib against the vertebrae as a consequence of severe side-to-side squeezing of the ribcage. The rib neck is bent over the transverse process of the vertebrae which cracks open the inner cortex of the rib with disruption close to the growth plate (14, 15). Such indirect forces are produced by squeezing the thorax from side to side (which may also be accompanied by vigorous shaking of the infant) when the infant is held by the chest. These posterior rib fractures are very rarely visible in a skeletal survey and are not associated with significant haemorrhage (Fig. 15). The damage is at the interface of the bone with the cartilaginous growth plate (Fig. 16), and so there is virtually no callus formation to produce an easily seen mass. If posterior rib fractures at the posterior growth plate are suspected, the pleura should be stripped and, if in any doubt, histology should be taken.

Skull Fractures in Infancy

Infants skulls are relatively soft and there are open sutures (spaces between the skull bones) that when compressed can overlap. Thus, the skull tends to bend and deform under compression making fracturing difficult, hence the extreme rarity of skull fractures as a birth injury. It takes an impact injury to fracture an infant skull and this would be of considerable force.

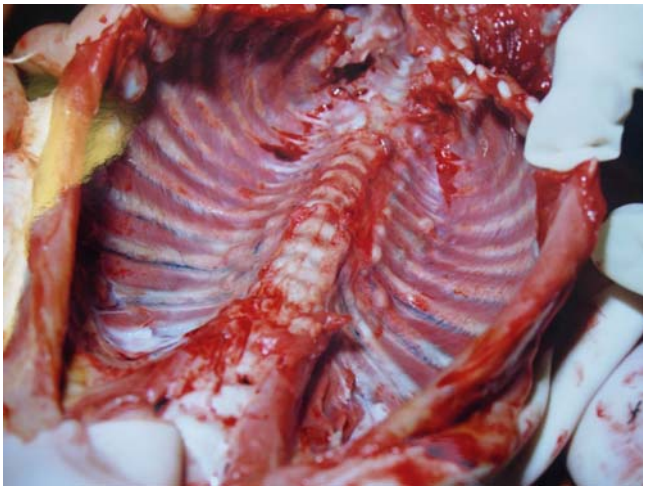


Fig. 15 Three posterior rib fractures and very numerous fractures of the posterior necks of the ribs, more noticeable on the left of the photograph, in a two-month-old infant

Upper Metaphyseal Tibial and Lower Metaphyseal Femoral Fractures in Infants

These types of fractures are extremely rare outside of non-accidental injury. It requires a twisting or gripping of the legs below the knee (often around the ankle) with such inappropriate force that there is shearing between the hard

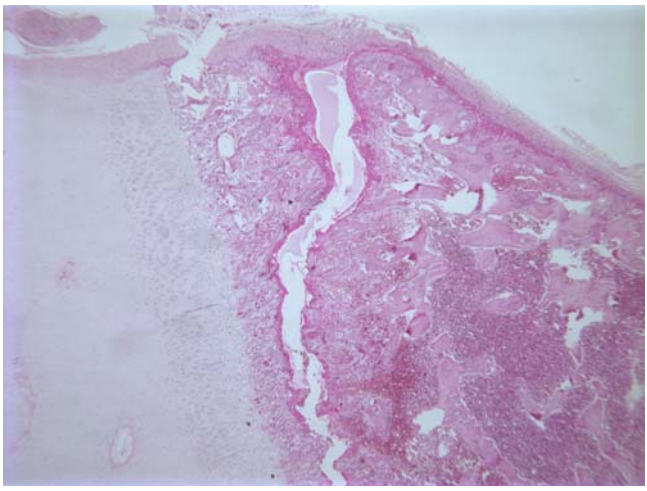


Fig. 16 Typical incomplete fracture of the posterior neck of rib in an infant with the fracture through the growth plate immediately beneath the cartilage of the growth plate. H&E

bone of the upper tibia and the more compliant cartilage of the tibial growth plate. This shearing force results in disruption of the weak new bone at the interface of growth plate and bone. Often this injury causes tearing of the periosteum (outer lining of bone) so that blood tracks between the periosteum and bone causing new bone to form on the outside of the tibia (external callus or periosteal reaction) for some distance beyond the actual fracture.

Avulsion or step fractures occur around the knee, particularly at the metaphysis of the distal femur where a twisting force results in avulsion of bone attached to an overstretched tendon (Fig. 17).

Wrist and Elbow Damage

These may be similar to the metaphyseal damage around the knee or may be periosteal damage without an actual fracture of the bone or a greenstick

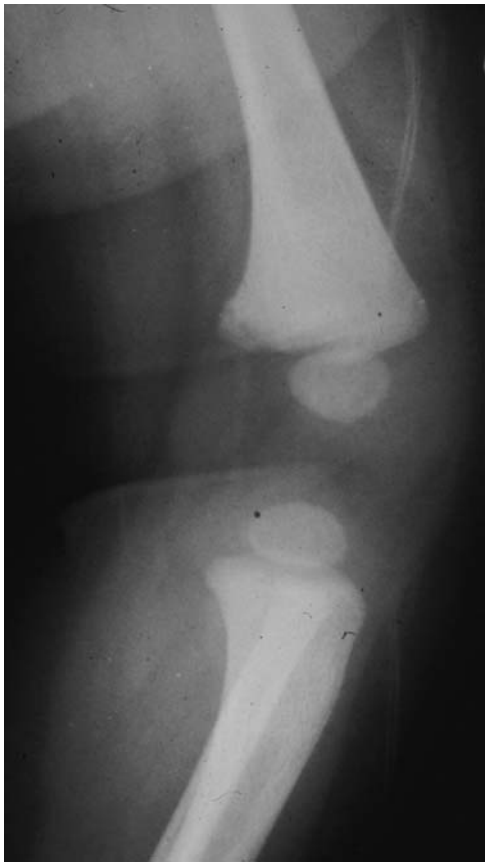


Fig. 17 Radiograph of an avulsion fracture of the posterior part of the distal femoral metaphysis

fracture. The mechanism of damage is very similar to metaphyseal fractures around the knee and is usually caused by twisting or gripping injuries.

Shoulder Fractures

These are normally caused by rotational forces, shaking or forceful pulling of the joint and are rarely seen in non-mobile infants in a non-accidental situation.

Pathology That May Cause or Contribute to a Fracture

Before attributing a fracture directly and exclusively to trauma, the possibility of a contributing pathology should be considered (Table 1).

Inherited and Congenital Diseases

There are four genetic subgroups of osteogenesis imperfecta with marked heterogeneity in clinical expression (16). An infant may have osteogenesis imperfecta and yet have normal radiographs in early infancy. Most patients with type 2 osteogenesis imperfecta die at or before birth. Osteogenesis imperfecta type 3 is a very severe form and is likely to have been diagnosed shortly after birth. However, mild forms of type 1 and type 4 may be missed, especially type 4 where the scleral colour is normal unlike the blue sclera of type 1 patients. Patients with osteogenesis imperfecta develop osteopaenia and their bones fracture very easily (brittle bone disease). The entire skeleton is affected. Often a fracture in a patient with osteogenesis imperfecta will produce an exceptionally large callus. A biopsy of bone in a patient with osteogenesis imperfecta will show hypercellularity of the bone with crowded and slightly enlarged osteocytes with an irregular lamellar pattern with varying amounts of woven bone. Where there is a lamellar pattern, the lamellae are thin. The growth plate may be normal or slightly splayed due to fracturing of the underlying supporting bone.

Osteopetrosis (marble bone disease) is a rare hereditary bone disease that causes sclerosis of the bone with narrowing of the medullary canal. It is due to abnormalities in osteoclasts formation and/or function and leads to abnormal bone remodelling. Although the bone is thick, it is not structurally sound and therefore the bone fractures more easily (compare with Paget's disease of bone).

Patients with neurofibromatosis and Gauchers disease may also sustain fractures more easily.

Table 1 Causes of pathological fracture

Inherited and congenital diseases
Osteogenesis imperfecta
Osteopetrosis
Gaucher's disease
Enchondromatosis
Metabolic bone disease and endocrine disturbances
Hyperparathyroidism
Osteoporosis (generalized/localized)
Osteomalacia
Vitamin C deficiency
Cushing's disease/steroid therapy
Paget's disease
Bone infection
Tumours and tumour-like conditions of bone — benign and malignant

Metabolic Bone Disease and Endocrine Disturbances

Osteoporosis is the most common metabolic bone disease (17, 18). In autopsies, the most common type of osteoporosis is primary or involutional osteoporosis, which is generalised bone loss and is associated with age, more common and often more severe in women. It is a generalised reduction in mineralised bone, which results in the affected individual having a greater propensity to fracturing a bone. The smaller the body mass the more likely the individual is to suffer osteoporosis in later life. The more severe the osteoporosis the less force that is required to cause a fracture, and in individuals with severe osteoporosis, there may be spontaneous fractures, particularly vertebral crush fractures and femoral neck fractures. Histology of osteoporosis is singularly unexciting, and it shows normally mineralised bone, which is lamellar in type, but there is an absolute reduction in the amount of bone. Osteoporosis affects the medullary bone more severely than cortical bone, although the latter is affected where osteoporosis is moderate or severe or in very elderly patients. Osteoporotic femoral neck fractures are common because most of the strength of the femoral neck relies upon medullary bone rather than cortical bone, hence its regular fracturing due to osteoporosis. Indeed in patients with severe osteoporosis, the femoral neck may fracture spontaneously. Clearly, the force required to fracture a severely osteoporotic bone is much less than that of a normal bone. In assessing osteoporosis, the bone from the fracture site is not ideal. The standard method of assessment of osteoporosis is to measure the trabecular bone volume (TBV) (19). This can be by simple histomorphometric technique (Fig. 18), and there are tables available listing the trabecular bone volume in

different age groups that are separated into male and female. These tables are based on measuring the TBV from bone biopsies of the iliac crest. If the degree of osteoporosis is an important component in the autopsy case, then an iliac crest bone sample should be taken for histomorphometric measurement so an exact trabecular bone volume can be given.

Patients with osteomalacia (rickets in children) also have an increased tendency to bone fracturing (20). However, in this case, the trabecular bone volume is normal, but the amount of mineralised bone is dramatically reduced, thus reducing the strength of the affected bone. If this is suspected, then a sample of iliac crest should be obtained for bone morphometry, ideally on undecalcified sections. In children, examination of the growth plate in rickets will show widening of the epiphysis with a disordered growth plate, an increase in the number of cartilage cells and loss of the regular columnar arrangement.

Hyperparathyroidism results in a marked increase in bone turnover with loss of mineralised bone, both generally and localised. Such a condition would increase the likelihood of bone fractures, and again an iliac crest biopsy would allow such a condition to be diagnosed. Vitamin C deficiency (scurvy) is a very rare cause of bone disease in developed countries. Vitamin C is important for collagen synthesis without which there is no cross-linking of the collagen. This results in irregular growth plates in children with areas of sub-periosteal haemorrhage, which may be misinterpreted as non-accidental injury. In both children and adults, there is an increased susceptibility to fracturing and these fractures heal more slowly.

Paget's disease of bone (Osteitis Deformans) is a relatively common disorder of bone remodelling (21). It can affect a single bone or multiple areas of the skeleton. It affects patients over the age of 40 years although the commonest incidence is patients over 60 years of age. The disease is due to the uncontrolled overactivity of osteoclasts, which results in rapid bone remodelling. Where new

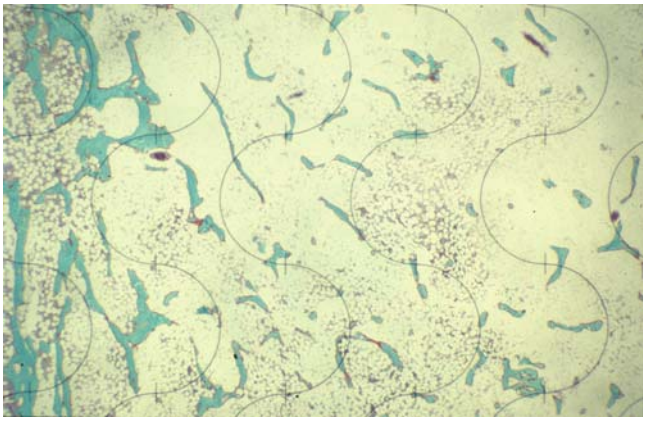


Fig. 18 Graticule for measuring trabecular bone volume (TBV) in osteoporotic medullary bone

bone forms in an area of Paget's disease, it does so in a haphazard fashion rather than along the lines of stress (Fig. 19). Therefore, patients with Paget's disease of the bone may predispose to fracture either because of the loss of bone in a localised area or because the bone has formed in an irregular and haphazard fashion and not along the lines of stress. If Paget's disease is suspected of being implicated in a fracture, then the bone tissue 1 cm on either side of the fracture site should be sampled rather than just the fracture.

Localised Bone Disease

The traditional view of a pathological fracture is a fracture through a localised abnormality of bone such as bone infection, a tumour-like condition of bone (Fig. 20), benign and malignant bone tumours and bone metastases, which are the most common in adults. A sample taken immediately adjacent to, but not at, the fracture site should establish the presence of these conditions.

Summary

There are situations where histology may be required to confirm unequivocally the presence of a fracture. In infant skulls, and posterior ribs in infants, histology may be required to confirm the presence of a fracture. The histology of a fracture can assist in the ageing of the fracture and can also assist in determining whether all the fractures are of the same or different ages. Histology of the fracture site, or in the case of possible metabolic bone disease, an iliac crest biopsy, may be required to determine if there was any inherent

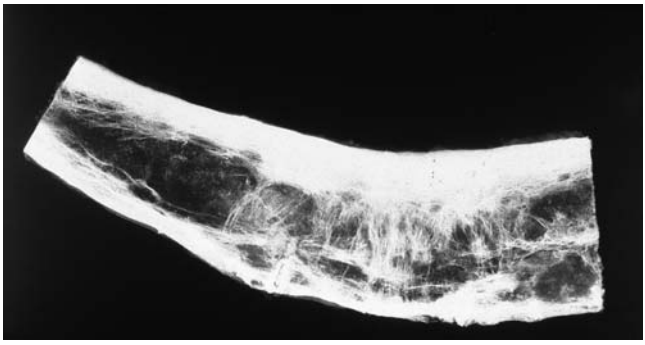


Fig. 19 Specimen radiograph of a Pagetic tibia showing the irregular bone resorption and bone formation with a crack fracture of the anterior cortex (*top right of centre*)

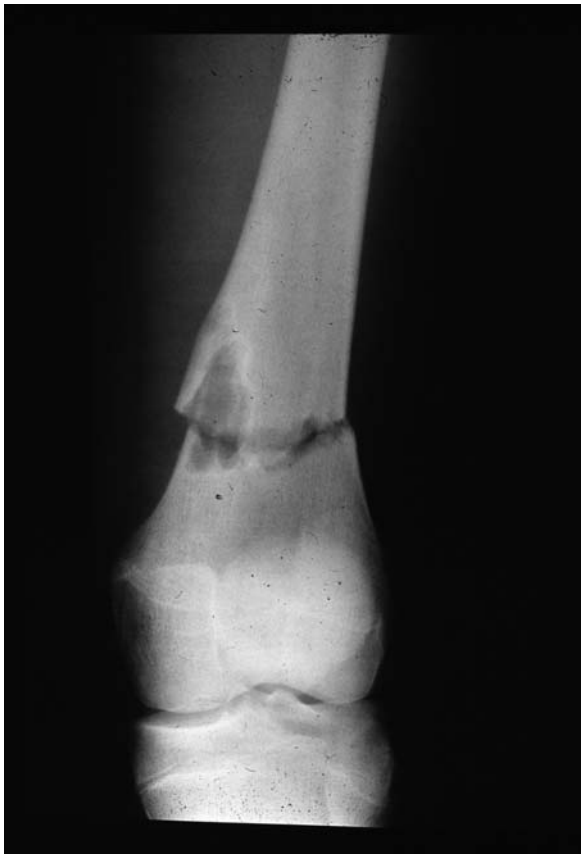


Fig. 20 Radiograph of a pathological fracture in a teenager through a fibrous cortical defect

pathology which may have caused, or contributed to, the fracture and what force would therefore have been necessary to cause the fracture.

References

1. Silverman FN. The bones: normal and variants. In Silverman FN, Kuhn JP, eds. Caffrey's Pediatric X-ray Diagnosis: An Integrated Imaging Approach, vol. 2, 9th edn. St. Louis: Mosby, 1993, pp. 1465–1529.
2. Oni O, Gregg PJ. Terminology, description and classification. In Gregg PJ, Stephens J, Worlock P, eds. Fractures and Dislocations: Principles of Management. Blackwell Science, 1996, pp. 29–36.
3. Plunkett J, Plunkett M. Physiologic periosteal changes in infancy. Am J Forensic Med Pathol 2000; 21: 213–216.
4. Jacobsen FS. Periosteum: its relation to pediatric fractures. J Pediatr Orthop 1997; 6: 84–90.
5. Malcolm AJ. Basics of fracture healing. In Gregg PJ, Stevens J, Worlock P, eds. Fractures and Dislocations. Blackwell Science, 1996, pp. 71–79.

6. Goodship AE, Lowes TJ, Harrison L. The biology of fracture repair. In Hughes S, McCarthy I, eds. Sciences Basic to Orthopaedics. WB Saunders, 1988, pp. 128–143.
7. Brighton CT, Hunt T. Early histological and ultrastructural changes in medullary fracture callus. J Bone Joint Surg 1991; 73: 832–847.
8. Wolff J. The Law of Bone Remodelling (translated by Maquet P, Furlong R). Springler, 1986.
9. O'Connor JF, Cohen J. Diagnostic Imaging of Child Abuse. Baltimore: Williams and Wilkins, 1987, pp. 112–118.
10. Laor T, Jaramillo D, Oestrich AE. Musculoskeletal system. In Kirks DR, Griscom NT, eds. Practical Pediatric Imaging: Diagnostic Radiology of Infants and Children, 3rd edn. Philadelphia: Lippincott-Raven, 1998, pp. 335–342.
11. Betz P, Liebhardt E. Rib fractures in children—resuscitation or child abuse? Int J Leg Med 1004; 106: 215–218.
12. Shouldice M, Huyer D. Non-accidental rib fractures. In Recent Advances in Paediatrics, number 19. 1994; Chapter 5, pp. 63–76.
13. Carty HML. The radiological features of child abuse. Curr. Paediatr. 1995; 5: 230–235.
14. Kleinman P, Marks S, Spevak M, Richmond J. Fractures of the rib head in abused infants. Radiology 1992; 185: 119–123.
15. Kleinman P, Schlesinger A. Mechanical factors associated with posterior rib fractures. Paediatr Radiol 1997; 27: 87–91.
16. Shapiro JR, Primorac D, Rowe DW. Osteogenesis imperfecta. Current concepts. In Bilezikian JP, Raisz LG, Rodan GA, eds. Principle of Bone Biology. Academic Press, 1996, pp. 889–902.
17. Silver JJ, Einhorn TA. Osteoporosis in ageing. Clin Orthop 1995; 316: 10–20.
18. Riggs BL, Melton LJ. Involutional osteoporosis. N Engl J Med 1986; 314: 1676–1684.
19. Malcolm AJ. Metabolic bone disease. Curr Diagn Pathol 2002; 8: 219–225.
20. Teitelbaum S. Pathological manifestations of osteomalacia and rickets. Clin Endocrinol Metab 1980; 43–62.
21. Milgram JW. Radiological and pathological assessment of the activity of Paget's disease of bone. Clin Orthop 1977; 127: 43–54.

The Relationship of Body Weight and Sudden Death

James A.J.(Rex) Ferris

Introduction

The extremes of body weight, both high and low, are known to be associated with increased morbidity and mortality and in this chapter the pathology and mechanisms of sudden death in both anorexia nervosa and morbid obesity will be considered.

Anorexia Nervosa and Bulimia Nervosa

The enigma of sudden death and dieting has been a well-recognised phenomenon in medical practice since early in the twentieth century. However, it was not until the death of the internationally renown singing star Karen Carpenter in 1983 that the public at large became aware of the dangers of anorexia nervosa. Anorexia nervosa and its partner condition, bulimia nervosa, are especially common in young white women who are hyper-achievers, ballet dancers, fashion models and gymnasts and are sometimes popularly referred to as the "slimmer's diseases".

Although in 1975 Karen Carpenter's body weight was reported to have fallen to 36 kg (80 lbs), at the time of her fatal cardiac arrest on February 4, 1983 she weighed 49 kg (108 lbs) and was 1.62 m (5'4") in height. This was a body mass index (BMI) of 18.7 and is at the lower end of what is usually considered to be the "healthy" body weight range. A detailed account of the struggles of Karen Carpenter and her obsession with weight loss is well documented by Adena Young (1). This is a classical and typical story of a young woman's struggle with her self-image including medically directed "water diets" and thyroid medication. In 1990 the generally accepted medical view was that in anorexia nervosa, the immediate danger was related to the effects of voluntary starvation,

G.N. Rutty (ed.), *Essentials of Autopsy Practice*,

including hypophosphataemia, bone marrow failure, cardiac decompensation and shock. In patients with bulimia nervosa there was often significant fluid and electrolyte abnormalities resulting in hypovolaemia, secondary hyper-aldosteronism and depletion of total body potassium resulting in cardiac dysrhythmias (2). Older pathology texts reported that there was extreme loss of heart mass with mucoid degeneration of the epicardial fat and microscopic examination of the myocardium in these victims, and as with other cases of extreme starvation, usually showed diminution of myofibre size with an apparent accumulation of lipofuscin pigment at the poles of the nuclei (Fig. 1). This entity was often referred to as "brown atrophy" of the heart and appears to correspond to an exaggerated senile change in the myocardium (3).

Ravaldi et al. in 2003 reported that the death rate in anorexia nervosa and bulimia nervosa was up to 30 times greater than that of age-matched women (4). In their cases, bradycardia, hypotension and mitral valve prolapse were frequently encountered and the risk of sudden death was substantially linked to QT prolongation which in turn could be linked to hypokalaemia in bulimia nervosa and starvation-derived anatomical remodelling of the heart in anorexia nervosa. They found that the principal risk factors were the duration of the illness > 10 years, chronic hypokalaemia, plasmatic albumin which was chronically less than 3.6 g/dl and an absolute QT interval greater or equal to 600 ms.

The incidence and significance of prolongation of the QT interval was at that time unclear, however, in 2004 Takimoto et al. (5) studied 179 women over 18 years of age with eating disorders and excluded from their series those women with abnormal plasma electrolytes and those taking medications that might influence their electrocardiograms. The patients eligible for inclusion in this study were divided into four groups. One group comprised 43 patients with anorexia nervosa (restricting type), a second group comprised 35 patients with anorexia nervosa

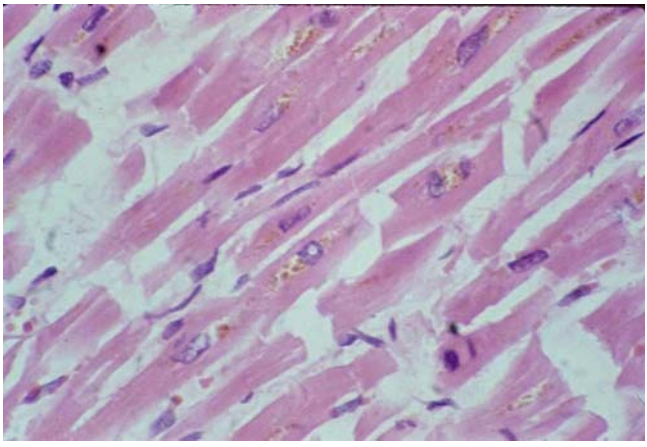


Fig. 1 Myocardial para-nuclear lipofuscin pigment in anorexia nervosa

binge/purging type, a third group 63 patients with bulimia nervosa purging type and a fourth group of 23 cases with non-purging bulimia nervosa.

QT dispersion was measured in each group as the difference between the longest and shortest QT intervals, and the QT intervals and QT dispersion in each patient group was compared with those in a control group. QT interval and QT dispersion were significantly longer in all the eating disorder groups than in a similar aged control group, and QT interval prolongation and QT dispersion were significantly correlated with the rate of loss of body weight, particularly in the bulimia nervosa cases.

Although the eating disorders of anorexia nervosa and bulimia nervosa are associated with the highest mortality rate of any psychiatric condition (6), much of this mortality results from cardiovascular complications such as dysrhythmias related to prolonged QT interval with or without electrolyte disorders, hypotension and bradycardia (6).

Structurally the heart is atrophic with small atrophic myocytes and prominent para-nuclear lipofuscin pigment (Fig. 1). These patients have low cardiac output and demonstrate increased peripheral vascular resistance despite the presence of hypotension. Treatment of such eating disorders has intrinsic cardiovascular risks and such patients may manifest dysrhythmias, tachycardia, congestive cardiac failure and sudden death. It is apparent that the management of such cases requires electrocardiographic monitoring of QT interval and QT dispersion.

It is also important to realise that patients with severe anorexia nervosa and bulimia may have other related and unrelated potentially fatal disorders. For example, Derman and Szabo recently described a 36-year-old woman with long-standing anorexia nervosa and a BMI of 12.5 who died suddenly with multiple bilateral pulmonary thrombo-emboli and bilateral deep vein thrombosis (7). In another case reported by Garcia-Rubira et al. a 39-year-old woman with long-standing anorexia nervosa developed chest pain and was found to have a myocardial infarction associated with coronary atherosclerosis apparently indicating that starvation does not protect against atherosclerosis (8).

Obesity

Obesity is becoming a global epidemic in both adults and children. It is associated with numerous co-morbidities such as cardiovascular diseases, type 2 diabetes, hypertension, strokes, certain cancers and sleep-disordered breathing. As a result of these co-morbidities there is a significant reduction in life expectancy and in particular a significant association with premature sudden death in relatively young adults (9).

Not only is obesity a significant risk factor for sudden premature death, but many of the medical and surgical treatments used for such obesity carry with them an inherent risk of sudden death.

Although morbid obesity, usually defined as a BMI of 40 or more, is associated with a high incidence of sudden unexpected death DuFlou et al. in 1995

found little consistency in the mechanisms of death (10). Although heart weights were increased they found that these remained constant as a percentage of body weight. In their series of 28 patients, only myocyte nuclear area was an independent predictor of obesity and morbid obesity related cardiomyopathy, which was characterised by cardiomegaly, left ventricular dilatation and myocyte hypertrophy in the absence of interstitial fibrosis. Dilated cardiomyopathy was the most frequent cause of death, 10/28–36% with only six ischaemic heart deaths (21%). Personal experience indicates a much lower incidence of dilated cardiomyopathy. However, obesity is associated with high chronic cardiac workload due to the need to supply more blood to peripheral tissue and this high cardiac output is maintained by an increase in stroke volume and high heart rate sustained by an increase in ventricular mass. This increase in left ventricular mass also implies an increase in non-muscular tissue that plays a role in the development of electrical abnormalities, heart failure and sudden death (11).

Even in the absence of cardiac dysfunction, obese patients are at risk of dysrhythmias and sudden death. In 1995 Baharti and Lev reported the presence of significant pathological findings in the conduction system in sudden death of obese young people (12). However, these abnormalities appear not to be reflected in detailed electrocardiographic studies. In 1999 Bilora et al. reported that obese patients presented a shorter PQ, a prevalence of left cardiac axis, a higher heart rate, a longer QT, but not QTc compared with normal males. They found no correlation between QTc (QT corrected interval) and obesity (13). Similarly Girola et al. in 2001 concluded that the QTc intervals did not correlate with BMI and no association was found between QTD and anthropometric parameters reflecting body fat distribution such as age, BMI, waist measurement or abdominal sagittal diameter (14). QTD is defined as the difference between the maximum and minimum QTc across a 12-lead electrocardiograph.

Obesity and Hypoventilation Syndrome

This syndrome is defined as a combination of morbid obesity, chronic hypoventilation which in turn leads to pulmonary hypertension, cor pulmonale and is frequently associated with sleep apnoea and a significant risk of sudden death. This syndrome is often called the "Pickwickian syndrome" after the account in Charles Dicken's *Pickwick Papers* where Mr. Pickwick described Joe, an extremely obese servant-boy and carriage driver to Mr. Tupman, "…..and on the box sat a fat and red-faced boy, in a state of somnolency…Joe! – damn that boy, he's gone to sleep again …" (15). Recent data indicate that this is an under-recognised and under-treated syndrome (16) and because of the world-wide obesity epidemic it is important that physicians should be able to recognise and treat such obesity-related conditions.

The major respiratory complications of obesity include an elevated demand on the breathing mechanisms, reduced respiratory muscle efficiency and

diminished respiratory compliance. These problems are further exacerbated by posture and body position and can lead to significant levels of hypoxia in the prone position. The major circulatory complications are increased total and pulmonary blood volumes, high cardiac output with elevated left ventricular and end-diastolic pressures. Patients with morbid obesity commonly develop hypoventilation and sleep apnoea syndromes with attenuated hypoxic and hypercapnoeic ventilatory responsiveness (17).

Whether or not morbid obesity significantly contributes to asthma and airway hyper-responsiveness is uncertain. In a recent study published in 2006 by Sood et al. using a methacholine challenge test in 1725 subjects, only a very weakly significant interaction was found between asthmatic status and body mass index (18). Similarly Vlaski et al. in 2006 have been unable to establish a significant association between obesity and other symptoms of asthma or atopic eczema (19).

Chen et al. in 2006 have found that obesity is likely to have a larger effect on non-allergic asthma (20). They have suggested that the greater prevalence of non-allergic asthma in women may explain the stronger obesity–asthma association seen in women compared with men and children who have a greater prevalence of allergic asthma. Chinn (21) has concluded that no study in Western society has found objective evidence for the association between obesity and asthma. Studies have been found to vary in their definitions of obesity and whether or not the symptoms of asthma have been doctor diagnosed or simply parent reported. A personal review of the lung histology of a series of sudden death in morbidly obese individuals (Table 1) clearly indicates a significant increase in the amount of smooth muscle present in the alveolar walls (Fig. 2) with no significant increase in hyalinisation of the basement membranes of the bronchi or eosinophilic infiltration (22). These findings would tend to support the fact that asthmatic symptoms in the morbidly obese are as a result of chronic hypoxia and pulmonary hypertension and not as a result of an allergic response.

Table 1 Comparison of the amount of alveolar wall smooth muscle in a series of age-and sex-matched controls and morbidly obese (*N*–18)

Controls		Morbidly obese	
BMI	Alveolar smooth muscle	BMI	Alveolar smooth muscle
23.6	+	45.6	++++
23.8	++	50.0	++++
24.3	+	50.1	+++
25.2	+	54.6	++++
26.0	+	55.5	+++
26.7	+	57.7	++
29.7	+	67.4	+++
30.8	+	< 70	+++
33.8	++	< 70	++++

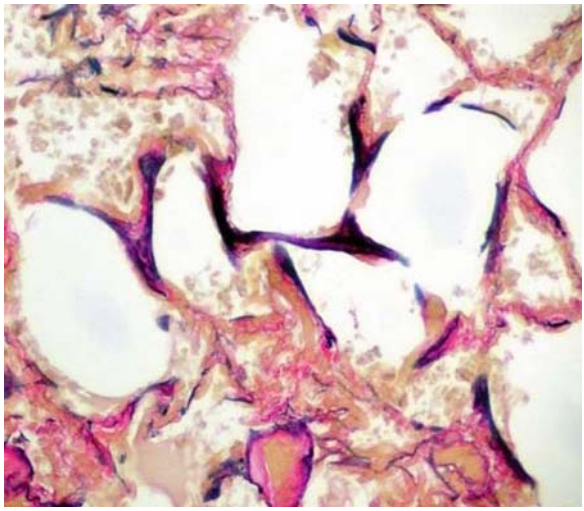


Fig. 2 Marked hypertrophy of alveolar wall smooth muscle in morbid obesity (EVG)

A recent review of this topic by Shore in 2007 indicates that not only does obesity increase the prevalence, incidence and possibly the severity of asthma, but there seems little doubt that weight reduction and physical activity are effective means of reversing virtually all the respiratory complications of morbid obesity (23).

Management of Morbid Obesity and Sudden Death

The medical management of patients with severe morbid obesity appears of itself to be associated with a relatively high level of morbidity and mortality. Liquid protein diets used for the control of morbid obesity since the 1960s have been associated with an increased risk of sudden death. Although this was thought to be related to prolongation of the QT interval occurring in the absence of structural abnormalities of the heart, Surawicz and Waller found that weight loss, starvation and dieting methods other than liquid protein diets were not associated with an increased incidence of sudden death (24). Sudden death related to such liquid protein diets and protein malnutrition appears to remain an enigma.

Papaioannou et al. have reported how the use of surgical vertical banded gastroplasty for the management of morbidly obese patients appears to be followed not only by significant weight loss but by a significant shortening of the QTc interval (25). The effect of this is to reduce the incidence of malignant ventricular dysrhythmias and sudden death associated with the long QT syndrome.

When in 2003 the US Air Force adopted a policy strongly discouraging the use of ephedra, the use of dietary supplements containing ephedrine-type

alkaloids to enhance athletic performance and stimulate weight loss was seriously questioned (26). Naik and Freudenberger in 2004 reported two cases of young apparently fit and healthy young men who developed ephedra-associated cardiomyopathy and myocyte toxicity (27).

It is clear that the extremes of weight loss and weight gain are associated with a substantial risk of sudden death. Anorexia and bulimia nervosa are now recognised as medical disorders requiring significant medical and psychological management. It is also clear that the current world-wide obesity epidemic requires similar multidisciplinary management because of not only the risk of sudden death but the morbidity from a wide variety of other related disorders including diabetes, heart disease, hypertension and respiratory failure. It is also apparent that the medical management of these conditions also carries a significant risk of sudden death.

References

1. Young A. Battling Anorexia: The Story of Karen Carpenter. http://atdpweb.soe.berkeley.edu.
2. Comerci GD. Medical complications of anorexia nervosa and bulimia nervosa. Med Clin North Am 1990; 74(5): 1293–1310.
3. Cappell DF. Muir's Text-Book of Pathology, 7th edn. London: Arnold, 1958.
4. Ravaldi C, Vannacci A, Ricca V. Cardiac complications of anorexia nervosa. Recent Prog Med 2003; 96(6): 267–270.
5. Takimoto Y, Yoshiuchi K, Kumano H, Yamanaka G, Sasaki T, Suematsu H, Nagakawa Y, Kuboki T. QT interval and QT dispersion in eating disorder. Psychother Psychosom 2004; 73(5): 324–328.
6. Casiero D, Frishman WH. Cardiovascular complications of eating disorders. Cardiol Rev 2006; 14(5): 227–231.
7. Derman T, Szabo CP. Why do individuals with anorexia die? A case of sudden death. Int J Eat Disord 2006; 39(3): 260–262.
8. Garcia-Rubira JC, Hidalgo R, Gomez-Barrado JJ, Romero D and Criz Fernandez JM. Anorexia nervosa and myocardial infarction. Int J Cardiol 1994; 45(2): 138–140.
9. Poirier P, Giles TD, Bray GA, Hong Y, Stern JS, Pi-Sunyer FX, Eckel RH. Obesity and Cardiovascular disease: pathophysiology, evaluation and effect of weight loss. Circulation 2006; 113(6): 898–918.
10. DuFlou J, Virmani R, Rabin I, Burke A, Farb A, Smialek J. Sudden death as a result of heart disease in morbid obesity. Am Heart J 1995; 130(2): 306–313.
11. Contaldo F, Pasanisi F Finelli C, de Simone G. Obesity, heart failure and sudden death. Nutr Metab Cardiovasc Dis 2002; 12(4): 190–197.
12. Baharti S, Lev M. Cardiac conduction system involvement in sudden death of obese young people. Am Heart J 1995; 129(2): 273–281.
13. Bilora F, Vettore G, Barbata A, Pastorello M, Petrobelli F, San Lorenzo I. Electrocardiographic findings in obese subjects. Minerva Gastroenterol Dietol 1999; 45(3): 193–197.
14. Girola A, Enrini R, Garbetta F, Tufano A, Caviezel F. QT dispersion in uncomplicated human obesity. Obes Res 2001; 9(2): 71–77.
15. Dickens C. The Pickwick Papers, Chapter IV—The Field Day and Bivouc. Oxford: Oxford University Press, 1986.
16. Olson AL, Zwillich C. The obesity hypoventilation syndrome. Am J Med 2005; 118(9): 948–956.

17. Parameswaran K, Todd DC, Soth M. Altered respiratory physiology in obesity. Can Respir J 2006; 13(4): 203–210.
18. Sood A, Verhulst SJ, Varma A, Eagleton LE, Henkle JQ, Hopkins-Price P. Association of excess weight and degree of airway responsiveness in asthmatics and non-asthmatics. J Asthma 2006; 43(6): 447–452.
19. Valski E, Stavric K, Isjanovska R, Seckova L, Kimovska M. Overweight hypothesis in asthma and eczema in young adolescents. Allergol Immunopathol (Madr) 2006; 34(5): 199–205.
20. Chen Y, Dales R, Jiang Y. The association between obesity and asthma is stronger in nonallergic than allergic adults. Chest 2006; 130(3): 890–895.
21. Chinn S. Obesity and asthma. Paediatr Respir Rev 2006; 7(3): 223–228.
22. Ferris JAJ. Microscopic Evidence of Pulmonary Hypertension in Morbid Obesity. Unpublished research.
23. Shore SA. Obesity and asthma: implications for treatment. Curr Opin Pulm Med 2007; 13(1): 56–62.
24. Surawicz B, Waller BF. The enigma of sudden cardiac death related to dieting. Can J Cardiol 1995; 11(3): 228–231.
25. Papaioannou A, Michaloudis D, Fraidakis O, Petrou A, Chaniotaki F, Kanoupakis E, Stamatiou G, Melissas J, Askitopoulou H. Effects of weight loss on QT interval in morbidly obese patients. Obes Surg 2003; 13(6): 869–873.
26. Miller SC. Safety concerns regarding ephedrine-type alkaloid-containing dietary supplements. Mil Med 2004; 169(2): 87–93.
27. Naik SD, Freudenberger RS. Ephedra-associated cardiomyopathy. Ann Pharmacother 2004; 38(3): 400–3.

Fatal Sexual Abuse in Childhood

Roger W. Byard and Terence Donald

Introduction

Deaths from sexual abuse in infancy and childhood are uncommon; however, the approach to the investigation and autopsy of such cases follows the general principles for the assessment of homicide in this age group. The evaluation of homicides in infancy and early childhood presents an array of difficulties that are not present in older children and adults. Disparity in size between the victims and their attackers means that children can be manipulated and controlled with less force and may therefore not manifest as many signs of physical injury. Conversely, their relative physical weakness renders them vulnerable to attack, with less ability to defend themselves, and so injuries may be quite extensive involving many different organs and structures. The autopsy assessment may, therefore, be complicated by a paucity of physical signs of trauma or alternatively by an abundance of injuries that may be of various ages and due to a variety of mechanisms. Attempting to determine a cause of death when there are no definitive autopsy findings may not be possible, just as trying to unravel the pattern of assault in the face of overwhelming numbers of injuries may be difficult. The inability to interview the child victim in fatal cases further complicates assessment.

Another feature of immature human beings is their unique and evolving anatomy that requires an understanding of developmental stages for accurate interpretation. Nowhere is this of more significance than in the genital area of young females, where marked developmental changes occur in the anogenital region throughout the whole of the prepubertal period (1). It is not until genital maturity is reached (Tanner stage 5) that no further developmental changes will occur. These changes have now been well described, both for the hymen and for more external structures (Fig. 1) (2).

G.N. Rutty (ed.), *Essentials of Autopsy Practice*,

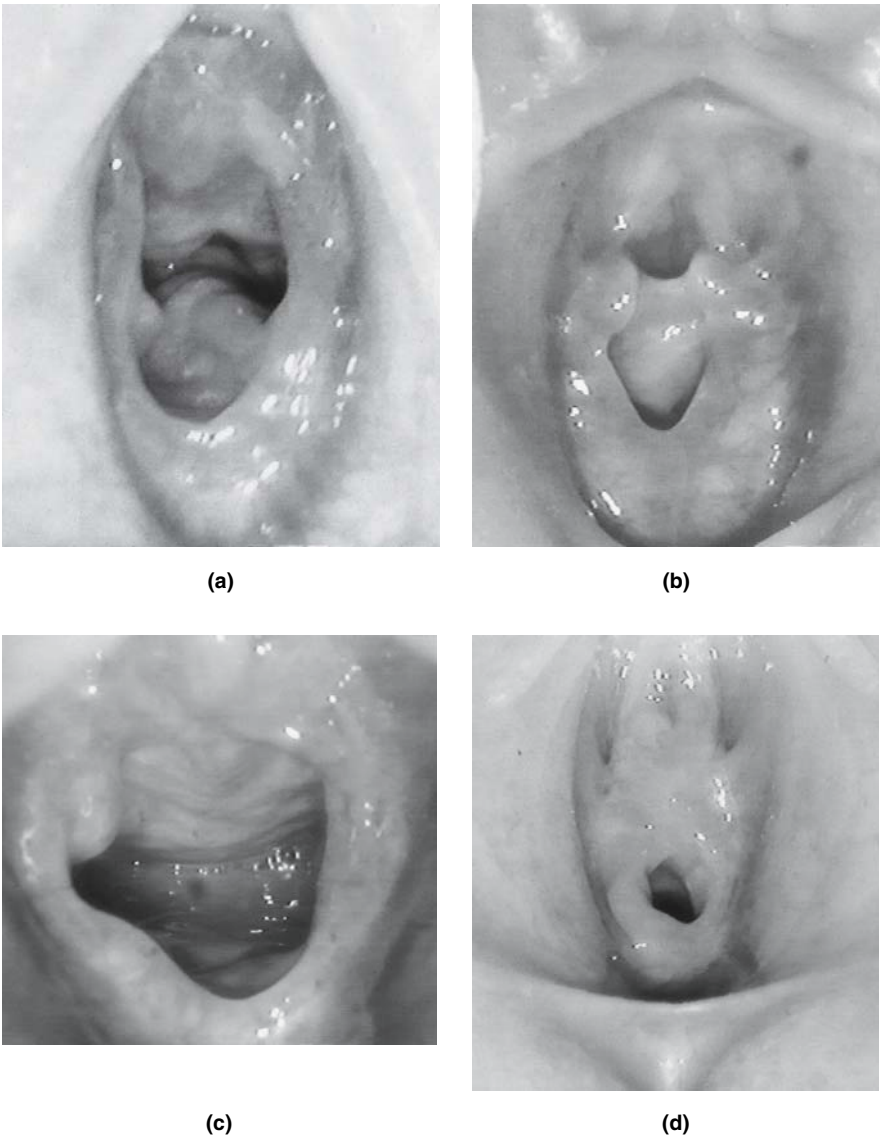


Fig. 1 (**a**) Normal prepubertal cresentic hymen with prominent intravaginal ridges at 4 and 9 o'clock. (**b**) An 18-month-old girl with a normal cresentic hymen with an anterior opening and deficiency of hymenal tissue at 12 o'clock. (**c**) A 6-year-old girl with a normal prepubertal hymen. There is a prominence at 9 o'clock and an intravaginal ridge at 11 o'clock. (**d**) The circumferential hymen of a 5-year-old girl with a folded edge and intravaginal ridges at 2 and 10 o'clock. There are periurethral ligaments present symmetrically on either side of the urethra

The importance of a clear knowledge of the features of normal development in children is one of the major reasons for ensuring a multi-professional approach to any child fatality. Cooperation between the examining forensic pathologist, paediatric pathologists, paediatric radiologists and paediatric forensic physicians is a vital component in the successful evaluation of such cases.

Definition

A variety of definitions of child abuse and child sexual abuse exist. Sexual abuse has been defined by the National Center for Child Abuse and Neglect as "contact or interaction between a child and an adult, when the child is being used for sexual stimulation of that adult or another person. Sexual abuse may be committed by a person under the age of 18, when that person is either significantly older than the victim, or when the abuser is in a position of power or control over that child" (3). Another definition is that it is "the involvement of dependent, developmentally immature children and adolescents in sexual activity that they do not fully comprehend, to which they are unable to give informed consent, or that violate the social taboos of family roles" (4, 5). Such activity includes not only genital, anal or orogenital contact between the child and the perpetrator but also noncontact abuse such as exhibitionism, voyeurism or using a child in pornographic films (1, 6, 7).

