INTENSIVE CARE MEDICINE

ANNUAL UPDATE 2007

JEAN-LOUIS VINCENT
EDITOR

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Common Abbreviations

ALI	Acute lung injury
APACHE	Acute physiology and chronic health evaluation
ARDS	Acute respiratory distress syndrome
BAL	Bronchoalveolar lavage
CABG	Coronary artery bypass grafting
CBF	Cerebral blood flow
CNS	Central nervous system
COPD	Chronic obstructive pulmonary disease
CRP	C-reactive protein
CSF	Cerebrospinal fluid
CT	Computed tomography
CVP	Central venous pressure
DIC	Disseminated intravascular coagulation
EKG	Electrocardiogram
HSP	Heat shock protein
ICAM	Intercellular adhesion molecule
ICP	Intracranial pressure
ICU	Intensive care unit
IFN	Interferon
IL	Interleukin
LDL	Low density lipoprotein
LPS	Lipopolysaccharide
MAP	Mean arterial pressure
MAPK	Mitogen-activated protein kinase
MCP	Monocyte chemoattractant protein
MOF	Multiple organ failure
MRI	Magnetic resonance imaging
NF-κB	Nuclear factor-kappa B
NO	Nitric oxide
NOS	Nitric oxide synthase
PAF	Platelet activating factor
PAI	Plasminogen activator inhibitor
PAOP	Pulmonary artery occlusion pressure
PEEP	Positive end-expiratory pressure
RBC	Red blood cell
ROS	Reactive oxygen species
SAPS	Simplified acute physiology score
SIRS	Systemic inflammatory response syndrome

SOD	Superoxide dismutase
SOFA	Sequential organ failure assessment
SvO_2	Mixed venous oxygen saturation
TBI	Traumatic brain injury
TNF	Tumor necrosis factor
VILI	Ventilator-induced lung injury

Biomarkers

The Diagnosis of Sepsis: The Present and The Future

A.S. McLean, B. Tang, and S.J. Huang

Introduction

Sepsis is a complex syndrome characterized by systemic inflammation in response to infection. It is a significant health problem with an estimated 750,000 new cases in the USA annually [1]. It is also the third leading cause of death in developed countries, equalling the number of fatalities from acute myocardial infarction. The incidence of sepsis has increased in recent decades and is predicted to continue to rise. The high sepsis-related mortalities and the burden on healthcare systems means there is an urgent need to improve the diagnosis and management of sepsis patients.

Despite medical advances and the introduction of novel therapeutic approaches, little progress has been made in sepsis diagnosis. While our understanding of sepsis pathophysiology has expanded, this has yet to be translated into clinical practice, such as earlier detection or more accurate identification of sepsis patients.

The Diagnosis of Sepsis in General

The Difficulties in Sepsis Diagnosis

By far, the most important issue in sepsis diagnosis lies in the differentiation of sepsis from other non-infectious causes of systemic inflammation (commonly known as systemic inflammatory response syndrome, SIRS) [2]. Presently available diagnostic approaches are neither sensitive nor specific enough to distinguish patients with sepsis from those with SIRS. Physical signs of SIRS such as leukocytosis, raised temperature or heart rate are commonly found in critically ill patients. As a result, there is considerable overlap in the clinical manifestation of SIRS from any cause (e.g., surgery or trauma) and those with an infective origin (e.g. sepsis).

Microbiological culture is routinely used to distinguish infection from other non-infectious cause of SIRS. However, the poor sensitivity and a delay of up to forty-eight hours render it a less useful diagnostic tool. Poor yield due to prior antibiotic therapy and lack of correlation with host-response are the other setbacks for microbiological cultures. Added to these is the difficulty in interpretation of the results due to the mixing of colonizing organisms with pathogens and in the assessment of the importance of organisms normally of low virulence [3].