Prevalance

Child sexual abuse is found in all cultures and societies and has been documented in 2–62% of women and 3–16% of males, depending on the criteria that have been used (8). It has been reported that one-third of women and one-sixth of men will have experienced some form of sexual abuse by adulthood (4). In the USA, it has been estimated that more than 200,000 children are sexually assaulted each year with a prevalence rate of 45 cases per 100,000 children (9). In New Zealand, a prospective study of over 1000 children, 10.4% (17.3% of females and 3.4% of males), reported having experienced child sexual abuse before the age of 16 years (10).

Scene Examination

Victims of fatal sexual assault in childhood may be found at the scene of a homicide, may have been removed to another site with attempts made to conceal the body or may have been taken to hospital alive and subsequently died there of their injuries. In cases of in-hospital deaths, evaluation and documentation of anogenital injuries with appropriate sampling will most likely already have been undertaken according to in-house protocols by paediatric forensic physicians. On occasions, however, cases will still arrive at the mortuary with minimal or inadequate assessments.

When a victim has been found dead in the community with suspicious features, the case should be treated as a potential homicide, with all of the usual police and forensic procedures invoked. The death scene must be handled very carefully, with great care taken to meticulously document the circumstances of the body and its immediate surrounds. Extreme care must also be taken not to contaminate the body or scene with foreign DNA. Clothing specimens and the body must be evaluated in good light so that trace evidence is not lost.

The scene will be managed by police crime scene officers who will have set up a perimeter with an entrance point where a designated officer will control and document access and egress. The role of the forensic pathologist will be to try to determine the nature of the injuries, the cause of death and if possible the time of death. Circumstances may be suboptimal for these types of evaluations, particularly if the body has been concealed, as the lighting may be poor and the physical environment difficult. For example, examinations may be limited if bodies have been hidden in drainage ditches or pipes, in abandoned, structurally unsound buildings or in shallow graves. Under these circumstances, removal of the body to the mortuary may be the only means of obtaining appropriate access.

Determining time of death is not an easy task and becomes less accurate when the interval between death and examination is longer. Although standard nomograms are provided to assist with this, they do not apply to infants and very young children and rely upon relatively stable and undisturbed environmental temperatures. When sexual assault is suspected, it is preferable not to take a rectal temperature, as it may not be possible to definitely exclude the prospect that any injuries subsequently found in the rectum, such as superficial mucosal tears, were caused by the insertion of a glass thermometer. Alternative means of temperature assessment, such as inner ear probes, may be considered. Lividity and rigor mortis are of even less use in helping to determine a precise time of death and can provide broad indications only.

Concealment of a corpse and/or dumping of a body outdoors are associated with additional problems. Rain or water may result in the loss of valuable DNA evidence, although swabbing is always worthwhile, as positive results have been reported even after hours of submersion in water (11). Concealment of a corpse may also increase the likelihood of decomposition, which complicates postmortem assessment.

If there has been an injury that is associated with excessive bleeding, such as a stab wound, or blunt trauma to the head with deep scalp lacerations, care must be taken in transporting the body to avoid contaminating the clothing or body surfaces with the victim's blood, as this may obliterate traces of an offender's DNA. Wrapping of the victim's hands in paper bags is a useful method of ensuring that trace material such as hairs or skin under the nails is not dislodged and lost in transit to the mortuary.

Clothing

Assessment of clothing may be a vital part of the evaluation of cases of sexual assault. In fact the first indication that a sexual assault has occurred may be the absence of underwear, or the incorrect placement of clothing when a body has been redressed after death in an attempt to disguise what has occurred. While clothing will be handled by crime scene police officers, examination of the clothing for defects during the autopsy may help in the interpretation of wounds. Clothing may need to be dried if it is covered in blood or other fluids prior to storage to prevent mildew and fungal destruction of materials.

Autopsy Assessment

Although sexual abuse in children rarely leads to death (8), because of its prevalence, anogenital evaluation should be a routine part of the autopsy examination of children. Sexual molestation in the context of a child's family or perpetrated by an individual well known to the child rarely causes any significant physical injury. Therefore, most physical examinations of the genitals in sexually molested children of this type are reported as normal (12–16). Sometimes mild to minimal trauma may result from genital fondling or penetration outside of the hymen by a finger or other object; however, because injuries in this area heal quickly, no abnormality is likely to be found by the time medical examination occurs. Conversely, violent sexual assaults invariably cause significant genital injury, particularly in prepubertal children, and this is the group that is likely to comprise the majority of fatal sexual assaults in children.

As with all forensic cases, a meticulous examination of the external surfaces of the child's body and the bodily orifices is an essential part of the autopsy examination. This includes particular attention to the mouth, breasts, genitals, perineal region, buttocks and anus. In girls, the examination should include the inner aspects of the thighs, labia majora and minora, clitoris, urethra, periurethral tissues, hymen, hymenal opening, the vestibule (including the fossa navicularis) and posterior fourchette. In boys, this should include the inner aspects of the thighs, penile shaft, foreskin and glans and scrotum. In both boys and girls, the perineum and anus should also be carefully inspected in good light (7).

In cases of sexual assault with homicide, injuries associated with the sexual attack are usually non-lethal, with death being caused by other forms of trauma such as strangulation, or cranial injuries. Cases do occur, however, where death has resulted directly from the sexual assault. For example, haemorrhage and sepsis have resulted from forceful penetration of small vaginas and rectums with laceration of tissues, and small intestinal evisceration has been reported in a 4-year-old boy following foreign body insertion into the anus. Rarely fatal peritonitis may follow rectal perforation (17, 18). Possible causes of vulvar and vaginal bleeding are listed in Table 1.

Table 1 Cause of vulval and/or vaginal bleeding in prepubescent girls

Trauma
Foreign body
Vulvovaginitis
Urethral prolapse
Tumours
Haemangioma
Sarcoma botryoides
Clear-cell adenocarcinoma
Endodermal and mesonephric carcinomas
Lichen sclerosis et atrophicus
Endometrial bleeding
Neonatal oestrogen withdrawal
Early puberty
Exogenous oestrogen exposure
Ovarian neoplasia
Hypothalamic–pituitary axis conditions

Problems

Decomposition

Decomposition almost always complicates the assessment of possible injuries as skin discoloration and slippage masks wounds, and haemolytic staining of tissues may simulate bruising. Decomposition is also associated with loss of trace evidence and with contamination and loss of foreign DNA material. An associated problem is that of post-mortem animal depredation that may take the form of insect larval activity centred on wound and body orifices, thus altering the most significant areas that require evaluation (19). Ants, millipedes and cockroaches may create skin lesions that resemble abrasions and that may also passively ooze blood. Rodent activity is relatively easy to identify with characteristic double-grooved nibbles of the finger and toe tips, the nose and ears. Larger predators such as cats, dogs and foxes may consume significant amounts of skin and tissues. The end result of decomposition, skeletonisation, precludes definitive assessment of soft tissue injuries.

Artefacts

Over-interpretation of the significance of normal anatomical variants has occurred in the past (Fig. 2), with confusion regarding post-mortem anal dilatation and exposure of the anal mucosa and pectinate line (20–24). In a study of 65 deceased children aged from birth to 17 years, McCann et al. found that anal dilation and exposure of the pectinate line, resembling fissuring, was a

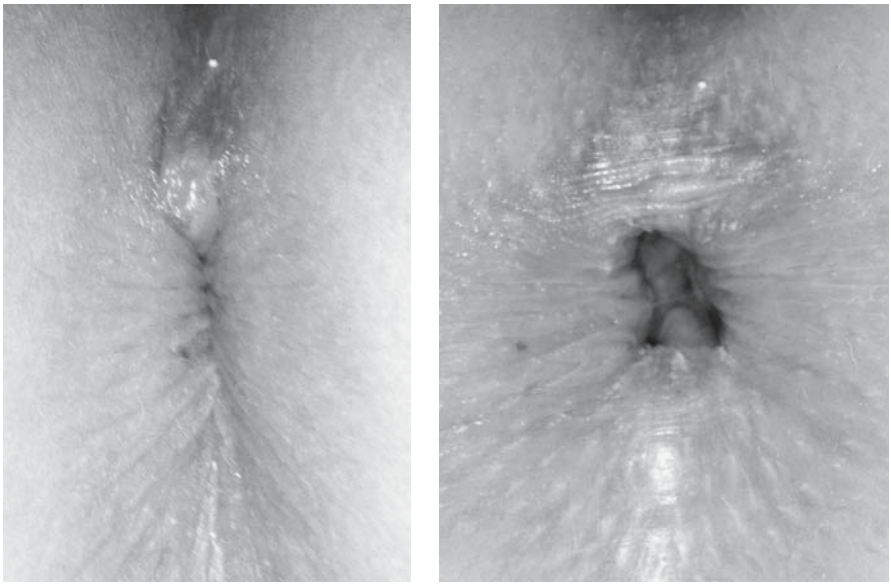


Fig. 2 (**a**) Anterior anal fold at 12 0'clock. (**b**) Separating the buttocks flattens the fold, an example of the effect of diastasis ani

common post-mortem finding. He did not regard anal dilatation as abnormal. Anal laxness may be more obvious in children with certain medical conditions such as chronic constipation, Crohn disease, myotonic dystrophy and cerebral injuries (22, 25, 26).

Considerable overlap also occurs in the dimensions of the hymenal opening in prepubertal children who have or have not experienced a penetrating injury (27–31). Thus, while the hymenal diameter in non-abused children at different ages and in different examination positions (i.e. knee-chest, traction, separation) has been recorded in living children (32), these measurements are not helpful in deciding in isolation whether or not a child has suffered a penetrating injury to the area.

Gardener, in a colposcopic study of 79 prepubertal girls aged between 3 months and 11 years 7 months with no evidence of sexual abuse, found a number of features that had previously been described in association with sexual abuse. These included increased vascularity in 44%, midline avascular areas in 27%, ragged posterior fourchette epithelium in 18%, notch configuration of the posterior fourchette in 10%, tethering between the hymen and perihymenal tissues in 14%, hymenal bumps between the 3 and 9 o'clock positions in 11% (Fig. 1c) and asymmetry of the hymen in 9% (21). McCann et al. reported similar findings in 93 prepubertal girls aged between 10 months and 10 years with no evidence of abuse. There was vestibular erythema in 56%, periurethral bands in 51% (Fig. 1c), labial adhesions in 39% (Fig. 3a) and midline avascular

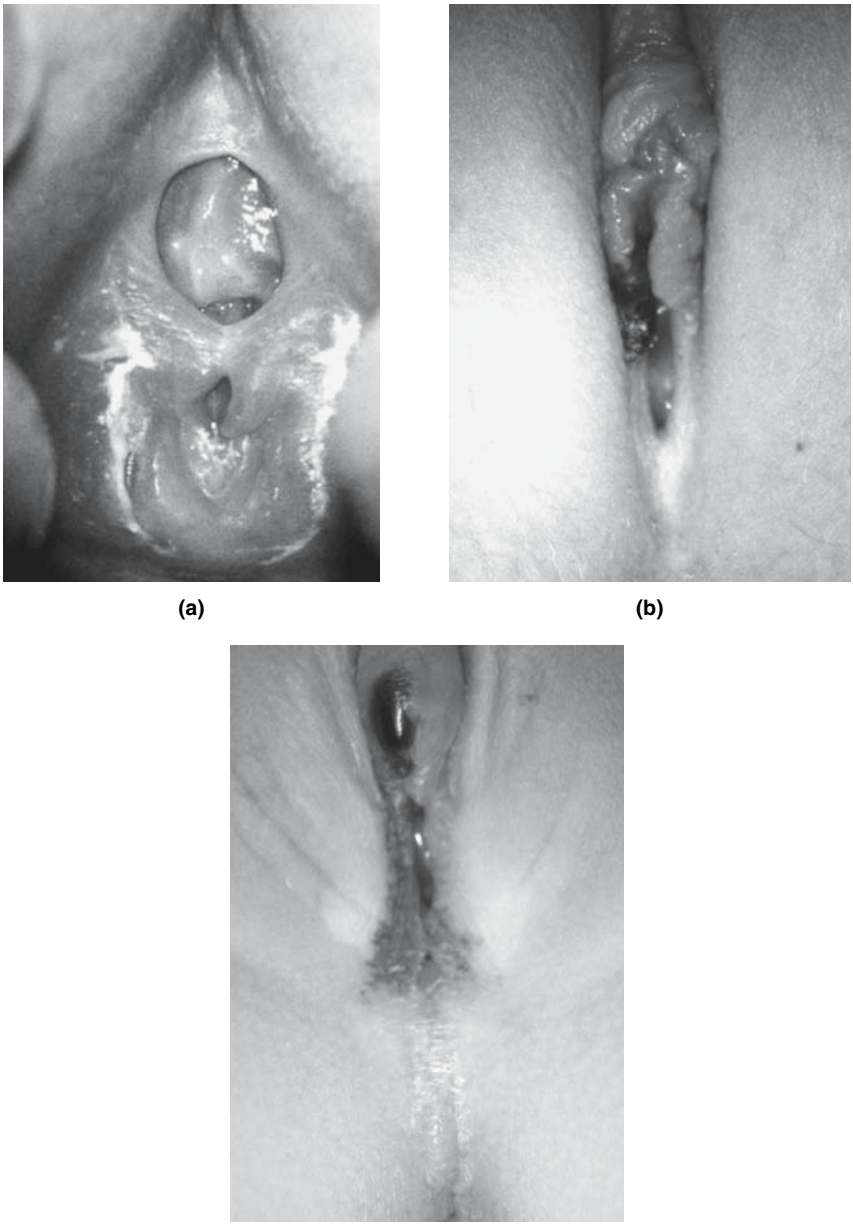


Fig. 3 (**a**) A three-year-old girl with central labial fusion and an anterior circular anterior opening with a smaller, slit-like posterior opening. (**b**) A 5-year-old girl with lichen sclerosis et atrophicus with a bullous haematoma over the lower part of the right labium minorum. (**c**) A 7-year-old girl with lichen sclerosis et atrophicus with abrasion, swelling and haemorrhagic blisters

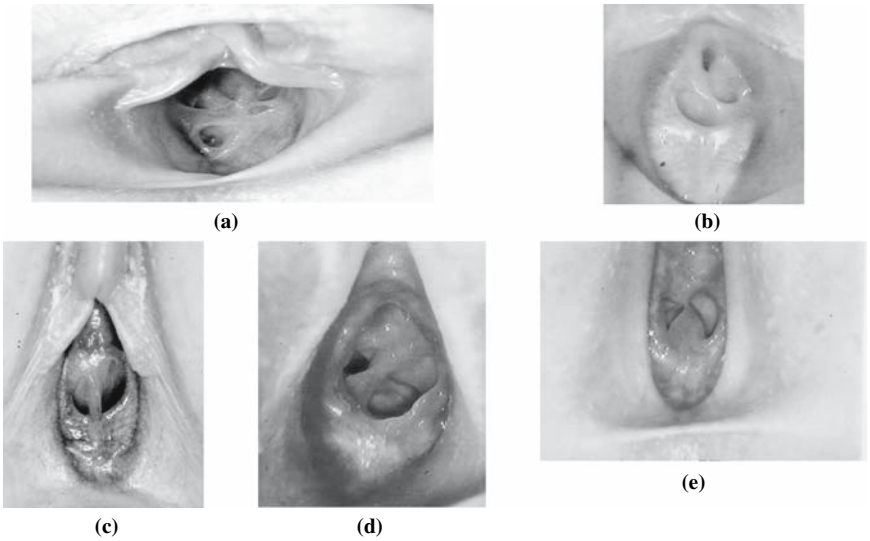


Fig. 4 (**a**) A 2-year-old girl with a normal cribriform hymen showing multiple openings. (**b**) A 3-year-old girl with an anteriorly placed septum producing two anterior openings in the hymen. (**c**) A 5-year-old girl with a midline septum of the hymen. (**d**) An 8-year-old girl with a septum arising from the posterior part of the hymen attached to the anterior vaginal wall. (**e**) A 8-year-old girl with a recently "broken septum" held to the anterior vaginal wall by surface tension

areas in 26% (33). Hymenal clefts, bumps ridges and tags (Fig. 4) have been described in a "significant" number of newborns (2, 9). Periurethral ligaments (Fig. 1d) that were once considered to be synechiae derived from injury are now considered normal structures, and other findings present in many children include lymphoid follicles in the hymen and surrounding tissues, hymenal mounds and projections, midline hymenal remnants and intravaginal longitudinal ridges (Fig. 1a) (2, 33).

Colposcopy

Examination of the anogenital region with the aid of the colposcope in fatally abused children provides an excellent means of visualising the area and documenting injuries. The colposcope illuminates and magnifies the areas being examined and enables both video and photographic recording of findings (33). One of the benefits of using a more structured examination approach, including the recording of the examination, has been to establish that certain findings that were once considered abnormal (as detailed above) are found in a significant number of normal, non-abused children (34).

Photography

As colposcopic facilities will not be available in every medical examiner's office and forensic facility, it is important to ensure that all external and internal abnormalities are fully documented by adequate photographs (35). Numbering of injuries during the autopsy prior to photography may assist in the correlating of findings for subsequent reports. Incising of possible bruises with photography and sampling for histology will also assist in differentiating lividity and erythema from genuine bruising, as this may be a query that is subsequently raised.

Samples

After an external examination has been performed that includes careful inspection of the entrances to the vagina and anus, a number of samples need to be taken that include swab and smears from the mouth, pharynx, nipples, skin, neck, hands, vulva, vestibule, vagina, endocervix and anus, with possible sampling of fingernails, head hair and pubic hair (the latter if the victim is post-pubescent). An approach to the handling of clothing and specimens is detailed in Table 2. Scanning of the body and clothes with an ultraviolet Wood's lamp may help to identify stains that can be swabbed to check for semen (36). Samples for faecal contamination of the penis should be taken if there is an indication that the victim had been forced to penetrate another's anus.

Although the presence of seminal fluid confirms that sexual contact has taken place, this is not found all that often and may be extremely scanty. Failure to ejaculate or ejaculatory dysfunction may characterise the perpetrator, and a previous vasectomy will result in failure to identify spermatozoa. Sperm survival is also reduced in prepubertal girls due to the lack of cervical mucus (37). In post-pubertal rape victims, prostatic acid phosphatase has been found 22 h, and sperm 12 h, after the event (37) (or even longer than this in our experience). Routine blood, fluid and tissue samples will be needed to be submitted for toxicological evaluation as the child victim may have been sedated to assist in removal from his or her home, to expedite the rape or to facilitate confinement.

Extragenital Injuries

General Injuries

As noted, infants and younger children generally do not have significant extra-genital injuries after non-fatal sexual assault (36). However, in the older child, a range of injuries may be present that reflect the nature of the assault with scattered bruises, abrasions and lacerations. In fatal cases, there may also be

Table 2 Sampling protocol for sexual assault

1. Clothing
Photographed on the body and documented
Removed without damaging or extending any existing tears or cuts
Handed to crime scene police officers (separate labelled paper bags)
Hairs or fibres on clothing photographed and either removed or retained with clothing
2. Swabs, smears and samples
General
External swabs before internal
External swabs moistened with distilled water
Internal swabs used dry (soaked in body secretions/fluids)
Each swab smeared on glass slide
Air dried prior to sealing
Labelled with name, case number, site and time
Swab and smear mouth, pharynx, nipples, skin, neck, hands, vulva, vagina, endocervix, anus
Specific
Mouth—along gum line, behind teeth and under tongue
Pharynx—tonsillar fossae
Vulva—introitus and labia
Low vagina—1–2 cm in depth
High vagina—upper vagina and posterior fornix
(Note—mark sample "? saliva ? semen" if oral sex is suspected)
Endocervix—canal (if there has been a delay between the assault and examination)
Anus—perianal
Rectum—first clean perianal area with swab soaked in distilled water to remove any semen that may have leaked from the vagina
Penis—shaft and glans
Skin—bite marks, nipples, neck and hands, plus any areas of possible semen or saliva (swabs for saliva require a wet swab, then a dry swab and also a control)
Other
Faecal matter—swabs from the penile shaft and glans
Vaginal washings—with buttocks elevated, wash out vagina with 20 ml of normal saline in a syringe and submit for semen analysis
Pubic hair combings—onto paper for foreign hair or other trace material
Fingernail cuttings/swabs—right and left hands separately—nails swabbed and then cut
Controls
Blood spots on paper for victim's DNA reference sample
Head hair—at least 20 hairs plucked with the roots
Pubic hair—at least 15 hairs plucked with the roots
Saliva control—swab moistened with distilled water from an area away from likely oral contact
3. Routine samples for toxicology
As per local toxicology laboratory requirements/protocols

bruising around the neck with abrasions from fingernail scratches in cases of manual strangulation, or circular parchmented ligature marks if a rope or cord has been used. Blunt head trauma may have resulted in extensive lacerations of the scalp, with or without underlying skull fractures. Circumferential reddening, abrasions and bruises around the wrists and ankles may indicate forcible restraint with ligatures, and abrasions at the corners of the mouth may result from gagging.

More Specific Injuries

Certain other types of injury may reflect the sexual motivation of the attack.

Breasts: These may show oval "fingertip" bruising from grasping or squeezing, with fingernail scratches from the assailant. Bite marks if present should be swabbed. In sadistic attacks, the nipples may have been bitten or cut off.

Thighs: There may be bruising of the inner thighs from forcing the legs apart.

Back: Pressure of the assailant on the victim may have caused abrasions or markings on the buttocks or upper back that may be covered with vegetation or material from the rape scene. This material should be sampled, particularly if it is thought that the body may have been subsequently moved.

Mouth and lips: There may be bruising of the inner lips and gums from forceful kissing, or from blows with a hand.

Skin: Bite marks may be centred around the breasts, neck and buttocks. These may appear as semicircular bruises or abrasions that may be able to be matched to an assailant's dental arcade. Bites may also show round to oval suction lesions with scattered petechial haemorrhages. Linear abrasions may be present if the teeth have been dragged across the skin surface, and deep lacerations may occur in particularly savage attacks. Bites should be swabbed for DNA as soon as possible and then examined by a forensic odontologist. Careful photographs and measurements should also be made to facilitate comparisons with possible perpetrator's dentition at a later stage. In particularly vicious sexual murders, there may also be mutilation of the victim, with multiple stab wounds centred around the breasts and perineum, with or without foreign body insertion into the anus or vagina. Patterns may have been cut into the skin.

Anogenital Injuries

Injuries to the perineum may indicate a variety of possibilities including rape prior to homicide, genital mutilation from foreign objects, either pre- or post-mortem or anogenital injury as part of an overall pattern of assault, such as from kicking or stomping. The first indication on external examination of sexual assault may be swelling and bruising of the perineal area or blood seepage. The likelihood of significant trauma occurring from penetration

increases as the age of the child decreases and reflects mismatch in the size of the genital and hymenal opening size and the penetrating object. Injuries may arise from digital, foreign body or penile penetration of the vagina, intra-crural penile friction, lingual stimulation of the genital area and digital, foreign body or penile penetration of the anus (24). Injuries may heal very quickly without significant residual abnormalities or complications (36). Old, healed injuries may also be difficult to assess and may be represented by subtle scarring of the fourchette, navicular fossa or hymen, or thickening and scarring of the anal verge (24). As has been emphasised, it is important to avoid over-interpretation of normal morphological variations (26). Genital findings in abused children have been classified by Muram (Table 3) and Adams (9, 38, 39). While the use of classifications in helping to establish whether or not a child has been sexually molested is limited, they do provide general guidance to the practitioner.

The types of lesions that may occur are listed below.

Vulva: There may be reddening, bruising or abrasions of the labia and vestibular area with lacerations that range from superficial skin tears to deep wounds linking the vagina with the anus. Abrasions of the vestibule may also result from scratching by a perpetrator's fingernails. Swelling and petechial haemorrhages may also be present (24).

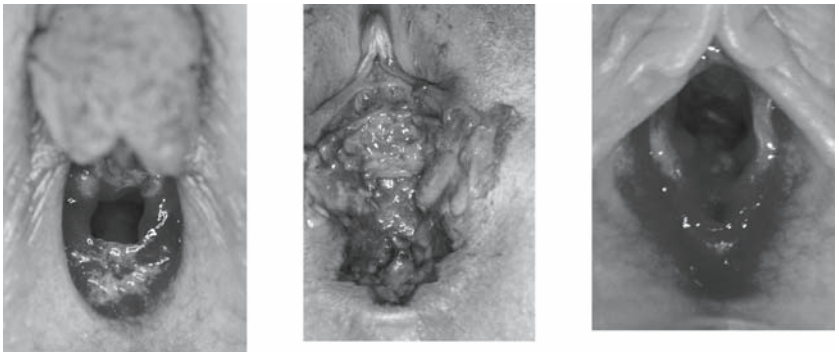
Hymen: While the hymen may stretch in post-pubertal children and may not rupture during forced penetration, infants and younger children may show hymenal rupture with laceration and bleeding (Fig. 5). This is most likely when the penetrating object exceeds the size of the hymenal opening. Hymenal injury is strongly suggestive of penetrating sexual molestation, even though a penetrating injury can occur from incidents of trauma that are not sexually motivated (Fig. 6).

In infancy, the hymen is thick and elastic due to hormonal effects. While this gives it a degree of resilience to trauma, the small calibre of the entrance to the infant vestibular area means that penetration into the vestibule could cause injury, depending on the size of the penetrating object. As the effects of maternal hormones decline, the hymen thins out and becomes more vulnerable to injury. Penetration of prepubertal girls with this type of hymen, therefore, usually results in tearing. Tears, which extend the full width of the hymen from the opening to the vestibular wall, are called transections and are usually found in the posterior aspect of the hymen (Fig. 6). Lacerations to the hymen caused by penetration may be partial or complete. In infants and young girls

Table 3 Genital findings in cases of sexual abuse in girls

Category 1	Normal appearing genitalia
Category 2	Non-specific findings: erythema, discharge, small lacerations/fissures of posterior fourchette
Category 3	Specific findings: recent or healed lacerations of the hymen or vagina, bite marks, venereal disease
Category 4	Definite findings: sperm

Adapted from [38]

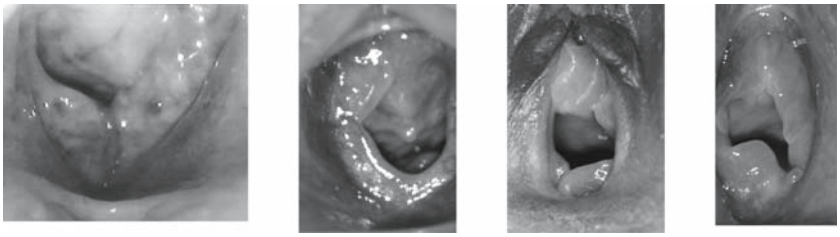


(a) (b) (c)

Fig. 5 (**a**) A 7-year-old girl who complained of oral sexual contact in her genital area from an adult male demonstrating a swollen and haemorrhagic hymen. (**b**) A 3-year-old girl who had a soft-drink bottle forced into her genital area 7–10 days previously showing an untreated, infected second degree tear. (**c**) A 6-year-old girl who was attacked by an adult male while she was waiting at a bus stop after school. A recent (within 2 h) injury due to digital penetration is present in the posterior part of the hymen at 6 o'clock with swelling and haemorrhage

penetrated by an adult male penis, the laceration may extend through the navicular fossa onto the skin surface at the position of the posterior fourchette (40, 41).

In adolescence, the hymen is again thickened and elastic due to the effect of pubertal hormones. Whether or not injury occurs from penetration depends primarily on the hymenal opening size, the degree of elasticity and the state of relaxation of the genital tissues. In this age group, tears may be



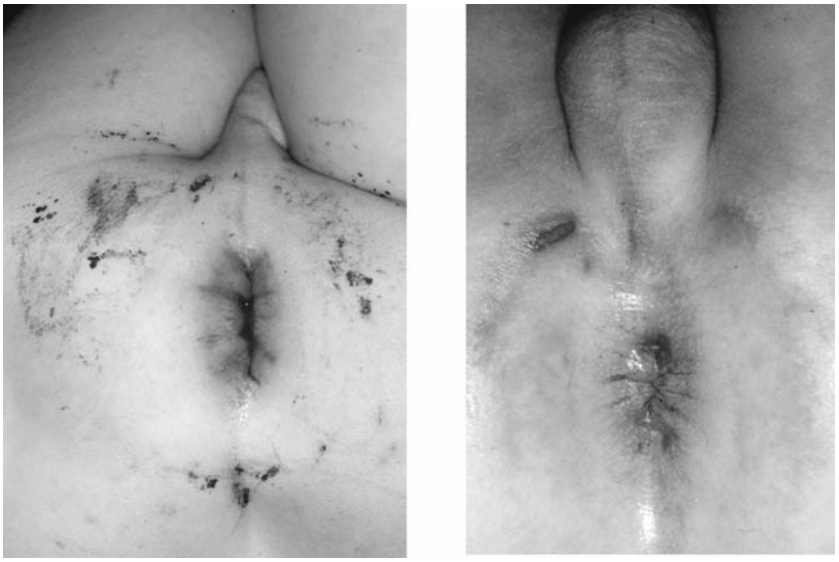
(a) (b) (c) (d)

Fig. 6 (**a**) An 11-year-old girl with a recent penetrating injury to the hymen. Swelling and areas of hymenal haemorrhage and bruising are present with transection at 6 o'clock. (**b**) A 7-year-old girl with a notch at 9 o'clock from digital penetratation that occurred 3 months previously. (**c**) A 6-year-old with a thickened prepubertal hymen. A scar and cleft at 8 o'clock are due to previous transection secondary to a penetrating injury. (**d**) An 8-year-old girl with a thickened hymen and a cleft at 5 o'clock from a previous transection secondary to a penetrating injury

partial or complete (transection) and tend to occur more laterally (4 and 8 o'clock) than in younger ages due to the protective effect of the posterior vaginal ridge (36).

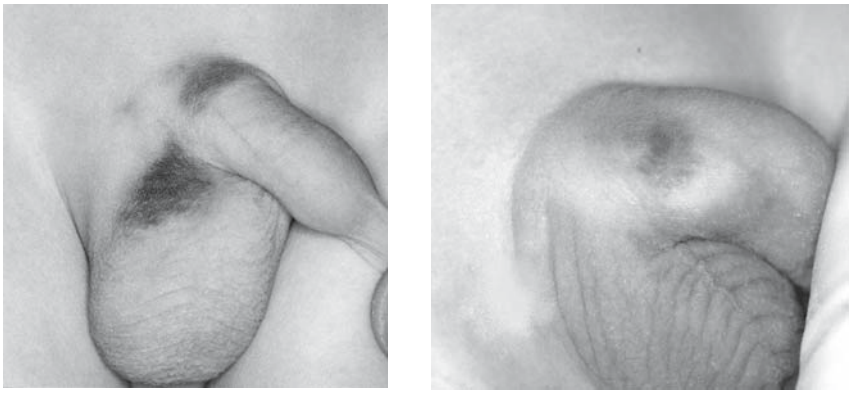
The appearance of an acutely lacerated hymen in the very young is of a "v"-shaped tear with clean edges (Fig. 6a). Bleeding may be insignificant. The laceration becomes covered with a mucoid inflammatory infiltrate that disappears after about 7–10 days (42, 43). In adolescents, the edges of the lacerations are less regular and there is swelling of the hymen with petechial haemorrhages, abrasions and bruising. Hymenal lacerations may heal well and may leave minimal scarring (Fig. 6b–d). Alternatively, no healing may occur and the free edges of the laceration may reabsorb leading to a very narrow or absent rim of hymen where the laceration meets the vaginal wall. Attempted dating of recent injuries to the hymen and the evaluation of possible old injuries are most appropriately undertaken by paediatric forensic physicians with colposcopic evaluation.

Vagina: Again the amount of injury depends on the degree of trauma and also on the disproportion between the size of the penetrating object or adult penis and the infant/child's vaginal canal. In older children, there may be



(a) **(b)**

Fig. 7 (**a**) The recent sexual assault of an 18-month-old boy with penile penetration of his anus resulting in swelling and bruising of his perianal area with a slit-like anal opening and radially distributed abrasions and lacerations. The assault occurred 3 h before the examination. (**b**) The recent sexual assault of a 3-year-old boy with penile penetration of his anus showing an abrasive injury laterally, and at 12 o'clock, from penile friction. The anus is swollen and bruised, with circumferential lacerations



(a) (b)

Fig. 8 (**a**) Bruising of the base of the penis with tracking into the upper part of the scrotum in an 18-month-old boy. (**b**) A 2-year-old boy with a probable insect bite to his penile shaft

minimal injury with slight erythema of the mucosa, whereas in infants there may be significant lacerations and tearing which is often in the posterior vaginal wall. Foreign bodies such as knives, broom handles or screwdrivers may have been inserted into the vagina causing very significant injuries with blood loss.

Anus:– Although there may be perianal bruising from forcing the buttocks apart to assist with anal penetration, there are often few signs of injury to suggest that sodomy has occurred. This may in part be due to the ability of the anus to dilate, even in the young. Injury is also less likely if a lubricant has been used. The injuries manifest as bruising, radial fissuring to the anal verge and swelling of the anal opening (Fig. 7). More severe injuries, including perforation into the peritoneal cavity, are more likely to occur with foreign body insertion.

Penis: The penis is not usually damaged during sexual assault in childhood although there may be swelling associated with bite mark or scratches. The usual scenario where injuries to the penis occur involves physical abuse with hitting, pinching or twisting, sometimes associated with attempts to toilet train. Alternative causes of reddening and swelling, such as insect bites, should be considered (Fig. 8).

Other

Pregnancy may be found in victims of sexual assault and may have initiated the lethal attack in an attempt to obscure evidence of rape.

Special Dissections

Pelvic Exenteration

Once the vulva, vagina and anus have been carefully examined from the exterior, with recording and photographing of injuries, special dissection needs to be undertaken. This consists of a circular incision around the perineum inside the rim of the pelvis. Some pathologists also remove the anterior part of the pelvis to facilitate en bloc removal of the pelvic organs. The organ block is then accessible to detailed dissection. This commences with opening of the vagina with scissors along the anterior aspect, placing the cut to avoid any injuries. This enables the vagina to be opened in its entirety so that any mucosal lesions or injuries can be clearly seen, examined, measured and photographed. This also gives good exposure to the cervix and enables the uterus to be opened. The block can then be turned over and the anus and rectum can be opened in a similar fashion posteriorly, again avoiding any injuries with the scissor cut, and a similar assessment made.

Venereal Diseases

The presence of a sexually transmitted disease in an infant or a prepubertal child may be an indication of previous sexual contact, and microbiology swabs for gonorrhoea should be taken if this is suspected. Blood tests for syphilis, HIV and hepatitis B can also be ordered. Perinatal transfer of gonorrhoea, chlamydia, trichomonas, genital herpes, venereal warts, syphilis and vaginal candidiasis has, however, been documented (36).

In the post-natal period, gonorrhoea is taken as evidence of probable sexual contact, as is syphilis, if it is primary or if there has been no neonatal exposure. Chlamydia, trichomonas, venereal warts (which may first appear after vertical transmission up to 5–6 years of age) and type 2 genital herpes infection may indicate that a child has experienced sexual contact although autoinoculation and nonsexual contact have also been reported (36, 44).

Differential Diagnosis

A number of conditions may mimic the physical abnormalities that are usually associated with sexual molestation, and detailed texts illustrating normal anogenital anatomy in childhood are available (1). Causes of vaginal bleeding in prepubertal girls that are not due to sexual assault (Table 1) include group A streptococcal and Shigella sp. vaginitis, urethral prolapse,

tumours, lichen sclerosis et atrophicus (Fig. 3b and c) and intravaginal foreign bodies (37).

Anogenital Injury Not due to Sexual Abuse

Straddle-type injuries in girls may result in labial bruising that may be unilateral and not usually associated with trauma to the hymen (Fig. 9). The underlying mechanism is compression of soft tissues between the pubic symphysis and rami, and the object being straddled causing bruising and injury to the anterior aspect of the labia majora and minora, the lower urethra, the clitoris and the mons pubis. The injuries do not usually extend into the more internal genital structures and are often linear in configuration (45). Injuries to the posterior fourchette may also occur following a fall or with forceful separation of the legs during gymnastics or from falling on to an object such as a roller blade (46). In a study of 87 girls attending a paediatric emergency department with genital injuries, 74 (85%) were due to straddle injuries, 5 were due to accidental penetrating injures and 3 were due to stretch injures. Two girls had self-inflicted scratch injuries and sexual abuse was alleged at presentation in three cases (47).

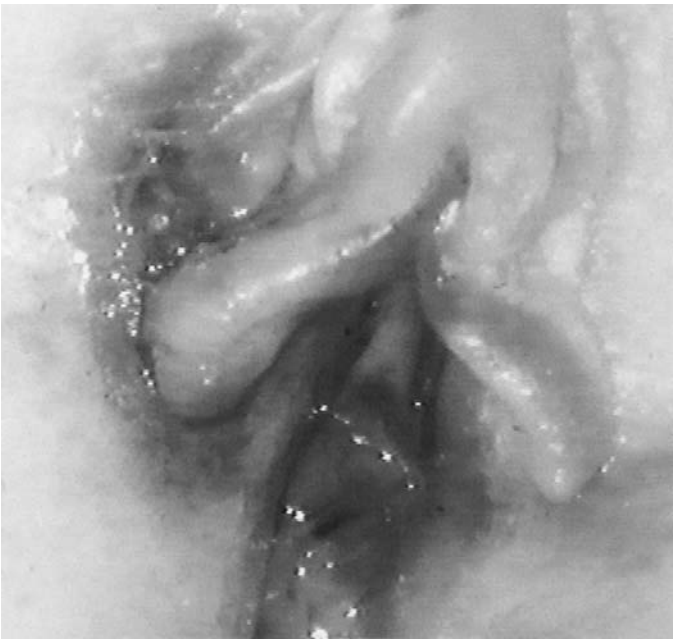


Fig. 9 A 4-year-old girl who fell astride the edge of a bath as she was climbing out. Bruising is present on the right labia minorum with laceration and is also present over the urethral area betweeen the labia in the 12 oã clock position of the vestibule

As with most types of significant trauma, medical assistance is usually sought immediately and a consistent and plausible history of the incident of injury is volunteered. The findings on examination should also be in keeping with the history of trauma.

Skin Conditions

Erythematous rashes around the perineum may be caused by a variety of irritating materials or substances, such as washing detergents, and may result in a child scratching and producing significant genitocentric excoriations.

Haemangiomas of the labia may be mistaken for bruising; however, this can be dispelled readily at autopsy by incising the lesion and demonstrating no subcutaneous extravasation of red blood cells. Histology can be used to confirm the presence of a benign vascular lesion.

Lichen sclerosis et atrophicus (Fig. 3b and c) is an inflammatory skin condition characterised by the formation of white plaques with skin atrophy. Fifteen percent of cases of this chronic disorder occur in children where the presentation may include itching and genital bleeding. The condition involves only the skin of the perineum and not the mucosa of the hymen or vestibule. The areas frequently affected are the anus, posterior fourchette and clitoris. The lesions bruise easily and are vulnerable to infection. They are also pruritic and scratching may again produce lesions that raise the possibility of abuse (48). Henoch-Schonlein purpura may appear on the genitals before the more characteristic rash appears elsewhere (49).

Vulvovaginitis

Young girls are susceptible to vulvovaginitis due to poor hygiene in association with a thin vaginal mucosa that is less resistant to infection. Vulvovaginitis may be caused by non-specific polymicrobial infection related to poor hygiene or the presence of a foreign body (e.g. toilet paper). The area may also become secondarily infected from a urinary tract infection, or there may be specific primary venereal infection due to sexual abuse/assault (48).

Foreign Bodies

Foreign bodies left within the vagina produce foul, blood-stained discharges with considerable inflammatory reaction. The material often consists of fragments of toilet paper (48).

Tumours

Haemangiomas are benign tumours that may result in bleeding if there is traumatic disruption of the delicate microvasculature. Less commonly sarcoma botryoides or embryonal rhabdomyosarcoma may occur in young girls and result in vaginal discharge and bleeding. Other tumours in older children that may present in a similar manner are endodermal carcinoma, mesonephric carcinoma and clear-cell adenocarcinoma, the later associated with maternal exposure to diethylstilbestrol (48).

Endometrial Bleeding

Another cause of blood within the vagina is endometrial bleeding. This may occur in neonates as a result of maternal oestrogen withdrawal, or it may be a normal finding in older girls with an early menarche. Exposure to exogenous oestrogen in medication may cause endometrial bleeding, as may certain ovarian lesions such as functional ovarian cysts and granulosa cell tumours. Children with central nervous system conditions ranging from tumours to previous skull fractures and meningitis or encephalitis have all had documented precocious puberty, possibly related to some ill-understood effect on the hypothalamic–pituitary axis. Finally, children with McCune Albright syndrome who have cutaneous pigmentation and polyostotic fibrous dysplasia also have precocious puberty (48).

Congenital Anatomical Variants

Hymen: A range of congenital variations may alter the morphology of the hymen, including cribriform and imperforate hymens and asymmetry of the hymenal orifice. The hymen may be septate or show evidence of remnants of a septum such as a small tag over the posterior part of the hymenal opening.

Perianal tissues: Smooth, wedge-shaped areas in the anterior or posterior midline of the anal verge may be confused with scarring. These are, however, present in 26% of non-abused children and represent normal splitting of the underlying external anal sphincter. Known as diastasis ani, there is often an overlying dimple or depression associated with the muscle defect (32).

"Perianal grooves" have been confused with healed lacerations or fissures. They are congenital variations that cause shallow depressions that run along the median raphe from the fossa navicularis to the internal anal sphincter. These grooves result from failure of skin fusion over the perineal body during intrauterine life. They are not traumatic in origin, are usually completely asymptomatic and eventually become obliterated by normal skin (32). Perianal

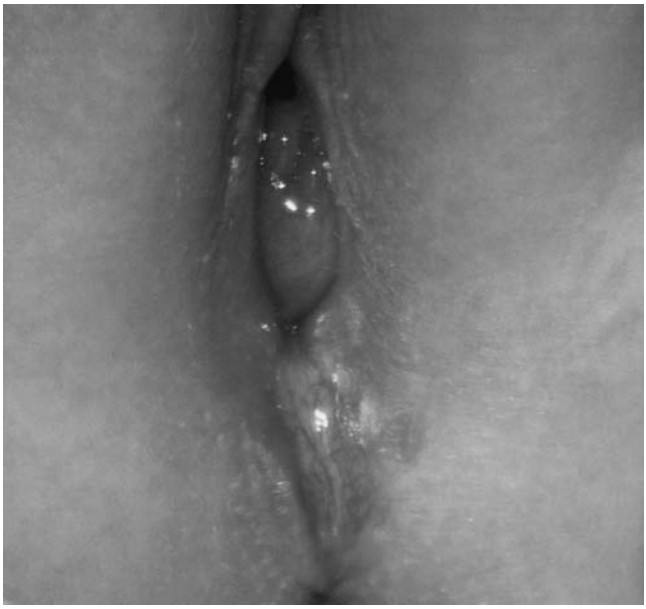


Fig. 10 A 6-month-old girl with a congenital midline fusion defect which stretches from the posterior fourchette to the perineum

skin folds are also lesions that may be confused with healed injuries. These are always found in the midline and are most often anterior to the anus (Fig. 10).

Urethral Prolapse

Urethral prolapse or caruncle is seen as a reddish mass at the external urethral orifice and may present with apparent vaginal bleeding or staining of underpants with blood (50). It is often difficult to diagnose, particularly when small.

Summary

While sexual abuse/assault is not often associated with a lethal outcome in childhood, cases do occur where a victim has died either as a result of injuries inflicted during sexual abuse or as a consequence of a subsequent homicidal assault. All such cases need to be evaluated meticulously, with multi-professional interaction including the involvement of forensic paediatricians. Lesions may be subtle, and misinterpretation of normal developmental variations as

well as signs of recent or old injury may occur. Proper photographic or video documentation of lesions and injuries is necessary using colposcopy and standard cameras. Comprehensive forensic sampling and testing is also required with the development and use of appropriate scene and autopsy protocols to facilitate the standardisation of investigation and documentation (51).

References

1. Giardino AP, Datner EM, Asher JB, eds. Sexual Assault. Victimization Across the Life Span. A Clinical Guide. St. Louis: GW Medical Publishing Inc., 2003.
2. Berenson AB. Normal anogenital anatomy. Child Abuse Negl 1998; 22: 589–596.
3. Muram D. Rape, incest, trauma: the molested child. Clin Obstet Gynecol 1987; 30: 754–761.
4. Krugman RD. Recognition of sexual abuse in children. Pediatr Rev 1986; 8: 25–30.
5. Kempe CH. Sexual abuse: another hidden pediatric problem. Pediatrics 1978; 62: 382–389.
6. Finkel MA, DeJong AR. Medical findings in child sexual abuse, Chapter 9. In Reece RM, ed. Child Abuse, Medical Diagnosis and Management. Baltimore, Williams and Wilkins, 1996, pp. 185–247.
7. American Academy of Pediatrics Committee on Child Abuse and Neglect. Guidelines for the evaluation of sexual abuse of children. Pediatrics 1991; 87: 254–260.
8. Johnson CF. Child sexual abuse. Lancet 2004; 364: 462–470.
9. Muram D. The medical evaluation in cases of child sexual abuse. J Pediatr Adolesc Gynecol 2001; 14: 55–64.
10. Fergusson DM, Lynskey MT, Horwood LJ. Childhood sexual abuse and psychiatric disorder in young adulthood: I. Prevalence of sexual abuse and factors associated with sexual abuse. J Am Acad Child Adolesc Psychiatry. 1996; 35: 1355–1364.
11. Sweet D, Shutler GG. Analysis of salivary DNA evidence from a bite mark on a body submerged in water. J Forensic Sci 1999; 44: 1069–1072.
12. Berenson AB, Chacko MR, Constance M Wiemann CM, Clifford O Mishaw CO, Friedrich WN, James J Grady JJ. A case-control study of anatomic changes resulting from sexual abuse. Am J Obstet Gynecol 2000; 182: 820–834.
13. Finkel MA. Anogenital trauma in sexually abused children. Pediatrics 1989; 84: 317–322.
14. Muram D. Anal and perianal abnormalities in prepubertal victims of sexual abuse. Am J Obstet Gynecol 1989; 161: 278–281.
15. Adams JA, Harper K, Knudson S, Revilla J. Examination findings in legally confirmed child sexual abuse: it's normal to be normal. Pediatrics 1994; 94: 310–317.
16. Adams JA, Knudson S. Genital findings in adolescent girls referred for suspected sexual abuse. Arch Pediatr Adolesc Med. 1996; 150: 850–857.
17. Orr CJ, Clark MA, Hawley DA, Pless JE, Tate LR, Fardal PM. Fatal anorectal injuries: a series of four cases. J Forensic Sci 1995; 40: 219–221.
18. Press S, Grant P, Thompson VT, Milles KL. Small bowel evisceration: unusual manifestation of child abuse. Pediatrics 1991; 88: 807–809.
19. Byard RW, James RA, Gilbert JD. Diagnostic problems associated with cadaveric trauma from animal activity. Am J Forensic Med Pathol 2002; 23: 238–244.
20. Emans SJ. Sexual abuse in girls: what have we learned about genital anatomy? J Pediatr 1992; 120: 258–260.
21. Gardner JJ. Descriptive study of genital variation in healthy, nonabused premenarchal girls. J Pediatr 1992; 120: 251–257.

22. McCann J, Reay D, Siebert J, Stephens BG, Wirtz S. Postmortem perianal findings in children. Am J Forensic Med Pathol 1996; 17: 289–298.
23. McCann J, Voris J, Simon M. Labial adhesions and posterior fourchette injuries in childhood sexual abuse. Am J Dis Child 1988; 142: 659–663.
24. Paul DM. The pitfalls which may be encountered during an examination for signs of sexual abuse. Med Sci Law 1990; 30: 3–11.
25. Reardon W, Hughes HE, Green SH, Woolley VL, Harper PS. Anal abnormalities in childhood myotonic dystrophy—a possible source of confusion in child sexual abuse. Arch Dis Child 1992; 67: 527–528.
26. Robinson R. Physical signs of sexual abuse in children. Br Med J 1991; 302: 863–864.
27. Wissow LS. Child abuse and neglect. N Engl J Med 1995; 332: 1425–1431.
28. Berenson AB, Chacko MR, Wiemann CM, Mishaw CO, Friedrich WN, Grady JJ. Use of hymenal measurements in the diagnosis of previous penetration. Pediatrics 2002; 109: 228–235.
29. Heger AH, Ticson L, Guerra L, Lister J, Zaragoza T, McConnell G, Mary Morahan M. Appearance of the genitalia in girls selected for nonabuse: review of hymenal morphology and nonspecific findings. J Pediatr Adolesc Gynecol 2002; 15:27–35.
30. Kellogg ND, Menard SW, Santos A. Genital anatomy in pregnant adolescents: "normal" does not mean "nothing happened". Pediatrics 2004; 113: e67–e69.
31. Pugno PA. Genital findings in prepubertal girls evaluated for sexual abuse—a different perspective on hymenal measurements. Arch Fam Med 1999; 8: 403–406.
32. McCann J, Wells R, Simon M, Voris J. Genital findings in prepubertal girls selected from non-abuse: a descriptive study. Pediatrics 1990; 86: 428–439.
33. McCann J. Use of the colposcope in childhood sexual abuse examinations. Pediatr Clin North Am 1990;3 7: 863–880.
34. O'Connell BJ. Evaluation of the sexually abused child, including the role of colposcopy. J Low Genit Tract Dis 2001; 5: 87–93.
35. Ricci LR. Photodocumentation of the abused child. Chapter 10. In Reece RM, ed. Child Abuse, Medical Diagnosis and Management. Baltimore, Williams and Wilkins, 1996, pp. 248–265.
36. McCann J, Rosas A, Boos S. Child and adolescent sexual assaults (childhood sexual abuse). Chapter 29. In Payne-James J, Busuttil A, Smock W London, eds. Forensic Medicine: Clinical and Pathological Aspects. Greenwich Medical Media, 2003, pp. 453–468.
37. Paradise JE. The medical evaluation of the sexually abused child. Pediatr Clin North Am 1990; 37: 839–862.
38. Muram D. Classification in genital findings in prepubertal girls who are victims of sexual abuse. Adolesc Pediatr Gynecol 1988; 1: 151–152.
39. Adams JA. Evolution of a classification scale: medical evaluation of suspected child sexual abuse. Child Maltreat 2001; 6: 31–36.
40. Emams SJ, Woods ER, Flagg NT, Freeman A. Genital findings in sexually abused, symptomatic and asymptomatic girls. Pediatrics 1987; 79: 778–785.
41. Bays J, Chadwick D. Medical diagnosis of the sexually abused child. Child Abuse Negl 1993; 17: 91–110.
42. McCann J. The appearance of acute healing and healed anogenital trauma. Child Abuse Negl 1998; 22: 605–615.
43. McCann J, Voris J. Perianal injuries resulting from sexual abuse: a longitudinal study. Pediatrics 1993; 91: 390–397.
44. Ross JDC, Scott GR, Bussutil A. Condylomata acuminata in pre-pubertal children. Med Sci Law 1993; 33: 78–82.
45. West R, Davies A, Fenton T. Accidental vulval injuries in childhood. Br Med J 1989; 298: 1002–1003.
46. Wynne JM. Injuries to the genitalia in female children. S Afr Med J 1980; 57: 47–50.

47. Pierce AM, Robson WJ. Genital injury in girls—accidental of not? Pediatr Surg Int 1993; 8: 239–243.
48. Muram D. Vaginal bleeding in childhood and adolescence. Obstet Gynecol Clin North Am 1990; 17: 389–408.
49. Muram D, Levitt CJ, Frasier LD, Simmons KJ, Meritt DF. Genital injuries. J Pediatr Adolesc Gynecol 2003; 16: 149–155.
50. Bays J, Jenny C. Genital and anal conditions confused with child sexual abuse. Am J Dis Child 1990; 144: 1319–1322.
51. Byard RW. Sudden Death in Infancy, Childhood and Adolescence, 2nd edn. Cambridge: Cambridge University Press, 2004.

Elder Abuse and Gerontocide

Klaus Püschel

Introduction

The ever-expanding growth of the geriatric population increases the likelihood of elder abuse and neglect to occur, both in public and private settings. The fact that the elderly control 70% of the Europe's wealth marks them as tempting targets for deceitful relatives, swindlers, burglars and violent perpetrators.

Epidemiological studies concerned with the "greying phenomenon" estimate that the world-wide population of individuals older than 65 years will surpass 1 billion people in 2030. This trend reflects a drastic increase in the global population. Even in many third-world countries the life expectancy has increased to 80 years. Concomitant with the growing geriatric population, demands on medical and public health services are expected to increase (1). A considerable growth in the long-term health care costs of older adults will be attributable to a greater prevalence of chronic diseases, various disabilities and illnesses related to injuries as well as mental dysfunction. By 2040, the elderly will comprise 20% of the world's population. The estimated number of individuals 80 years of age and older, will double or triple. Hence, the age-pyramid of developed countries, whose shape today may be compared to that of a pine tree, will transform its silhouette to reflect a figure with a diminishing base, representing populations of children and young adults, and an expanding apex comprised of the middle and older adult populations (Fig. 1).

Violence against the elderly poses a public health problem with serious consequences (2–8). Incidences of elder maltreatment in well-developed countries are estimated to occur in 5–10% of the aging population. The following chapters will focus on violence and crimes against the elderly, specifically as they relate to German-speaking regions.

G.N. Rutty (ed.), *Essentials of Autopsy Practice*,

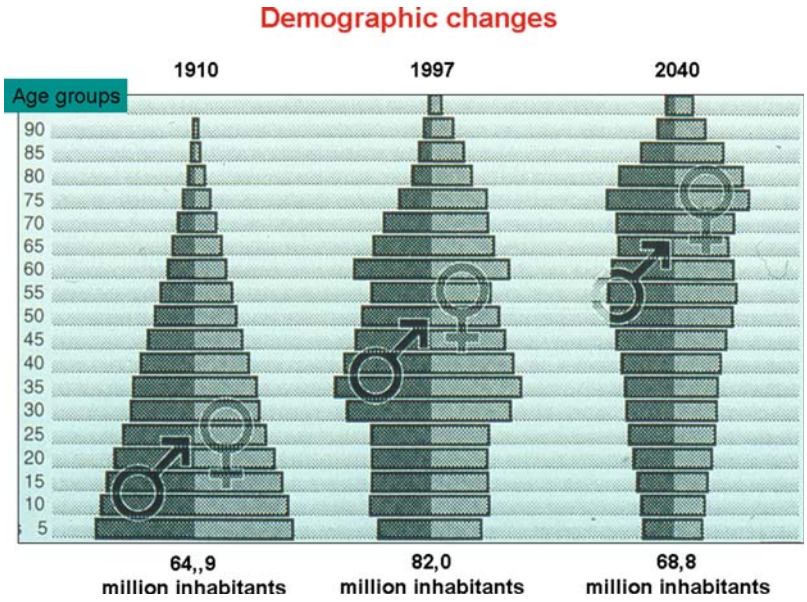


Fig. 1 Age-pyramid: demographic changes projected for 2040

Crimes Against the Elderly

Violence and Public Crime

Crime statistics compiled by law enforcement agencies, as well as criminological studies estimating the unofficial numbers of elderly victims, point towards the fact that the risk and danger of being victimized in a public environment are, statistically speaking, low for adults older than 60 years of age. These observations apply not only to crimes involving bodily contact, such as robbery and aggravated assaults, but also to property crimes, such as theft and burglary. Social scientists have focused their research efforts on the epidemiological aspects and risk-factor assessments, whereas clinical guidelines and diagnostic tools have been primarily developed by health care specialists. Forensic medicine has put its focus on the analysis of homicides amongst the elderly. The frequency, with which these violent offences occur, also remains relatively low as compared to other age groups. The criminal act itself does not appear to be carried out in a more brutal fashion than seen in homicides of other populations.

The comparatively low risk of victimization amongst older adults is partially derived from a circumstantial decrease in the number of opportunities for criminal activities to take place, as well as from the heightened sense of caution pervasive amongst older adults. As part of the aging process, they remain in the

close vicinity of their familiar surroundings, e.g. apartment and neighbourhood. They venture out less frequently into unknown territories, e.g. unfamiliar settings. As a result, the number and variability of situations in which the elderly may be victimized are effectively reduced. Older adults are aware of their increasing vulnerability as part of their decreased mobility and increased risk of morbidity. They develop a sense of caution, not fear as often thought, which indirectly aids in the avoidance of risk behaviours associated with victimization. Due to this vulnerability in the elderly, even minor injuries may lead to severe incapacitation and slowed healing or rehabilitation processes.

Maltreatment by Caregivers

The multi-factorial based retreat from social interactions by the elderly and the accompanying social isolation may negatively affect this population. Social isolation poses a risk for self-neglect amongst the elderly. It may further contribute to interpersonal conflicts amidst the constricted circle of individuals or caregivers with whom older adults interact. Secondary products of these escalading conflict situations range from elder neglect and psychological or physical abuse to extreme forms of violence against the elderly, such as the phenomenon of "genocide".

An estimation as to the extent of the problem proves to be difficult amongst this victimized population because many older adults have poor communication skills due to illness, disabilities or dementia. Others may only be able to communicate with the help of their caregiver, the potential or actual perpetrator. In these instances, the clinician or investigator must be able to distinguish between factual and fictional abusive events and consider contributory factors, such as the person's cognitive state, frailty and degree of social isolation. The question remains: how can health care professionals, specifically family physicians who may be first to intervene, better recognize the signs and symptoms of elder abuse and maltreatment as well as potentially arising caregiver conflict situations in an outpatient setting? (9) Even though many older adults have the opportunity to report the abuse, they often chose not to, because they fear retaliation, e.g. becoming even more isolated. Furthermore, they harbour an intense sense of shame regarding their abusive family member or caregiver that is reciprocated by other family members and caregivers who know about the abuse. Hence, the crimes are not reported and the impenetrability of the situation remains.

Regular and comprehensive medical care could prevent elder maltreatment and neglect. However, the extent and implementation of such a concept is reliant upon the benevolence and cooperation of caregivers, their institutions and family members. Health care professionals should further be more sensitized to important aspects of elder maltreatment and abuse to be able to identify problem areas more easily. On the other hand, instances of health care

personnel maltreating or abusing older adults have been identified. Here, additional conflicts may arise due to matters of loyalty, dependency and responsibility amongst the health care professionals themselves and the institution employing them. Finally, these complicating aspects reduce the probability of cases of maltreatment and abuse against the elderly to surface and to be reported.

Forms of Maltreatment: Elder Abuse Definition

Next to the subtle long-term effects of psychological abuse, signs and symptoms of physical abuse are often impossible to be readily recognized in an objective manner. Through the aging process itself, a phenomenology of injuries and illnesses develop in the older adult. Even after having obtained a correct history of events by law enforcement, the injuries themselves or even the individual's death do not necessarily prove causality of the injury mechanism. The multi-morbid disposition of the elderly (10–15) must be factored into the analysis of any injuries, the potential injury mechanism and death investigation. Hence, a detailed documentation and description of all injuries, especially those that are discrete and subtle in nature, are essential for the appropriate categorization and differentiation of "natural or accidental" versus "non-accidental" injuries (16–19).

Older adults experience a multifaceted phenomenology of different forms of violence against them. The different forms of violence may also be displayed in different types of crime settings, e.g. in a public environment or at the home, as part of family or interpersonal violence. Only in rare instances is sharp force trauma used against the elderly. Generally, older adults become the victim of blunt force trauma. Injuries sustained from these brutal acts range from haematomas or bruising of exposed skin, e.g. head, extremities, to patterned injuries such as tramline bruising of the inner upper arm caused by the perpetrator's tight grasp, to fractures, e.g. of the ribs which might easily remain unrecognized during a routine medical assessment. The overall poor health status of the affected, older adult must be factored in when establishing the causality of all injuries possibly due to violence. This necessary analytical process may act as a diagnostic barrier to elder abuse and, essentially, lengthen the time from the report of a possible abusive situation to the actual determination that one has taken place.

Specifically, older adults in need of constant medical care are at risk for elder neglect and may, for some time, not be identified as victims. Categorically, the attribution of responsibility for size and extent of decubiti, the grade of cachexia and dehydration or the development of infection among elderly patients is difficult. Establishing a diagnosis of abuse or neglect is further complicated by situations in which older adults have only remote access to health care facilities or receive medical or nursing care from providers who do not perform adequate

documentation or do not have proper clinical guidelines in place. Cases of inhumane treatment of the elderly by caregivers or family members may just be at the tip of the iceberg. Other subtle forms of violence, such as medication errors (medication not given or the wrong dosage for sedation administered) can only be detected through a complex chemical and toxicological analysis. In practice, these offences may only be discovered as part of an autopsy of a non-accidental death.

The American Medical Association (20) provided the following standard definition of elder abuse:

> "Abuse" shall mean an act or omission which results in harm or threatened harm to the health or welfare of an elderly person. Abuse includes intentional infliction of physical or mental injury, sexual abuse or withholding of necessary food, clothing, medical care to meet the physical and mental needs of an elderly person by one having care, custody or responsibility of an elderly person.

The following characteristics of elder abuse maybe differentiated: active physical abuse, physical neglect, sexual abuse, killing of elderly people (gerontocide, genocide), as well as non-physical abuse such as psychological humiliation and financial abuse (Fig. 2). Additionally, the health care provider or investigator might need to consider a possible differential presentation/diagnosis mimicking forms of elder abuse. Several of these above-mentioned factors or characteristics may, and often do, occur simultaneously.

"Maltreatment" or "mistreatment" is synonymous with elder abuse and neglect.

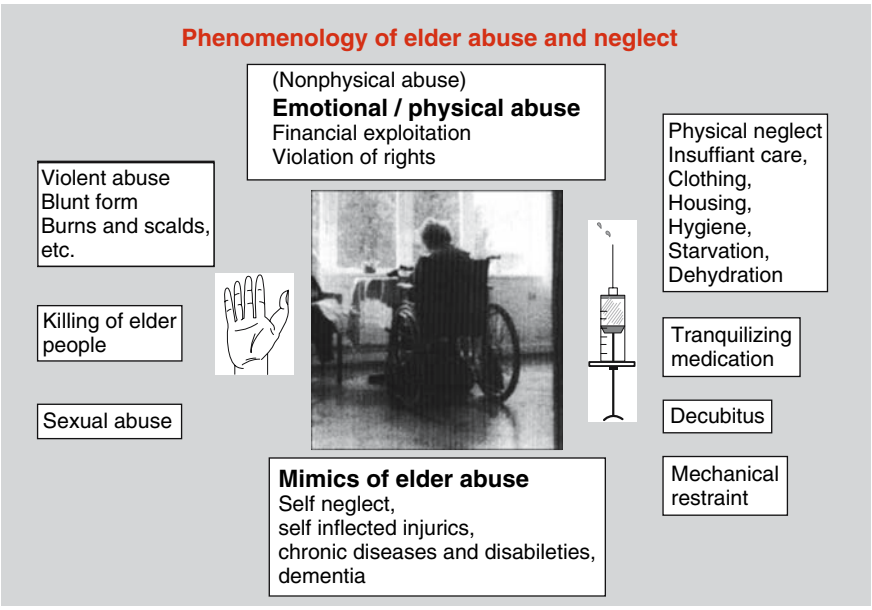


Fig. 2 Phenomenology of elder abuse and neglect

Elder maltreatment affects hundreds of thousands of older adults and does not differentiate between socioeconomic living standards. The number of unreported cases for elder abuse is similar to that of unreported cases of child abuse and neglect; however, the incidence rate of elder maltreatment appears to be slightly less than that of child abuse. Only in a select number of countries is the reporting of such abuse or other forms of family violence mandatory. It is estimated that approximately 5–10% of adults 65 years and older experience some form of maltreatment annually. Nearly half of them experience some type of moderate-to-severe abuse.

In 1992, the European Council published an international study, emphasizing that 1–8% of the elderly suffer injuries, deprivation and unnecessary danger at the hands of their own families and relatives. In Germany, an incidence and prevalence study aiming at conservatively estimating the number of unreported case of elder abuse was performed and similar results were obtained (Table 1).

Physical Abuse

Suggestions on how to extract a proper medical and personal history, investigate the surroundings or setting of the abuse, establish facts of the phenomenology and other aspects of the morphological investigation, as well as describe the characteristic physical findings and complete the consecutive technical and laboratory procedures are summarized in Table 2.

Blunt Force Trauma

The general physical appearance of injuries caused by blunt force trauma has been repeatedly, intensely and competently described in other textbooks. Nonetheless, special pathogenetic aspects, morphological peculiarities and characteristic patterns have to be taken into consideration concerning elderly victims. A differential diagnosis of accidental versus non-accidental injuries must be entertained and events of such nature as older, diseased, mentally and/or physically disabled adults collapsing, stumbling and falling, e.g. out of their

Table 1 Family and interpersonal violence against adults (>60 years)

	Victim (%)	Confidence interval (0.95) (%)
Physical violence	3.4	3.13–3.67
Neglect/abuse of medication	2.7	2.14–3.36
Chronic verbal abuse	0.8	0.45–1.15
Financial abuse	1.3	0.85–1.75

Table 2 Elder abuse and neglect: investigations and findings

Setting
Housing/living conditions (in private settings and nursery homes)
General physical appearance
Medication schedule, feeding, hydration
History
Consistency between the physical appearance, the pattern of injuries and neglect and the offered explanation. Prescribed medical treatment and nursing care
Physical examination
(Investigation of the naked whole body from head to feet with detailed documentation including exact measurements and photos (resp. video))
Head and neck
Multiple contusions, haemorrhages, abrasions to the skullcap, the eyes, ears, cheeks and lips (specifically those appearing to be of different stages or wound healing), traumatic alopecia
Ecchymosis of gingiva, uvula and palate may indicate forced feeding
Poor dentition including tooth fractures or ill-fitting dentures may suggest neglect
Retinal bleedings, orbital fractures or traumatic cataracts may suggest chronic abuse
Petechial conjunctival haemorrhages may suggest strangulation
Neck abrasions or contusions may suggest manual strangulation or strangulation by ligature
Skeletal
Acute or occult rib fractures
Fractures of long bones, immobilization, osteoporosis
Trunk
Acute injuries (haemorrhages, abrasions, other types of wounds, rib fractures) or occult blunt trauma of the chest, breast and mamilles, abdomen or back—especially of different onset or with a defined shape (for example bite marks)
Unhealed decubitus ulcers with infection or infestation of maggots
Extremities
Restraint-associated ligature marks that may include abrasions, lacerations
Burns, lacerations, abrasions, bruises, especially if patterned and of different age, may suggest recidivous violence
Immersion burns may be clearly delineated and must be differentiated between trauma and natural disease state, such as Raynaud?s disease, digital ischemia or vitamin C deficiency
Genitals
Ecchymosis of vulva, anus or scrotum may indicate forced sexual activities
Bite marks (i.e. on the breast or bottom) may indicate sexual abuse
Sexually transmitted diseases
General
Overall hygiene, pests, excrements. Clothing. Weight and height (body mass index). Marasm oedema, exsiccosis, body temperature. Mental state. Psychological, neurological and cognitive evaluation
Mobility; joint-contractions, disabilities, handycaps
Autopsy procedures and findings
Detailed documentation of the gastrointestinal tract (concerning nutrition)
Pre-existing consumptive or infectious disease
Traumatic lesions—Hemo-pneumothorax. Rupture of the spleen or intestine, laceration of the liver and/or intraabdominal blood

Table 2 (continued)

Bronchopneumonia as sequelae of neglect or trauma
Closed head injuries and intracerebral haemorrhages
Neuropathology (i.e. concerning dementia)
Vaginal and anal swabs; removal of all genital organs, subsequent documentation of any injuries
Histology, immunohistology (i.e. concerning the age estimation of injuries)
Consecutive technical and laboratory investigations
Roentgenology, CT (fractures, osteoporosis), ultrasound
Toxicology (i.e. concerning indicated medication, additional sedatives, alcohol toxic substances?)
Laboratory investigations (i.e. biochemistry of blood, urine, cerebrospinal fluid and vitreous humour concerning metabolism, malnutrition, dehydration, anaemia)
Microbiology (esp. in septic conditions)
Entomology (in case of severe neglect)
Stain and DNA investigations (indicates a suspected sexual abuse or assault)

bed while bumping against furniture, walls, etc. should also be considered (Fig. 3 and Fig. 4). Moreover, the intensity and morphological pattern of injuries may be influenced by pre-existing disease states such as neurological and dermatological diseases, diabetes mellitus, medications, specifically anti-coagulants, circulatory disorders and an altered skin structure and subcutaneous tissue. The sequelae of injuries are far more serious in the elderly and the regeneration and rehabilitative processes prove to be much more difficult and longer lasting. What one may consider as a superficial and trivial injury may transpire to be a significant impairment for the older adult.

Special aspects: Skin imprints caused by knuckles or fingers on the inner, upper arms are occasionally discernible and indicative of forceful slapping or fist pounding. Other imprints or abrasions, including those found in the sub-axillary regions and around the ankles may stem from frictional forces applied to the victim while he or she is being pulled by the axilla or feet. Parallel "railroad-track" bruise patterns are characteristic of having been caused by a firm, smooth-surfaced, cylindrical or round-shaped object, such as a rod, pipe or switch. Often, the exact placement of these contusions becomes valuable in distinguishing those injuries that may have resulted from a fall as opposed to having been caused by a deliberate violent act. Nonetheless, primary caregivers may attempt to "explain the injuries away" by stating that the victim simply fell down the stairs and hence, may actively conceal the actual culprit of the injuries. The most common sites where injuries may be found in the elderly are the face and neck, the thorax, the genitals and hands (Fig. 5). So-called defensive injuries are located on the supine aspects of the forearms and hands. Other common injuries include mucosal contusions of the gingiva, the palate and the uvula, which are often consistent with non-consensual feeding procedures. Dating bruises by their appearance, e.g. their size and colour is by no means an exact science and remains unreliable partially due to unique variations in the

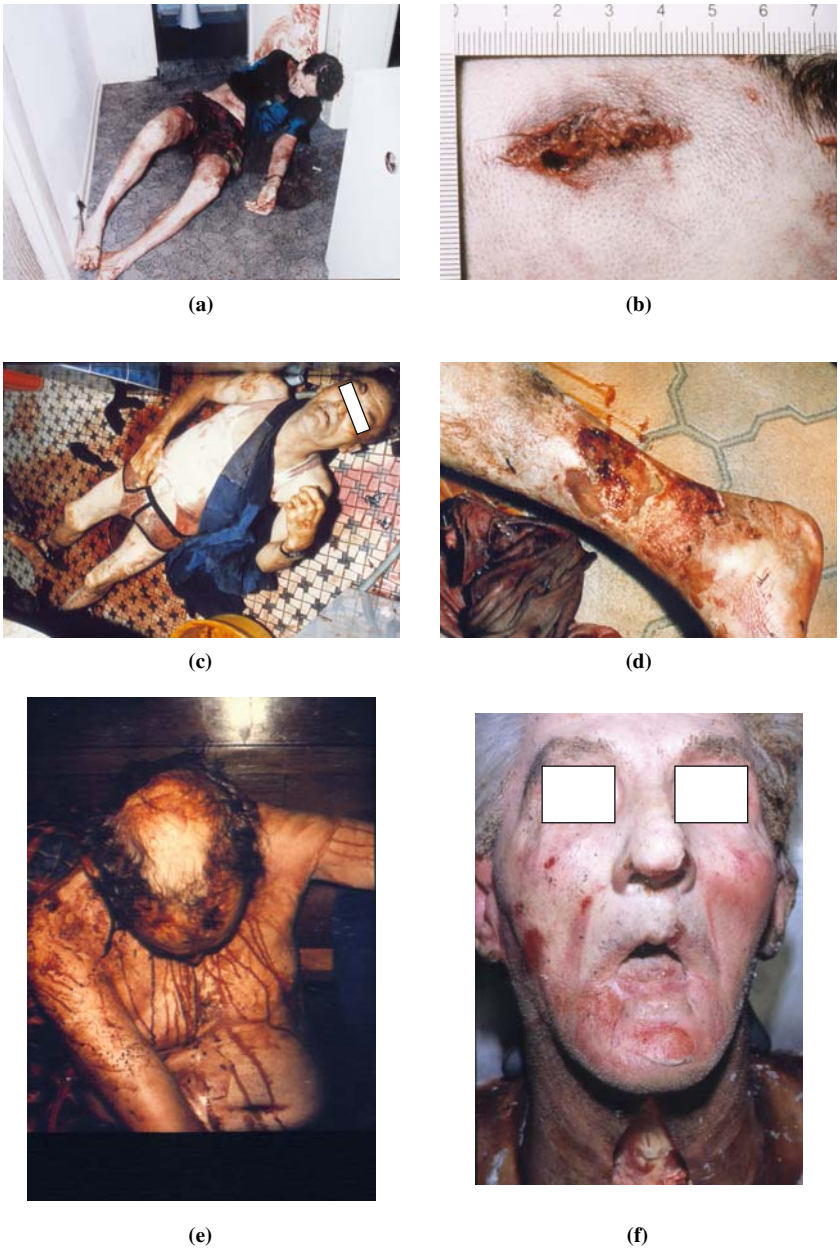


Fig. 3 Accidental death. (**a**) and (**b**) Fall while intoxicated, fatal haemorrhage from scalp laceration. (**c**) and (**d**) Fatal haemorrhage from lower leg ulceration, leg with erosive varices. (**e**) Fatal nose bleed while alcohol intoxicated. (**f**) Fatal second degree burn injuries and cinged facial and head hair after an apartment fire

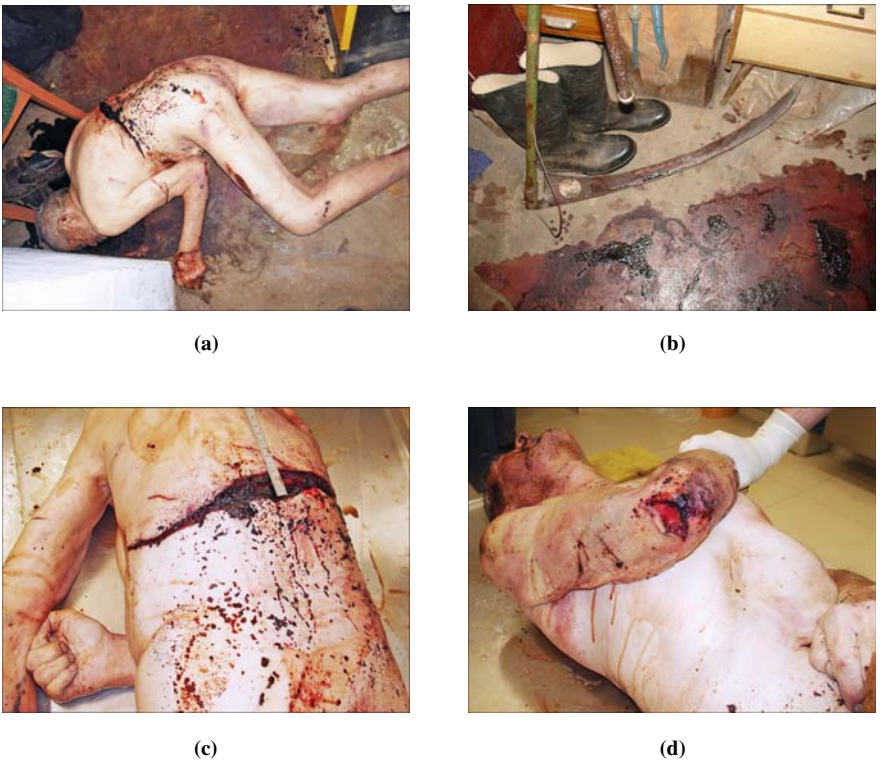


Fig. 4 Sharp force trauma. (**a**) Accident by falling onto a sickle with (**b**) horizontal, deep incision to individual's back. (**c**) Mainly vertically running trails of blood. (**d**) Additional lacerations to right elbow

skin type and tissue response of individuals to the injuries. In general, recently inflicted bruising is displayed in varying shades of red, blue or purple whereas later stages appear blue-green, yellow-green or brown. Histopathological examination of bruises to determine their age is limited to fatal cases and requires a battery of histochemical and immunohistological staining (21–27).

Injuries to the skin, specifically abrasions are caused by the impact of blunt objects or the impact against such surfaces so that frictional forces are applied during the oppositional movement of the two surfaces against each other. These types of wounds are quite common among the elderly and are augmented by an age-related loss of skin thickness, elasticity and tensile strength. These injuries are frequently observed on the upper extremities of the geriatric population residing in institutionalized settings. The multiplicity of such injuries, as well as an observable dissimilarity in the age and location of these injuries, such as those present on other part of the body besides the extremities, should be regarded as highly suspicious for violent abuse. At times, patterned injuries, such as abrasions, may more readily and accurately reflect the configuration of

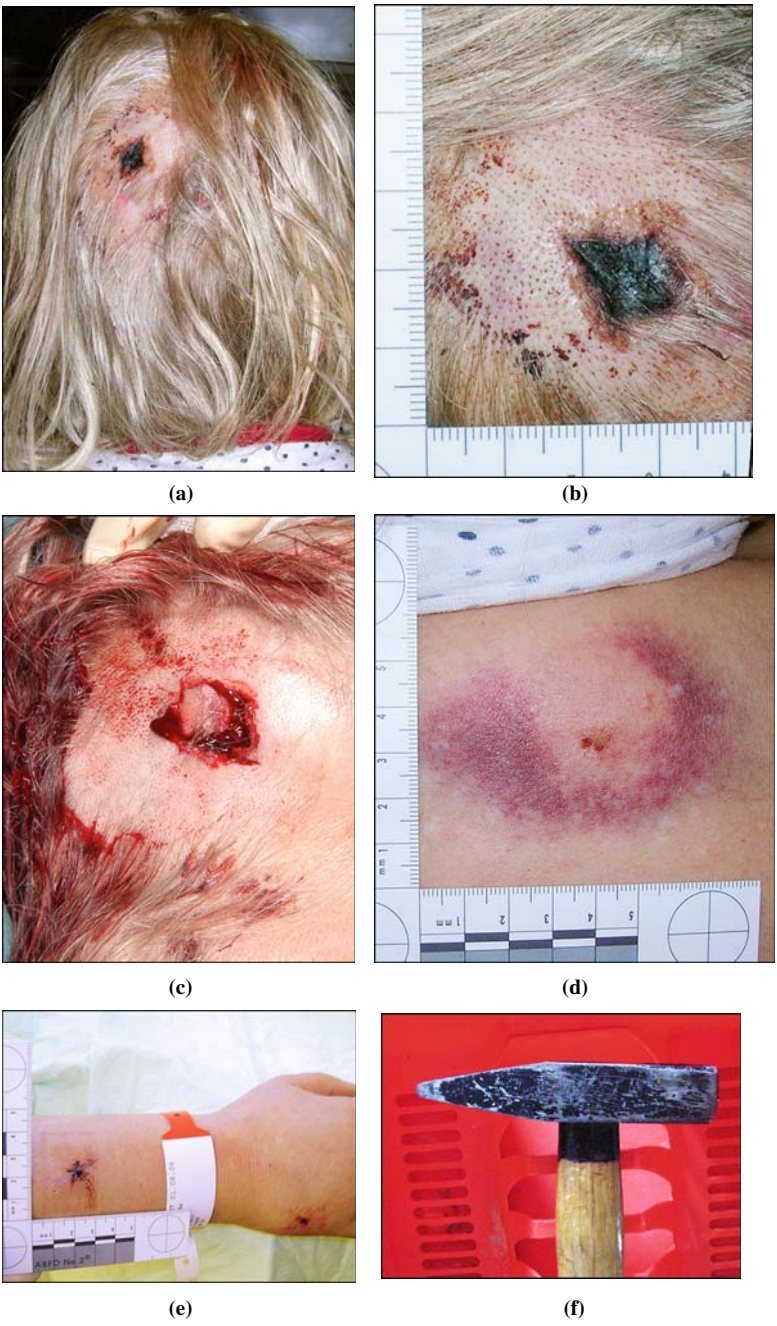


Fig. 5 Blunt force trauma: attack with a hammer. (**a**) and (**b**) Quadratic-shaped abrasion, partly lacerated, injury to back of head. (**c**) Additional quadratic-shaped lacerations on left forehead. (**d**) Patterned contusion on posterior right scapula. (**e**) Defence injuries to supine aspect of right hand and forearm. (**f**) Instrument (hammer) used during the attack

the causative instrument than, e.g. bruises. Once more, the dating of abrasions and lacerations, which is scientifically based on the dynamics of wound healing, has significant limitations. Often, fibroproliferative disorders predispose the elderly to the formation of keloids and hypertrophic scars.

Cutaneous bite marks are a special form of blunt force injury. They consist of abrasions, contusions, haematomas and lacerations or a combination thereof. Wounds inflicted by biting are arranged in two opposing semi-circular to oval patterns, representing approximately the size and shape of the canine and front teeth. Bite marks inflicted by humans are suspicious for sexual abuse and should be properly evaluated and documented by a forensic odontologist. Here, swabbing for trace evidence of saliva and a subsequent DNA analysis may prove very useful in identifying the perpetrator.

Bone fractures occur comparatively often in elderly individuals. Osteoporosis and a host of other biological changes, as well as prolonged immobility causing disease states such as advanced stages of cancer, result in the fragility of bones and enhance the possibility of fractures to occur. Pathological fractures due to cancer may occur at any skeletal site. Common age-related fractures, e.g. those due to falls, are found especially in the hips and wrists. Elderly women with osteoporosis commonly experience vertebral and hip fractures. In Northern Germany, a representative study of more than 10,000 postmortem examinations of elderly individuals prior to their cremation demonstrated that approximately 10% were the recipient of total hip replacements by means of an endoprotheses.

Sites and types of fractures that raise suspicion of current physical abuse of the elderly are categorized as follows: mid- and lower facial fractures, e.g. fractured or avulsed teeth, fractures of the mandible and zygoma; rib fractures; and spiral fractures of the extremities. Antemortem and postmortem radiographs, especially computerized tomography scans, facilitate the detection of fractures, the classification of type and age and the assessment of bone density (28). In regard to the timing of fractures: the health care specialist should apply caution during the evidentiary fact finding phase as there exists a plethora of individual variations in bone structure and composition.

Strangulation, Suffocation

A myriad of factors such as mental or physical disabilities, dementia, frailty and a deteriorating general health status often contribute to the reduced capability of the elderly to defend themselves against actual perpetrators. In many instances, little force is needed to terrorize, overpower and kill older adults. Often, external evidence of trauma is not readily apparent in the older population and evidentiary injuries, that would suggest blunt violence or a homicide to have taken place, are found only scarcely (29).

Generally, deceased elderly individuals exhibiting no external signs of trauma are expected to have died of natural causes related to the geriatric aging process. Due to this phenomenon, the assumption must be made that the estimated number of unknown elder abuse cases is even greater than research has shown so far (30, 31).

With the exclusion of intentional intoxications, the primary mechanism of injury associated with unreported cases of elder abuse remains suffocation. Here, suffocation entails the occlusion of the naso- and oropharyngeal openings, manual strangulation or strangulation by ligature, and other forms of compression of the neck, e.g. choke hold, and/or the thorax (Fig. 6).

Specifically, cases of manual and ligature strangulation may be underreported as these methods produce little physical evidence. For example, extensive formation of petechial haemorrhages of the conjunctiva, buccal mucosa and face (Fig. 7) may not be present. Especially in the frail and elderly population, the blood circulation of the carotid arteries may be stopped quickly, so that the formation of petechial haemorrhages is minimized or simply circumvented (32).

In comparison to other methods, the occlusion of the naso- and oropharyngeal opening represents an easy and untraceable murderous method for the perpetrator to use on individuals who are confined to the bed: he or she puts a hand, often gloved, pillow or other suitable instrument over the victim's external airways. This act leaves little to no physical evidence on the victim's body except for occasional redness, minor abrasions and/or bruising of or around the nose and mouth (Fig. 8). Petechial haemorrhages that can be attributed to a naso- and oropharyngeal occlusion were noted in less than 10% of known cases.

Hence, injuries that are easily identifiable as having caused a violent death such as defence wounds or other pattern injuries may not be found amongst frail, elderly victims.

Therefore, a careful and impeccable autopsy technique should be employed for the deceased elderly. The external inspection of the body should encompass a detailed examination of even the most discrete injuries to the face, specifically those of and around the nose and mouth as well as injuries to the anterior and posterior neck (Figs. 6 and 8). As in paediatric autopsies, the same dissection technique for each individual layer of soft tissue and musculature of the neck should be employed. It may be necessary to apply the same technique to the soft tissue and musculature of the face, the upper extremities (e.g. defence wounds) and back (e.g. injuries due to friction and reposition of the body).

Suffocation with the use of a plastic bag is a widely chosen suicide method of the elderly because the necessary instrument, alas the plastic bag, is commonly available. Pro-euthanasia organizations recommend this method and refer to these plastic bags as "exit bags". This method is generally employed along with the self-administration of a specific "drug cocktail", usually a combination of psychotropic medications.

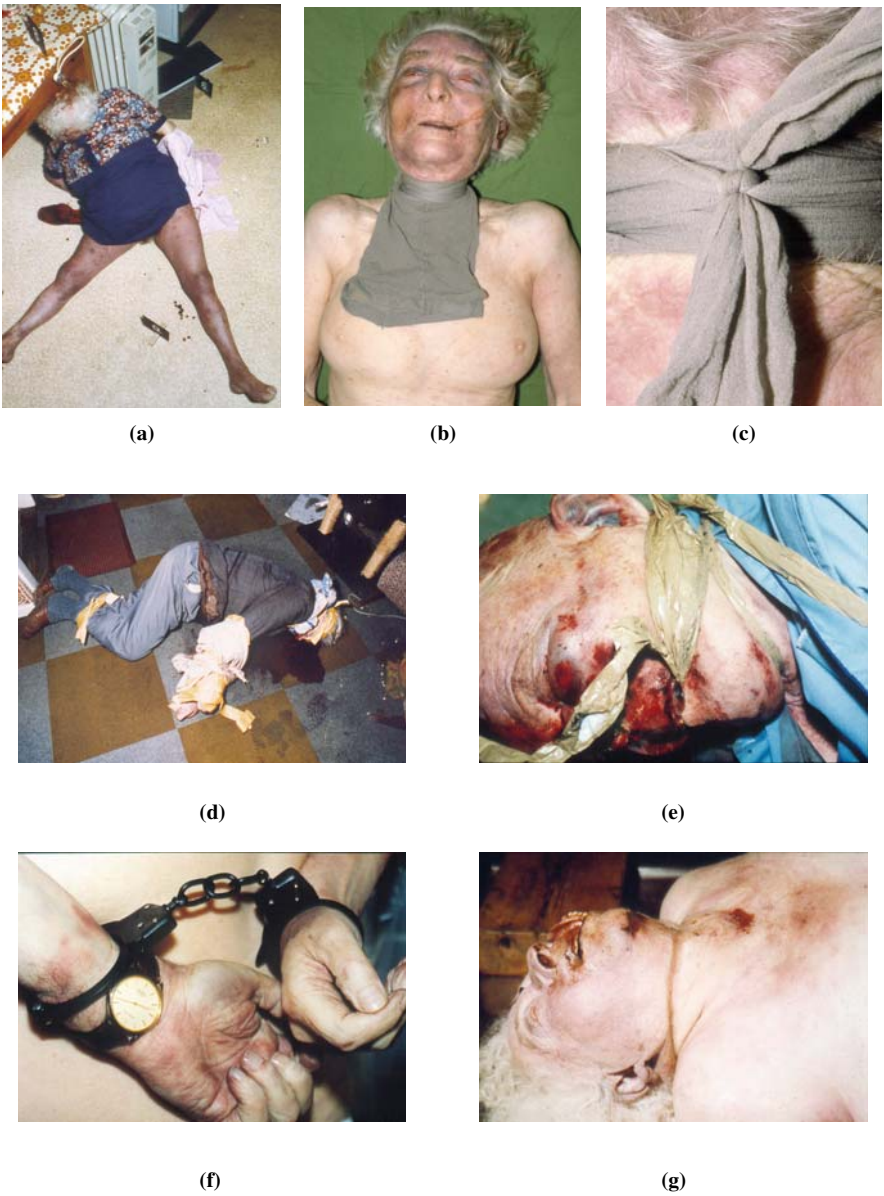


Fig. 6 Killing of the elderly. (**a**) Homicide by hanging. (**b**) and (**c**) Homicide by ligature strangulation: double-knott at posterior neck with entangled hair pieces. (**d**) Victim of a robbery, bound and gagged. (**e**) Gagging by tape applied to front of mouth. (**f**) Handcuffed victim. (**g**) Manual and ligature strangulation

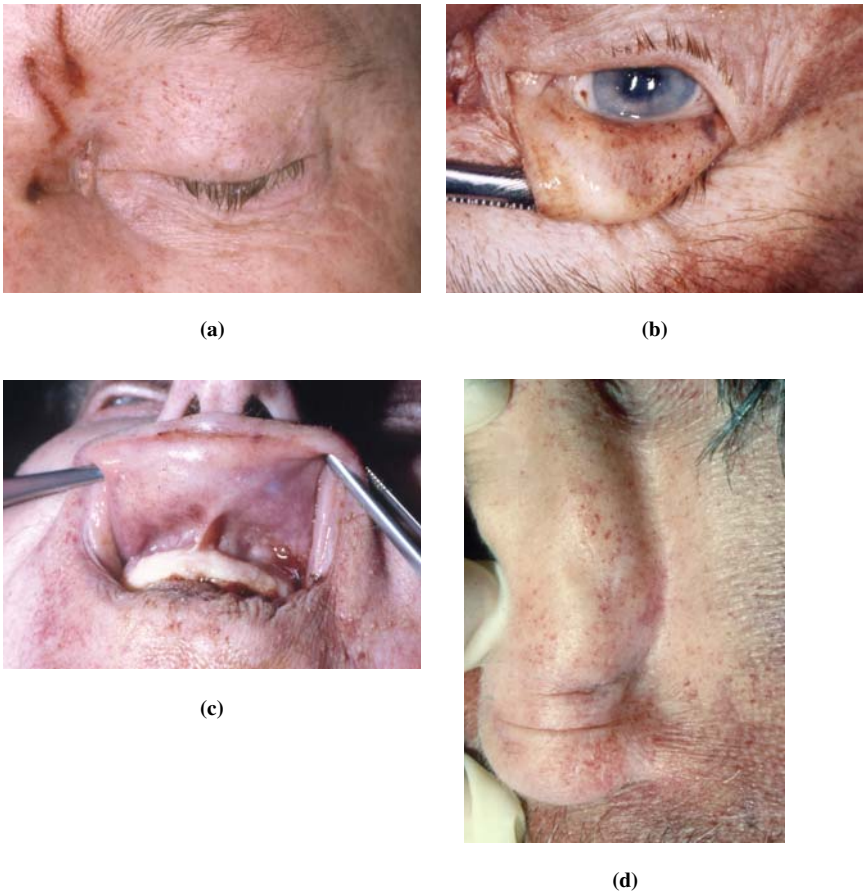
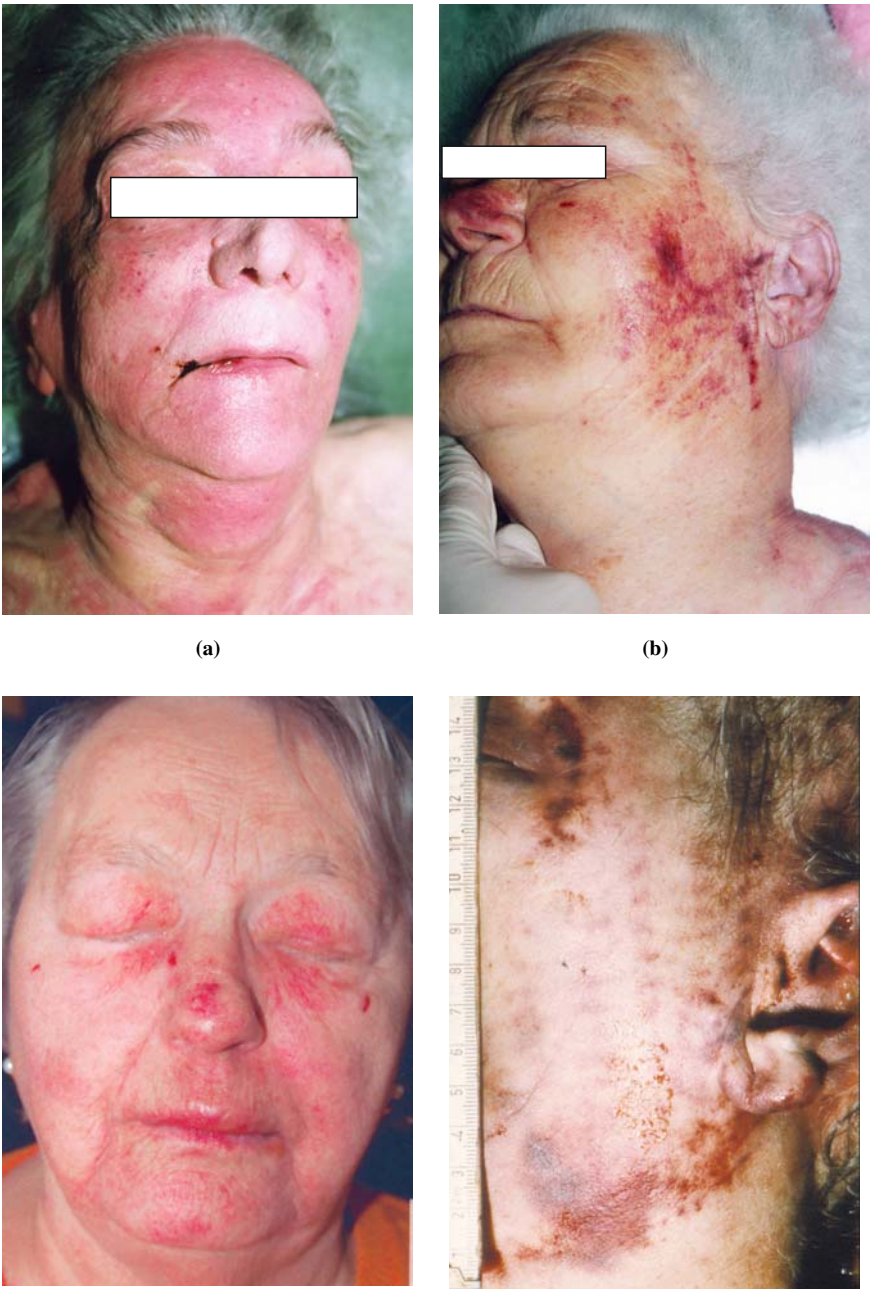


Fig. 7 (**a**–**d**) Petechial haemorrhages on exterior eye lid and conjunctiva, less prominent on buccal mucosa and post-auricular skin

Burns and Scalds

Elderly individuals suffer and die from complications of burns and scalds at a higher rate than any other population. However, the relationship of this kind of elder abuse is less transparent in comparison to evidence-based patterns of child abuse. In the elderly, burns attributed to abuse are frequently present bilaterally on the palms, soles of the feet and hips. Cigarette burns and scalding by hot water during forced showers occur comparably often in children and the elderly. Of course, these injuries have to again be differentiated from accidental injuries. Some retrospective studies suggest that burns may represent a marker of neglect and abuse amongst the elderly. It is recommended that first-line caregivers, such as nurses and social workers regard these types of burns as



(a) **(b)**

(c) **(d)**

Fig. 8 (**a**) and (**b**) Violence to the face. (**a**) Occlusion of naso- and oropharyngeal openings using gloved hands and a pillow. (**b**) Superficial lacerations and minor haemorrhages to the victim's face. (**c**) Smothering with an ether-containing towel. (**d**) Blunt force trauma to facial cheek due to perpetrator kicking the victim, profile of perpetrator's shoe visible

suspicious factors for abuse. In rare cases, thermal body burns or scalds may mimic decubiti (Fig. 9a) (33–35). However, thermal lesions may also be caused by malfunctioning electric blankets or the improper use of such devices.

At the Institute of Legal Medicine in Hamburg, postmortem examinations were carried out for 45 elderly individuals (>60 years) who died as a result of

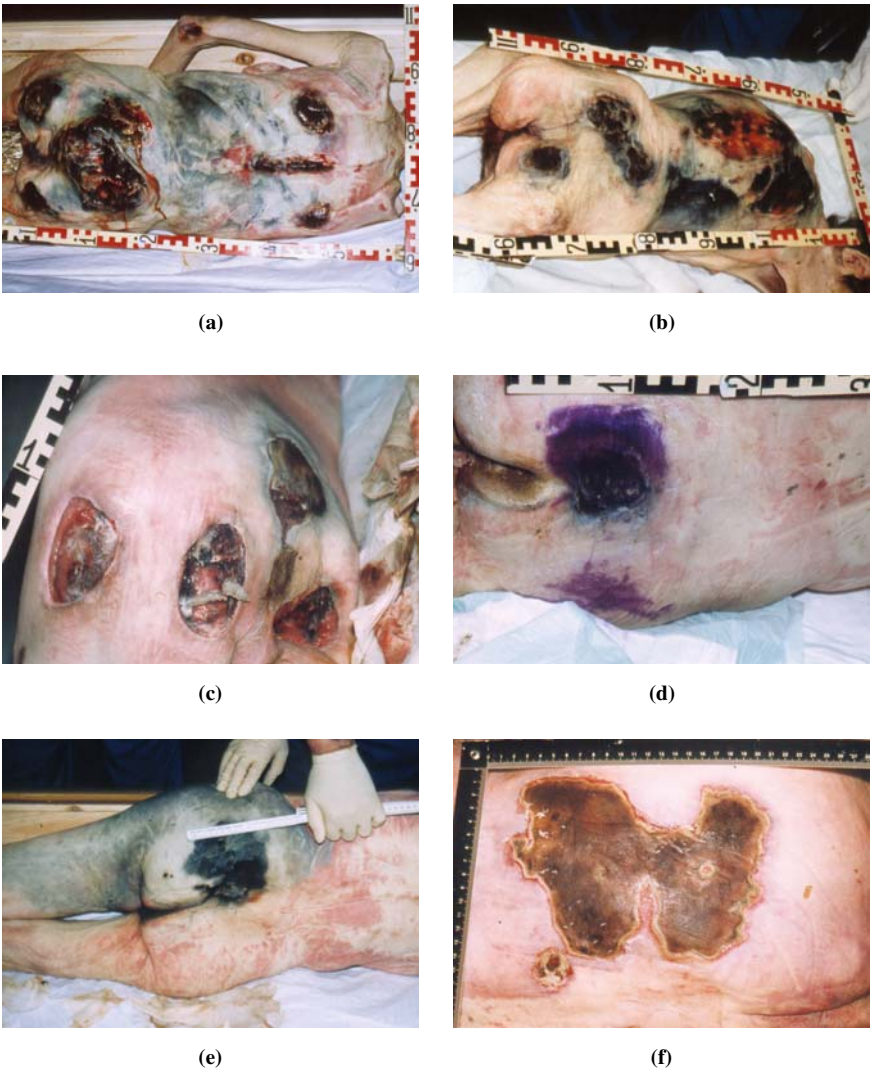


Fig. 9 Advanced grade decubiti. (**a**–**b**) Decubiti of buttocks, back (specifically over bony prominences of the spine), and shoulder blades. (**c**) Multiple decubiti present on buttocks. (**d**) Inadequate treatment of decubiti with simple disinfection of the skin. (**e**) Extensive tunnelling of the sacral decubiti resulting in eschar of left buttocks. (**f**) Differential diagnosis of thermal injuries on buttocks due to an electric heating blanket

sequelae of full-thickness burns (36). This retrospective study evaluated not only the phenomenology of accidents, suicides and murder cases it further quantified the burn surface area, depth and location as well as investigated those factors associated with carbon monoxide or cyanide gas poisoning. Severe burn injuries were the most common cause of death (41%). In the majority of cases, the burn shock resulted in the individual's death within the first 2 h. Accidents generally occurred at home and were frequently related to the individual's level of alcohol intoxication or chronic disease. Specific injury patterns could not be differentiated from this sample.

Physical Neglect

Neglect of the elderly may be intentional (active) or unintentional (passive). Intentional neglect aims at punishing or harming the elderly. Forms of this abuse include withholding items of necessity, such as utensils for hygiene, food or medication. Unintentional neglect can be caused by the individual's or the caregiver's physical or psychological impairment. There are different patterns and grades of neglect. Extreme cases present with:

- Marasmus (severe loss of body weight): atrophy of the subcutaneous fatty tissue, the skeletal muscles and the parenchymatous organs.
- Exsiccosis: dry fatty tissue, skin folds remaining elevated, dry and scabby tongue.
- Signs of hypothermia: low body temperature, cold-associated erythema.
- Poor physical appearance: soiling with excrements over the entire body, uncut, claw-like toe and finger nails, matted head hair.
- Superinfections and decubiti: on the back, the shoulders, the ischial tuberosities, the elbows and heels with concomitant osteomyelitis.
- Infectious skin diseases: exanthema, ulcerations.
- Insect infestations: e.g. fleas or lice, especially in combination with decubiti, found also in head hair or the anogenital region.
- Immobilization: contraction of joints.

Additional findings during autopsies include the atrophy of the inner organs, an empty small intestine, hard dry stool in the colon, anaemia, osteoporosis, a decrease of fatty tissue and sepsis as a result of infectious diseases. Other findings include stress-related changes such as the loss of fat in the cortex of the adrenal glands, brown bowel syndrome and erosive gastritis.

Here, laboratory studies can be very helpful in diagnosing starvation, malnutrition and dehydration as markers of neglect (37).

Self-neglect may coincide with intentional (physical) neglect of the elderly by a caregiver. A comprehensive analysis of the social history, shaping the risk factors for victims and their caregivers, was performed by Ortmann et al. (38). Initially, the victims had a dominant personality while the abusers, here their

adult-children, were strictly controlled and formed by their parent. Generally, the victims exhibited some specific risk factors such as living together with the abuser, being socially isolated and dependent upon the abuser's care giving. Upon encountering increased health challenges, victims often refuse outside help and become more dependent upon their abuser. Finally, the victims relinquish all control over their income and the administration and availability of any money for their personal use to the abuser. These generally unemployed abusers live socially isolated and are financially and in some form psychologically dependent on their victims. In these cases, a psychological disorder could not be established, yet the abusers displayed diminished social skills and competence.

Legal medicine is predominantly involved in fatal cases of the elderly with regard to the external postmortem examinations and autopsies. However, most practising physicians do not routinely consider abuse of the elderly a possibility, even if the corpse presents without obvious findings of such abuse. Mild and moderate signs of neglect are often ignored and attributed to multi-morbidity factors that pre-existed amongst these patients. Nonetheless, the medico-legal expert can be of great service and assist in the examination and identification of injuries in living and deceased elderly individuals to affirm or refute cases of suspected abuse (39). The expert should do so only in close collaboration with other departments such as internal medicine, neurology and gerontology to name a few.

Starvation and Dehydration

Malnutrition and its extreme form of starvation result from inadequate and insufficient consumption of essential nutrients. Among the elderly, the most common type of malnutrition stems from a lack of protein in their diets. Several age-related conditions contribute to the underweight and malnourishment of the elderly, such as edentia and poor-fitting dentures, loss of appetite, dementia, immobility, commonly prescribed psychotropic drugs that inadvertently repress the desire to eat, neurological diseases, other diseases associated with a wasting effect of the body. Inefficient mastication or deglutition may prompt the caregiver to either force-feed or to not feed the elderly individual at all.

Hence, starvation, malnutrition and dehydration are symptoms of neglect. During the examination of potentially forensic cases, grave illnesses and those diseases associated with a severe wasting of the body have to be differentiated from signs and symptoms of neglect. Often, malnutrition initiates a vicious cycle, predisposing the elderly to vitamin-deficiencies, anaemia, immunological weakness, infections, decubiti and impaired healing. A loss in body weight of more than 40% may be fatal.

Dehydration frequently accompanies malnutrition. Elderly individuals are prone to dehydration because of the alterations occurring in their control of

electrolyte and fluid homeostasis. Illness, especially infections or diarrhoea, may result in dangerous states of dehydration. Dehydration may also be a symptom of neglect. Caregivers, who do not supply the elderly with a balanced fluid intake, risk the elderly's life. A loss of 10–15% of the individual's body weight, due to dehydration taking place within a short period of time, is life-threatening.

Thus, early stages of starvation and dehydration are potentially fatal for the older adult. The question arises as to how long an elderly individual may live without receiving any food or water before death occurs. An older adult may live without water for only a few days and without food potentially a few weeks. During an autopsy and in addition to the assessment of the overall health status, body weight and the visceral mass, the forensic specialist should document and take samples of the entire gastrointestinal tract's contents. A diagnosis of dehydration further includes sunken eyes, dry skin and mucosa, as well as faecal impaction. Laboratory studies, including the "dehydration pattern in vitreous humor chemistry panel" in addition to other histological investigations, legally warrants a diagnoses of starvation and dehydration (37).

Medico-legal Aspects of Pressure Sores

The prevention and treatment of pressure sores (decubiti) is a challenge for geriatric, as well as post-operative and intensive care units. In Germany, the estimated incidence of decubiti within the general population is 0.5%, or approximately 400,000 cases. In the clinical setting, approximately 50% of patients are at risk for developing a decubitus. Among home care patients, this rate is significantly less (30–40%). In Hamburg, the prevalence of pressure sores among residents of nursing homes ranges from 0 to 25%. This finding is thought to be correlated with the variability in sample cases derived from individuals who presented with various pre-existing diseases and disabilities, as well as a randomness in their self-initiated prophylaxis, care and professional therapy of such diseases. About 1.3% of patients develop new pressure sores during hospital treatment in Germany, whereas in US 1.9% of patients do.

Forensic pathologists are increasingly confronted with cases of professional negligence (40–44) in the prevention of pressure sores and cases of suspected malpractice (Table 3). Experts discussing how pressure sores may be effectively avoided by standardized nursing protocols such as frequent mobilization, nutritional and fluid intake control, proper bedding such as air mattresses as well as regularly scheduled repositioning of patients, have not yet come to a final consensus. At what point should high-graded pressure sores be regarded as an act of malpractice? Challenges arise when a patient's risk of developing a decubitus has never been classified according to standardized scales (e.g. Norton, Braden, Waterlow).

Although treating practitioners had already certified an individual's natural death, some of the advanced grade pressure sores, which are diagnosed during the postmortem examinations, are subject for debate as the correct documentation of the manner of death has clearly not been indicated in these cases. To clarify, advanced grade decubitus expose subcutaneous muscle or bone tissue. The regional prevalence rate of such advanced grade decubiti in a representative sample indirectly implicates the nursing and other health care professions as omitting essential care, and hence, of malpractice. In Hamburg, forensic pathologists are often confronted with this issue, as they perform external postmortem examinations at the central crematory on a daily basis. A fact to consider is that approximately 70% of Hamburg's deceased are cremated and by law, must be examined by a forensic pathologist prior to being cremated (45).

Apart from public health care-oriented considerations, every deceased individual with an advanced grade decubitus must be thoroughly examined and results discussed before coming to the conclusion that a suspected act of malpractice has taken place. If septicaemia is proven (46) to be the underlying cause of death, a causality must be ensured that it may be truly decubitus-related. Exclusions of independent infectious foci as the original source of septicaemia, e.g. respiratory or urogenital diseases, may fall short of providing the necessary argument for malpractice.

A systematic investigation of more than 6500 deceased individuals at the Hamburg-Öjendorf Crematory in 1998 (45) revealed that the prevalence rate for decubitus grades III and IV was 0.9 and 1.1%, respectively (Figs. 9 and Fig. 10). Since 2000, this decubiti prevalence rate, drawn from the same sample site, has decreased significantly. This phenomenon is particularly true for fatalities that occurred in nursing homes. Round table discussions with public health experts, health authorities and nursing professionals rather than judicial inquiries introduced significant changes in the standard and quality of care. In conclusion, the Hamburg study demonstrated that forensic pathologists have the opportunity to implement postmortem monitoring systems for controlling the effectiveness of quality assurance programmes for decubiti prophylaxis at other institutions. In addition, the findings demonstrated that decubitus-related malpractice may be suspected if septicaemia, suppurative osteomyelitis and the absence of competitive causes for the death have been established by a variety of available morphological and toxicological tests. Decubiti remain a complex issue for forensic pathology in times of epidemiological and demographic changes of the world-wide population.

Mechanical Restraints

In the field of geriatric psychiatry, the therapy for confused patients suffering from severe disorientative states and/or symptoms of brain damage presents particular challenges. Many fatalities have been reported at different

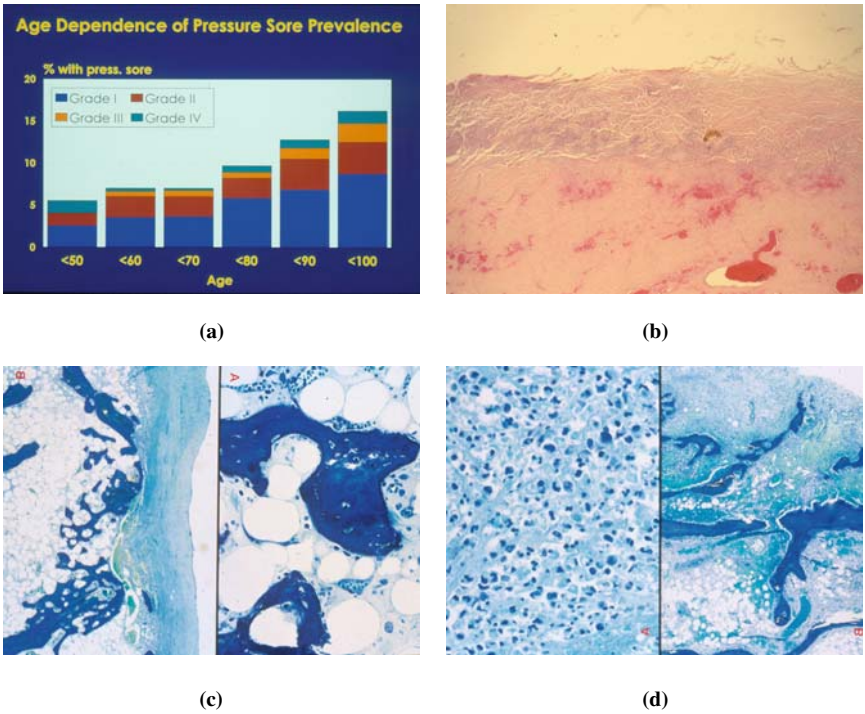


Fig. 10 Decubitus prevalence and micromorphology. (**a**) Age dependence of decubiti prevalence in the elderly. (**b**) Histology (HE) of a severely infected decubitus. (**c**–**d**) Histology of decubiti (toluidine-blue, not decalcified) with superficial scaring osteomyelitis, and infection of the soft tissues mechanical restraints

institutions due to physical restraint that were improperly employed (47–49). Restraint-related causes of death involved accidental strangulation by patients getting caught between the bedrails and mattress, as well as cases of positional asphyxia that occurred while the patient was immobilized with an abdominal or thoracic restraint belt or covered with a special protective blanket. Similar accidents may also happen with the use of restraints attached to a chair. A retrospective reconstruction of the event has to be performed using autopsy findings as well as other documentation describing the scene in which the patient was found. Here, patterned blunt injuries of the skin on the individuals' trunk and neck, including any furrows created by the restraint straps correlated with the respective restraint situation. Physical markers of restraint include abrasions and contusions encircling the wrists or ankles and corresponding scars on healing. A considerable number of unreported deaths must be assumed to have taken place in such a manner. A precise documentation of the indications, physician's orders and implementation of such restraint devices must be reviewed, as well as at the positioning of the body, etc. upon discovery of the

scene. Physical restraints have to be exclusively ordered by the treating physician and are restricted in their use to specific situations wherein a potential harm to the patient or other individuals can be reasonably expected. Detailed guidelines concerning the basic indications for restraint usage in nursing homes and hospitals are necessary to optimize the protection of every patient's personal freedom. "Safe" restraint procedures should include a three-point fixation of legs, trunk and upper extremity.

Sedatives

It has been shown that elderly patients often practice or are the recipient of polypharmaceutical measures and, as a result, become exposed to extremely high dosages of opioid analgetics, neuroleptics and benzodiazepines. The sedative effects of these drugs dampen "unwanted" activities of troubled, restless and noisy patients, but may also lead to their immobilization, helplessness, drowsiness and mental deterioration. Then, the risk of developing decubiti increases (50), and the road for respiratory and urogenital problems is paved.

Suicide Amongst the Elderly

Between 1985 and 1999, 5731 suicides occurred in Hamburg, Germany. Of the total number of suicides, 2336 were committed by adults over the age of 60 years. The incidence rate for Hamburg's population was calculated to be 17.9 suicides per 100,000 individuals and an astounding 40.5 per 100,000 for adults, 60 years and older. This in-depth analysis demonstrated that the suicide rate rapidly accelerates during the aging process and peaks among the very old. A distinctive surge in the number of suicides occurred for individuals 70 years and older.

A chosen suicide method may reveal general information about the nature and personal characteristics of the suicide itself. However, this statement does not necessarily apply to the elderly. Often, the aging process entails the partial or complete immobility of older adults. As a result, the instruments needed to complete the suicidal act, e.g. a gun, are difficult to obtain and the older adult faces a limited number of options. The most common suicide methods (itemized in a descending order) employed by older adults in Hamburg are: hanging or ligature strangulation, drug intoxication, suicidal jump from heights or in front of a train, gun shots and drowning (Fig. 11 and Fig. 12). Hanging is the preferred method to commit suicide by men (36%), whereas women primarily choose drug intoxication (31%). The rate of drug intoxication cases remains relatively constant among all age groups older than 60 years. Interestingly, shot guns are primarily employed by adults 65–69 years of age. The incidence rate of older adults who jump in front of trains lessens with increasing age, whereas the

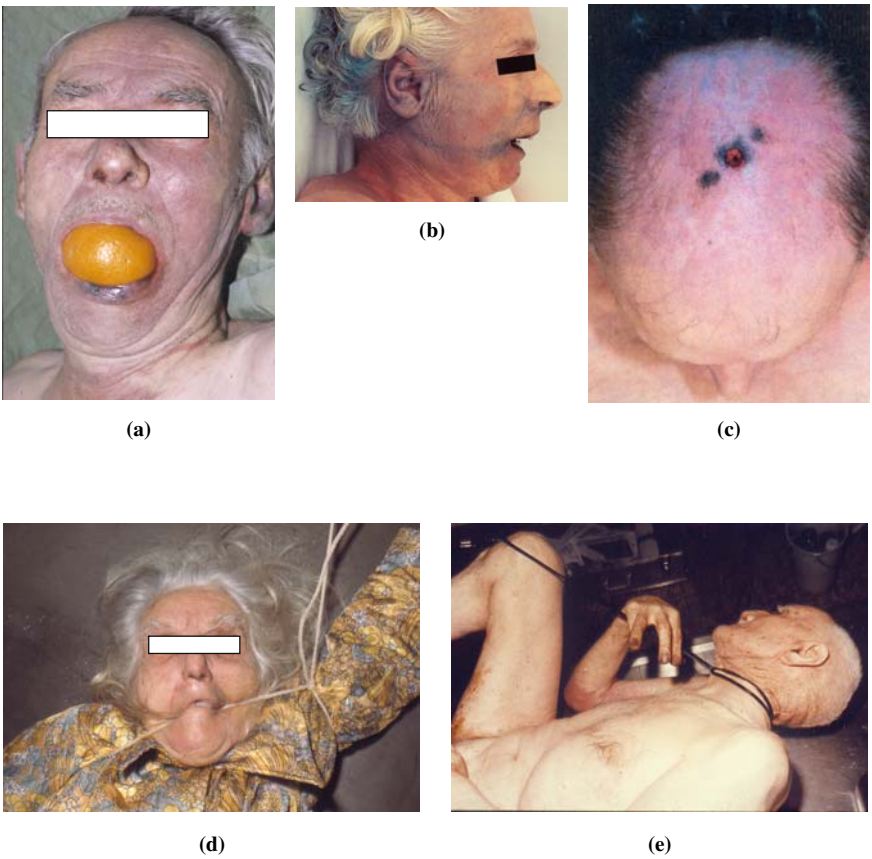


Fig. 11 Suicide. (**a**) Suffocation by self-gagging activities with orange and plastic bag. (**b**) Fatal pesticide ingestion. (**c**) Injury due to captive bolt gun. (**d**) Atypical hanging method. (**e**) Ligature self-strangulation

incidence rate of suicidal jumps from heights increases for this population. In Germany, cases of carbon monoxide and pesticide poisoning have declined over recent years. However, the number of suicides due to self-inflicted sharp force trauma, e.g. stab wounds and incisions, has doubled.

Despite regional differences the overall incidence rate of suicides increases with age. In addition, the root cause for suicides amongst the elderly is often multifaceted and only in rare instances, mono-causal. Somatic (30%) and psychological (48%) illnesses, restlessness, having nothing to look forward to and loneliness are affirmed most often as the motive. Police records often indicate "depression" as the actual motive. Yet, this definition should only apply to individuals diagnosed with a manifested psychiatric illness. "Depression" as a motive remains overused or is often used incorrectly in a need to simplify the causality, while only tangentially touching upon the true reason for the suicide.

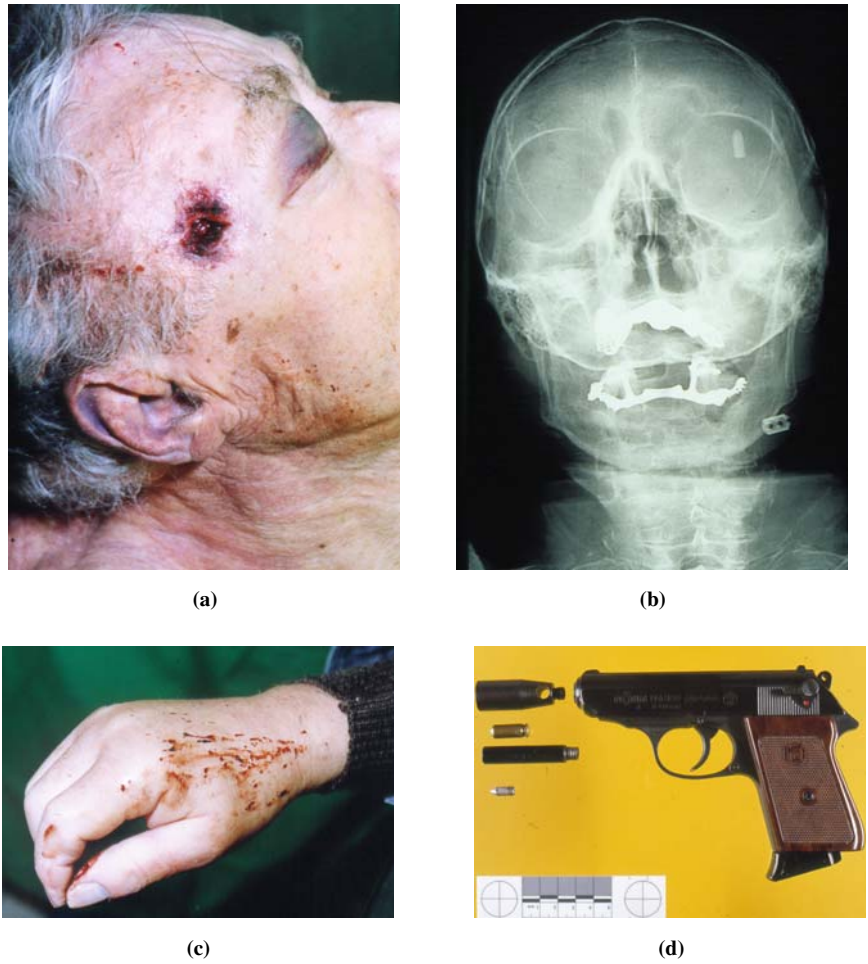


Fig. 12 Suicide with manipulated gas pistol. (**a**) Entrance wound to right temporal area. (**b**) Self-made bullet projected behind left orbit on x-ray. (**c**) Back-splatter on right hand. (**d**) The manipulated weapon

Sexual Abuse

Generally, the prevalence of sexual abuse amongst the elderly remains unknown as investigative studies have not been performed and estimated numbers of incidences are only vague guesses at the actual rate of occurrence. Known sexual assaults of elderly women rarely take place in secluded, public areas (Fig. 13). However, elderly homosexual men comparatively often encounter alterations with male prostitutes; they are robbed while sustaining various injuries during the attack.

Sexual assault and abuse may occur more often in semi-private and less in public settings. Incidences of the old, helpless, mentally ill, handicapped and

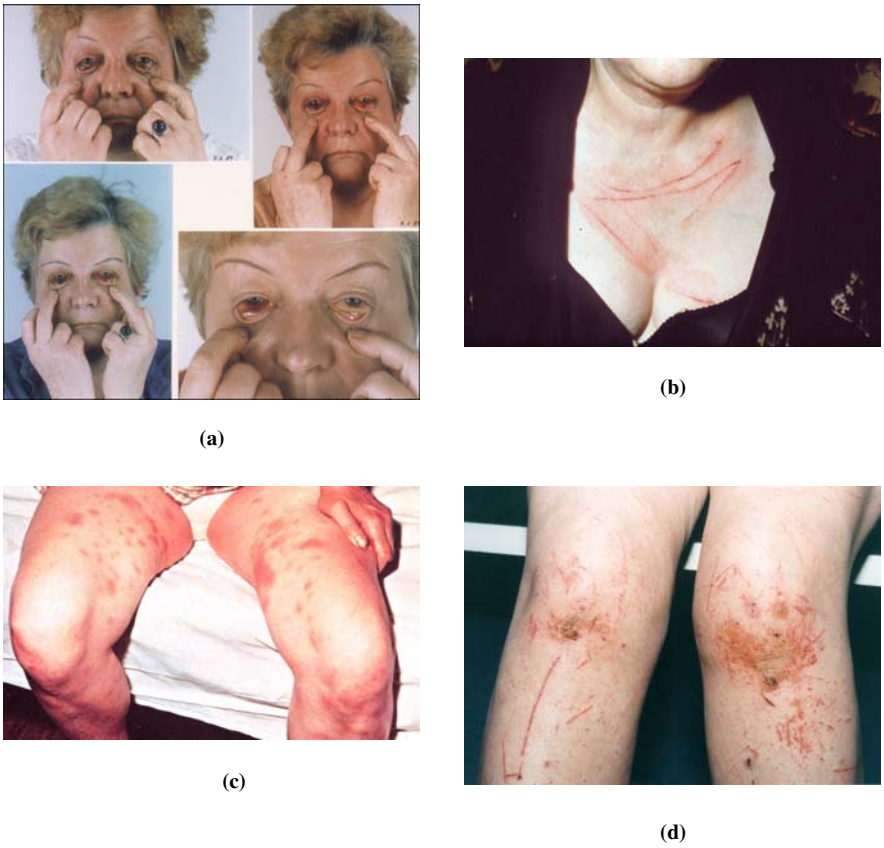


Fig. 13 Clinical forensic medicine. (**a**) Conjunctival petechiae: staging during a 1-week time frame. (**b**) Self-inflicted superficial scratches with a needle in the unclothed area superior to the sternum. (**c**) Haematomas to inner, upper thighs due to forceful separation of legs during a sexual assault. (**d**) Superficial abrasions and scratches to bilateral knees by crawling on a rough surface post-assault

even comatose patients being sexually abused have been reported. Perpetrators who have some degree of mental retardation or are diagnosed as sexually deviant are implicated in many cases of elder abuse. In some of these cases the abuser may have tried to nurture his fetish of gerontophilia and/or necrophilia (51).

Elder sexual abuse and molestation encompasses all coerced sexual contact with the victim's genitalia, breast, mouth or anus taking place by force or threat and against the elderly's will (Fig. 14). Acts of kissing, fondling or improper handling during daily acts such as grooming are additional examples of such abuse. Many times, elderly individuals with physical or cognitive deficiencies are easier targets and more readily victimized. The forensic specialist, after having considered the victim's sexual history, should view a diagnosis of a sexually transmitted disease by means of swabs taken from the oral and anogenital region as highly suspicious of

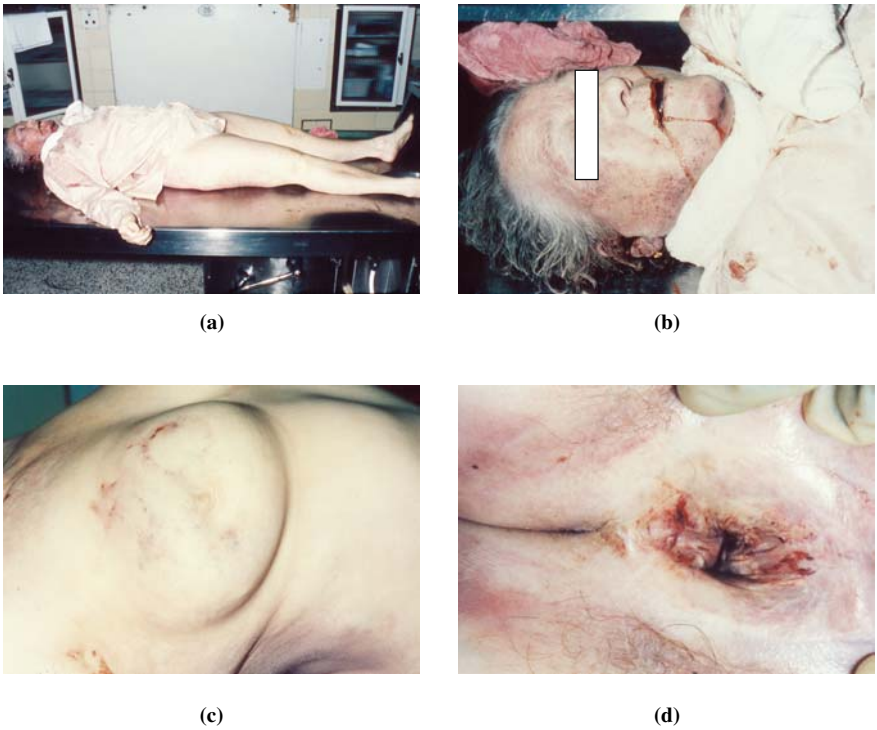


Fig. 14 Sexually motivated femicide. (**a**) Cyanosis, naked lower part of the body. (**b**) Manual strangulation. (**c**) Bite marks to breasts. (**d**) Abrasions, lacerations and haemorrhages to the victim's vaginal area (introitus)

abuse. In cases of attempted or completed digital or penile penetration, abrasions and lacerations of the anogenital region occur comparably often due to the dry and rigid mucosal genital tissue in the elderly. Bite marks, especially of the breast, nipples and bottom should be regarded as highly suspicious for sexual abuse.

In cases of suspected sexual abuse, a collaboration of multi-disciplinary departments and forensic professionals is imperative for a successful investigation. Experts in the fields of gynaecology, dermatology and forensic medicine may be especially valuable in the fact-finding process.

Killing of the Elderly (Gerontocide, Genocide)

A growing population of older individuals contributes to the risk for victimization, as well increases the need for preventive measures. A heightened fear of bodily harm, sexual offences, misuse, exploitation and even violent fatalities can significantly impact an older individual's perception of their quality of life. Television and newspapers convey often a false impression that violence against

elderly citizens is increasing both quantitatively and qualitatively. From 1983 to 2002 all homicides of individuals 65 years and older that occurred in Hamburg and the surrounding areas were analysed in relation to the individual's cause of death, crime or death scene, living circumstances, sex and age distribution, motivation of the perpetrators, etc. Accidental deaths and cases of medical malpractice were excluded.

A total of 185 homicide cases were evaluated (52) of which approximately two-thirds were females (63.8%) and one-thirds males (36.2%). Of these victims, 36.8% were 65–74 years old, 40.7% 75–84 years and 20.5% 85 years and older. Causes of death included (in a descending order): sharp force trauma ($n = 43$), delayed effects of injuries sustained ($n = 34$), blunt force trauma ($n = 30$), strangulation ($n = 15$), suffocation ($n = 12$), intoxication ($n = 5$), fire ($n = 5$), fatal gun shot wound ($n = 4$) and starvation ($n = 1$). The following motivations of the perpetrators could be differentiated: robbery ($n = 76$), interpersonal conflict ($n = 7$), spontaneous conflict or conflicts ensued by an argument ($n = 16$), euthanasia ($n = 10$), sexual assault ($n = 8$), psychopathy of the perpetrator ($n = 18$), concealment of another crime ($n = 10$), contract killings ($n = 1$) and unknown motivation ($n = 39$). The crime scene was the residence of the victim in 123 cases, private property ($n = 7$), unrelated apartment site ($n = 2$), public environment ($n = 11$), a nursing home ($n = 13$) and at a hospital twice.

Criminalistic and criminological analyses of homicide cases that involve elderly victims demonstrate special aspects of the personality, motivation and aggression by perpetrators, as well as victimological aspects of the elderly. Results indicate that serial killings of elderly patients by medical professionals occur in hospitals, nursing homes and, specifically, intensive care units. The study further ascertains that sexual assaults of the elderly also take place, yet are rare. The majority of victims lived alone and the crimes most often took place in the evening hours at the victim's residence. In a relatively high proportion of cases, the perpetrators were familiar with the victim, so that the elderly victims readily opened the door to their assailant. Robbery dominates as the single most often-mentioned motive for elder homicide amongst the sample group of perpetrators. Rarely did the perpetrators have to break into the house. Most assailants were diagnosed with having a severe psychological disorder, such as borderline personality disorder, schizophrenia or depression. The assailants predominantly used knives, heavy objects, towels, pillows and ropes to kill their defenceless victims. Fatal gun shot wounds remain less of an occurrence because guns are not readily available in Germany. Self-defence injuries were found in only a small number of victims. The victims generally exhibited a combination of various patterned injuries including those caused by fixation. A rather high proportion of victims died because of medical complications after their hospitalization. Many of the victims suffered from organic diseases that at times contributed to the cause of death. In one case, an older couple, a 70-year-old woman and a 68-year-old man, died without any obvious signs of injuries, although both had been handcuffed together. Both individuals had a

previously established history of cardiac disease and underwent extreme psychological stress resulting in their death. The medico-legal investigation of these cases may sometimes turn into a difficult task when morphological and toxicological evidence of deliberate killings must be compared to the multi-morbidity and overall health status of the elderly victims.

A great challenge to the resolution of elder homicides continues to be the hidden number of homicide cases associated with an insufficient medico-legal investigation of the deceased during the external examination. A false classification of the cause and manner of death made by the treating physician contributes to this dilemma. Experts calculate that the proportion of unknown offences against the health and life of elderly people is by far the highest of all age groups and involves a wide range of social groups. Society, law enforcement, as well as the judicial system, and especially medical professionals who are responsible for the accurate diagnosis and therapeutic interventions, simply seem to need more education with respect to recognizing elder neglect, abuse and violent offences against this frail population. They are prone to assume a natural cause of death even when the circumstances are unclear, suspicious or even quite obviously the result of illegal actions.

Nonetheless, the so-called fear-victimization paradox has to be emphasized. The realistic expectancy of becoming the victim of a life-threatening attack does not correspond with the impression created by the media and often, fixated within the mindset of the older adults. Our own data demonstrate that, for the last two decades in Northern Germany, i.e. in the metropolitan area of Hamburg and its surroundings, there exists a slight decrease in the number of violent killings of elderly people.

The following generalized conclusions and initial approaches for preventive measures may be drawn from our study of gerontocides: a large number of estimated cases are simply not recorded, and their general prevalence does not seem to increase. Another important aspect for effectively investigating elder homicide cases is the proper and thorough securing of any evidence—which remains difficult. Our study should help in establishing a victim profile, yet, the fear-victimization paradox should not be forgotten. The increased risk associated with social isolation may further increase the vulnerability of the elderly as it is caused by age-related senility and other related handicaps. In most instances, the crime scene is the victim's personal residence and often perpetrators belong to the victim's close circle of known individuals. The perpetrator's motivation is most often that of his own financial enrichment and elderly individuals should be aware of these risks.

According to German criminological research studies, elderly individuals may be specifically endangered by underprivileged individuals or criminally known offenders. In light of the epidemiological development of a growing population of older adults, predictions of violence against the elderly and their abuse will remain a complicated and ever-present problem of our society. In this context, the above-mentioned findings can only serve as a provisional and

temporary appraisal of the potential for a new spectrum of violence and violation of human rights.

Assisted Suicide, Active Euthanasia and Serial Killings

In medicine and law, the ethical values confront one another: "death with dignity", i.e. "allowing someone to die" versus the "right to life", i.e. "preservation of life" (53). An individual's right to self-determination conflicts with the health care professionals' obligation to preserve life. Challenges to this ethical dilemma such as active euthanasia have been legally resolved in only a few countries, e.g. the Netherlands. Dedicated organizations have been implemented in many countries, e.g. Switzerland aiming to support seriously ill patients and their deeply rooted and openly expressed will to die.

An analysis of 32 cases of euthanasia and assisted suicide of elderly individuals demonstrated that in seven cases of active euthanasia the individuals were killed by insulin injections ($n = 1$) or administration of digitoxin ($n = 4$). In one case, the individual was shot in the head and in another case the individual's throat was cut. In the remaining 25 cases of suicide, a third party provided the instructions or the means for a completion of the suicide. Most of the deceased lived alone and suffered from multiple diseases. In most cases the persons killed themselves at home: in 10 cases by ingestion of cyanide and in the other cases, by ingestion of various medications. The suicide was generally affirmed by self-written testimonies placed by the individual at the scene.

A completely new criminological phenomenon among homicides has developed within the last few years: The serial killing of patients by nurses or doctors in hospitals and—less frequently in—nursing homes. Some characteristics of these specific murder sprees differ from those found in traditional homicides: the scene of the killing is within the professional domain of the perpetrator and the perpetrator is the one person who has the professional and ethical duty to save lives. Most victims are primarily unsuspecting as well as totally helpless, weak and often, seriously ill. The scene of the crime is generally intensive care units within medical institutions. From a criminological point of view, the precise motive for the killings cannot be deduced. The motivational pattern of the perpetrators varies and from a psychiatric point of view, is often highly complex. The actual number of cases continues to be unknown because they are very difficult to investigate due to the victim's complex and multi-morbid medical history. In these cases, death often does not come as a surprise. Preferred killing methods are overmedicating the individual with opiates, glycosides or insulin, administering air embolisms via the intravenous catheter, suffocating the patient and intravenous push injections of potassium chloride (31, 52, 54).

In Hamburg, we observed a very unusual streak of killings. The assistant director of a local nursing care facility, a 30-year-old, tall man, visited several

elderly female patients and killed five of them within 9 days. He put his large hands that were padded with socks over the naso- and oropharyngeal openings of his victims and simultaneously compressed their neck. He then finalized these gruesome acts by smothering his victims with a cushion. This scenario is a typical case of a masked homicide. Autopsy findings of blunt force trauma to the face, signs of suffocation and additional lesions to the victim's face and neck were not readily apparent and generally rare. The perpetrator was only caught because his sixth victim called for help and a neighbour came to her rescue. The victim later identified the perpetrator.

The most famous mass-murderer of elderly people was Harold Shipman, who practiced for 24 years as a well-known and respected family doctor in the little town of Hyde near Manchester, UK. He was sentenced to lifelong imprisonment for killing 15 of his elderly patients with injections of morphine. However, it is assumed that he killed an estimated 200–300 of his patients. The media called him "Dr. Death".

Non-physical Abuse and Neglect

During a forensic investigation, forensic specialists sometimes rely on their intuition and become suspicious of cases of non-physical abuse or neglect of the elderly. However, further investigative work should primarily be performed by other professionals such as social workers and family physicians familiar with the individuals and their living situations.

Non-physical abuse includes the psychological humiliation, financial exploitation and violation of individual's rights. Such forms of elder maltreatment are frequently difficult to prove and prosecute. The affected elderly are often lonely and abandoned. Non-verbal and verbal threats or insults are debilitating components of this type of abuse and occur among families as much as they do within institutionalized health care environments.

Differential Presentations; Mimics of Elder Abuse

Self-neglect occurs when the presumptively competent elderly refuse assistance and care from relatives, friends or medical professionals to look after them. Self-neglect may sometimes go as far as threatening the patient's safety or health. Non-specific but typical signs and symptoms of self-neglect include poor personal hygiene, dehydration and insufficient nutrition; hazardous, unsanitary or unclean living conditions with inadequate housing often further characterize the situation.

Neglect and subtle signs of elder abuse are often difficult to diagnose because of the common presence of chronic, degenerative or debilitating diseases. Approximately 80% of all elderly individuals, 65 years of age and older, suffer

from at least one chronic disease, and about 50% suffer from at least two chronic conditions. This aspect is specifically true for cardiorespiratory diseases and neoplasms. In addition, the prevalence of Alzheimer's disease as well as forms of dementia doubles every 5 years for individuals 65 years of age and older. Common manifestations of such cognitive disorders include urinary or faecal incontinence and a plethora of behavioural disorders.

Self-inflicted injuries to the skin and false accusations made to the police are often encountered amongst female adolescents but may also occur amongst the elderly who pretend to be alleged victims of rape (Fig. 13b), violence or indecent exposures. Conclusive evidence that the injuries were self-inflicted and have not been produced by a third party must be obtained by means of a systematic analysis of the distribution and configuration of the injuries. Self-inflicted wounds are usually found on the face, the arms and the chest. They are almost never inflicted over painful areas, i.e. the eyes, the lips and the mamma. They are superficially located, equal in shape as they are most often linear and parallel in their appearance, sometimes even symmetrical. Needles, knives or scissors are primarily used to inflict these types of wounds. In these cases, the underlying factor for the self-inflicted excoriation appears to be the effort to attract attention to the individual's current situation. The most important point in recognizing these purposefully self-inflicted injuries is to keep the specific injury patterns and motivational factors in mind and refer individuals for assistance.

Summary: A Sensitive Approach Without Taboos

Publicizing the subject matter of "violence against the elderly" and sensitizing individuals to it may provoke scandalous fallout and newly defined ethical dilemmas. Surely, the public should be informed about this acute health care challenge. There is a substantial need for a concrete and well-organized social and medical network, incorporating victim advocate programmes as well as alternative resources for family members or other caregivers to ask for guidance or to receive temporary respite care for their loved one. Professional health care practitioners should attend and receive topic-related continuing education programmes, be familiar with referral systems and sites and contribute to "supervision" conferencing meetings. Victims and their families should receive similar opportunities. Chronic stress experienced by caregivers may lead to a "burn out" syndrome. If this "burn out" is not properly verbalized, identified and dealt with, caregivers tend to employ more or less violent strategies to resolve this conflict. Then, elderly patients are sedated or fall victim to chemical restraints. They receive verbal threats or are forced to perform physical tasks under severe intimidation and, in extreme cases, are murdered. Nonetheless, victims should not be stigmatized and potential perpetrators should not be accused of a crime until proven guilty. Due to the type of situation and the emotional charge it is filled with, individuals involved may overreact and never

fully recover from the mistakes made. With a careful analysis of the situation, a sensitive and thorough investigation, libel charges may be avoided and justice be served.

References

1. Moon A, Williams O. Perceptions of elder abuse and help-seeking patterns among African American, Caucasian American, and Korean-American elderly women. Gerontologist 1993; 3: 386–395.
2. Johnson TE, Boccia AD, Strayer MS. Elder abuse and neglect: detection, reporting, and intervention. Spec Care Dentist 2001; 21: 141–146.
3. Jones JS. Elder abuse and neglect: responding to a national problem. Ann Emerg Med 1994; 23: 845–848.
4. Lachs MS, Pillemer K. Abuse and neglect of elderly persons. N Engl J Med 1995; 332: 437–443.
5. Lachs MS, Williams CS, O'Brien S, Pillemer KA, Charlson ME. The mortality of elder abuse. JAMA 1998; 280: 428–443.
6. Lachs MS, Pillemer K. Elder abuse. Lancet 2004; 364: 1263–1272.
7. Pillemer K, Finkelhor D. The prevalence of elder abuse; a random sample survey. Gerontology 1998; 28: 51–57.
8. Shields LBE, Hunsaker DM, Hunsaker, JC, III. Abuse and neglect: a 10-year review of mortality and morbidity in our elders in a large metropolitan area. J Forensic Sci 2004; 49: 122–127.
9. Gruszecki AC, Edwards J, Powers RE, Davis GG. Investigation of elderly deaths in nursing homes by the medical examiner over a year. Am J Forensic Med Pathol 2004; 25: 209–212.
10. Paveza GJ, Cohen D, Eisdorfer C. Severe family violence and Alzheimer's disease: prevalence and risk factors. Gerontologist 1992; 32: 493–497.
11. Payne BK, Cikovic R. An empirical examination of the characteristics, consequences and causes of elder abuse in nursing homes. J Elder Abuse Negl 1995; 7: 61–74.
12. Pillemer K, Moore DW. Abuse of patients in nursing homes. Findings from a survey of staff. Gerontologist 1989; 29: 314–320.
13. Quinn MJ, Tomika SK. Elder Abuse and Neglect, 2nd edn. New York: Springer, 1997, pp. 49–179.
14. Swagerty DL, Jr., Takahashi PY, Evans JM. Elder mistreatment. Am Fam Phys 1999; 59: 2804–2808.
15. White SW. Elder abuse: critical care nurse role in detection. Crit Care Nurs Q 2000; 23: 20–25.
16. Aravanis SC, Adelmann RD, Breckmann R, et al. Diagnostic and Treatment Guidelines on Elder Abuse and Neglect. Chicago, IL: American Medical Association Press, 1992.
17. Collins KA, Bennet AT. Elder abuse. In Froede RC, ed. Handbook of Forensic Pathology, 2nd edn. Northfield, IL: College of American Pathology, 2003, pp. 257–263.
18. Dyer CB, Conolly MT, McFeeley P. The clinical and medical forensics of elder abuse and neglect. In Bonnie RJ, Wallace RB, eds. Elder Mistreatment: Abuse, Neglect, and Exploitation. Aging America/Panel to Review Risk and Prevalence of Elder Abuse and Neglect. Washington, DC: National Academies Press, 2003, pp. 339–381.
19. Gray-Vickrey P. Recognizing elder abuse and neglect. Nursing 1999; 29: 52–53.
20. AMA Council on Scientific Affairs. Elder abuse and neglect, JAMA 1987; 257: 966–971.
21. Janssen W. Forensic Histopathology. Berlin: Springer, 1984.
22. Oehmichen M, Kirchner H (eds). The Wound Healing Process—Forensic Pathological Aspects. Lübeck: Schmidt-Römhild, 1996.

23. Hausmann R. Timing of cortical contusions in human brain injury. Morphological parameters for a forensic wound-age estimation. In Tsokos M, ed. Forensic Pathology Reviews, Vol. 1. Totowa, NJ: Humana Press, 2004, pp. 53–75.
24. Hausmann R. Age determination of brain contusions. Forensic Sci Med Pathol 2006; 2: 85–93.
25. Betz P, Lignitz E, Eisenmenger W. The time dependent appearance of black eyes. Int J Leg Med 1995; 108: 96–99.
26. Betz P, Tübel J, Eisenmenger W. Immunohistochemical analysis of markers for different macrophage phenotypes and their use for a forensic wound age estimation. Int J Leg Med 1995; 107: 197–200.
27. Betz P, Eisenmenger W. Morphometrical analysis of hemosiderin deposits in relation to wound age. Int J Leg Med 1996; 108: 262–264.
28. Fenton SJ, Bouquot JE, Unkel JH. Orofacial consideration for pediatric adult, and elderly victims of abuse. Emerg Med Clin North Am 2000; 18: 601–617.
29. Brinkmann B, Fechner G, Püschel K. Identification of mechanical asphyxiation in cases of attempted masking of the homicide. Forensic Sci Int 1984; 26: 235–245.
30. Heinemann A, Püschel K. Zum Dunkelfeld von Tötungsdelikten durch Erstickungsmechanismen. Arch f Krim 1996; 197: 129–141.
31. Rueckert S. Tote haben keine Lobby. Die Dunkelziffer der vertuschten Morde. Hamburg: Hoffmann and Campe, 2000.
32. Giles TE, Williams AR. The postmortem incidence of senile ecchymoses. Am J Forensic Med Pathol 1994; 115: 208–210.
33. Bird PE, Harrington DT, Barillo DJ, McSweeney A, Shirani KZ, Goodwin CW. Elder abuse: a call to action. J Burn Care Rehab 1998; 19: 522–527.
34. Bowden ML, Grant ST, Vogel B, Prasad JK. The elderly, disabled and handicapped burned through abuse and neglect. Burns Incl Therm Inj 1998; 14: 447–450.
35. Tsokos M. Ungewöhnliche Differenzialdiagnose von Dekubitalulzera. Tägl Prax 2002; 43: 795–800.
36. Sanchez-Hanke M, Püschel K. Brandtodesfälle im fortgeschrittenen Alter Z Gerntol Geriat 1996; 29(3): 185–190.
37. Madea B. Death as a result of starvation: diagnostic criteria. In: Tsokos M, ed. Forensic Pathology Reviews, Vol. 2. Totowa, NJ: Humana Press, 2005, pp. 3–23.
38. Ortmann C, Fechner G, Bajanowski T, Brinkmann B. Fatal neglect of the elderly. Int J Leg Med 2001; 114: 191–193.
39. Rickert CH, Karger B, Varchim-Schultheiß K, Brinkmann B, Paulus W. Neglect-associated fatal Marchiafava-Bignami disease in a non-alcoholic woman. Int J Leg Med 2001; 115: 90–93.
40. Heinemann A, Tsokos M, Püschel K. Medicolegal aspects of pressure sores. Leg Med 2003; 5: 263–266.
41. DiMaio VJM, DiMaio TG. Homicide by decubitus ulcers. Am J Forensic Med Pathol 2002; 23: 1–4.
42. Hunsaker DM, Hunsaker JC. Elder abuse. Challenges of clinical forensic specialists and forensic pathologists in the 21st century. In Tsokos M, ed. Forensic Pathology Reviews, Vol. 4. Totowa, NJ: Humana Press, 2006, pp. 25–62.
43. Tsokos M, Heinemann A, Püschel K. Pressure sores: epidemiology, medicolegal implications and forensic argumentation concerning causality. Int J Leg Med 2000; 113: 283–287.
44. Türk EE, Tsokos M, Delling G. Autopsy-based assessment of extent and type of osteomyelitis in advanced-grade sacral decubitus ulcers. A histopathologic study. Arch Pathol Lab Med 2003; 127: 1599–1602.
45. Püschel K, Tsokos M (eds). Krematoriums-Leichenschau. Research in Legal Medicine, Vol. 22. Lübeck: Schmidt-Römhild, 2000.
46. Tsokos M. Pathology of sepsis. In: Rutty GN, ed. Essentials of Autopsy Practice. London: Springer, 2006, pp. 39–85.

47. Mohr WK, Mohr BD. Mechanism of injury and death proximal to restraint use. Arch Psychiatr Nurs 2000; 14: 285–295.
48. Saternus KS, Kernbach-Wighton G (eds). Fixierung erregter Patersonen. Todesfälle in Klinik und Gewahrsam. Lübeck: Schmidt-Römhild, 2003.
49. Woodford NWF. Injuries and death resulting from restraint. In Rutty GN, ed., Essentials of Autopsy Practice. London: Springer, 2006, pp. 171–188.
50. Püschel K, Heinemann A, Krause T, Anders A, von Renteln-Kruse W. High-grade decubitus ulcers in the elderly. A postmortem case–control study of risk factors. Forensic Sci Med Pathol 2005; 1: 3–193.
51. Cooper GM, King MR. Interviewing the incarcerated offender convicted of sexually assaulting the elderly. J Forensic Nurs 2006; 2(3): 130–133.
52. Püschel K, Garbe V, Heinemann A. Killing of elderly people. In Sames K, Sethe S, Stolzing A, eds. Extending the Lifespan. Biotechnical, Gerontological and Social Problems. 2003.
53. Bosshard G, Jermini D, Eisenhart D, Bär W. Assisted suicide bordering on active euthanasia. Int J Leg Med 2003; 117: 106–108.
54. Oehmichen M (ed.). Lebensverkürzung, Tötung und Serientötung—eine interdisziplinäre Analyse der Euthanasie. Lübeck: Schmidt-Römhild, 1996.

Air Crash Investigation for Histopathologists

Graeme Maidment

Introduction

Fatal aircraft crashes are fortunately relatively rare events; in the UK, there are on average around 20 each year, the vast majority involving light aircraft or gliders. This means that unless a pathologist specialises in aviation pathology, the chances of them being called upon to deal with more than one or two cases in their career is very small. The aim of this chapter is to provide a broad overview of aviation pathology in order to assist the general pathologist, should they be faced with having to perform an autopsy on an aviation-related fatality.

The Legal Framework

In England and Wales, Her Majesty's coroner is required to inquire into deaths which are violent or unnatural, or where the death is sudden and the cause of death is unknown; deaths associated with aircraft crashes invariably fall into one or both of these categories. The coroner must attempt to ascertain who has died, where and when they have died and by what means they came about their death, and will appoint a pathologist to perform autopsies on the deceased on his or her behalf in order to assist in answering these questions. In other parts of the world procedures vary, but most countries have a legal official whose duties are at least in part similar to those of the coroner and who will have authority over the bodies.

Aircraft accidents are also subject to other statutory investigations. The International Civil Aviation Organization (ICAO), an Agency of the United Nations, in Article 26 of its Convention (1) requires the state in which an aircraft accident occurs to institute an inquiry into the accident and that the inquiry should, as far as the laws of that state allow, follow recommended

G.N. Rutty (ed.), *Essentials of Autopsy Practice*,

ICAO procedures as detailed in Annex 13 of the ICAO Convention. In the United Kingdom, the responsibility for complying with the ICAO requirements regarding accident investigation rests with the Chief Inspector of Air Accidents, who is the head of the Air Accidents Investigation Branch (AAIB), a branch of the Department for Transport. The AAIB is granted powers under the Civil Aviation (Investigation of Air Accidents) Regulations 1996 and is tasked with performing investigations to determine the causes and circumstances of accidents with a view to preserving life and avoiding future accidents. It is not the purpose of the AAIB's investigation to apportion liability or blame.

In particularly serious accidents, the Secretary of State may order a public inquiry, in which case the AAIB inspectors will present their evidence to the inquiry. If the accident is a purely military one, it will be investigated by a military Board of Inquiry; the purpose of the Board is similar to that of the AAIB, and in some cases the Board may call on the AAIB for expert assistance.

The accident may be subject to other strands of investigation and scrutiny. The police will almost always initially have primacy at the crash site, and although procedures vary between local police forces, most will start by treating the crash site as a potential crime scene; whether they subsequently continue to handle the police investigation as they would with a suspicious death will depend on local policy as well as the circumstances of the individual accident. In a large-scale crash, the police will set up a Disaster Victim Identification team, and will coordinate the identification efforts. Even when there are no suspicious circumstances, it is prudent for the investigation, including the autopsies, to be performed to high forensic standards; there is always the possibility that the accident may become the subject of criminal or civil litigation at some future time, and the evidence derived from the autopsy may come under critical legal scrutiny. The accident will almost certainly attract a degree of media coverage, and in the higher profile accidents this may be detailed and prolonged; books are written and documentaries are made about aircraft accidents many years after they happen, and the conduct of the investigation may well be looked at in detail.

The Role of the Pathologist

If the investigation of the accident is to be thorough, there must be good cooperation between the various interested agencies, and indeed they are mutually dependent upon one another. In a fatal crash, the pathologist plays a pivotal role in assisting the various investigating authorities to perform their functions. The pathologist is appointed by the coroner (or equivalent legal officer) and performs the autopsies on their behalf; the autopsy reports remain the property of the coroner. The accident investigators will require the post-mortem findings in order to complete their investigation, not least in case there was a medical cause for the accident; however, the pathologist should not disclose the autopsy findings without the permission of the coroner. Conversely,

the coroner will need the accident investigators' findings to arrive at the appropriate inquest verdict. If the crash is potentially suspicious, the Police will also need the post-mortem findings, but once more these should be obtained via the coroner. In practice, there is usually excellent cooperation between the various parties; on the rare occasions when conflicts arise, it is usually due to a lack of understanding of the roles and responsibilities of the different agencies.

The autopsy in a fatal aircraft crash serves a number of functions. Primarily, it fulfils the legal requirements of the coroner in helping to establish the identity of the deceased and in providing a cause of death. Additionally, the autopsy findings may provide the following: evidence which helps in the reconstruction of the crash sequence; evidence of pre-existing medical or toxicological factors which could have caused or contributed to the accident; epidemiological data for the analysis of accidents; information on survivability, such as the effectiveness of harnesses or other safety equipment. This information may promote flight safety in the future by allowing modifications to aircraft or equipment design, the medical criteria for pilot certification or emergency procedures such as the adoption of the brace position (2).

The technicalities of the aviation autopsy are no different from any forensic autopsy performed on a victim of trauma. The difference comes in the interpretation of the findings, which requires an understanding of the aviation environment. Even if the pathologist performing the autopsy has no experience of aviation pathology, as long as they perform a thorough forensic autopsy and document their findings in detail, their findings will be of use to the accident investigators.

The Crash Site

While there may be some benefit in the pathologist attending the scene of the crash, in the author's experience it is rarely necessary. If the scene can be reached quickly then attendance may be beneficial; however, given that aircraft crashes often occur in remote locations, it can take some considerable time for the pathologist to attend, and during that time the bodies are exposed to the effects of the environment and to predators, and are also potentially exposed to the view of onlookers and the media. It is important that the location of all the bodies and body parts be accurately mapped and recorded and ideally photographed; this is primarily a police responsibility. Once this has been done, there is much to be said for recovering and refrigerating the bodies as soon as possible; the preservation of the pathological evidence, as well as the dignity of the deceased, usually outweighs any benefit of delaying body recovery for the arrival of the pathologist. The accident investigators will usually recover the aircraft wreckage for detailed examination; if the pathologist needs to examine the wreckage, for example to determine how individual injuries have been caused, this can be done at a later date, and indeed this is often much more fruitful after the autopsy has been performed than at the scene of the crash with the bodies in situ.

Identification

The principles involved in identification are no different in an aircraft accident from those employed in any death, although the challenges may be somewhat greater, depending on the number of fatalities and the degree of trauma. Various methods may be employed; ultimately the coroner must be satisfied as to the identity of the deceased. In a low-speed light aircraft crash, the injuries may be no worse than those sustained in a car crash and may allow the deceased to be identified by facial recognition by relatives or associates, as is the norm with routine coronial autopsies. This should only be considered if facial injuries are slight; with more severe facial trauma, the process may not only be unduly upsetting for the relatives but lead to erroneous identifications being made.

Higher speed crashes produce a greater degree of traumatic injury, and in some cases there may be total body fragmentation. Fire is a not uncommon consequence of a powered aircraft crash and may lead to burning of the deceased. As it may take some time to recover the bodies from a crash in a remote location, decomposition and predator damage may be considerable. All of these factors hinder identification.

Ideally, identification should rely on a method that is considered unique enough to definitively establish identity by itself; these include odontology, fingerprints and DNA. All of these require antemortem references for comparison. Less unique criteria may be useful, such as tattoos and jewellery, but again these require reliable antemortem information to be available. These are largely matters for the police and the coroner, but the pathologist plays a vital role in providing an accurate and detailed description of the body, in exposing the dentition and in taking appropriate samples for DNA analysis.

It is useful to distinguish between "closed" and "open" disasters. In the former, the number of individuals involved and their identities are known, and so the identification process is one of matching the remains to the individuals known to be involved. An aircraft crashing into a remote location is an example of a closed disaster; in theory, at least, it is generally known who was on board, and as long as no one on the ground was struck by the aircraft, the disaster is "closed", although the possibilities of errors in the passenger manifest or of stowaways should always be considered. If an aircraft crashes into a populated area, then the disaster becomes "open"; the number of people affected on the ground will not initially be known, nor will their identities.

Aircraft crashes can cause body fragmentation, with the production of many hundreds or even thousands of body parts of varying size, some just being tiny fragments of bone or soft tissue. Modern techniques allow virtually all such remains to be analysed for their DNA profiles, thus potentially allowing the vast majority of the human remains to be identified and reunited. However, this is an extremely costly, time-taking and labour-intensive exercise. In a high-energy open disaster, it may be necessary for DNA analysis to be performed on virtually all the recovered remains; the total number of individuals involved in

the crash is unknown, and any one of the small tissue fragments may be all that is recovered of a deceased person. In a closed disaster, the situation is somewhat different; once the identities of all on board have been established by whatever means, then the analysis of DNA from the smaller remains is not necessary for the coroner to fulfil his or her duty, although it would allow all the remains to be reunited for burial or cremation. In practice, in this situation, most coroners take a pragmatic view and set criteria in terms of the size or type of the tissue to be sampled for analysis. Generally, if the relatives are sensitively made aware of the nature of the problem and kept informed of progress, most are sympathetic to this approach.

Radiology

Consideration should be given as to whether the remains should be X-rayed. This should be considered essential if there is any chance that the accident was caused by an explosion, sabotage or other terrorist activity. Radiology may be the only way that the presence of bullets or shrapnel from an explosive device is identified; if present, they must then be retrieved for forensic examination. In other contexts radiology is of more limited value. It is able to demonstrate the extent and nature of fractures, but this information can be obtained during a thorough autopsy. Radiology can be of assistance in the identification of remains; implanted surgical prostheses may be identified, and if the remains are fragmented, X-rays can assist in finding fragments of dentition or of jewellery that may aid identification. It clearly relies on portable X-ray equipment being available, together with the staff to operate the machinery and interpret the images, and this precludes the use of radiology in many cases.

The Approach to the Autopsy

As with any autopsy, it is highly desirable for the pathologist to have access to as complete a history of the accident as possible before undertaking the post-mortem. Information regarding the crash should be available from the police, the coroner or the accident investigators, although at the time of the autopsy the details may be sketchy. The pilot may have made radio calls declaring an emergency, or eyewitnesses may have seen evidence of mechanical problems with the aircraft; this information can be invaluable in deciding whether medical incapacitation of the pilot was a possible cause of the accident. If the pathologist has not visited the crash site, they should be briefed on what was present and avail themselves of any photographs that were taken. In particular, they should note the position of the bodies in relation to the aircraft, whether harnesses were used and whether they had remained intact, and whether there was evidence of a fire. It is useful to know how the bodies were recovered;

aircraft are often mangled, and the rescue services may have to cut through metal to extricate the bodies. The possibility of post-mortem injuries being produced during this process should be appreciated. The length of time between the crash and the recovery of the bodies, together with the ambient temperature, can be useful information when interpreting toxicology results.

It is also important to have access to the past medical history of the pilot. For most types of flying, the pilot will require some sort of valid medical certificate. For sporting activities such as gliding, this may be no more than a self-certification of fitness to fly, which may be countersigned by the pilot's general practitioner. Commercial and military flying requires more rigorous periodic medical examination and certification of aircrew. Ideally, the past medical history should be obtained both from the medical regulatory authorities and from the general practitioner.

The body should be transported to the mortuary fully clothed, and the clothing and associated flying equipment should be examined and described as part of the autopsy. Not only may the clothing aid identification but damage to the clothing may give clues as to injury causation. Helmets and specialised military flying clothing and equipment should be retained for examination by appropriate specialists.

Patterns of Injury

In the great majority of fatal crashes of all types of aircraft, the cause of death of the occupants is given, not surprisingly, as multiple injuries (3–5). However, in a small proportion of accidents, the autopsy yields other causes of death (6, 7), and even when death is due to multiple injuries, the nature, severity and pattern of those injuries can produce useful information for the reconstruction of the accident and the making of safety recommendations. It is therefore important that a full, detailed autopsy be performed on the fatalities and that all the injuries, both external and internal, be documented in detail.

The injuries sustained in an aircraft crash can usually be considered as being due to deceleration, impact, penetration or fire. When an aircraft crashes, the occupants will obey Newton's laws of motion, and will continue to travel with the same velocity as they were before the crash until their motion is arrested, either by restraint systems or by impact with a solid structure; the deceleration will produce a force equal to the product of mass and deceleration. Physical injury results when the stress imposed on a body tissue by the imposing force produces a degree of deformation (or strain) which exceeds the viscoelastic yield point of the tissue; the degree of injury is therefore not simply a function of the magnitude of the applied force, but it depends on the stresses which the force produces, the duration of the force and its variation with time, the nature and orientation of the stressed tissue and the interaction of other stresses (8).

The decelerations experienced during an aircraft crash are usually expressed as multiples of *G*, 1*G* being the acceleration due to gravity, i.e. 9.8 m s^{-2}. Standard aeromedical terminology defines decelerations of the body in three orthogonal axes: *Gx* in the anteroposterior axis, *Gy* in the transverse lateral axis and *Gz* in the longitudinal caudo-cephalic axis. The pattern of observed injuries may allow estimates to be made of the decelerations sustained by the body in these axes. It is important to appreciate that the deceleration of the occupant will not be the same as that of the aircraft as a whole, and indeed the crushing of the aircraft structure at impact will produce different deceleration profiles in different parts of the aircraft. Nonetheless, the information derived from the injury patterns may be vital for the accident investigators in their attempts to reconstruct the nature of the impact (particularly if the aircraft wreckage is unavailable for study, as may occur if the aircraft crashed into the sea) and for recommendations to be made with regard to increasing the crashworthiness of aircraft or the provision of safety equipment.

Head Injury

Head injuries are very common in aircraft accidents, with fractures of the skull and facial bones being identified in approximately half to two-thirds of all fatalities. In one series, head injury was given as the sole cause of death in 22% of accidents (6); this raises the possibility that at least some of these crashes may have been potentially survivable if the head injury could have been avoided or reduced in magnitude.

Head injury often results from the head striking the instrument panel; adequate upper torso restraint may prevent this, and the torso should be carefully inspected for abrasions and bruises which may confirm that a harness was used. However, even if an adequate harness was worn, distortion of the aircraft may still result in the head striking internal structures in the cockpit. A helmet may provide a high level of head protection, but many head injuries result from facial impacts, and these are difficult to protect against. The facial soft tissue injuries should be documented in detail, and ideally a facial dissection should be performed and the injuries to the facial skeleton documented, although when extensive bony and soft tissue facial injuries are present the dissection can be extremely difficult and may render facial reconstruction by the mortuary technician impossible; in such cases, careful palpation often reveals the extent of facial bony injury.

Fractures of the cranial vault and of the base of the skull are common. The former are commonly caused by direct impact, and may well be associated with abrasions or lacerations, the characteristics of which may indicate the nature of what has been struck. Occasionally projecting controls or switches penetrate the skull bones and cause penetrating brain injury. The dura should be stripped to look for subtle fractures of the skull base, although often they are all too

obvious. Basilar skull fractures may result from impact forces being transmitted through the mandible and temporomandibular joints; this often results in a transverse "hinge" fracture in the middle fossae. Crashes with a high *Gz* component can result in forces being transmitted up the spinal column, causing a "ring" fracture circumferentially around the foramen magnum; this is not uncommonly seen in crashes where the aircraft is in a relatively flat spin or in helicopter crashes which exhibit relatively large vertical decelerations. In higher speed accidents, there may be very extensive "eggshell" fracturing of the cranium, with loss of bone and often loss of brain tissue.

Fatal head injury can occur in the absence of skull fractures, as a result of deceleration producing shearing forces within the cranium, leading to traumatic axonal injury, subarachnoid haemorrhage or subdural haemorrhage. Again, helmets may help to prevent this form of injury by producing a finite stopping distance for the head, thus reducing the peak deceleration it experiences.

Loose items within the aircraft can cause head injury by secondary impact with the occupants (9); this may well be with the back of the head, either as a consequence of the conservation of forward momentum of the loose object or by an object falling vertically on to the head of a passenger who has assumed a brace position. Overhead lockers in passenger-carrying aircraft are a potent source of heavy objects that may produce such head injuries.

Spinal Injuries

Injuries to the spinal column are common and can arise from compression, shearing or, in the cervical spine, hyperextension or hyperflexion. The thoracic spine is the region most commonly injured, but any segment of the spinal column is susceptible. *Gz* forces of around 20–30*G* can produce compression fractures of the vertebral bodies. Horizontal decelerations of the order of 200–300*G* can cause shearing through the vertebral body. Fracture dislocation of C1 and C2 may in some circumstances be caused by the loop formed from the lower edge of a helmet and the retaining strap (10). Ejection seats are specifically designed to impart a significant *Gz* acceleration to the occupant in order for them to clear the aircraft, and wedge compression fractures of the thoracolumbar vertebrae are not uncommon, even in successful ejections.

Thoracic Injuries

Fractures of the ribs are the commonest of all internal injuries, being seen in over 80% of fatalities. It is usual for rib fractures to be multiple, and flail segments of the thoracic cage are often produced. The location and number of rib fractures should be documented; sometimes the pattern of rib fractures will correspond to the position of a restraint harness. Transverse fractures of the

body of the sternum are not uncommon. In some cases where there is a high *Gz* deceleration, these may be caused by downward flailing of the head resulting in the chin forcefully striking the sternum; associated injuries to the chin would be expected, and sometimes there may be underlying injury of the heart, the so-called chin-sternum-heart syndrome, initially described in parachutists (11). Haemothorax and pneumonthorax are extremely common, and the volume of blood in the chest cavities should be documented.

Visceral thoracic injury may be caused in a number of ways. Lung contusion is common, as is aspiration of blood from injuries to the face and upper airways. The sharp ends of broken ribs can cause lacerations of the lungs and heart. The lungs are supported only by their hilar attachments; this means that during a deceleration, once the movement of the body as a whole has been arrested either by restraint or impact, the lungs will continue to move and place a strain on their hilar attachments. Haemorrhage around the lung hilum is very common; in some cases there is transection of the pulmonary veins and arteries, but often the exact source of the haemorrhage is difficult to identify. In more extreme decelerations, there may also be transection of the bronchi, leading to the lung lying free within the pleural cavity. The hemidiaphragms may be ruptured by decelerative forces.

The heart is similarly free to move, only being supported within the pericardial sac by its arterial and venous attachments. The ascending aorta is firmly tethered posteriorly to the spinal column, and movement of the heart due to deceleration often leads to rupture of the aorta at the junction of the distal arch and the upper thoracic segment. In the more severe cases, with deceleration of the order of 80–100*G*, complete transverse circumferential transaction can occur, with intimal "ladder" tears being observed with lesser decelerations. Vertical lacerations of the aorta are more likely to be produced by fractured ribs.

A sudden rise in intracardiac pressure as a result of a compression force acting on the chest or abdomen can produce endocardial laceration, or in more severe cases atrial or ventricular rupture. The heart may also rupture as a result of direct compression between the sternum and the thoracic spine. Ventricular rupture classically occurs in the anterior wall of the right ventricle, parallel with and close to the interventricular septum. Rupture of the heart is seen in between a third and half of all fatal accidents.

Abdominal and Pelvic Injuries

Deceleration produces torsional and shearing strains within the solid organs of the abdomen that frequently lead to injuries; they may also be subject to direct compression. Varying degrees of rupture of the liver are seen in nearly half of all fatalities, and often the degree of internal parenchymal disruption is much greater than one would expect from the external damage to the capsule.

Rupture of the liver is often accompanied by rupture of the spleen; splenic rupture without concomitant rupture of the liver is surprisingly rare and probably relates to the spleen's slightly more fluid parenchyma having a greater viscoelastic yield point. The kidneys are relatively well supported in their perirenal fat, and their posterior location affords protection from direct impact injury; nonetheless, they are ruptured in about a quarter of accidents, and some degree of perirenal haemorrhage is seen much more commonly.

The hollow viscera are much more resistant to rupture than the solid organs. The stomach is particularly durable, and even in crashes where there is considerable fragmentation of the body, it is not unusual for the stomach to remain intact. The intestines often show contusion, and the pattern of contusion may correspond to the position of the lap belt of a restraining harness (which during the dynamics of a crash may not retain its normal position over the pelvis). In more extreme cases, the small intestine or transverse colon may be crushed against the anterior spine and possibly transected. The mesentery often shows bruising and sometimes fenestration, again usually as a result of the action of a lap belt harness.

The pelvis very often shows evidence of disruption, with distraction of the symphysis pubis and disruption of the sacroiliac joints; sometimes more extreme fracturing of the pelvic bones is seen. The bladder is ruptured in approximately one-third of the cases where there is disruption of the pelvis but is extremely rarely ruptured in the absence of bony pelvic trauma. Perirectal haemorrhage is common. The prostate and the uterus are both very resistant to the effects of trauma and in a highly disrupted body may be extremely useful in confirming the sex of the deceased. Lacerations of the perineum are seen in cases with a significant *Gz* component to the deceleration.

Injuries to the Limbs

Soft-tissue and bony injuries of the lower and upper limbs are very commonly seen and may be caused either by transmitted decelerative forces or by direct impact, either by intrusion of components of the airframe or by flailing of the limbs. The nature of any soft tissue injury associated with a fracture may give useful information as to the mechanism of causation, and these should be carefully recorded.

Fracture dislocations of the ankle joint, often compound, are among the most commonly observed bony injuries; whether the fractures have occurred in inversion or eversion may provide clues as to the direction of the lateral forces, and therefore the attitude of the aircraft at impact. The tibia and fibula may be fractured by the legs flailing forwards and impacting structures in front of them, such as the lower spar of the seat in front in passenger aircraft. In light aircraft, the engine firewall may be driven backwards into the cockpit on impact, causing lower leg injuries.

Fractures of the femur may be caused by the knees striking structures in front of them, with the impact forces then being transmitted axially up the femur, leading to femoral and pelvic fractures; this mechanism is often seen in car crashes. In aircraft crashes, the additional presence of significant *Gz* deceleration also produces the possibility of femoral fractures resulting from a bending moment being applied to the femur by the front transverse spar of the individual's seat (12).

Injuries to the upper limbs may also be caused either by flailing or by transmitted force, and the latter has special significance, as they may provide useful information as to who was controlling the aircraft at the time of the accident.

Control Injuries

Many aircraft are provided with dual controls; if more than one pilot was on board at the time of the crash, then it becomes important to attempt to deduce which of the pilots was flying the aircraft. While the details of controls vary from aircraft to aircraft, commonly, the pilot will be required to hold a control stick or yoke, and sometimes a throttle, in his hand such that the control is grasped in the palm and the forearm is held approximately horizontally. If the aircraft is brought to an abrupt halt by an impact, the pilot's hands will continue to move forwards; the forces from the control may then be transmitted through the pilot's hands and arms. Classical control injuries of this sort consist of abrasions and lacerations on the palm of the hand, particularly linear lacerations between the thumb and the first finger, sometimes with avulsion of the thumb (13); fracture dislocations of the wrist, fractures of the distal third of the forearm and posterior fracture dislocations of the elbow may also be produced. If the controls are damaged, fragments of them may be present on the hands, or alternatively on gloves if they were worn. Hand injuries produced by flailing of the arms tend to be found on the dorsum of the hand rather than on the palm.

The pilot's feet operate the rudder pedals, and in a crash, these too may produce control injuries in the form of bruises on the soles of the feet and transverse fractures of the metatarsals. Imprints on the soles of the footwear may reflect the structure of the rudder pedals.

Control injuries may not only indicate which pilot was at the controls but also confirm that the pilot was indeed actively attempting to fly the aircraft at the time of the collision; this can be important if incapacitation of the pilot due to medical or other factors is a possibility.

Great caution must be exercised in the interpretation of possible control injuries. They are only produced when the crash forces are such that sufficient strain is produced in the tissues to cause the classical injuries. Even when there is little doubt that the pilot was not incapacitated, classical control injuries are observed in only a very small minority of pilots; there are a number of possible

reasons for this, including the pilot releasing his hands from the controls in the last seconds before impact. The absence of control injuries must therefore be treated with circumspection. It is extremely rare to see classical control-type injuries in passengers, but it should be borne in mind that if a passenger were to rest their feet on the bar of the seat in front of them, or to grasp a solid structure with their hands, injuries very similar to control injuries could be produced. In high-speed impacts where very extensive injury and fragmentation is produced, the specificity of control-type injuries markedly reduces.

Notwithstanding their limitations, the presence of classical control injuries can be evidentially compelling, particularly if they are unequivocally present in one pilot and not in another who otherwise has similar patterns of injury.

Injury Scoring

In order to compare the patterns of injury of multiple fatalities, it is useful be able to grade the severity of injuries that an individual has sustained. This has a number of uses. In an accident with a number of people on board, comparing the injury severity with their seating position in the aircraft may assist in reconstructing the accident and in highlighting particular mechanisms of injury causation with regard to aircraft design. Furthermore, if the aircraft wreckage is unavailable for study, for example, because the aircraft has been lost at sea, the patterns of injuries in the deceased (and indeed in any survivors) may allow the pattern of physical damage to the aircraft to be estimated. This may be particularly useful if there is any possibility of an explosion on board the aircraft having caused the crash, as those passengers seated close to the source of the explosion would be expected to have a different pattern of injuries compared to those who were more remote from it. Injury severity scoring also allows comparison of injury trends over time and provides a means of determining whether improvements in aircraft and safety equipment design are having the desired effect.

The Abbreviated Injury Scale (AIS) was first published in 1971 (14) as a tool to help car crash investigators to standardise the frequency and severity of injuries and has subsequently been regularly updated by the Association for Advancement of Automotive Medicine. It consists of an extensive catalogue of injuries to all regions of the body, which are graded from 1 to 6, 1 being minor injury, ranging through moderate, serious, severe, critical, with 6 being maximum, or fatal. The AIS is now very widely recognised and is regularly applied clinically to traumatic injury cases.

A number of injury scoring systems have been derived from the AIS, principally to predict clinical outcome in injured patients. The Injury Severity Score (ISS) (15) divides the body into six regions, namely head or neck, face, chest, abdominal or pelvic contents, extremities or pelvic girdle and skin, and is calculated as the sum of the squares of the highest AIS value in each of the three

most severely injured body regions. The ISS scores range from 1 to 75 (although the scale is non-linear, and not all numbers from 1 to 75 can be attained by the formula); a score of 75 results either from three AIS 5 injuries, or from a single AIS 6 injury, as that is fatal in itself. The ISS suffers from the fact that it does not take into account the effect of multiple injuries within a single body region, and a New Injury Severity Score (NISS) has been proposed (16), which is the sum of the squares of the three highest AIS injuries, regardless of their body region, i.e. all three injuries may be in the same region of the body.

While AIS, ISS and NISS are potentially useful in the clinical situation, they are limited in their use at post-mortem, particularly in aircraft crashes where the degree of trauma and multiplicity of injuries may be much greater than in motor vehicle accidents. It is possible to assign AIS scores to each injury, but the number of injuries often makes this a laborious task. The ISS or NISS values are often of limited use in fatal accidents, as the presence of fatal injuries automatically produces a value of 75, thus masking any differences in the patterns of injury. Looking at the maximum AIS value for each separate body region is a more profitable approach. Simpler injury severity scales have been used in fatal aircraft accidents (17), but these lack the universal acceptance of the AIS.

Unless they are involved in research, it is highly unlikely that a non-specialist pathologist would be required to perform injury severity analysis. However, unless the injuries are comprehensively documented in detail, it will be impossible for anyone else to do so at a later date.

Causes of Death Other Than Multiple Injuries

While multiple injuries due to blunt force trauma are by far the commonest cause of death in aircraft crashes, occasionally other causes of death are given, and these often produce more questions for the accident investigator to attempt to address.

Fire

In approximately a third of powered aircraft crashes there is a fire; the majority of these are post-crash fires, as the fuel tanks and lines become breached, but a small proportion of fires start in flight. If the deceased are burned, the pathologist must attempt to determine whether they were alive during the fire and whether they died of the effects of the fire rather than of any injuries they may have sustained.

The toxicological determination of the carboxyhaemoglobin saturation is the most helpful test, but the results can be difficult to interpret. In the absence of respiration during fire, carboxyhaemoglobin levels in non-smokers can reach

3% and those in smokers may be up to 10% (18). Values above 10% therefore can be taken to indicate that the individual has been exposed to carbon monoxide during life, but the converse is not necessarily true. Aircraft fires can be extremely intense, and death can occur due to the thermal effects, the presence of other toxic compounds or anoxia before a high level of carboxyhaemoglobin is attained. In one series, half of aircrew who died due to the effects of fire had carboxyhaemoglobin levels less than 10% (19). It must also be borne in mind that carbon monoxide may be present in the cockpit as a contaminant rather than as a result of fire. Hydrogen cyanide may also be present in a fire atmosphere and may be rapidly incapacitating. It can be useful to analyse blood for cyanide levels, but as those levels may either increase or decrease in the post-mortem period, their interpretation can be problematic.

The presence of soot in the airways below the vocal cords and in the oesophagus also indicates vitality during the fire; this may only be visible on histology. Histological examination of the lungs may also help determine whether the pilot was alive at the time of impact; bone marrow emboli and fat emboli (which require special stains such as Oil Red O to render them visible) may be seen in the lungs following bony injury, provided the circulation remains functioning for a finite time thereafter, probably of the order of at least 30 s. The number of fat emboli increases with the length of the agonal period (20). The presence of fat or bone marrow emboli therefore implies either that there has been a period of survival following the crash (irrespective of the presence of fire) or that the individual has sustained a bony injury prior to the crash, implying that something has happened during the flight sufficient to cause such an injury. The interpretation of sections of highly traumatised lungs stained for fat emboli can be difficult, and only intravascular fat should be regarded as representing unequivocal fat emboli.

A number of artefacts are associated with burning; these include linear splits in the skin, particularly over the skull and extensor surfaces of the limbs, thermal damage to bones that may mimic fractures and extrusion of blood and brain tissue into the extradural space, where it may be misinterpreted as an extradural haematoma. While the artefactual patterns can usually be recognised, histology of skin and bone lesions or toxicological analysis of a suspected extradural haemorrhage may be necessary to confirm their true nature.

One artefact that can mimic burning is seen when a body lies in aviation fuel, a not uncommon scenario following a crash. Redness of the skin with slippage of the epidermis may be seen, and the appearance may be misinterpreted as superficial burning if its true origins are not appreciated.

If an aircraft occupant dies of the effects of a post-crash fire, this implies that they must have survived the blunt force trauma of the crash forces, and this raises the question as to why they were unable to escape from the aircraft before succumbing to the effects of the fire in what may have been a potentially survivable accident. The presence of head injuries may have rendered the individual unconscious, or lower limb injuries may have impeded their escape

from the aircraft; it is important that all such injuries be documented in detail, as only then may lessons be learned which may help to prevent a similar tragedy in the future. It may also be of some comfort to the relatives if the pathologist is able to say that the deceased was probably unconscious during the fire, although clearly this should only be postulated if the pathological and witness evidence supports it.

Drowning

A proportion of aircraft accidents occur at sea, and while the occupants may die of the injuries produced by the crash forces, others show evidence at autopsy of having drowned; findings may include the classical signs of froth in the nose, mouth and airways and waterlogged hyperinflated lungs. As with deaths in fires following survivable crashes, the question must then be asked as to why, when the occupants have clearly survived the crash, have they subsequently drowned. Injuries may prevent egress from the aircraft, but the availability of quick-release harnesses and doors, flotation devices and protection from hypothermia may also play a role in survival; again without a thorough autopsy, interpretation of the sequence of events following the crash is likely to be impossible.

Natural Disease

In a very small number of cases, pilots of dual control aircraft have died of natural causes and the other pilot has safely landed the aircraft; the autopsy is then merely directed at determining the cause of the sudden death. A much more common scenario is the finding of natural disease in the body of the pilot of an aircraft which has crashed, and its significance must then be assessed.

The medical standards which pilots must meet vary dramatically depending on the type of flying which they are undertaking and the type of pilot's licence they hold. For certain classes of light aircraft, gliders and microlights, often all that is required is for the pilot to complete a certificate of medical fitness to fly, which is countersigned by the pilot's general practitioner. Commercial and military aircrew undergo much more stringent periodic medical examination by qualified aviation medicine examiners, but no screening medical can detect all possible conditions which could potentially interfere with the ability to safely control an aircraft. It is therefore extremely important for the pathologist to conduct a full autopsy on the aircrew in all fatal accidents with a view to finding any evidence of natural disease which could have caused or contributed to the accident. It must be remembered that a condition need not be one that could necessarily cause unconsciousness or death; a condition which causes distraction at a critical phase of flight may be sufficient to cause an accident. In

approximately 1.5–2.1% of all fatal aircraft accidents, medical conditions are identified which have probably caused or contributed to the accident (19, 21), and in a further 1.4% of cases conditions which could possibly be implicated are identified (19).

As with the general population, coronary artery atheroma is a common finding in pilots, and its degree and severity tend to increase with age. In a recent study, atheroma occluding at least 50% of the lumen of a coronary artery was found in almost 20% of the pilots examined (19), and this value is similar to that found in previous studies (22). In the absence of acute changes such as coronary thrombosis, deciding whether coronary artery disease (or indeed any medical condition) may have played a role in the accident relies heavily on detailed knowledge of the circumstances and history of the flight and requires close cooperation with the accident investigators. If the recorded flight path is erratic with no evidence of the pilot attempting to control the aircraft or to make radio calls, then the possibility of medical incapacitation is strongly raised. Conversely, if the pilot reports engine problems and is clearly attempting to make an emergency landing, then it would seem appropriate to regard the finding of coronary artery atheroma as merely coincidental. Unfortunately, the majority of cases where natural disease is found fall into the grey area between these two extreme scenarios.

Assessment of the coronary arteries can be made more difficult by the presence of traumatic damage to them, which is often seen following a crash. Traumatic haemorrhage tends to be periadvential, at least in part, rather than being into an atheromatous plaque. The presence of haemosiderin within an atheromatous plaque implies prior haemorrhage at that site, at least a few days before the accident.

Of course other conditions, both within the heart and within other organs, could cause or contribute to an accident, and their assessment at autopsy does not differ from any routine post-mortem, with the exception that the pathologist must be alert for conditions that could cause incapacitation rather than death. For example, certain central nervous system lesions, while not being fatal in themselves, could act as epileptogenic foci and may therefore prove fatal to someone piloting an aircraft. Some conditions, such as focal myocarditis, may only be observed microscopically, and there are good arguments for performing histology on the pilots.

In studies of in-flight medical incapacitation of double-crewed aircraft which have not resulted in an accident, neurological problems are the commonest cause of loss of consciousness (23, 24). The fact that this finding is not mirrored in fatality statistics may reflect the lack of morphological correlates of some neurological disorders, and the difficulty of demonstrating subtle changes in the brain without retaining, fixing and submitting the brain for expert neuropathological assessment. In the current climate, it is impossible to justify retaining the brain in most cases, but if the history of the flight suggests medical incapacitation and no cause is found at autopsy, consideration should be given to performing a formal neuropathological examination of the brain.

Toxicology

A full toxicology screen should be performed on all pilots and other crew members killed in aircraft crashes, looking for alcohol, drugs, both illicit and therapeutic, carbon monoxide and other potential volatile cockpit contaminants. Consideration should be given to performing toxicology, particularly for carbon monoxide and cyanide, on a representative proportion of, if not all, passengers.

The nature and quality of samples which may be obtained for toxicology are often limited by the extent of the trauma to the body. If possible, samples of peripheral blood (ideally from two different sites), urine and vitreous humour should be taken. Ideally, 2 ml aliquots of each of these fluids should be preserved in tubes containing at least 1% fluoride as a preservative, and 10 ml of blood and urine should be retained in plain tubes with no preservative. Retaining blood in a tube with EDTA, ideally filled to the brim to eliminate any headspace, can be useful for analysis for carbon monoxide and volatiles. Bile may be useful, particularly if the other body fluids cannot be obtained, and it too should be distributed between plain and fluoride tubes. Blood from the chest cavity may be used for preliminary screening, but it is unsuitable for quantitative analysis; the laboratory must be notified of the site of origin of blood samples. Analysis of the stomach contents may assist in interpreting the timing of the ingestion of various substances.

Sometimes the degree of trauma is such that no body fluids can be obtained (25). Samples of liver and skeletal muscle, or if these are unavailable, lung, kidney or brain can be analysed. Liver is easier to digest during analysis than muscle but is less suitable for quantification. Psoas muscle and quadriceps are often readily accessible, with the latter often being less contaminated. Ideally, a toxicology laboratory should be used which has experience in the processing and interpretation of specimens from aviation accidents. The degree of trauma and contamination of the specimens is such that specialised handling is often needed, and artefactual results are common; for example, a false positive screening test for amphetamines is very often obtained from traumatised tissue samples.

Positive toxicology results are not at all uncommon; in a recent study from the USA, drugs or alcohol were found in 52% of pilots dying in aviation accidents (26).Of these, the vast majority were prescription or non-prescription therapeutic drugs, but ethanol was found in 6.4% of the fatalities, and controlled substances were found in 9.8%. A similar study in the UK found controlled substances in only 1.5% of the fatalities (27).

Whenever a positive toxicology result is found, a number of questions must be addressed. Firstly, the veracity of the result must be scrutinised. While modern analytical techniques, particularly gas chromatography–mass spectrometry, are able to reliably identify the nature of the vast majority of substances, the problems of contamination and post-mortem redistribution mean that the

quantification of the amount of drug present may be unreliable. This is particularly so when there has been extensive trauma or decomposition. Alcohol is particularly problematic, as it may be produced after death as a by-product of bacterial putrefaction, at a concentration of up to 150 mg/dl (28). The most reliable indication of genuine antemortem ingestion of alcohol is good concordance of results between multiple samples of different body fluids. Vitreous humour is extremely useful, as it is relatively sterile and does not readily support post-mortem production of alcohol, although there is disagreement as to how reliable post-mortem vitreous is in predicting blood ethanol levels (29–31). Urine is also usually relatively sterile and the presence of alcohol in the urine has generally been taken to indicate antemortem ingestion, although it has been shown that artefactual post-mortem alcohol can be demonstrated in urine, particularly in bodies which have been immersed in water (32). A rise in the alcohol level on repeat analysis of a specimen a few days after the first analysis confirms that post-mortem alcohol production is ongoing. Bacteria tend to produce a range of other alcohols in addition to ethanol, and their presence on analysis is another pointer to post-mortem generation of alcohol. It is possible to culture specimens and test whether the organisms which are grown are capable of producing alcohol, although this approach is now rarely used. As the metabolism of 5-hydroxytryptamine is influenced by the presence of ethanol, it has been proposed that the ratio of 5-hydroxytryptophal (5-HTOL) and 5-hydroxyindoleacetic acid (5-HIAA) in the urine may be of use in confirming recent antemortem alcohol ingestion (33), although autopsy experience with the technique is limited. Even if these various strategies are employed, it remains the case that it is sometimes impossible to resolve the issue as to how much of the detected alcohol represents genuine ingestion.

The second question which arises from a positive toxicology result for drugs or alcohol is whether the substance has in some way caused or contributed to the accident, or whether its presence is merely coincidental. This relies on knowledge of the likely effects and side effects of the compound, a reliable estimation of its concentration in the blood and correlation with the history of the flight. Clearly, alcohol or psychoactive drugs may impair performance, but other therapeutic drugs may also have more subtle but potentially significant side effects, such as the drowsiness produced by some antihistamines. Even if a mechanical or operational cause of the accident is discovered, it may be the case that the presence of drugs or alcohol may have impaired the ability of the pilot to react to the unanticipated situation. It is certainly not unheard of for pilots to self-medicate with over-the-counter medication which is not cleared for use in flight; unless the toxicology screen looks for such drugs at their normal therapeutic concentrations, significant evidence may be missed at the autopsy.

It is also necessary to consider why the pilot had taken the drug and whether the condition or symptoms for which the drug had been taken was causal or contributory to the accident. Even something as innocuous as the common cold may have flight safety implications, either by causing a general decrease in performance or more specifically by impairing vestibular function and

predisposing to spatial disorientation in flight. As with natural disease discovered at autopsy, the interpretation of the significance of therapeutic drugs relies heavily on knowledge of the circumstances of the accident and requires close cooperation between the pathologist and the air accident investigators.

As previously mentioned, toxicology for carbon monoxide can not only be useful in indicating whether the individual had been alive at some point during a fire but may also point to contamination of the cabin air by products of incomplete combustion from the engines or other sources. Even only moderately raised levels of carboxyhaemoglobin may be sufficient to produce impairment of pilot performance which could contribute to an accident; this may particularly be the case, given the reduced partial pressure of oxygen in the atmosphere at altitude. In the event of a post-crash fire, differentiating between carbon monoxide due to the fire and that due to cabin contamination may be impossible, but if multiple fatalities are involved comparing their carboxyhaemoglobin levels with their seating positions in the aircraft and the location of their bodies following the crash may allow meaningful conclusions to be drawn. Carbon monoxide should ideally be measured by a gas chromatographic method, as this is best able to cope with putrefied blood.

Suicide

While not being something that can be demonstrated at autopsy, the possibility that the crash may have been caused deliberately as a means of the pilot committing suicide should always be considered, although the circumstances of the flight usually allow this to be readily discounted. However, a small proportion of crashes are unquestionably deliberate (34, 35). Sometimes a suicide note leaves no doubt as to the pilot's intentions, but in other cases, autopsy findings such as positive toxicology or evidence of previous attempts at self-harm may support the possibility of suicide, if the circumstances suggest it. Clearly, knowledge of the past history of the individual and their psychological state prior to the flight are important in allowing the coroner to arrive at the correct verdict.

Summary

The thorough investigation of fatal aircraft accidents is very much a team effort; the pathologist is able to provide a small but essential piece of the investigative jigsaw puzzle. Occasionally the autopsy provides evidence of the cause of the accident, but even negative findings are of immense use in the reconstruction of events. Full interpretation of the findings requires close cooperation with all the investigating authorities, and the pathological findings should ideally be

interpreted by someone with experience in aviation pathology. However, the initial requirement is for a thorough autopsy, performed and documented to forensic standards, and this is no more than is routinely expected of pathologists performing any sort of medicolegal autopsy. The autopsy provides a one-off opportunity to obtain information which may help to enhance flight safety in the future and hopefully prevent occurrences of similar accidents.

References

1. International Civil Aviation Organisation. Convention on International Civil Aviation. DOC 7300/8. 2000.
2. Brownson DM, Wallace WA, Anton DJ. A modified crash brace position for aircraft passengers. Aviat Space Environ Med 1998; 69(10): 975–978.
3. Wiegmann DA, Taneja N. Analysis of injuries among pilots involved in fatal general aviation airplane accidents. Accid Anal Prev 2003; 35(4): 571–577.
4. Chalmers DJ, O'Hare DP, McBride DI. The incidence, nature, and severity of injuries in New Zealand civil aviation. Aviat Space Environ Med 2000; 71(4): 388–395.
5. Taneja N, Wiegmann DA. Analysis of injuries among pilots killed in fatal helicopter accidents. Aviat Space Environ Med 2003; 74(4): 337–341.
6. Li G, Baker SP. Injury patterns in aviation-related fatalities. Implications for preventive strategies. Am J Forensic Med Pathol 1997; 18(3): 265–270.
7. Shkrum MJ, Hurlburt DJ, Young JG. Fatal light aircraft accidents in Ontario: a five year study. J Forensic Sci 1996; 41(2): 252–263.
8. Raddin JH. Principles of crash survivability. In Injury Prevention in Aircraft Crashes: Investigative Techniques and Applications. AGARD lecture series 208. North Atlantic Treaty Organization, 1998.
9. White BD, Firth JL, Rowles JM. The effects of structural failure on injuries sustained in the M1 Boeing 737/400 disaster, January 1989. Aviat Space Environ Med 1993; 64: 95–102.
10. Cogswell SC. Aviation pathology. In Injury Prevention in Aircraft Crashes: Investigative Techniques and Applications. AGARD lecture series 208. North Atlantic Treaty Organization, 1998.
11. Simson LR. "Chin-sternum-heart" syndrome: cardiac injury associated with parachuting mishaps. Aerosp Med 1971; 42(11): 1214–1217.
12. Brownson P, Rowles DM, Wallace WA, Anton DJ. The mechanism of femoral fracture in an impact accident. Aviat Space Environ Med 1998; 69(10): 971–974.
13. Krefft S. Who was at the aircraft's controls when the fatal accident occurred? Aerospace Med 1970; 41: 785–789.
14. Rating the severity of tissue damage: I. The Abbreviated Injury Scale. JAMA 1971; 215: 277–280.
15. Baker SP, O'Neill B, Haddon W, Long WB. The injury severity score; a method for describing patients with multiple injuries and evaluating emergency care. J Trauma 1974; 14(3): 187–196.
16. Osler T, Baker SP, Long W. A modification of the injury severity score that both improves accuracy and simplifies scoring. J Trauma 1997; 43(6): 922–925.
17. Hill IR. The Air India jumbo jet disaster in Kalishna—injury analysis. In Caddy B, ed. Uses of Forensic Sciences. Edinburgh: Scottish Academic Press, 1987, pp. 120–145.
18. Mayes RW. Measurement of carbon monoxide and cyanide in blood. J Clin Pathol 1993; 46: 982–988.

19. Cullen SA. The contribution of pathology to the investigation of fatal aviation accidents. MD thesis. University of Liverpool, 2002.
20. Mason JK. Pulmonary fat and bone marrow embolism as an indication of ante-mortem violence. Med Sci Law 1968; 8: 200–206.
21. Booze CF. Sudden inflight incapacitation in general aviation. Aviat Space Environ Med 1989; 60: 332–335.
22. Underwood Ground KEA. The prevalence of coronary atherosclerosis in healthy United Kingdom aviators. Aviat Space Environ Med 1981; 52(11): 696–701.
23. McCormick TJ, Lyons TJ. Medical causes of in-flight incapacitation: USAF experience 1978–1987. Aviat Space Environ Med 1991; 62: 884–887.
24. Martin-Saint-Laurent A, Lavernhe J, Casano G, Simkoff A. Clinical aspects of inflight incapacitations in commercial aircraft. Aviat Space Environ Med 1990; 61: 256–260.
25. Chaturvedi AK, Smith DR, Soper JW, Canfield DV, Whinnery JE. Characteristics and toxicological processing of post-mortem pilot specimens from fatal civil aviation accidents. Aviat Space Environ Med 2003; 74(3): 252–259.
26. Chaturvedi AK, Craft KJ, Canfield DV, Whinnery JE. Toxicological findings from 1587 civil aviation accident pilot fatalities, 1999–2003. Aviat Space Environ Med 2005; 76(12): 1145–1150.
27. Cullen SA, Hill IR. Aviation pathology and toxicology. In Rainford DJ, Gradwell DP, eds. Ernsting's Aviation Medicine. London: Edward Arnold, 2006, pp. 517–533.
28. Corry JEL. Possible sources of ethanol ante- and post-mortem: its relationship to the biochemistry and microbiology of decomposition. J Appl Microbiol 1978; 44: 1–56.
29. Jones AW, Holmgren P. Uncertainty in estimating blood ethanol concentrations by analysis of vitreous humour. J Clin Pathol 2001; 54(9): 699–702.
30. De Martinis BS, de Paula CM, Braga A, Moreira HT, Martin CC. Alcohol distribution in different post-mortem body fluids. Hum Exp Toxicol 2006; 25(2): 93–97.
31. Honey D, Caylor C, Luthi R, Kerrigan S. Comparative alcohol concentrations in blood and vitreous fluid with illustrative case studies. J Anal Toxicol 2005; 29(5): 365–369.
32. Cullen SA, Mayes RW. Alcohol discovered in the urine after death: ante-mortem ingestion or post-mortem artefact? Med Sci Law 2005; 45(3): 196–200.
33. Hagan RL, Helander A. Urinary 5-hydroxytryptophal following acute ethanol consumption: clinical evaluation and potential aviation applications. Aviat Space Environ Med 1997; 68: 30–34.
34. Cullen SA. Aviation suicide: a review of general aviation accidents in the UK, 1970-96. Aviat Space Environ Med 1998; 69(7): 696–698.
35. Bills CB, Grabowski JG, Li G. Suicide by aircraft: a comparative analysis. Aviat Space Environ Med 2005; 76(8): 715–719.

Bioterrorism

Sebastian Lucas

Introduction

Although bioterrorist attacks have taken place over the last two decades, it is still an area of ignorance amongst UK medical staff, including pathologists. This is paradoxical since it is pathologists and anatomical pathology technologists (APTs) in the mortuary who are more likely to be involved closely than many other medical and paramedical staff. The purpose of this chapter is to outline some of the history of bioterrorism (BT), the responses of government, what the likely agents are and their clinical pathologies, medico-legal aspects and what everyone in mortuary work should be prepared for. By definition, much is uncertainty, although the expert consensus is that all industrialised countries could experience a BT attack sooner or later. Following the initiative of the USA, many countries (including the UK) have drawn up plans and produced guidance documents. These have been freely adopted here, accompanied by personal observations where there is no agreed evidence base.

Biowarfare and, to a lesser extent, bioterrorism have been with us for hundreds of years, but the modern industrialised world has only woken up to the actuality in the last decade (1)—see Box 1 and Box 2. Although the potential was beginning to be addressed in the 1990s (and really only in the USA) at government and institutional levels (2), it was the 2001 episode of anthrax-contaminated letters in the mail (in the USA) that truly focused minds. The civil and criminal investigation responses to that event are well documented (3), but the critical role of pathology in that investigation needs to be emphasised (4–6).

Bioterrorism is defined as "the use or threatened use of biologic agents against a person, group, or larger population to create fear or illnesses for purposes of intimidation, gaining an advantage, interruption of normal activities, or ideologic activities. The resultant reaction is dependent upon the actual

G.N. Rutty (ed.), *Essentials of Autopsy Practice*,

Box 1. Historical examples of the utilisation of this mode of attack include (9, 10):

- Fourteenth century, Black Sea city of Kaffa: catapulting plague-infected human cadavers into the city under siege (biowarfare)
- Fifteenth century, South America: the conquistador Pizarro introduced smallpox naturally into a naïve population and furthered the infection rate by presenting the local population with infected clothes (inadvertent biowarfare and bioterrorism)
- 1930s, China: experiments with anthrax (fatal) on humans and subsequent autopsy analysis (biowarfare research)

These examples also indicate some of the agents most prominent in BT considerations: anthrax, plague, smallpox. Their virulence and mortality rates are well attested.

Box 2. More recently, the significant BT events have been:

- USA 1984: contamination of salad bars with *Salmonella typhimurium*, apparently for local political purposes (3, 10); 751 persons affected; no deaths.
- Iraq 1990s: following the first Gulf war in 1991, it became evident that the country had prepared and stockpiled vast quantities of botulinum toxin, aflatoxin and anthrax spores (8, 21, 31). These were never found—presumed to have been destroyed on site—by the subsequent USA and Europe science teams that went in search of weapons of mass destruction.
- Japan 1995: when the attack on the Tokyo metro with the neurotoxic sarin gas by the Aum Shinrikyo cult was investigated, it emerged that members had visited Zaire in order to obtain samples of Ebola virus for BT purposes (10). They were not successful. They had also attempted to disseminate *Clostridium botulinum* toxin, unsuccessfully (31).
- USA 1996: contamination of food in a laboratory staff rest room with *Shigella dysenteriae*; motive unknown (3). Twelve persons became ill; no deaths.
- USA 2001: about 10 gm of anthrax spores was sent in multiple envelopes by post, by person(s) unknown, which resulted in 22 cases. The presumed intended targets were news media and government personnel (36). The index case had anthrax meningitis (4). Twenty of the victims handled mail, and one person was probably accidentally infected by indirect letter-to-letter contamination. Eleven cases were cutaneous anthrax (all survived); 11 cases were inhalational or meningeal anthrax of whom five died (3, 5, 23); 32,000 people received antibiotic prophylaxis.

event and the population involved and can vary from a minimal effect to disruption of ongoing activities and emotional reaction, illness, or death" (7).

Bioterrorism should be distinguished from state-initiated biowarfare research projects and applications. The governments of the UK, USA, Germany, USSR, Japan and Iraq, to list only the most publicised examples, have all supported research into the potential use of biological agents in order to harm enemy military and/or civilian personnel in the twentieth century. They have officially ceased such activities now, but much important information has been gleaned from accidents and other events during this phase of activity.

It was the 1990s events in Japan and Iraq that forced everyone to think of BT as a serious possibility that could affect any country, no matter how well prepared militarily for conventional or nuclear attack. In 1997, the *Journal of the American Medical Association* devoted a whole issue to BT, which reached a wide audience (8, 9).

The Bioterrorism Potential: The Anthrax Scenario

Partly because of the relatively simple process of disseminating the agent in a BT attack, and partly because there has already been a BT attack using anthrax spores, this is the infection that most planning scenarios have considered the most likely in the future. In a relatively densely populated country such as the UK, with conurbations of up to 8 million people, deliberate release could leave up to 5 million people exposed, particularly if it was multiple simultaneous attacks in urban areas.

The actual lethal dose is not known although experiments indicate a LD_{50} (50% of those infected develop disease and die) of 10,000 spores; the elderly may require a smaller dose, as suggested by one patient in the USA during the anthrax-by-post attack in 2001 who had no connection with primarily infected mail.

Perhaps 10% (= 500,000 people) would be at significant risk of infection (quantified as >0.1% risk), and 2% (= 100,000 people) would be at high risk of infection (>1%). Thus of those exposed about 7500 could become infected without prophylactic antibiotic therapy. As determined from the 1979 Sverdlovsk episode (see Box 4), the risk of infection would extend to 50 km downwind.

As a result, a large number of people would present to hospitals with respiratory disease and fever over the following week or so. How they would be managed will depend on:

- Whether the attack was overt or covert
- How many present and to how many health centres
- How rapidly the diagnosis is made in those living
- How rapidly the diagnosis is made from those dying, if not made before death
- The facilities available at the health care centre (hospital), e.g. ICU
- The polices drawn up in preparation for such events

Once it became public that an anthrax BT event had occurred, which would probably be within the usual incubation period time of a week, a larger number of people within the target zone (including the downwind area) would present to health care centres or contact health phone lines for advice. They can be grouped as:

- Well but worried
- Chronically ill with another disease, but perceive themselves at high risk from anthrax
- Symptomatic although the cause is more likely be something else than anthrax
- Symptomatic due to anthrax

There will also probably be a greater number of people who could not have been exposed directly (for geographical reasons) but who are concerned for their health and those who have been in contact with people within the target zone since the attack. The exact numbers involved here are unknowable, but would almost certainly swamp the health centres and resources, and divert resources from the normal disease management processes.

There would be demands for antibiotic prophylaxis and vaccination (although in this case there would be no available vaccines for the public). Without stockpiles, there would not be enough antibiotics to go round, let alone the health care staff to distribute them. Public anger at perceived ill preparation on the part of the government and the health systems could result in public disorder, with extra demands on the forces of law and order.

Because anthrax spores (which are viable for years) would be distributed over the ground, through air conditioning systems (probably), and get into immediate water supplies, infra-structures such as public transport and schools would be closed for decontamination. This would disrupt the social economy as well as the business and financial economies, and reduce the active workforce (child-minding, workplace closed) and so on.

From this brief scenario, it will be seen that the objectives of terrorists—to inflict harm upon a population and disrupt the social systems—will have been achieved. The message that emerges from consideration of the possible effects of a BT attack is the need to focus on several key issues, so as to minimise the effects on individuals, the wider public, the health systems and the economy. These include:

1. Preparation of evidence-based, or at least expert opinion, plans for all reasonable eventualities
2. Awareness among front line staff of the clinical features that should raise suspicion of a BT incident
3. Robust and rapid means of establishing or refuting the diagnosis of a BT-related infection in life
4. Similar means for autopsy diagnosis
5. Plans for reference centre involvement and epidemiological surveillance

6. Plans for the in-hospital management for such diagnoses or suspected patients, both for the first and then subsequent likely cases
7. Plans for advice, prophylaxis and/or vaccination as appropriate for health staff and for the wider public
8. Plans for the disposal of the dead resulting from a BT attack
9. Plans for the medico-legal systems' involvement in dealing with the dead

The Means and Cost of Terrorism

The four physical means of terrorism (conventional weaponry, biological, chemical and nuclear) have very different consequences for societies and their preparations for such events. Of the four means, BT is the most insidious. Assuming a BT attack is covert (not announced—see below), it would be days to weeks before anything was noticed and by then the effects would be widespread (regionally, nationally or even internationally), and then it would be up to individual doctors and health centres to notice the effect and notify the authorities. Hence the attention is now being paid to new methodologies of potential disease surveillance and diagnosis (see below). Chemical attacks have effects within minutes to hours and will be restricted in their radius.

These four main means for terrorists to cause harm and disrupt society are listed in Table 1, along with the estimated costs. The cost is the calculation of the expense to kill 50% of the population in a given area per square kilometre (10).

The Motives for Bioterrorism

The purposes of terrorism in general are varied and mainly beyond the scope of this chapter; hereon the focus is only on BT. From the events so far documented, the purposes include local personal grudges, a desire to influence voting patterns, a doomsday cult's activity and the simply unknown (the 2001 anthrax attack). Of more concern in the twenty-first century (and since the attack on the World Trade Center in New York in 11 September 2001) have to be the effect of

Table 1 Relative costs of the different modes of terrorism to kill civilians

The weapon	Cost in US dollars to kill 50% of a population
Conventional arms and weapons	2000
Nuclear weapons	800
Chemical weapons	600
Biological agents	1

international conflict, particularly in the Middle East and augmented by the 2003 Iraq invasion, and the perceived anti-Islam stand of (particularly) the USA and Europe. Broadly, the purposes of BT include:

- To cause morbidity and mortality
- To disrupt health services
- To induce fear in the population

The consequences of this are, presumably in the minds of bioterrorists, to

- Disrupt society
- Force change of government and/or government policies

The Potential Agents for Bioterrorism

There is no ideal bioweapon for terrorist purposes. One can draw up a list of criteria that would characterise the ideal agent, including:

- Easily available from other laboratories
- Easily prepared from local materials
- Safe to generate and weaponise
- Easily disseminated as an aerosol of 1-5 μm size particles
- Safe to disseminate
- Long lasting and stable in the environment to prolong infectivity
- Readily transmitted from person to person (contagious secondary spread)
- High infectivity, virulence and mortality rate
- No effective treatment of those with clinical disease
- No effective prophylaxis for infected, asymptomatic people (chemotherapy and/or vaccine)
- Major public health impact
- Cause public panic and social disruption
- Require special action for public health agencies

In the late 1990s, the Centers for Disease Control and Prevention (CDC), the federal public health institute in the USA, consulted experts and drew up a consensus list of the most likely and dangerous agents that bioterrorists might use. They comprise the Category A list (2, 7).

Category A

- Smallpox (variola)
- Anthrax (*Bacillus anthracis*)
- Plague (*Yersinia pestis*)
- Tularaemia (*Francisella tularensis*)
- Botulism toxin (*Clostridium botulinum*)
- Viral haemorrhagic fevers (Ebola, Lassa, Congo-Crimea haemorrhagic fever, Marburg viruses)

Following the 2001 anthrax attack, these were consolidated. Two further categories of infective agents were then considered that might be used in BT attack, but carried a lower mortality than the Category A list agents. One stimulus to including these was the perception at the CDC that the diagnostic capabilities for these agents needed to be improved and expanded nationally.

Category B

- Q fever (*Coxiella burnettii*) (11)
- Brucellosis (*Brucella* spp)
- Glanders (*Burkolderia mallei*)
- Arthropod-borne encephalitis (Venezuelan, eastern and western)
- Water- and food-borne gut pathogens (*Salmonella* and *Shigella* spp, *E. coli*, cholera (*Vibrio cholerae*), *Cryptosporidium parvum*)

A further Category C list has been compiled by the CDC, of emerging or re-emerging pathogens that might be engineered for mass dissemination, are easily available and have potentially high mortality. They include:

Category C

- Nipah virus
- Hantaviruses
- Tick-borne haemorrhagic fevers
- Tick-borne encephalitis
- Yellow fever virus
- Multi-drug resistant tuberculosis (*M. tuberculosis*)

Obviously none of these lists is exclusive and exhaustive, but represent the consensus of the most likely possibilities for BT. Table 2 summarises many of the Category A infection characteristics.

Availability, Weaponisation and Dissemination of the Proposed Agents

With the exception of smallpox (see below), all the agents in the three categories cause disease naturally and are present in nature, globally or locally. In addition, there will be freeze-dried preparation in many laboratories. Apart from smallpox, the most difficult to obtain by potential terrorists would be Ebola, as it is still not clear in which animal reservoir the virus resides in the wild in central Africa (12). But there are many isolates in several laboratories. To obtain smallpox would involve a source within one of the two laboratories (in USA and Russia) known to house isolates under secure conditions—unless, secretly, a scientist previously working there has already taken samples with him or her.

Table 2 Properties of biological agents in Category A (data from multiple USA and UK public health sources)

Agent/ disease	ACDP hazard group	Survival in nature	Infectious dose	Transmission	Incubation period	Person to person transmission	Case fatality (%)	Specific therapy
Smallpox	4	2 days	10–100	Contact; inhalation	10–16 days	Yes	15–95	?
VHF	4	2 days	Unknown	Inhalation; inoculation; ingestion	1–21 days	Yes	20–90	+/−
Anthrax	3	40 years	LD_{50} ~10,000, but may be 10–100	Inhalation; inoculation; ingestion	1–10 days, but may be up to 6 weeks	Not in life, but possible during autopsy	20–90	Yes
Plague	3	4 h	100–500	Inhalation; inoculation by infected flea	1–4 days via inhalation	Yes, from pneumonic plague	33–95	Yes
Tularaemia	3	Long	10–50	Inhalation; inoculation; ingestion	2–5 days	Not in life but possible at autopsy	30–50	Yes
Botulinum toxin	3	2 days	~1 ng/kg	Ingestion	3–4 days	No, although it is just possible from an externally contaminated cadaver	?	No

To establish a laboratory capable of manufacturing BT agents on a large scale is not difficult, and the estimated cost of setting up the facility is about $100,000 only (1, 10).

The various means of dissemination of BT agents include:

- Aerosol dispersion
- Contamination of food
- Contamination of water supplies
- Contamination of milk tankers
- Direct inoculation into people

The consensus is that aerosol dissemination is the most likely for mass BT attacks. The two previous gut pathogen BT attacks contaminated food and were targeted locally and specifically; widespread harm did not result. Similarly milk tanker contamination would have limited, brief and local effect only. Most of the Category A list pathogens are not disseminated by water, and those that could be would require vast amounts to be placed in reservoirs to overcome the dilution effect. Direct inoculation is simply inefficient, and is detectable.

Aerosol Dispersion

All the Category A list agents can potentially be disseminated in a fine particle aerosol of sizes 1-5 μm. This is invisible and small enough to be inhaled and passed to the alveoli without filtering and capture. The means of spreading the agents are various, and include:

- Paint-sprayers
- Fogging machines that are used to disseminate insecticides
- Hand-held perfume atomisers
- Hand-held drug delivery devices (like asthma inhalers)
- Airplanes, as for crop-dusting

Basic Definitions in BT Terminology and the Public Health Organisations' Responses

The UK Health Protection Agency (HPA) has issued, from 2005 with updates, interim guidelines for action in the event of deliberate release of a range of chemical, nuclear (radioactive) and biological agents (see their website www.hpa.org.uk/infections/topics_az/deliberate_release/menu.htm for updates). For the biological agents, they include comprehensive sections on Biology of the agent, Epidemiology, Transmission, Communicability, Clinical features, Mortality, Antimicrobial sensitivity, Clinical procedures, Treatment, Infection

control, Immunisation, Decontamination, Protection of health care workers, Post-exposure prophylaxis, Laboratory diagnosis, Public health procedures and contact names and addresses. Autopsy is considered briefly, in terms of what samples to take for diagnosis but also stressing that they should not be performed if the infection is suspected.

However, there is more detail in an earlier HPA document "Initial investigation and management of outbreaks and incidents of *unusual illnesses* – a guide for histopathologists" (2004) (13). As well as specific advice on what samples to take and where to refer them for confirmation, the role of the *coroner*[1] is acknowledged in the autopsy process; and there is the guidance not to perform an autopsy "on any patient recognised as having an unusual illness until expert advice has been sought". An update on microbiological sampling has been issued in 2006 (14). This encompasses the possibilities of genuinely new infections (see below) and enables the full panoply of molecular diagnostics to be applied to material for rapid identification of known and unknown infections.

Unusual illnesses are described as being in/of:

1. Patients presenting with signs and symptoms which do not fit any recognisable clinical picture
2. Known aetiology but not usually expected to occur in the UK or setting where it has been observed
3. Known aetiology that does not behave as expected, e.g. failure to respond to standard therapy
4. Unknown aetiology

An *outbreak* is said to occur where:

- The number of cases is greater than the number expected over a given time period.
- One or more cases are linked by epidemiological, toxicological, microbiological or radiological features.

Obviously, one case of a serious unusual illness such as inhalational anthrax is of public health concern, but would be termed an *incident*. Whilst an outbreak or incident of unusual illness may be the result of natural or accidental processes, they could be due to *deliberate release*; this may or may not have had an underlying malign intent. Deliberate release may be *overt*, where it is immediately evident that release has occurred (e.g. a phone call from perpetrators to the police), although the nature of the release may or may not be clear. A deliberate release may also be *covert*, with the first indication of a release being the presentation of people with unusual illnesses—alive and/or dead.

[1] Coroners are part of an independent national medico-legal system for investigating unexpected deaths, potentially unnatural deaths and deaths of unknown cause. They are not integrated into the British health service.

Thus health professionals have a crucial role to play in the identification of covert releases. Previously unrecognised syndromes may also be due to *new* or *emerging* or *re-emerging* conditions. New infections would be those previously unknown or known in animals but not known to affect man (probably all significant human infections arose in this way, from animal to man transmission). Emerging infections are those previously described but of low natural prevalence and incidence, but now becoming more widespread. Re-emerging infections are previously described, but considered eradicated or of such low natural prevalence as not to pose a risk to man, but are now found to be occurring more frequently.

Acute incidents are those where recognition of an event occurs rapidly, within minutes to hours. *Delayed incidents* are those where presentation of affected persons is delayed by hours, days or weeks: this is the situation in all biological agent incidents.

These descriptions and definitions are presented to demonstrate how public health authorities are preparing themselves and the wider network of medical and paramedical personnel. All the points could be criticised for vagueness or over-inclusion (e.g. this version of unusual illness would cover HIV disease with its complex presentations), but that is less important than the fact of consciousness-raising and placing the concepts before a broader audience.

Mortuary Plans and Provisions

BT cases can arrive at any mortuary, whether hospital or free-standing local authority (public mortuary), with prior known diagnosis or of unknown cause of death before autopsy. What happens once it is established that a BT outbreak, with mortality, is definite or likely will depend on scale. If there are only a few identified or suspected cases, then they will probably be handled at the place of arrival or in a specialist referral unit for autopsy.

However, if the outbreak is larger and is designated as a "disaster", then many local authorities will institute established plans and concentrate all cases in one designated public mortuary in an area, or go further and create a temporary "resilience mortuary". For example, in London, the coroners and local authorities have drawn up plans for designating disaster mortuaries as that most geographically and facility appropriate. Each such mortuary will have a maximum agreed capacity, and if this is exceeded, a resilience mortuary will be created to concentrate all the cases on one site.

This could be an existing public (or even hospital) mortuary, but is more likely to be a free-standing entity for logistic reasons. Because such BT outbreaks are by definition homicide and by the nature of the infections, there is the need for a multi-agency investigative team, forensic science access, identification teams, high inter-agency communications traffic, all-hours vehicular access, relatives' communication and viewing areas, media communication centre, etc. The

investigations could go on for weeks depending on the scale and cadence of the BT attack. All these factors, in the event of a major attack, would overwhelm most existing hospital and public mortuaries, and—critically—prevent them from undertaking their normal day to day activities (normal mortality and funereal requirements do not stop because of terrorism). The utility of this approach was demonstrated in the aftermath of the July 2005 London terrorist bombing, where a temporary mortuary facility and communications centre was erected in a territorial army campus in central London, and all the above activities could proceed smoothly; it was then taken down when all the bodies had been studied and prepared for disposal (London resilience plan, May 2005).

The Disease That May Present as Bioterrorism

Smallpox and anthrax are described in some detail, and the other four infections in Category A are more briefly depicted. Colour images of the clinical and gross and histopathological features of all the infections are available for viewing and downloading on the USA CDC and the UK HPA websites. Table 2 depicts the essential data on infectivity and contagiousness.

Smallpox

Smallpox is one of the six Category A infections considered most likely to be used in a BT attack. The following fairly full account of the disease—its presentation, investigation and management and its potential impact and autopsy issues—is presented as an exemplar of the aspects of which an involved pathologist needs to be aware (15–17).

Smallpox was declared extinct in 1980 by the WHO. The USA and Russia have maintained reference stocks of the virus (in one institution each) (16), and there are no known illicitly held smallpox virus stocks. Thus the possibility of deliberate release is low; however, the likely consequences are so severe that contingency plans are prepared against the event. The number of people who have active immunity from routine vaccination programmes prior to 1980 is low since the complete efficacy of the vaccine is believed to be not more than 5 years' duration.

Variola is a DNA virus. There is no natural animal reservoir. Transmission is usually from droplet aerosols of infected persons, inhaled into the respiratory tract of another. Direct skin-lesion-to-skin contact can transmit, as can contact with infected body fluids. The most infectious period in a patient is after the incubation period, during the first week of the rash, when the virus is released from the respiratory tract. Several of the critical facts of smallpox are in the Table 1.

Clinical Features

During the asymptomatic first week, there is viraemia and dissemination to the lymphoreticular system. A second viraemia commences on about day 8 from infection and is associated with the characteristic illness:

- Sudden high fever
- Macular rash 1–3 days later in the oropharynx, then face, forearms and trunk
- The rash becomes popular 2 days later, then vesicular after another 1–2 days. Typically it is more severe on the face and extremities—centrifugal pattern
- The vesicles become pustular after another 2–3 days, forming scabs 5–8 days after the onset of the rash
- The scabs separate leaving characteristic pitted scarring, most prominent on the face.

That is the typical pattern. Smallpox can have less common atypical patterns that have caused late diagnosis through confusion. The two forms are

- Haemorrhagic smallpox—with haemorrhage into the mucosal and skin lesions.
- Malignant smallpox—the lesions do not develop to the pustular stage but remain soft and flat.

Differential Diagnosis of Smallpox

With no natural cases of smallpox for >30 years, few doctors will recall the clinical features from personal experience. The classic differential has been chickenpox (varicella-zoster virus, VZV). Other skin rashes that could be confused with smallpox, and almost inevitably will do so in the event of an outbreak, when the threshold for suspecting smallpox will be lowered, include (15):

- Monkeypox
- Generalised vaccinia
- Herpes simplex virus
- Molluscum contagiosum
- Measles
- Parvovirus (B19)
- Rubella
- Enteroviral infections
- Hand-foot-and-mouth disease
- Syphilis
- Impetigo
- Drug eruptions and Stevens–Johnson syndrome
- Atypical forms of skin lesions in immunosuppressed persons (e.g. anergic cutaneous cryptococcosis in advanced HIV disease can resemble herpetic and smallpox rashes in the pustular stage)

For haemorrhagic smallpox, the differential diagnosis is:

- Meningococcal sepsis
- Acute leukaemia

For malignant smallpox:

- Haemorrhagic chickenpox

The World Health Organisation has produced training materials to help health care workers recognise smallpox and its differential diagnosis; see the WHO website www.who.int/emc/diseases/smallpox/slideset/index/htm.

Treatment

There are no proven antiviral drugs effective against smallpox (there has been no need to develop any against an eradicated infection, until now). However, cidofovir is active against other orthopox viruses, is active against variola in vitro and would be tried if cases arose. It has to be administered IV and is potentially nephrotoxic.

Vaccination

Stocks of smallpox vaccine have inevitably been depleted since 1980, but are now being regenerated precisely because of the threat of BT. It is most effective before exposure to smallpox, but vaccination does reduce the clinical attack rate if given after exposure. Previously vaccinated people may have an accelerated response in the development of the vaccination pustule. Vaccine "effectiveness" means reducing the attack rate to $<10\%$ and mortality to $<1\%$.

In the UK, stocks of vaccine are limited and only a few health care professionals, mostly laboratory staff, and emergency service personnel have been vaccinated; the supply is controlled by the Department of Health. No pathologists or APTs have been vaccinated at the time of writing. A further problem is that of vaccine complications. Apart from generalised malaise, specific complications include (18)

- Generalised vaccinia
- Progressive vaccinia
- Post-vaccination encephalitis
- Fetal vaccinia
- Myopericarditis

None of these is common, but are sufficiently notable to advise a cost–benefit approach as to who needs vaccination in the event of a definite or suspected smallpox BT attack. A further issue, not seen in the days before smallpox

eradication, is the susceptibility of HIV+ persons to a greater complication rate than non-infected persons. Whether potential vaccinees should have an HIV test or be screened by questioning for HIV risk factors and their own knowledge of their HIV status awaits clarification (19).

Diagnostic Procedures and the Autopsy

In life, there are protocols produced by the HPA on clinically suspecting smallpox and how to confirm or exclude the disease. In the laboratory, the diagnosis will be made by real-time PCR; electron microscopy identifies the virus but the morphology is similar to that of other orthopoxviruses (15).

For those who die, the HPA guidance is fairly specific (17):

- If the diagnosis is already known there is no requirement for autopsy.
- If a case is suspected but examination procedure can be kept to a minimum of invasiveness, to reduce the likelihood of infection to pathologists and APTs, there is no need to open the cadaver to prove smallpox.
- All staff involved in an autopsy must be immunised.
- Full respiratory protection must be used, in addition to the standard universal precautions in dress (see below).
- Skin samples, both uninvolved and obviously abnormal can show the virus through standard virological techniques
- Postmortem blood through cardiac puncture is a useful source of virus
- The samples must be transported to the reference centre laboratory in leak-proof secondary containers, complying with the UN602 standard packaging, and labelled BIOHAZARD.
- Bodies should be cremated, and not buried or expatriated, and thus disposed of rapidly. They must be placed in double body bags (not cotton shrouds).
- Bodies should not be embalmed
- Funeral director staff must be fully informed and involved with disposal, and should also be vaccinated.

However, this does not immediately answer the question of what to do when the likelihood of smallpox is actually low, and a fuller, invasive, autopsy will or might be needed to identify the proper cause of death. Following the previously published Royal College of Pathologists' guidelines on autopsy practice for Hazard Group 4 serious communicable diseases (20), it is recommended that the results of rapid virological studies, transported promptly to the reference centre, on the skin and blood material are awaited. If smallpox is excluded, and any similarly hazardous infection likewise, the autopsy can proceed under standard conditions. If smallpox is proven, but for forensic reasons there is a need for further examination, then the autopsy can take place in a mortuary designated suitable for such diseases, if the body is not there already.

The Pathology of Smallpox (6)

Skin. The rash has been described above. Histopathologically, there is intra-epidermal oedema, ballooned epidermal cells and necrosis. The characteristic Guaneri bodies are intra-cytoplasmic granular, basophilic viral inclusion bodies in epidermal cells. There is marked dermal inflammation. Similar inclusions are seen in the mucosal epithelium.

Heart. In fatal cases of smallpox, there is a myocarditis.

Lung. In fatal cases there is often a direct smallpox pneumonitis and secondary bacterial infections.

The Impact of Smallpox

In the absence, so far, of a smallpox BT event, any estimation of its impact on society is imprecise. It will depend on the number of people directly affected and how rapidly the health authorities diagnose the infection and act to limit its spread. However, it is worth reiterating what happened in Yugoslavia in 1972 when a person returned from the haj in Mecca having acquired smallpox infection there (1). The country had continued an active vaccination programme although there had not been a case since 1927. See Box 3.

What would happen during a smallpox outbreak in an industrialised country now is unpredictable, particularly where government does not have a similar built-in control over its population as pertained during the communist era in Yugoslavia. In a covert BT attack with smallpox, there would be no warning that such a febrile rash could be smallpox, and 7–10 days would lapse after the attack before any presentations. Because of delays in diagnosis, it might be another few days before the diagnosis was suspected and made (though now the diagnosis would be made virologically within 24 h once a sample was presented to a reference centre). Suppose 100 patients in a large city developed the disease, presenting to different hospitals. Coping with them using isolation precautions, with the exposed health care workers and with the friends and relatives of the cases would probably bring the hospitals to a halt in the discharge of their normal function.

The Impact in the Mortuary

What would happen in the mortuary should a case be autopsied, without anyone realising at the outset that it was smallpox, is also unpredictable. If the pathologist or APT suspects the diagnosis, then the diagnostic and logistic procedures outlined above should be followed. But the staff are unlikely to have been already vaccinated (as required by the guidance) and there will probably be a degree of panic. Also there may not be established and available protocols between the mortuary, the microbiology department and the reference centre as to how to proceed in the evaluation of suspected smallpox. Occupational health

Box 3. The 1972 Yugoslavia smallpox outbreak

The index patient developed an undiagnosed fever. Friends and relatives came to visit him. Two weeks later, 11 of them had developed fever and a rash; no clinicians consulted diagnosed smallpox.

One of the 11 secondary cases developed atypical haemorrhagic smallpox; he was taken from a local clinic to a larger hospital, to an ICU in a third institution, and died without a diagnosis. Two days later, the first case of smallpox was diagnosed. Now, a month after the index patient's return, 150 people were ill with the disease, and before the first diagnosis had been made. The patient with haemorrhagic smallpox had transmitted the infection and the disease, in transit through hospitals, to 38 other people.

The cases of smallpox were distributed over the country. Neighbouring countries closed their borders with Yugoslavia. The government launched a nation-wide vaccination campaign, and 20 million persons were vaccinated. Hotels and residential blocks were taken over as quarantine centres, and all people exposed to cases of smallpox interned, totalling 10,000 people who spent two weeks or more therein.

The Yugoslavia outbreak stopped 9 weeks after the index patient became ill; 175 persons had developed the disease and 35 died. And this was in a generally well-vaccinated population. One commentator noted that "it was, in fact, a small outbreak" (1).

units (in a hospital environment) will be involved and vaccines would have to be obtained from the Department of Health rapidly. In a public mortuary environment, there are usually no such on-site health advice facilities, and tissue sampling is also usually more problematic.

If the diagnosis was not suspected by the end of the gross autopsy examination, there are two possibilities:

1. The diagnosis is not made at all, no tissue having been retained and another cause of death provided
2. The diagnosis becomes evident later on histopathological and/or microbiological analyses

If only histopathology is used, it is likely to be days to weeks before the diagnosis is suspected and confirmed (the viral inclusion bodies in the skin can be confirmed by immunocytochemistry). Thus the opportunity to protect the staff by vaccination will either be lost or made perhaps too late, unless the diagnosis becomes retrospectively evident for other, clinical, reasons and/or from other similar patients.

Once the first case has been identified at autopsy, then the information will pass rapidly to all other mortuaries in the country. It is likely that only the few

designated mortuaries will then undertake even the skin sampling procedure for diagnosis in subsequent suspected cases, following a reasonable fear among the unvaccinated APT and pathology staff nationally. Unfortunately, at the time of writing, there are no such designated mortuaries in the UK; only a few anecdotally known to be capable of undertaking the relevant procedures safely.

The conclusion is that, despite preliminary planning and published guidance, the first time that such a BT attack takes place, the autopsy pathological process will be messy, probably chaotic and possibly late. The role of the medico-legal authorities has not been considered in this scenario, but as coronial jurisdictions are independent, there may be no consistent approach, at least in the initial phases of a BT attack. It will be a learning experience, and it is to be hoped that no staff become infected accidentally as a result.

Deliberate Release?

Any confirmed case of smallpox signifies deliberate release.

Anthrax

This is the only one of the Category A list pathogens yet to have been used in a BT attack (21). It is an aerobic gram-positive bacillus that is readily grown on artificial media. Once the growth is saturated, it forms spores, which also happens when bacilli in man are exposed to air. Infection is by the spores, which measure 2 μm. In nature, many animals are normally infected, form spores in the soil, where they can remain viable for decades (22).

There are three routes of infection for *Bacillus anthracis*: inhalational, cutaneous and gastrointestinal. However, the clinical presentation expands to include meningitis (half the autopsied victims of the Sverdlovsk inhalational anthrax outbreak (Box 4) also developed meningitis; the first case in the USA 2001 anthrax attack presented with meningitis). From the forensic epidemiological viewpoint (see below), an index case of anthrax meningitis should suggest deliberate release.

Clinical Presentation

For inhalational anthrax, the incubation period is usually between 2 and 10 days. The initial symptoms are non-specific fever, non-productive cough and malaise. Then sudden shortness of breath, hypotensive shock, stridor and cyanosis develop, and within a day or so death occurs, despite intensive care. Radiologically the critical finding is widening of the mediastinum and pleural effusions.

Box 4. The Sverdlovsk episode, 1979 (1, 21).

There was an accidental release of a small quantity (estimates vary from 1 to 100 gm) of anthrax spores into the atmosphere from a military bioweapons facility at Sverdlovsk (ex-Ekaterinberg) in the then USSR. Within a radius downwind of 4 km, 77 persons became ill and 66 died, from official announcements. It is suspected that more were infected, perhaps 300, with 100 deaths.

The modal incubation period (IP) was 9–10 days, but the longest IP was 43 days. Irrespective of the IP, death from inhalational anthrax followed 1–4 days from the beginning of symptoms.

Animals were affected up to a radius of 50 km from the source, causing death in sheep and cows. Anthrax spores reside viable on earth for decades.

The clinical suspicion of inhalation anthrax is raised in a previously healthy person if there is:

- Rapid onset of severe unexplained febrile illness of febrile death
- Rapid onset of severe sepsis not due to a predisposing illness, or respiratory failure with a widened mediastinum
- Severe sepsis with gram-positive rods or *B. anthracis* identified in the blood, chest effusions or cerebro-spinal fluid, and assessed not to be a contaminant.

Pathologically, this is not a pneumonia or pneumonitis, but a haemorrhagic effusion involving the mediastinal lymph nodes (hence the mediastinal widening on chest X-ray) and pleura. There is necrosis of the lymph nodes with immunoblast proliferation, abundant gram-positive bacilli, but little acute inflammation. The lung parenchyma shows oedema and haemorrhage and acute lung injury (acute respiratory distress syndrome, ARDS) in some cases. If the patient has been treated with chemotherapy for 72 h or more, the gram-positive rods may not be visible, but immunohistochemically are still evident (23).

The meningitis is also haemorrhagic. On CSF examination it is like other acute bacterial meningitides except that the gram-positive bacilli are plentiful.

Cutaneous lesions developed in half of the 2001 USA anthrax patients. These are ulcers that become eschars. The clinical differential diagnosis has included skin haematoma and non-specific erosions (23). Histologically, there is epidermal and dermal oedema and necrosis, acute inflammation and characteristic gram-positive rods; treatment will reduce their number, but immunocytochemistry will identify the antigens.

Autopsy in cases of known or suspected anthrax in the UK is discouraged (22). But if they take place, then full protective clothing and equipment are to be utilised (see below).

Deliberate Release?

This should be considered with a single confirmed case of inhalational anthrax or a single confirmed case of cutaneous anthrax in someone who does not routinely have contact with animals or animal hides, two or more cases of suspected anthrax that are linked in time and place.

Plague

Plague is caused by *Yersinia pestis*, a gram-negative cocco-bacillus. It is a zoonotic infection widespread globally (24, 25).

In the UK, the last outbreak was in 1919. Infection is usually transmitted by the bites of infected fleas, but human-to-human transmission can occur through infectious respiratory droplets. The common natural plague infection is bubonic, i.e. from fleas, with lymphadenopathy, sepsis and secondary pneumonia. Pneumonic plague is the likely outcome from BT attack, as the bacilli can be stored and aerosolised readily.

The incubation is 1–4 days, and once pneumonia has developed, transmission to others is possible from sputum; this phase could last up to 3 days after commencing antibiotic therapy.

The clinical suspicion of plaque is raised by the following clinico-pathological presentations, in previously healthy persons, especially of two or more cases that are linked in time and place (24):

- Sudden onset of severe unexplained febrile respiratory illness
- Unexplained death following a short febrile illness
- Sepsis with gram-negative cocco-bacilli identified in clinical specimens

From the autopsy pathology perspective, this and tularaemia are the potential BT diseases that most mimic other septic conditions commonly seen in the mortuary (see Table 3). Other clinical patterns that could present are pharyngeal infection, meningitis and septic shock syndrome.

The gross pathology of primary pulmonary plague is acute pneumonia. Histologically there is alveolar oedema, acute inflammation and abundant bacilli. Septicaemic plague results in skin vasculitis with numerous bacilli, vascular obstruction, skin infarction and gangrene (4). Lymphadenopathic plague shows large haemorrhagic and necrotic nodes, with abundant bacilli (6).

Autopsy in cases of known or suspected plague are discouraged in the UK (24), but if the standard precautions are used (see below) the risks to staff are minimal.

Tularaemia

Francisella tularensis type B is endemic across northern Europe, but the more virulent type A strain, which would be used in BT, is restricted to North

Table 3 The pathological syndromic approach to surveillance for BT agents (expanded from Guarner and Zaki (4) and Nolte et al. (7))

Clinico-pathological syndrome (biopsy or autopsy)	Common causes, including infections of public health interest	Potential bioterrorism illness
Vesicular skin rash	Varicella, immunological blistering disorders	Smallpox
Diffuse haemorrhagic skin rash	Measles, rickettsioses, meningo-coccaemia, dengue, toxic shock syndrome, entrovirus, other thrombo-cytopaenias, leukaemia	Viral haemorrhagic fever, smallpox
Community-acquired pneumonia	*Strep. pneumonia, Legionella*, influenza, hantavirus pulmonary syndrome, tuberculosis, other bacterial and viral pneumonias	Plague, tularaemia, Q fever
Haemorrhagic mediastinitis and pleural effusion	Carcinoma and mesothelioma, pulmonary leptospirosis	Anthrax (if gram+ve rods present, highly likely)
Sepsis syndromes, including disseminated intra-vascular coagulation (35)	Streptococcal and staphylococcal infections, meningococcaemia, malaria, leptospirosis, yellow fever, rickettsioses, tuberculosis, haemophagocytic syndrome, lymphoma, HIV	Plague, tularaemia, viral haemorrhagic fever, anthrax
Haemorrhagic meningitis	Herpes simplex encephalitis	Anthrax (if gram+ve rods present, highly likely)
Encephalitis, meningitis	Viral, bacterial, fungal and parasitic meningitis and encephalitis	Venezuelan equine encephalitis, Nipah virus
Swallowing, muscle movement, eye movement and breathing difficulties	Myasthenia gravis, Eaton–Lambert syndrome, Guillain–Barre syndrome, rabies	Botulinum toxin
Hepatitis, fulminant hepatic necrosis	HBV, HCV, septic shock	Brucellosis, viral haemorrhagic fevers
Haemorrhagic colitis	Bacillary dysentery, infarction	*E. coli, Shigella*, gastrointestinal anthrax
Pharyngitis, epiglottitis	Common viral and streptococcal sore throat	Viral haemorrhagic fever (Lassa)

America where it is zoonotic. It is a gram-negative bacillus, transmitted by arthropod (tick) bites, contact with infectious animal material and inhalation of infected aerosols, e.g. damp hay. The minimum infective dose may be as low as 10 organisms (26, 27).

Clinically, BT tularaemia will be pneumonic or septicaemic, and the mortality is 30–50%. Gentamicin is the treatment for cases, but for prophylaxis cirpofloxacin is indicated.

Clinical suspicion of tularaemia arises with:

- A severe unexplained febrile illness or febrile death in a previously healthy person
- Severe unexplained respiratory illness in an otherwise healthy person
- Severe unexplained sepsis or respiratory failure not due to a predisposing illness
- Severe sepsis with unknown gram-negative cocco-bacillary species that fails to grow on standard blood agar, identified in blood or cerebro-spinal fluid (26).

Person-to-person transmission does not occur in life, but transmission during an autopsy is possible. The UK HPA discourages autopsy of suspected cases, but if one is required for diagnostic or forensic reasons, then universal precautions and FFP3 mask respiratory protection as minimum is required.

Pathologically there is a pneumonia and multi-organ failure from septic shock. Histology shows necrotising haemorrhagic pneumonia and reactive necrotic lymph nodes, with abundant bacilli which can be proven to be *F. tularensis* by immunocytochemistry in fixed material if fresh microbiological studies have not already identified the infection (4). The relevant samples to take include lung tissue, blood and lymph node.

Viral Haemorrhagic Fevers

Deliberate release of these viral agents may not actually occur as they are difficult to weaponise by aerosolisation; only in experimental animal situations has aerosol transmission been proven. However, because clinical person-to-person transmission is frequent, some of them have high mortality, and when known to the public, they are included among Category A pathogens. The four agents considered are (28, 29):

- Lassa fever
- Crimea/Congo haemorrhagic fever (CCHF)
- Ebola virus
- Marburg viruses

Lassa fever presents insidiously as malaise, headache and a sore throat. Ebola infection starts as an acute fever and diarrhoea (which may be bloody) and vomiting. Marburg is similar to Ebola. CCHF starts abruptly with fever and malaise. All the VHFs develop into multi-organ failure (but the lung is not usually affected) with haemorrhages from gut, pharynx and skin. The mortality rates vary with that for Ebola (50–90%) the highest.

The differential diagnosis includes falciparum malaria, yellow fever, dengue and gram-positive and gram-negative bacterial septic shock syndromes.

Treatment is with intensive care as appropriate. Ribovirin is effective in Lassa fever and might be useful for CCHF, but is ineffective in Ebola and Marburg infections.

Pathologically, the organ injury is more necrosis than inflammation, notably in the liver and kidney (30). Gut haemorrhage is common. Intra-cytoplasmic viral inclusions are seen in the liver in Ebola and Marburg (6). Immunohistochemical and electron microscopical analysis shows vast amount of virus in most organs. Skin punch biopsies also show virus with this technique, which can be used to screen potential cases without a full autopsy (4).

The UK guidance on autopsy is that these are not allowed for known or suspected VHF disease (28). This follows from guidelines issued by the Advisory Committee on Dangerous Pathogens (mainly concerned with management of living patients) which are enforceable by the Health and Safety Executive. Nonetheless cases of undiagnosed suspected imported VHF have been examined at autopsy in the UK, using proper respiratory protection; they have excluded VHF and provided other diagnoses (personal observations). The only two definite cases of VHF in the UK over the last decade were not autopsied (28).

Botulinum Toxin

The clostridial neurotoxins are among the most potent lethal substances known (31). The human LD_{50} is about 1 ng/kg. Botulinum toxin, in nature, is usually ingested in contaminated food. It causes muscle paralysis and respiratory failure. Apart from failed attempts in Japan to use it for BT purposes, it is known that Iraq had prepared some 19,000 L of toxin, and weaponised much of it, in the 1980s.

The toxin could be disseminated either in food or liquid (e.g. milk) or aerosolised for airborne transmission. Natural toxicity results in neurological symptoms from 12 to 36 h (range 8 days to 8 days) after ingestion (32). These comprise progressive motor paralysis and difficulties in breathing. The management of cases is ventilation; vaccination is ineffective as it takes about 12 weeks to undergo. Vaccination for those predicted to be exposed is possible. The supply of antitoxin is limited everywhere. Diagnosis is currently by an in vivo mouse assay, tested against serum, faeces or respiratory secretions. Clinically the diagnosis is made by electromyography.

Differential diagnosis of botulism: this is limited to myasthemia gravis, Eaton–Lambert syndrome, Guillain–Barre syndrome and some cases of rabies.

The role of the autopsy pathologist in suspected botulinum toxin attack is limited. Gathering samples—if not already collected in life—is important, although it is unlikely that there will be detectable toxin in cases of inhalational botulism. There is also the differential diagnoses and evaluating co-morbidities that may have contributed to death.

Surveillance for Bioterrorism

There has been much discussion on the optimum means of identifying BT attacks, either before they have affected anyone or as early as possible after people become ill. The four basic modes are (1):

1. Regional syndromic surveillance for unusual diseases
   - Monitoring reported cases of fever, diarrhoea, rash, respiratory tract infections
2. Data mining
   - Analysis of school and work absentees
3. Regular air sampling from sentinel sites
   - For example, detecting anthrax and tularaemia agents in the air
4. Local reporting of unusual cases
   - For example, PUO + pustular rash = ?smallpox
   - Pneumonia + haemoptysis = ?plague
   - Fever, SOB, mediastinal widening on chest X-ray, rapid demise = ?anthrax

The present consensus is that local reporting systems are the most likely to be useful and efficient in real time for identifying BT. The pathologist has a role both as surgical biopsy and autopsy diagnostician, and the most important aspects are (i) being aware of the differential diagnoses that include BT agents and (ii) being able to pursue the possibilities so as to confirm and exclude them. Table 3 presents the commoner clinico-pathological scenarios that may present to the mortuary that include the possibility of a BT-related disease, alongside the commoner infectious and non-infectious causes of those presentations.

Needs and Standards of Autopsy Practice

In the circumstance of a pandemic bird influenza epidemic (33), it is agreed that once the initial cases have been identified from a combination of clinical pre-mortem investigation and autopsy investigation, the presenting syndrome will have been described and clarified. Subsequent cases will then be identified syndromically and will not require positive identification of the infecting agent by laboratory analysis in life or from autopsy. It is agreed that the syndromic diagnosis will suffice for medico-legal purposes and death certification.

However, deaths from proven bioterrorist attacks are homicide (7, 6), so consideration will need to be given to the degree of autopsy examination if there

are many cases; compromise may affect the stringency of evidence in individual cases required to bring a successful prosecution against alleged perpetrators at a criminal trial. Here, forensic epidemiology comes into play.

Forensic Epidemiology

Epidemiologists have been investigating the patterns and causes of outbreaks of infectious disease (and other diseases) with increasing sophistication for more than a century. But the recent concept of "forensic epidemiology" has emerged when public heath investigations overlap with criminal investigations; and BT attacks are an evident cause for this. Because the training and experience of epidemiologists is quite different from that of criminal investigators, there are different priorities and thus conflicts when the two groups interact, and the new cadre of forensic epidemiologists is intended to bridge the gap. Forensic epidemiology is defined as:

> the use of epidemiological methods as part of an ongoing investigation of a health problem for which there is suspicion or evidence regarding possible intentional acts of criminal behaviour contributing to the health problem (3).

In the USA, there is now a standard training programme in forensic epidemiology being rolled out (see CDC website: www.bt.cdc.gov/). In brief, the important issues when investigating an outbreak of infectious disease focus on:

1. Understanding how public health investigations proceed
    i. Defining exposed populations
    ii. Providing prophylaxis to exposed persons
2. Identifying the source, i.e. perpetrators or reservoir
3. Recognising that certain unusual or unnatural findings in a disease investigation may suggest intentional (deliberate) or covert action
4. Identifying procedures and mechanisms to communicate suspicions of intentionality to law enforcement officials
5. Understanding how a public health investigation differs from and is similar to a criminal investigation
6. Assessment and credibility of a threat
7. The laws surrounding entry into and obtaining samples within homes and workplaces
8. Establishing chain of custody of evidence
9. How to conduct concurrent public health and criminal investigations

A little thought about what happens during an autopsy in cases when there is no initial consideration of criminality or homicide, particularly regarding points 3, 4, 5 and 8 in the above list, exemplifies the lack of relevant training and the problems likely to ensue for pathologists in the event of a covert BT

attack that results in fatalities with no diagnosis. Further, in the UK, the general lack of training in, and experience of, infectious diseases for pathologists (as opposed to cancer, the bedrock of histopathology training) is a concern. The situation already exists whereby pathologists may refuse to undertake Hazard Group 3 infectious autopsy cases (e.g. HIV, TB, HCV) for fear of infection of themselves and their APTs. There is ethical debate as to the degree to which medical practitioners are expected to potentially put their lives at risk in managing patients. It seems likely that the continuing greater awareness of possible BT will affect the patterns of mortuary activity across the country, with such cases being more concentrated in a restricted number of centres with the resources and protocols to handle them.

UK Medico-legal Systems and Bioterrorism

Several organisations in the USA (e.g. New York City, Nov 2004) have made formal agreements for conducting joint public health and law enforcement investigations following a BT attack. The impression is that planning consideration for BT deaths is more advanced in the USA than in the UK, with a greater degree of central control and network organisation of the relevant medico-legal authorities (medical examiners and/or coroners) and laboratories. In the UK, the initial investigations of deaths will almost certainly take place under the coronial system (or procurator fiscal in Scotland), which is currently fragmented, under-resourced and non-standardised. There is statutory reform planned and it is hoped that considerations of terrorism (all forms) will feature more prominently in the contingencies and planning.

Fundamentally, if the likelihood of a BT-related infection is not highlighted at the commencement of an autopsy, there is currently no standardised approach to considering the possibility of BT and how to resolve the differential diagnosis among the pathologist community at large. Table 3 shows how similar BT infections are to many non-BT infections. It is not impossible that early cases may be missed through inadequate investigation.

Personal Protection for Pathologists and APTs During Autopsy in BT Cases

Historically, pathologists have acquired a wide range of diseases from working infected cadavers, including streptococcal sepsis, tuberculosis, tularaemia, erysipeloid fever, diphtheria, glanders, scrub typhus, systemic mycoses (blastomycosis, coccidioidomycosis), toxoplasmosis, HIV-1, hepatitis B and C, rabies, smallpox and viral haemorrhagic fever (34). Deaths among prosecutors from these infections—which include agents in the BT Category A–C lists—have occurred.

It is likely that the threat of BT will reinforce the historical trend amongst mortuary workers to utilise greater levels of personal protection against

accidental infection. Both in the USA and UK, the standard recommendation dress for all exposed staff for *all* autopsies is (20, 34):

- Surgical scrub suit
- Hat
- Water-impermeable gown covering arms, trunk and upper legs
- Plastic apron over the gown
- Respiratory protection—see below
- Eye protection
- Reinforced rubber boots
- Multiple glove layers, ideally latex gloves either side of cut-resistant neoprene glove

The respiratory protection prevents inhalation of aerosols and contamination of the mucosa by droplets. Standard surgical masks provide protection against droplet splashes, but finer aerosols readily get round the leaky sides. When there is a risk from inhaling a pathogenic aerosol, respirators that prevent nearly all particles $>=1$ μm getting into the lungs are necessary, following guidelines (34). There are two basic types:

- The modified disposable mask (N-95 respirator in USA; EN149 FFP3 respirator in UK)
- The powered air-purifying respirator (PAPR) with high-efficiency particulate air (HEPA) cartridge filters. These are ventilated hoods that cover the head; they may be part of a whole body suit or applied on top of a separate suit. In the UK, the standard PAPR is the EN12941 with PP3 filters.

The latter provides better protection for those with beards, and also protects against chemical toxic agents. It is intuitive that PAPR would be preferred when dealing with suspected BT agents such as smallpox and VHFs (34); but it should be emphasised that there is (as yet) no evidence base for such a statement, and there is no official public health service guidance on the issue, in either USA or UK. At present, it is a matter of preference and equipment resource. One downside to currently available PAPR is the somewhat reduced visibility of the dissection area compared with usual goggles or visor.

Generic Protocol for the Autopsy and Specimen Collection in Suspected Bioterrorism Cases (14)

1. Autopsies should be performed within 24 h of the patient's death, to increase the validity of culture and PCR results
2. Aseptic techniques must be applied as rigorously as possible to minimise contamination, from within and without the body
3. All samples should be collected in duplicate for

- Histopathology—fixed in formalin
- Microbiology—unfixed in sterile containers, fresh and frozen

4. Normally site-sterile samples (with no commensal flora to complicate interpretation) are preferred whenever possible, as long as they are relevant to signs and symptoms displayed
5. Tissues to be sampled

- Local inflammatory lesions or abscesses
- Liver
- Spleen
- Lung
- Heart
- Kidney
- Lymph nodes
- Bone marrow
- Other organs with gross pathological abnormality

6. The tissue fragments for microbiology should be 1 cc in size
7. Samples for microbiology should be both fresh and frozen at –70°C
8. Heart blood to be collected

- Whole in one tube
- Spun for postmortem serum in a separate tube

9. Cerebro-spinal fluid to be collected—fresh and frozen
10. Urine to be collected—frozen
11. Faeces and gut contents to be collected, for microbe and toxin detection
12. Cytological preparations from tissue smears done at the time of autopsy
13. All samples to be clearly labelled with patient's name, date of autopsy and site of sample
14. Chain of custody of sample evidence ensured

Once samples have been gathered, then microbiological analysis can be conducted through the local microbiology departments or directly with the HPA laboratory network.

This sample list is necessarily exhaustive and is hardly likely—or needed—to be followed in every instance. The nature of the case and the material actually available will determine what is taken. However, the important fact is that mortuaries undertaking autopsies of this nature must be equipped with necessary facilities and material. This includes:

- Good ventilation, lighting and water provision
- Sterile specimen containers and labels
- Formalin fixative
- Plentiful sterile instruments
- Centrifuge
- Freezer

- Storage facilities for specimens
- Anatomical pathology technologists (APTs) skilled for the task
- Appropriate personal protective equipment
- Protocols for safe practice in the mortuary
- Communication facilities for pathologist–microbiologist–other professional conversations

Disposal of the Cadaver

For all the likely used BT infections, the guidance on disposal of the cadaver is uniform (Table 4). Embalming is not to be done, and the body should be cremated rather than buried. Funeral directors must be informed about the case and associated hazards. There is no reason why relatives may not view the body after death so long as skin contact is not made if there is a possibility of skin contamination.

The degree of reconstruction of cadavers autopsied following BT infections is not defined in guidelines. But common sense indicates that for blood-borne infections the reduction of potential glove penetration by not using needle and thread is evident. As the cadavers are to be cremated, then binding them up with sticky tape is recommended.

Pacemakers, for all BT infections, should be removed (as for all cremation cases), treated with hypochlorite, bagged and disposed of (but not by incineration) (26).

Pathological Identification of BT Agents

The clinico-pathological syndrome is the starting point for consideration (4). Table 3 depicts some of the syndromes that could present as BT-related illness, with the other common causes of such disease. If a BT infection is suspected, confirming or excluding it depends on the scenario and what material is available. Fresh tissue for microbiological analysis is obviously optimal. But formalin-fixed, paraffin-embedded material can be precisely categorised in many cases, using H&E, empirical special stains, immunocytochemistry and (potentially) molecular diagnostics.

H&E stains identify viral inclusion bodies, but do not necessarily specify them. The histological special stains most useful in evaluation of BT infections and their differential diagnosis are: Gram, Grocott silver, Ziehl-Neelsen and Warthin-Starry (6). Immunocytochemical antibodies are not generally available for specifying the relevant infectious agents, at least in the UK. The USA Centers for Disease Control, however, does maintain a large panel of antibodies that can reliably identify most of the BT agents (and all those in Category A) (4). Molecular technology, including PCR, is in its infancy as regards identification

Table 4 Summary checklist of relevant issues if an autopsy is performed on a Category A infection case

	Anthrax	Smallpox	Plague	VHF	Tularaemia	Botulinum toxin
HEPA respiratory protection	Yes	Yes	Yes	Yes	Yes	No
Samples for microbiology	Lung, pleural fluid, spleen, lymph node	Skin, blood	Lung, spleen, lymph node	Blood, liver, spleen	Lung, spleen, lymph node	Blood, faeces
Samples for histopathology	Standard set	Standard set including skin	Standard set	Standard set	Standard set	Standard set
Vaccinate staff	No	Yes	No	No	No	No
Antibiotic prophylaxis for staff, assuming universal precautions used during autopsy	Yes	No	Yes	No. Only if direct skin contact with infected material	Yes	No
Antitoxin for staff	n/a	n/a	n/a	n/a	n/a	No
Decontaminate body and surfaces with hypochlorite	Yes	Yes	Yes	Yes	Yes	No
Embalm body	No	No	No	No	No	No
Cremate body	Yes	Yes	Yes	Yes	Yes	Yes
Autoclave instruments	Yes	Yes	Yes	Yes	Yes	No

(with high sensitivity and specificity) of BT-related infections in fixed material; but it is increasingly the gold standard for microbiological identification in fresh material (5).

Summary

Bioterrorism attacks have not yet occurred in the UK, but are possible and considered likely in some quarters. Patients will present live and dead, and pathologists (and APTs) are therefore in the front line of exposure. This chapter has depicted some of the infections considered more likely to be used in BT, what they look like clinico-pathologically and how pathologists can approach the differential diagnoses. Health and safety issues are also highlighted. It is possible that the first episodes of fatal BT in the UK will be managed erratically and much will be learned from the experience. More robust and all-embracing guidelines can then be produced.

References

1. Henderson DA, Borio LL. Bioterrorism: an overview. In Mandell GL, Bennet JE, Dolin R, eds. Mandell, Douglas and Bennett's Principles and Practice of Infectious Diseases. Pennsylvania: Elsevier Churchill Livingstone, 2005, pp 3591–3601.
2. Centers for Disease Control and Prevention. Biological and chemical terrorism: strategic plan for preparedness and response. MMWR 2000; 49: 1–14.
3. Goodman RA, Munson JW, Dammers K, Lazzarini Z, Barkley JP. Forensic epidemiology: law at the intersection of public health and criminal investigations. J Law Med Ethics 2003; 31: 684–700.
4. Guarner J, Zaki SR. Histopathology and immunocytochemistry in the diagnosis of bioterrorism agents. J Histochem Cytochem 2006; 54: 3–11.
5. Shieh W-J, Guarner J, Paddock C, et al. The critical role of pathology in the investigation of bioterrorism-related cutaneous anthrax. Am J Pathol 2003; 163: 1901–1910.
6. Marty AM. Anatomic laboratory and forensic aspects of biological threat agents. Clin Lab Med 2006; 26: 515–540.
7. Nolte KB, Hanzlick RL, Payne DC, et al. Medical Examiners, Coroners, and Biologic Terrorism. A guidebook for surveillance and case management. MMWR 2004; 53(RR08): 1–27.
8. Zilinskas RA. Iraq's biological weapons: the past as future? JAMA 1997; 278: 418–424.
9. Christopher GW, Cieslak TJ, Pavlin JA, Eitzen EM. Biological warfare: a historical perspective. JAMA 1997; 278: 412–417.
10. Aggrawal A. Terrorism: nuclear and biological. In Payne-James J, Byard RW, Corey TS, Henderson C, eds. Encyclopedia of Forensic and Legal Medicine. Oxford: Elsevier, 2005, pp. 277–290.
11. Parker NR, Barralet JH, Bell AM. Q fever. Lancet 2006; 367: 679–688.
12. Lahm SA, Kombila M, Swaenpoel R, Barnes FW. Morbidity and mortality of wild animals in relation to outbreaks of Ebola haemorrhagic fever in Gabon, 1994–2003. Trans R Soc Trop Med Hyg 2007; 101: 64–78.
13. Health Protection Agency Centre for Infections. Initial Investigation and Management of Outbreaks and Incidents of Unusual Illnesses, Version 3. Colindale UK: HPA, 2004, pp. 1–42.

14. Health Protection Agency Centre for Infections. Protocol for the Investigation of Microbiologically Unexplained Serious Illness and Death, Version 1. Colindale UK: HPA, 2006, pp. 1–17.
15. Moore ZS, Seward JF, Lane M. Smallpox. Lancet 2006; 367: 425–435.
16. Rotz LD, Cono J, Damon I. Smallpox and bioterrorism. In Mandell GL, Bennet JE, Dolin R, eds. Mandell, Douglas and Bennett's Principles and Practice of Infectious Diseases. Pennsylvania: Elsevier Churchill Livingstone, 2005, pp. 3612–3617.
17. Health Protection Agency Centre for Infections. Smallpox. Interim Guidelines for Action in the Event of a Deliberate Release, Version 5.2. Colindale, UK: HPA, 2005, pp. 1–24.
18. Auckland C, Cowlishaw A, Morgan D, Miller E. Reactions to small pox vaccine in naive and previously-vaccinated individuals. Vaccine 2005; 23: 4185–4187.
19. Bartlett JG. Smallpox vaccination and patient with HIV infection or AIDS. Clin Infect Dis 2003; 36: 468–471.
20. Royal College of Pathologists. Guidelines for Autopsy Practice. London: Royal College of Pathologists, 2002.
21. Lucey DR. Anthrax. In Mandell GL, Bennet JE, Dolin R, eds. Mandell, Douglas and Bennett's Principles and Practice of Infectious Diseases. Pennsylvania: Elsevier Churchill Livingstone, 2005, pp. 3618–3624.
22. Health Protection Agency Centre for Infections. Anthrax. Guidelines for Action in the Event of a Deliberate Release, Version 5.8. Colindale, UK: HPA, 2007, pp. 1–22.
23. Guarner J, Jernigan JA, Shieh W-J, et al. Pathology and pathogenesis of bioterrorism-related inhalational anthrax. Am J Pathol 2003; 163: 701–709.
24. Health Protection Agency Centre for Infections. Plague. Guidelines for Action in the Event of a Deliberate Release, Version 5.9. Colindale, UK: HPA, 2007, pp. 1–21.
25. Borio LL. Plague as an agent of bioterrorism. In Mandell GL, Bennet JE, Dolin R, eds. Mandell, Douglas and Bennett's Principles and Practice of Medicine. Pennsylvania: Elsevier Churchill Livingstone, 2005, pp. 3601–3605.
26. Health Protection Agency Centre for Infections. Tularaemia. Guidelines for Action in the Event of a Deliberate Release, Version 2.3. Colindale, UK: HPA, 2007, pp. 1–22.
27. Mitchell CL, Penn RL. *Francisella tularensis* (tularemia) as an agent of bioterrorism. In Mandell GL, Bennett JE, Dolin R, eds. Mandell, Douglas and Bennett's Principles and Practice of Infectious Diseases. Pennsylvania: Elsevier Churchill Livingstone, 2005, pp. 3607–3612.
28. Health Protection Agency Centre for Infections. Viral Haemorrhagic Fevers. Interim Guidance for Action in the Event of a Deliberate Release, Version 2.2. Colindale, UK: HPA, 2006, pp. 1–18.
29. Peters CJ. Bioterrorism: viral hemorrhagic fevers. In Mandell GL, Bennet JE, Dolin R, eds. Mandell, Douglas and Bennett's Principles and Practice of Infectious Diseases. Pennsylvania: Elsevier Churchill Livingstone, 2005, pp. 3626–3630.
30. Zaki SR, Greer PW, Goldsmith CS, et al. Ebola virus hemorrhagic fever: pathologic, immunopathologic and ultrastructural studies. Lab Invest 1996; 74: 133A.
31. Bleck TP. *Botulinum* toxin as a biological weapon. In Mandell GL, Bennet JE, Dolin R, eds. Mandell, Douglas and Bennett's Principles and Practice of Infectious Diseases. Pennsylvania: Elsevier Churchill Livingstone, 2005, pp. 3624–3625.
32. Health Protection Agency Centre for Infections. Botulism. Guidance for Action in the Event of Deliberate Release, Version 4.4. Colindale UK: HPA, 2006, pp. 1–19.
33. Department of Health & Health Protection Agency. Guidance for Pandemic Influenza: Infection Control in Hospitals and Primary Care Settings, 1. London UK: Dept of Health, 2005, p. 68.
34. Nolte KB, Taylor DG, Richmond JY. Biosafety considerations for autopsy. Am J Forensic Med Pathol 2002; 23: 107–122.
35. Lucas SB. The autopsy pathology of sepsis-related death. Curr Diagn Pathol 2007; in press.
36. Atlas RM. Bioterrorism: from threat to reality. Annu Rev Microbiol 2002; 56: 167–185.

The Chronological Dating of Injury

Frank R.W. van de Goot

Introduction

To estimate the age of an injury, i.e. the time between infliction and circulatory arrest (in the event of death) or fixation (in the living), remains one of the more difficult aspects within legal medicine. Although many authors have contributed to this subject, the gold standard has not yet been determined.

This does not mean that it is impossible to make any validated statement concerning the age of an injury to support any kind of answer in a court of law. The use of inflammatory mediators or cells and matrix proteins in injured tissue will provide some clues to make an estimation. Skin injuries in particular, as well as other injuries such as injuries to organs or other mesenchymal structures, could be analysed in this way.

According to classical pathology, the healing of injuries can be classified into five different phases (1):

- The first phase of coagulation and thus inhibition of blood loss initiates the process of wound healing.
- The second phase initiates inflammation to prevent infection and to induce necrosis and/or apoptosis in lethally injured tissue.
- The third phase initiates the removal of debris.
- The fourth phase initiates regeneration of newly formed tissue and granulation tissue formation.
- Finally, in the fifth phase, immature tissue will maturate into its definitive form. New epithelium will close the defect and scar tissue will be formed.

Nevertheless, it is important to realize that re-injury can occur during these phases, especially in older wounds, which interferes with the precise classification of wound age.

G.N. Rutty (ed.), *Essentials of Autopsy Practice*,

To prevent misdiagnosis of an estimated injury, it is of vital importance to combine the anamnestic information of the estimated time of injury with the macroscopic and microscopic analysis of the wound. Wound estimation strictly based on only one of these three methods of wound analysis prevents a clear distinction from being made between, e.g. 1 or 2 h, several hours and a day.

Collection of the Samples and Processing of the Tissue

For wound estimation, a clear photograph of the injury taken at right angles to the injury and containing a scale must always be present. This is necessary in case microscopic results contradict the expected age of the injury. The macroscopic examination of an injury can provide information on whether the samples taken are representative for the injury. Subsequently, wound tissue specimens can be taken of the wound edges or, preferably, the entire injury can be excised for further processing. If the entire wound is not included for microscopic analysis, representative samples should be preserved from the rest of the wound, especially if large surfaces of skin are involved or if certain parts of the injured tissue are not expected to be representative for wound estimation. In general, cut and stab wounds will be more or less homogeneous, while in contrast, bruises can enlarge over time, producing different wound healing phases.

Samples can be fixed in 4% formalin and processed for standard histological analysis, namely haematoxylin and eosin (H&E), Elastica van Gieson (EvG) and Perls iron staining. H&E is used for standard histopathological investigation, EvG for interpretation of the collagen and elastin structure and Perls for the determination of iron in or outside of macrophages.

Material for immunohistochemical analysis is treated on a similar basis. Notably, frozen material can be of significance, although many of the mediators suitable for frozen material have not yet been validated for medico-legal purposes. Microarray analysis also requires frozen material, although one has to keep in mind that the purity/quality of RNA in autopsy material is a limiting factor. Most importantly, a control sample of normal skin is analysed with every wound estimation.

Reporting of the Estimated Time of an Injury

Although the early stages of wound repair can be expected to be more or less the same, regardless of the exact location of the wound, the later phases can be influenced by, for example, mechanical influences such as scratching. It is therefore important that the conclusion of the wound analysis is not regarded as a pure fact. However, current methods of wound analysis are optimized so as to give a clear answer to the question of whether a wound occurred before death or is a post-mortem injury.

A statistical interpretation on a Bayesian scale (interpretation on a likelihood ratio basis) can provide enough accuracy to use wound determination for medico-legal purposes. For this, a working hypothesis is acquired if possible. This hypothesis can be formed during the anamnestic and/or macroscopic investigation. The conclusion will provide a likelihood ratio, which indicates that, given the results, hypothesis 1 is more likely, unlikely or almost certain in comparison with hypothesis 2. If both hypotheses ultimately appear to be wrong, a third hypothesis must be formed and compared with the initial hypotheses.

Finally, wound analysis as proposed in this chapter is generally related to inflammation. Therefore, it is of pivotal importance that conditions known to influence wound healing are also taken into account. This includes the use of steroids, drugs and/or alcohol. The same is true for diseases that affect the inflammatory response, especially those involving the liver, bone marrow or infection. Although these influences are likely to be of little importance during the early phases, i.e. the first hour after infliction of an injury, the effect on older injuries can be substantive. Furthermore, external factors such as heat, cold or putrefaction can make it almost impossible to estimate the time of an injury on a microscopic or immunohistochemical basis. Injury caused by for example fire or frostbite does not respond in the same way as tissue under normal circumstances. Standard H&E, Elastica van Gieson and Perls iron staining can then be used on these injuries, but only to provide one gross information.

Determination of the Estimated Time of Injury

Shortly after infliction of tissue damage, a cascade of reactions will take place. According to classical pathology, damaged tissue liberates several inflammatory mediators. Fragments of membranes, sodium urate and enzymes such as trypsin and many others will activate Factor XII (Hageman factor), inducing the transformation of plasminogen into plasmin. Plasmin subsequently induces fibrinolysis and complements activation, as well as the activation of coagulation (1).

These early aspects are not visible through standard light microscopy. It is of vital importance to realize that many processes will not stop immediately after circulatory arrest. In particular, several wound reactions will be prolonged. In general, within 10 min after injury, the blood vessels will show dilatation inducing an increase in permeability, eventually resulting in oedema. Blood clots can subsequently be formed within minutes. Although dilatation and an increase in permeability are vital signs, these reactions will also take place after death. Notably, only the appearance of oedema is considered to be strictly vital (due to its dependence on blood pressure). Blood clots can be formed in the living as well as after death (2). Although immunohistochemistry can be used to differentiate between vital and non-vital clots by determining thrombocytic activation, the histological differentiation between vital and non-vital clots can still be very difficult.

One of the first, non-obligate vital aspects of wound reaction is haemorrhage. However, post-mortem haemorrhaging can be inflicted as described by Princeloo and Gorden in 1951. Even severe haemorrhaging can be seen as a post-mortem phenomenon (3). Swelling and formation of oedema in the soft tissues and around small vessels is also an early vital sign, although formation of gases can also induce similar effects. Notably, oedema due to different diseases must also be excluded (Fig. 1 and Fig. 2).

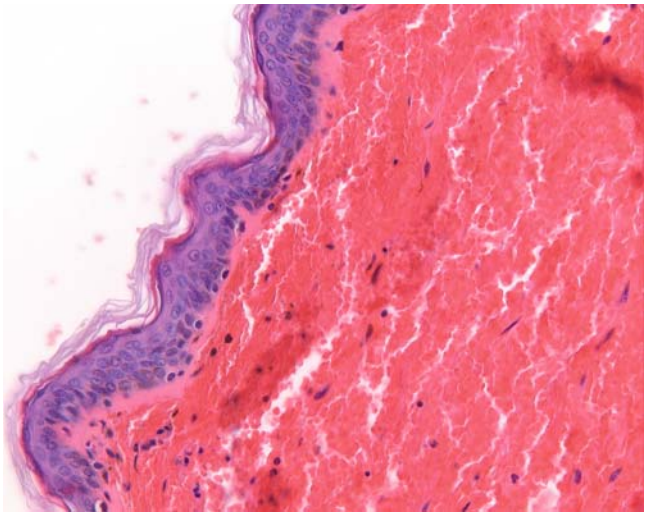


Fig. 1 A vital haemorrhage

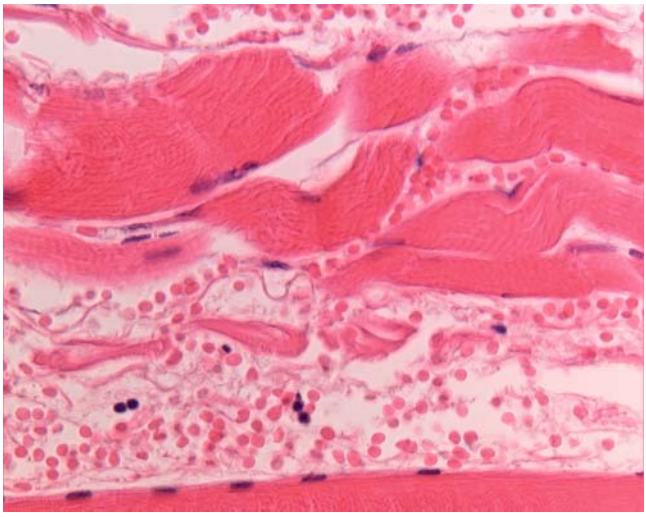


Fig. 2 Very recent vital haemorrhage

Table 1 Light microscopic appearances of mechanical-induced injuries

Time	Histological appearances
Post-mortum >30 min	No oedema, no PMN activity. If haemorrhaging occurs, no activity for F8. No or minor P-selectin activity, no or minor fibronectin or cathepsin activity
Antemortem seconds before death	Minimal oedema, no PMN activity. If haemorrhaging occurs, weakly positive for F8. Minor P-selectin activity, minor fibronectin or cathepsin activity
Early vital minutes before death	Haemorrhaging, exudate, swelling and some focal activity of polymorphonuclear (PMN) granulocytes. Activation of endothelium. Strongly positive for F8 in haemorrhage and on endothelium. Linear positivity for P-selectin on endothelium and moderate positivity for fibronectin and cathepsin-D
Vital 30 min to 1 h	After 30 min, the first PMN addition on the vascular wall. Diapedesis and migration after 30 min, up to 1 h. TNF-alpha/IL-1B. Within 1 h E-selectin clearly positive
Vital 2 h	Mast cell degranulation, PMN infiltration, disintegration of fibrin Clear migration of PMN. IL-6 after 60–90 min. TGF-beta after 1 h. ICAM after 1 h
Vital up to 4 h	Hyperaemia, many PMN and MN cells (first T-cells (CD3) than B cell (CD20))
	Clear PMN activity, deterioration of matrix. P-selectin positive around blood vessels and in the lumen. VCAM positive. IL-1a after 4 h. Early appearance of macrophages
Vital up to 12–16 hours	Extensive amount of MN cells, PMN disintegration. IL-8, MCP-1 and MIB-beta positive on granulocytes (4–6 h) massive positive on macrophages and fibroblasts after 1 day)
One day	Fibroblasts, capillary regeneration
Several days	Developing granulation tissue. CD55, CD59, IL-2 positive
Week	After 3–4 days, collagen III positive. To be followed by Collagen IV, V and VI
	After 4 days Collagen I in fibroblasts, After 4–5 days, alpha-SMA-positive fibroblasts Formation of scar tissue after a week
Up to 2 weeks and more	Active fibroblast, organizing collagen and flattening of the epidermis (absence of papillae). Formation and organizing of vessels. Decrease of cellular activity

The very early vital blood cell reaction, as depicted in Table 1, will be granulocyte extravasation, which can sometimes be seen within 10–30 min, and in most cases within 1–2 h after wound induction (Fig. 3). Neutrophils subsequently release free radicals and enzymes, resulting in secondary tissue damage (4).

Another type of blood cell reaction is characterized by extravasation of monocytes and subsequent transformation of monocytes into macrophages. These macrophages will subsequently phagocytize cell debris (5) (Fig. 4). This cell type can be demonstrated as early as 7 h after traumatization and will peak at 1–2 days (6–11).

It must be emphasized that this estimated time frame of wound healing again is not absolute. Walcher et al. reported attachment of granulocytes to the vessel wall as early as 8–30 min after injury infliction. However, one must always keep

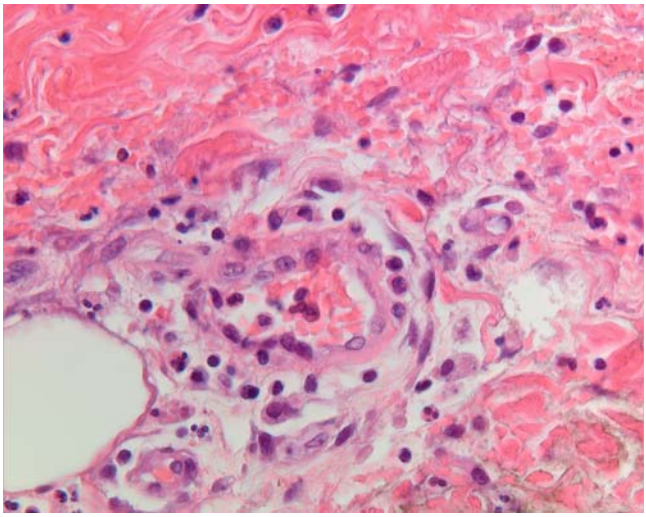


Fig. 3 Injury of 2 h old with infiltrate

in mind the possibility of post-mortem attachment of granulocytes to the vessel wall. Lendrum et al. and Fisseler-Eckhoff et al. (12) reported histological methods to differentiate between fibrin younger and older than 16 h. On the other hand, Wille et al. claimed time frame differentiation related to the presence of haemosiderin using the Puchtler and Sweat method and found a positive reaction of haemosiderin within 30 min after injury infliction.

Immunohistochemistry was already incorporated in the process of wound estimation in the early 1970s. Berg et al. demonstrated the appearance of

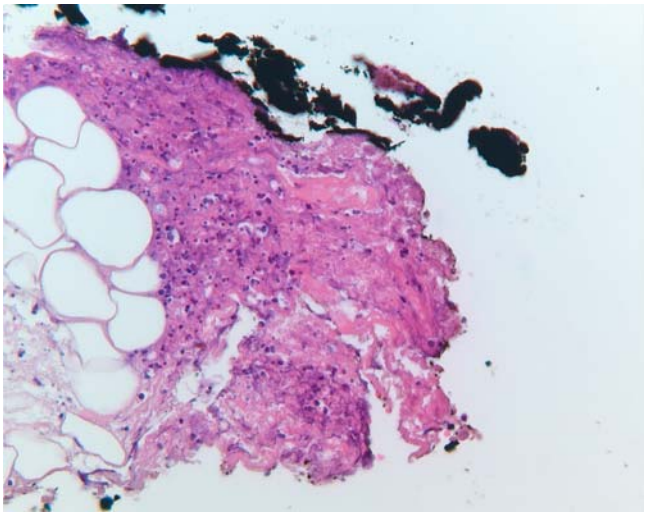


Fig. 4 Injury with necrosis, 1 day old

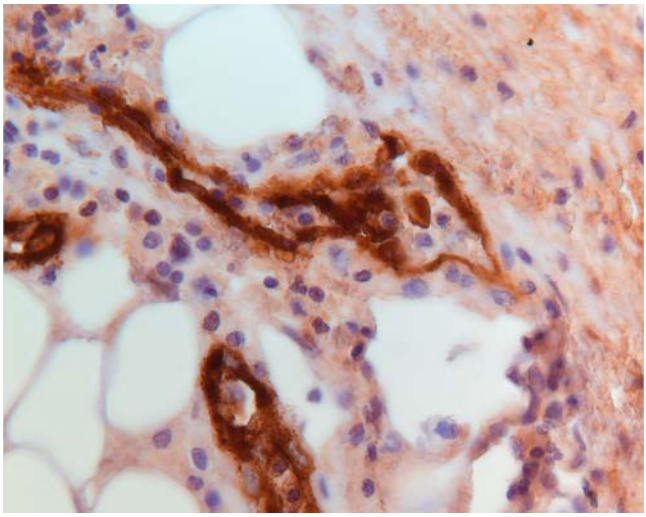


Fig. 5 Factor 8 on very recent injury

histamine and serotonin in the wound edges representing an early sign of vital injury. Furthermore, the detection of several proteinase inhibitors and lysozyme seemed to be most promising in judging wounds. Many other proteins, as discussed below, have since been proven useful (13–15), especially factor 8, an endothelial derived protein with a central role in the coagulation cascade. In vitally inflicted injury, endothelial cells will upregulate factor 8.

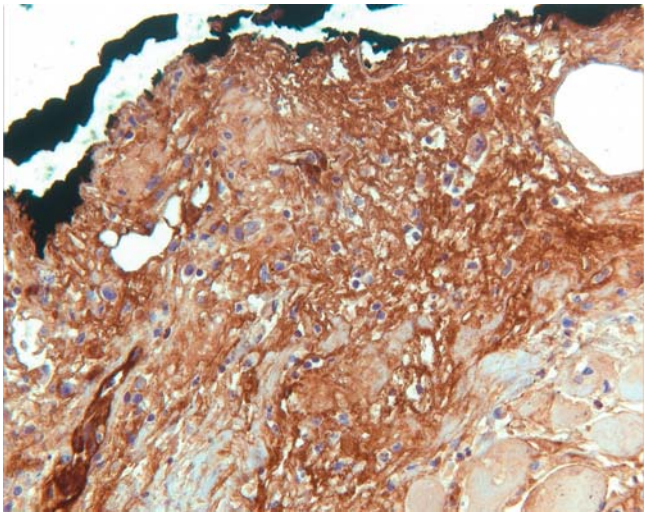


Fig. 6 Factor 8 on injury of 1 h old. Clear positivity of the endothelium

Post-mortem-inflicted injury will show no or minimal upregulation. Important is that the upregulation of factor 8 as well as some other proteins will continue for a short period after death (Fig. 5 and Fig. 6).

Adhesion Molecules

Recent studies have described the use of several adhesion molecules, e.g. P-selectin, E-selectin, VCAM-1 and ICAM-1, as markers for early wound reactions, especially on endothelium (see Table 2). Unfortunately, considerable variation in their expression pattern over time has been described (16).

P-selectin, an early adhesion molecule, is always present in the endothelium. In case of activation (i.e. injury), P-selectin will move from the cytosol to the endothelial plasma membrane surface. Therefore, in case of activation, the diffuse cytosolic expression of P-selectin will change into a more superficial layer of positivity, and after some time, due to endothelial deterioration, into a diffuse staining in the vessel lumen or surrounding the vessel. This shift in expression of P-selectin from the cytosol to the surface of the endothelium has been described from as early as 3 min up to 7 h after injury infliction. For E-selectin, this interval even varied from 1 h to 17 days after infliction, although E-selectin is not commonly present in the cytosol. ICAM-1, an intercellular adhesion molecule, showed strong positive pre-existing staining on the keratinocytes, especially on basal keratinocytes of the epidermis. Over time, staining intensity for ICAM-1 on endothelial cells, in particular in the vicinity of inflammatory infiltrates, was enhanced after wound induction. Strong positive staining on endothelium varied between 1.5 h and 3.5 days after wound infliction. Another adhesion molecule, VCAM-1, was detected on the endothelial surface, varying from 3 h to 3.5 days after injury infliction (17, 18) (Fig. 7).

Inflammatory Mediators and Extracellular Matrix Components

Inflammatory mediators used in forensic wound analysis are also summarized in Table 2 and will now be discussed shortly. *Fibronectin* is a 440 kDa glycoprotein and is pre-existing in basement membranes and interstitial connective tissue. Fibronectin, however, is also involved in angiogenesis during wound healing and in wound contraction. Strong fibronectin-positive reactions can be demonstrated in wounds at least 20 min old, while extensive networks of fibronectin can be detected at 40 min (20).

Fibronectin exists in different forms resulting in a molecular and functional diversity, related to alternative splicing of pre-mRNA. Under normal circumstances, endothelial cells and fibroblasts synthesize FN without the extra domain-A (ED-A Domain) (21). However, in tissue repair and pathological

Table 2 Different mediators in wound age determination

No.	Mediator	Estimated time indication
1	Factor VIII	Within minutes
2	P-selectin	3 min up to 7 h
3	Fibronectin	3 min to 8 h
4	Laminin	Early vital sign, but non-specific. After 36 h
5	Tenacin	Early vital sign, but non-specific. After 2–3 days
6	Cathepsine D	Within 30 min
7	TNF-A	Within 30 min
8	IL-1B	Within 30 min
9	MRP	After 30 min
10	TGF-B	After 1 h
11	E-selectin	1 h up to several days
12	IL-6	60–90 min
13	MPO	Within 1 h (according to PMN influx)
14	Defensin	One hour after infliction
15	ICAM-1	90–210 min
16	VCAM-1	180–210 min
17	CD-3	After 2–4 h (according to B-cell appearance)
18	CD-20	After 3–5 h (according to B-cell appearance)
19	IL-Ia	4 h
20	CD-68	After 10–16 h (macrophage appearance)
21	IL-8	4–12 h/1 day
22	MCP-1	4–12 h/1 day
23	MIP-alpha	4–12 h/1 day
24	IL-2	Several days
25	CD55	Days
26	CD59	Days
27	Collagen III	After 2–3 days
28	Collagen IV	After 3 days
29	Collagen V	After 4 days
30	Collagen VI	After 4 days
31	Collagen I	In fibroblasts after 4 days
32	Alpha-SMA	In fibroblasts after 5 days

circumstances such as fibrosis, the ED-A domain is expressed. Besides this, a difference in staining pattern of fibronectin can also differ between different injuries. In the case of acute fatal injuries (e.g. plane crashes or train accidents), a slight fibronectin positivity can be detected as single strands on the superficial sides of the wound bed if injury was inflicted during life. Conversely, in areas of active bleeding in fatal wounds (causing immediate or very rapid death), fibronectin can be detected in both diffuse and fibrous strand-like arrangements in haemorrhage. Notably, it has been observed that positivity can also be seen as an artefact at wound edges inflicted during sampling (22, 23).

Defensins are a family of 3–4 kDa antimicrobial and cytotoxic peptides, constituting more than 5% of the total cellular protein of human neutrophils.

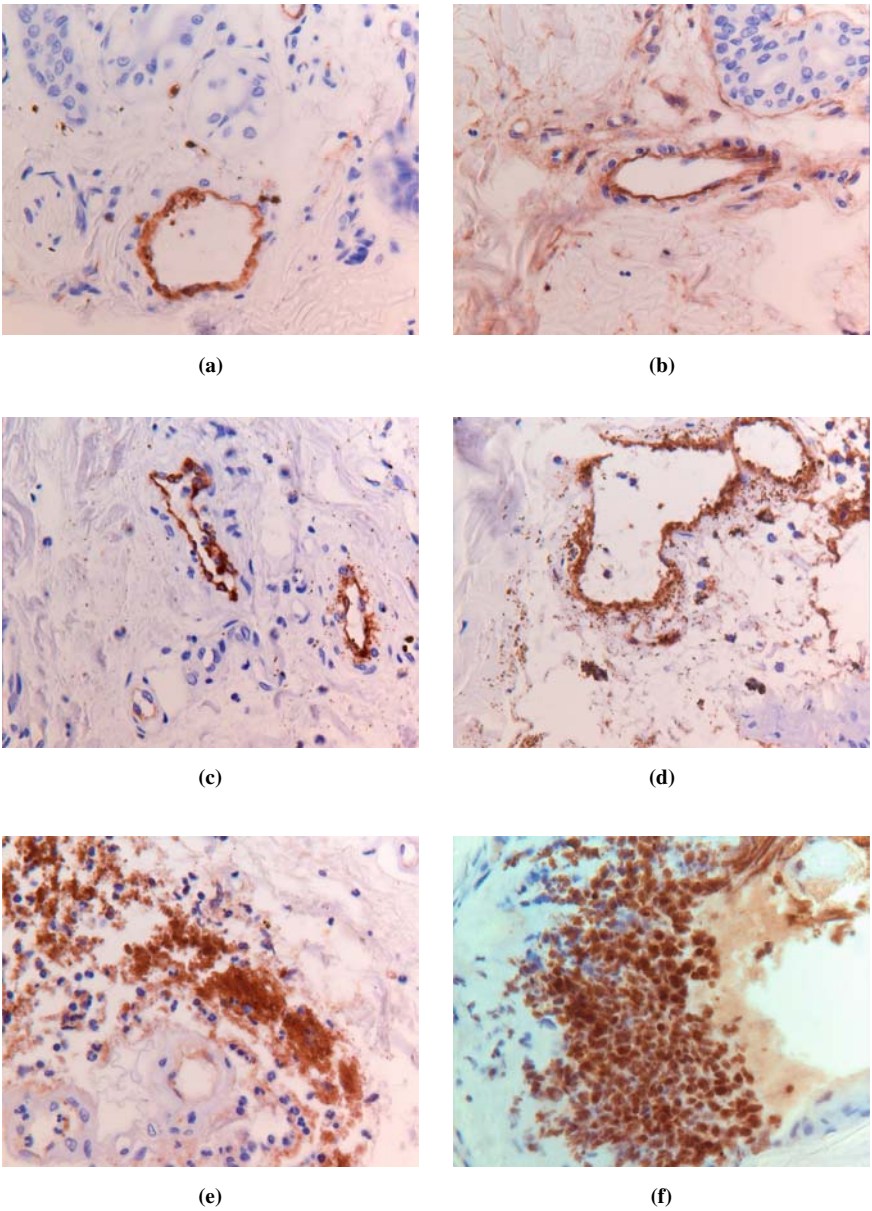


Fig. 7 Immunohistochemical staining on CD62-P (P-selectin). (**a**) Normal subcutaneous tissue. There is a normal expression. (**b**) 5–30 min after injury; first adhesion of a granulocyte. Typical linear expression of CD62-P. (**c**) 60 min after injury. Clear granulocytic adhesion and diapedesis. (**d**) 90 min. (**e**) 120 min after injury. Clear expression of CD62-P and clear infiltrate of granulocytes. (**f**) 12–24 h after injury. Clear positivity on granulocytes

Defensins have also been found in the epithelial surface of the intestine and the trachea. Defensin is strongly expressed at the wound margin in the first 1–2 h after wound infliction (24, 25).

MRP8 (8 kDa) and *MRP14* (13.2 kDa, migration inhibitory factor-related proteins 8 and 14) are calcium-binding proteins belonging to the S-100 protein family found in granulocytes and activated monocytes/macrophages. Fieguth et al. described the positive expression of MRP8, MRP14 and defensin in granulocytes in areas of active bleeding in wounds inflicted shortly before death, as well as reacting with intravasal granulocytes in adjacent undamaged tissue. Reactions positive for the above-mentioned antibodies could be seen after 20–30 min when granulocytes are expressing MRP8 and MRP14 (26–28).

An injury of 1 day old can be detected with a combination of interleukin 8 (IL-8), MCP-1 and MIP-Alpha. In this respect, the exact location of immunohistochemical positivity is important. Initially, in injuries 4–12 h old, only granulocytes are positive. However, after a prolonged period (1 day old), macrophages and fibroblasts will also stain clearly positive for these three antibodies. (29, 30).

Immunohistochemical staining of other extracellular matrix proteins like *Tenascin, Laminin* and the different types of *Collagen* have proved their use as well. Positive reactions for Tenascin or collagen type III indicate post-infliction intervals of at least 2–3 days, whereas vital reactions for collagen type V or VI occur at earliest 3 days after wounding. Collagen type I appears as spot-like fibroblast-associated reaction products in injuries aged 4 days or more, while typical string-like ramifying fibres indicate a post-infliction interval of at least 5–6 days. Fibroblast*s* positively staining for laminin or heparan sulfate can be detected in wounds with a survival time of approximately 1.5 days or more. Collagen type IV-positive fibroblasts occur at earliest 4 days after wounding, followed by alpha-smooth muscle actin expressing fibroblasts after 5 days or more (31–34).

Original basement membrane fragments (i.e. part of the damaged membrane) positive for laminin, proteoglycan or collagen type IV or VII indicate a wound age of at least 4 days. A complete restitution of the epidermal basement membrane in (moderate surgical) wounds can be observed at earliest 8 days after wound infliction, while the residual basement membrane fragments cannot be detected anymore after 13 days. A continuous staining of the basal cells of the newly formed epidermis with *cytokeratin 5* occurs 13 days after wounding, while cytokeratin staining disappears after 24 days.

Also useful are complement factors, vitronectin and decay-accelerating factor (CD55), for wounds of some days old, while protectin (CD59) can be detected in much older injuries.

The pro-inflammatory *cytokines* interleukin-1beta (IL-1beta), interleukin-6 (IL-6) and tumour necrosis factor-alpha (TNF-alpha) hold important functions in the early and late stages of inflammation, trauma and wound

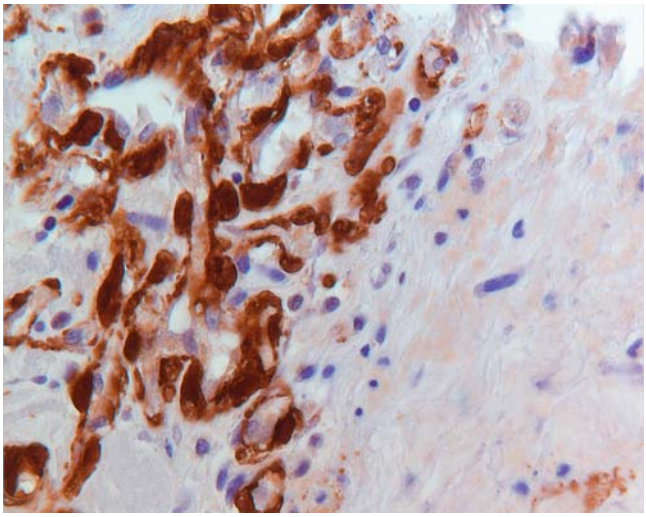


Fig. 8 SMA on injury of several days old

healing. TNF-alpha can be detected within 30 min after infliction of the injury, while IL-1beta and IL-6 are expressed after 15 and 20 min and are generally clearly expressed 60–90 min (Fig. 8 and Fig. 9). At the earliest, leukocytes reacting with IL-1beta and IL-6 appear after approximately 90 min up to 2 h (35, 36).

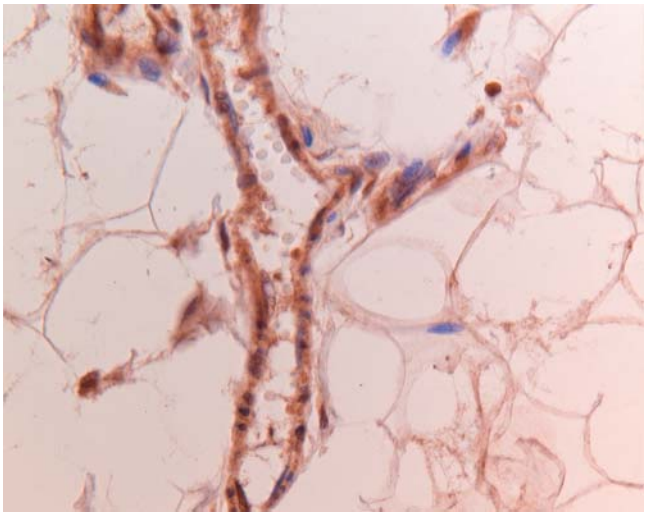


Fig. 9 IL-6, 2–4 h after injury

Inflammatory Cells

Many authors have described the use of inflammatory cell determination in wound analysis. Immunohistochemistry can be helpful in differentiating between the different cells. MPO (myeloperoxidase) can provide an impression of the number of neutrophilic granulocytes. CD3 and CD20 staining can be used for identifying T-lymphocytes and B-lymphocytes. CD131 staining can be used for detecting mast cells, while CD 163 or CD68 can detect monocytes and macrophages. Finally, CD138 has proved its use in detecting plasma cells (37, 38).

Summary

In order to investigate the estimated time of injury, it is very important to gain as much anamnestic and macroscopic information as possible. This needs to be confirmed or rejected on a histochemical or immunohistochemical basis. If conclusions are formed within the likelihood ratio of Baysian statistics, the conclusions can be of significant interest for medical legal purposes.

References

1. Robbins SL, Saunders Company WB. Pathologic basis of diseases. Eds, Cotran RS, Kumar V, Collins T, Robbins SL. 6th Edition, W.B. Saunders Company, London, 1999, Chapters 3 and 4, pp. 50–112.
2. Walcher K et al. Uber vitale reactionen. Vortrag auf der 18ten tagung der Deutsches Geselschaft fur Gerichtliche und sozial medizin. Heidelberg, September 1929.
3. Princeloo I, Gordon I. Postmortem dissection artefacts of the neck and their differentiation from antemortem bruises. S Afr Med J 1951: 358–361.
4. Knight B. Forensic Pathology, Chapter 4, 3rd edn, Arnold Hodder Headline Group, 2004, pp. 136–173.
5. Wille R, Elbert M, Cornely M. Zeitstudien uber haemosiderin (Vortrag).
6. Langlois. The aging of bruises. Forensic Sci Int 1991; 50: 227–238.
7. Bariciak ED, Plint AC, Gaboury I et al. Dating of bruises in children. Pediatrics 2003; 112: 804–807.
8. Maguire S, Mann MK, Sibert J et al. Arch Dis Child 2005; 90: 187–189.
9. Perper JA. Microscopic Diagnosis in Forensic Pathology, Chapter 1, 1st edn, Charles C Thomas Publishers. LTD, 1980, pp. 17–34.
10. Oehmichen M. Vitality and time course of wounds. Forensic Sci Int September 2004: 221–231.
11. Lendrum AC, Fraser DS, Slidders W, Henderson R. Studies on the character and staining of fibrin. J Clin Pathol 1962; 15: 401–413.
12. Fisseler-Eckhoff A, Muller KM. Lendrum (-MSB) staining for fibrin identification in sealed skin grafts. 1. Pathol Res Pract 1994; 190: 444–448.
13. Berg S, Ditt J, Friedrich D et al. Möglichkeiten der biochemischen Wundaltersbestimmung. Deutsches Zeitschrift fur Gerichtliche Med 1968; 63: 183–198.

14. Berg S, Bonte W. Praktische Erfahrungen mit der biochemischen Wundaltersbestimmung. Beitr Gerichtl Med 1971; 28: 108–140.
15. Oehmichen M, Schmidt V et al. Freisetzung von Proteinase-Inhibitoren als vitale Reaktion im frühen post-traumatischen. Intervall Z. Rechtsmed 1989; 102: 461–472.
16. Dressler J, Bachmann L, Koch R et al. Enhanced expression of selectins in human skin wounds. Int J Legal Med 1998; 112: 39–44.
17. Dressler J, Bachmann L, Kasper M et al. Time dependence of the expression of ICAM-1 (CD 54) in human skin wounds. Int J Leg Med 1997; 110: 299–304.
18. Dressler J, Bachmann L, Koch R et al. Estimation of wound age and VCAM-1 in human skin. Int J Leg Med 1999; 112: 159–162.
19. Hernández-Cueto C, Girela E, Sweet DJ. Advances in the diagnosis of wound vitality: a review. Am J Forensic Med Pathol 2000; 21: 21–31.
20. Amberg R. Time-dependent cytokine expression in cutaneous wound repair. In Oehmichen M, Kirchner H, eds.. The Wound Healing Process—Forensic Pathological Aspects (Research in Legal Medicine), vol. 13. Lübeck: Schmidt-Römhild, 1996, 107–121.
21. Straaten HM, Canninga-van Dijk MR, Verdonck LF et al. Extra-domain-A fibronectin: a new marker of fibrosis in cutaneous graft-versus-host disease. J Invest Dermatol 2004; 123: 1057–1062.
22. Betz P. Neue Methoden zur histologischen Altersbestimmung menschlicher Hautwunden (Arbeitsmethoden der Medizinischen und Naturwissenschaftlichen Kriminalistik). Lübeck: Schmidt-Römhild, 1996.
23. Fieguth A, Feldbrügge H, Gerich T et al. The time-dependent expression of fibronectin, MRP8, MRP14 and defensin in surgically treated human skin wounds. Forensic Sci Int 2003; 131: 156–161.
24. Ganz T, Lehrer RI. Defensins. Curr Opin Immunol 1994; 6: 584–589.
25. Kagan BL, Ganz T, Lehrer RI. Defensins: a family of antimicrobial and cytotoxic peptides. Toxicology 1993; 89: 131–149.
26. Odnik N, Cerletti J. Brüggen at ll. Two calcium-binding proteins in infiltrate macrophages of rheumatoid arthritis. Nature 1987; 330: 80–82.
27. Hessian PA, Edgeworth J, Hoog N. MRP8 and MRP14, two abundant Ca (2^{+})-binding proteins of neutrophils and monocytes. J Leuk Biol 1993: 197–204.
28. Fieguth A, Feldbrügge H, Gerich T et al. The time-dependent expression of fibronectin, MRP8, MRP14 and defensin in surgically treated human skin wounds. Forensic Sci Int 2003; 28, 131: 156–161.
29. Bosman FT, Stamenkovic I. Functional structure and composition of the extracellular matrix. J Pathol 2003, 2004: 423–428.
30. Engelhardt E, Toksoy A, Goebeler M, Debus S, Bröcker EB, Gillitzer R. Chemokines IL-8, GRO-A, MCP-1, IP-10 and mig are subsequently and differentially expressed during phase-specific infiltration of leukocyte subsets in human wound healing. Am J Pathol December 1998; 153(6).
31. Betz P, Nerlich A, Wilske J et al. Time-dependent pericellular expression of collagen type IV, laminin, and heparan sulfate proteoglycan in myofibroblasts. Int J Legal Med 1992; 105: 169–172.
32. Betz P. Immunohistochemical parameters for the age estimation of human skin wounds. A review. Am J Forensic Med Pathol 1995; 16: 203–209.
33. Ortiz-Rey JA, Suarez-Penaranda JM, Da Silva EA et al. Immunohistochemical detection of fibronectin and tenascin in incised human skin injuries.1. Forensic Sci Int 2002; 126: 118–122.
34. Betz P, Nerlich A, Wilske J et al. Analysis of the immunohistochemical localization of collagen type III and V for the time-estimation of human skin wounds. Int J Legal Med 1993; 105: 329–332.

35. Grellner W. Time-dependent immunohistochemical detection of proinflammatory cytokines (IL-1beta, IL-6, TNF-alpha) in human skin wounds. Forensic Sci Int 2002; 130: 90–96.
36. Kondo T, Ohshima T, Eisenmenger W, Immunohistochemical detection of chemokines in human skin wounds and its application to wound age determination. Int J legal Med April 2002; 116(2): 87–91.
37. Rathmell JC, Thompson CB. The central effectors of cell death in the immune system. Annu Rev Immunol 1999; 17: 781–828.
38. Lau SK, Chu PG, Weiss LM. CD163: a specific marker of macrophages in paraffin-embedded tissue samples. Am J Clin Pathol 2004; 122: 794–801.

Index

AAIB, Air Accidents Investigation Branch, 114
Abbreviated Injury Scale (AIS), 124–125
Abdominal and pelvic injuries, air crash, 121–122
Adhesion molecules (P-selectin/E-selectin/VCAM-1/ICAM-1), 174
Age-pyramid: demographic changes projected for 2040, 78
Air Accidents Investigation Branch (AAIB), 114
Air crash investigation
  autopsy, approach to, 117–118
  control injuries, 123–124
  crash site, 115
  death, causes
    drowning/fire/multiple injuries/natural disease, 125–128
  identification, 116–117
  injury scoring, 124–125
  legal framework, 113–114
  pathologist, role of, 114–115
  patterns of injury, 118–123
    abdominal and pelvic, 121–122
    head, 119–120
    limbs, 122–123
    spine, 120
    thorax, 120–121
  radiology, 117
  suicide of driver, 131
  toxicology, 129–131
AIS, Abbreviated Injury Scale, 124–125
American Medical Association, definition of elder abuse, 81
Anogenital injuries, child sexual abuse, 64–68
  external examination, indications, 64
  hymenal lacerations, 64
  lesions, types of
    vulva/hymen/vagina/anus/penis, 65
Anthrax, BT attack
  Bacillus anthracis, 152
  clinical presentation, 152–153
  inhalation of, 153
Anthrax scenario, 137–139
  Sverdlovsk episode (1979), 137, 153
Autopsy approach, aircraft crashes, 117–118
Autopsy practice, needs and standards, 158–159

Bayesian scale, time of injury, 169
Bioterrorism (BT), 135–136
  anthrax scenario, 137–139
  autopsy practice, 158–159
  cadaver, disposal of, 163
  definition, 135
  diseases, 146–157
    See also Smallpox
  forensic epidemiology, 159–160
  generic protocol, autopsy/specimen collection, 161–163
  mortuary plans/provisions, 145–146
  motives, 139–140
  pathological identification, agents, 163–165
  personal protection, pathologists, 160–161
  potential agents, 140–141
  Public Health Organisations/terminology, 143–145
  surveillance modes, 155, 158
  terrorism, cost of, 139
  UK Medico-legal Systems, 160
  weaponisation, 141
BMI, body mass index, 45, 47–48
Body fragmentation due to aircraft crashes, 116–117
  DNA analysis, 116–117

Body mass index (BMI), 45, 47–48
Body weight and sudden death, relationship, 45–47
  Anorexia Nervosa and Bulimia Nervosa
    "brown atrophy", 46
    "slimmer's diseases", 45–51
    "water diets", 45
  morbid obesity,
  see Morbid obesity and sudden death, management of
  obesity, *see* Obesity
Botulinum toxin, 157
  autopsy, role of pathologist, 157
  electromyography for diagnosis, 157
  natural toxicity, 157
BT, Bioterrorism, 135–136
BT attack, key issues, 138–139
  See also Anthrax scenario

Cadaver disposal, BT infections, 163
Causes of death, aircraft crashes, 125–128
CCHF, Crimea/Congo haemorrhagic fever, 156–157
Chronological dating, injury, *see* Injury, chronological dating
Control injuries, air crash, 123–124
Crash site, air crash, 114
Crimea/Congo haemorrhagic fever (CCHF), 156–157
Crimes, against elderly
  maltreatment by caregivers, 79–80
    reasons for non-report, 79–80
    regular/comprehensive medical care, 79
  violence and public crime, 78–79
    crime statistics, 78
    low risk of victimization, reasons, 78–79
  See also Elder abuse, characteristics of

Differential diagnosis, 69–70
  anogenital injury not due to sexual abuse, 69–70
  congenital anatomical variants, 72–73
    hymen/perianal tissues, 72
    "prianal grooves", 72
  endometrial bleeding, 72
  skin conditions
    erythematous rashes, 71
    lichen sclerosis et atrophicus, 71
  tumours, haemangiomas, 72
  urethral prolapse, 73
  vulvovaginitis, 71
Differential presentations; mimics of elder abuse, 107–108
Dissections, special, 69
  pelvic exenteration/venereal diseases, 69
Drug distribution/redistribution, determining factors, 6
Drug movement, blood vessel (stylised representation), 6

Elastica van Gieson (EvG), 168
Elder abuse, characteristics of
  physical, sexual abuse/physical neglect/killing, 81
Elder abuse and gerontocide, 77–109
  crimes, *see* Crimes, against elderly
  definition, elder abuse, 80
  demographic changes projected for 2040, 78
  differential presentations; mimics of elder abuse, 107–108
    self-inflicted injuries, 108
    self-neglect, 107
  "greying phenomenon", 107
  investigations and findings, 83–84
  non-physical abuse and neglect, 107
  physical neglect, 94–101
  sexual abuse, 101–103
  See also Phenomenology of elder abuse and neglect
Elder abuse and neglect: investigations and findings, 83–84
Endogenous substances, redistribution, 15
EvG, Elastica van Gieson, 168
Extracellular matrix components, 174–178
  defensins, 174–175, 176
  ED-A domain/fibronectin, 174–175

Fatal sexual abuse, childhood, 53–74
  anogenital injuries,*see* Anogenital injuries, child sexual abuse
  autopsy assessment
    vulval and/or vaginal bleeding (prepubescent), cause of, 58
  clothing, 57
  definition, 55
  extragenital injuries, 64
    general/specific, 62–64
  prevalance, 55
  problems, 58–62
    artefacts, 58–61
    colposcopy, 61
    decomposition, 58
    photography, 62
    samples, 62

scene examination
concealment of a corpse (dumping of a body outdoors), 56
police crime scene officers, 56
time of death, 56
special dissections,*see* Dissections, special
See also Postpubertal rape
Forensic epidemiology, 159–160
group 3 infectious autopsy cases, 160
Forms of maltreatment, elderly, 80–82
elder abuse, definition (American Medical Association), 80–82
"natural or accidental" *vs* "non-accidental," injuries, 80
physical abuse
blunt force trauma, cutaneous bite marks/bone fractures, 82–88
burns and scalds, 91–94
family/interpersonal violence against adults, 82
pro-euthanasia organizations, recommendations, 89
strangulation/suffocation, petechial haemorrhages, 88–91
See also Elder abuse, characteristics of; Phenomenology of elder abuse and neglect
Fracture, age of, 24–26
3–7 days, 27–28
7–14 days, 28–30
14–21 days, 30
factors, altering healing process, 33
avascularity, 33
displacement, significant, 33
local/systemic, 33
12 hours to 3 days, 26–27
perimortem fractures, 27
3–6 weeks, 30–32
6–12 weeks, 33
Fracture, conformation, 24–26
Fractures at autopsy, examination
age of fracture, *see* Fracture, age of
avulsion/comminuted fracture, 24
conformation of fractures, 24–26
definition, fracture, 26
greenstick fracture, 24
helical or spiral fractures, 24
infancy, *see* Fractures in infancy
pathology, *see* Pathology, contributing to fracture
radiological/pathological features, comparison, 34–35
transverse/oblique fractures, 24
Fractures in infancy
lower/upper metaphyseal tibial and femoral fractures, 37–38
avulsion or step fractures, 38
shoulder fractures, 39
wrist and elbow damage, 38–39
rib fractures
birth injury, 35–36
non-accidental rib fractures, 36
resuscitation, 36
skull fractures, 36–37

Gamma-hydroxybutyrate (GHB), 2, 11–12
Genital findings in cases of sexual abuse in girls, 65
Gerontocide/genocide, killing of elderly, 103–107
assisted suicide/active euthanasia/serial killings, 106–107
criminalistic/criminological analyses, homicide, 104
fear-victimization paradox, 105
maltreatment of elderly, forms of, 80–82
phenomenology of elder abuse and neglect, 81
physical neglect of elderly, intentional/unintentional, 97–99
See also Elder abuse, characteristics of
GHB, gamma-hydroxybutyrate, 2, 11–12
Greying phenomenon, 107

Hageman factor, 169
Head injury, air crash, 119–120
Healing of injuries, classification, 167
Health Protection Agency (HPA), 143–144, 146, 149, 156, 162
HPA, *see* Health Protection Agency (HPA)
Hyperparathyroidism, 42
Hypoventilation syndrome and obesity, 48

ICAO, International Civil Aviation Organization, 113–114
Identification, air crash investigation, 116–117
pathologist's role, 116
IL-8, interleukin 8, 177
Immunohistochemical stain, 176–178
collagen/cytokeratin 5, 177
IL-6/SMA, 178
Immunohistochemistry, 172–174
inflammatory cells, 179

Injury, air crash
patterns, 118–123
scoring, 124–125
Injury, chronological dating
adhesion molecules, 174
estimated time, 168–174
determination, 169–174
endothelium, positivity, 173
Puchtler and Sweat method, 172
reporting of, 168–169
healing of, classification, 167
inflammatory mediator, 174–178
mechanical-induced, 171
samples, collection, 168
immunohistochemical analysis, 168
stages of, 176
Interleukin 8 (IL-8), 177
International Civil Aviation Organization (ICAO), 113–114

Limb injuries, air crash, 122–123

Macroscopic examination of injury, 168
McCune Albright syndrome, 72
MDMA, 3,4-Methylenedioxymethamphetamine, 7, 12, 13
3,4-Methylenedioxymethamphetamine (MDMA), 7, 12, 13
Mimics of elder abuse, differential presentations, 107–108
Morbid obesity and sudden death, management of
liquid protein diets, 50
morbid obesity, definition, 47
surgical vertical banded gastroplasty, 50
use of ephedra, 50–51
MPO, Myeloperoxidase, 179
MRP8 (8 kDa)/MRP14 (13.2 kDa), 177
Myeloperoxidase (MPO), 179

National Center for Child Abuse and Neglect, 55
New Injury Severity Score (NISS), 125
NISS, New Injury Severity Score, 125

Obesity
circulatory complications, 49
dilated cardiomyopathy, 48
hypoventilation syndrome, definition, 48
non-muscular tissue, increase, 47–48
respiratory complications, 48
sleep apnoea syndromes, 49
Osteomalacia (rickets in children), 42
Osteoporosis, 40–42
trabecular bone volume (TBV), 40–41

Paget's disease of bone (Osteitis Deformans), 42
PAPR, powered air-purifying respirator, 161
Pathology, contributing to fracture
bone disease (metabolic) and endocrine disturbances, 40–42
hyperparathyroidism, 41
osteomalacia, 41
Paget's disease of bone (Osteitis Deformans), 41
inherited and congenital diseases, 39–40
osteopetrosis, 40
localised bone disease, 42
Patterns of injury, air crash, 114–123
See also Air crash investigation
Phenomenology of elder abuse and neglect, 81
Physical neglect of elderly, intentional/unintentional, 97–99
legal medicine, 95
medico-legal aspects, pressure sores, 96–97
pressure sores, prevention/treatment, 96–97
patterns/grades, extreme cases, 94
restraint-related (mechanical) causes, death, 98
sedatives, 99
self-neglect, 94
starvation/dehydration, malnutrition, 95–96
suicide amongst the elderly, 99–101
depression, 100
"Pickwickian syndrome", *see* Hypoventilation syndrome
Plague (*Yersinia pestis*), 154
Post-mortem haemorrhaging, 170
Postmortem toxicological redistribution, 1–17
animal studies, 6–7
drug redistribution using animal models, 8–9
artefacts associated with redistribution, 14
co-administration of drugs, 14
Shipman deaths, 14
case studies, 16
drug absorption and distribution administration, drugs, 3
lipid solubility/volume, distribution, 13–14
redistribution, definition, 3–4
contamination from gastrointestinal tract, 4–5

drug absorption and, 2–3
endogenous substances, 15
movement from another site, 5–6
tissue diffusion, 4
specific drug, 7–9
antidepressants, 9–10
antipsychotic drugs, 11
benzodiazepines, 11
cocaine, 11
Gamma-hydroxybutyrate (GHB), 11–12
ketamine, 12
MDMA and amphetamines, 13
morphine and opiates, 12–13
THC, 13
tissues, adipose/muscle/liver, 15
Postpubertal rape
scanning, ultraviolet Wood's lamp, 62
Potential agents, BT
acute incidents, 145
category A/B/C
aerosol dispersion, 143
delayed incidents, 145
dissemination, means of, 143
emerging/re-emerging conditions, 145
illnesses, unusual, 144
mortuary plans, 145–146
outbreak, 144, 145
Powered air-purifying respirator (PAPR), 161
Puchtler and Sweat method, time of injury, 172

QTD, 47–48

Radiograph, pathological fracture, 43
Radiological/pathological features of fracture healing, comparison, 34–35
Sampling protocol for sexual assault, 63
Smallpox, 149
clinical features, 147
complications, 148
diagnosis
differential, 147–148
procedures and autopsy, 149
forms, 147
impact, 150
pathology, 150
treatment, 148
vaccination, 148–149
Spinal injuries, air crash, 120
Suspected bioterrorism, 161–163

TBV, trabecular bone volume, 41
Terrorism, cost of, 139
Δ^9-tetrahydrocannabinol (THC), 2–3, 13
Thoracic (visceral) injuries, air crash, 120–121
Toxicology, positive, 129–130
Tricyclic antidepressants, 9
Tularaemia (*Francisella tularensis*), 154–156
clinical suspicion, 156

UK Medico-legal Systems and Bioterrorism, 160

Variola, 146
Venereal diseases, 69
VHF, *see* Viral Haemorrhagic Fevers (VHF)
Viral Haemorrhagic Fevers (VHF), 156–157

WHO, World Health Organisation, 146, 148