The Characteristics of a Good Diagnostic Test for Sepsis

These diagnostic challenges have led to a search for a biomarker that is pathognomonic of sepsis. An ideal biomarker for diagnostic purposes should have the follow-

Table 1. Biomarkers for Sepsis Diangosis

Marker	References
Tumor necrosis factor-α	[19]
Interleukin-6	[20]
Interleukin-8	[20]
Interleukin-10	[21]
Interleukin-12	[22]
Interleukin-18	[23]
C-reactive protein	[12]
CD 163	[24]
CD 5a	[25]
CD 14	[26]
Fibronectin	[27]
Human leukocyte antigen (HLA – DR)	[28]
CD 64	[29]
Triggering receptor expression on myeloid cells (TREM-1)	[30]
Procalcitonin	[10–12]

ing basic features: (1) specific and sensitive; (2) stable in blood samples; (3) the assay should be simple and easy to perform; (4) the results should be rapidly available; and (5) the costs should be reasonable and affordable.

A number of biomarkers have been claimed to be of value in the diagnosis of sepsis. These include various cytokines, acute-phase proteins, prohormones, cell receptors and other inflammation-related molecules (Table 1). Most of them cannot be regarded as 'good' biomarkers for sepsis, either because of technical difficulties in measurements or poor diagnostic performance or both. The fact that many of these biomarkers are associated with inflammatory processes further renders them useless because systemic inflammation has been an integral part of the measured biological activity. As a result, most of these biomarkers are confined to experimental stages and never eventuate in clinical applications. There seems to be one exception, however – Procalcitonin (PCT).

The New Era of Sepsis Diagnosis

Procalcitonin – The New Magic Bullet?

The relation between PCT and sepsis was first described in 1993 [4]. Serum PCT was found to be increased after intravenous injection of endotoxin, and was related to an increase in pro-inflammatory cytokines [5]. The increasing interest in PCT has resulted in a large body of literature assessing the diagnostic utility of this biomarker in a variety of clinical settings, including cardiothoracic surgery, burns units, pancreatitis and meningitis [6–8].

In the context of sepsis diagnosis, PCT has received some support from various studies which demonstrated that it is superior to other biomakers. PCT offers certain advantages as a marker for sepsis: (1) It has demonstrated better specificity than most other inflammatory markers; (2) it is relatively stable in blood sample; (3) the assay is readily available, relatively simple to carry out, and inexpensive; (4) the results are available quickly; and (4) its increase is prolonged during sepsis. However, on the downside, PCT has not been found to have a well defined role in the pathogenesis of sepsis. An abnormal PCT concentration, therefore, represents, at

best, a crude measurement of the underlying biological activity of sepsis. Further, emerging evidence casts doubt on PCT's ability to differentiate between sepsis and SIRS (poor sensitivity) [9].

Is PCT then a useful biomarker for sepsis? To answer this question, we have recently conducted a meta-analysis on the diagnostic performance of PCT in critical care settings [10]. In contrast to previous meta-analyses [11, 12], our analysis revealed that the diagnostic performance of PCT was lower than anticipated. For example, assuming a pretest probability of sepsis of 40% in adult ICU patients, using PCT would only raise the post-test probability to 66%. This is insufficient to influence treatment decisions (e.g., to start antibiotics). Conversely, if clinicians wish to stop antibiotics, using PCT would reduce the post-test probability to only 0.23, not quite enough to rule out an infection.

We can think of several explanations for the discrepancy between our meta-analysis and others, including: (1) over-interpretation of diagnostic odds ratios, (2) failure to address the problem of heterogeneity among studies; and (3) exclusion of medical patients. For example, in the meta-analysis by Uzzan et al., the authors suggested that PCT was a good diagnostic marker, based on a summary diagnostic odds ratio of 15.7 from 25 studies analyzed [11]. However, according to published guidelines, the magnitude of this odds ratio represents poor diagnostic accuracy [13]. Additionally, there was strong evidence of heterogeneity among studies included in the previous meta-analyses. Pooling of the outcome measures in the context of significant heterogeneity is often inappropriate and can adversely affect the validity of the analysis. It is also notable that medical patients were excluded from the meta-analysis by Uzzan et al. [11]. It is well known that medical patients often have more co-morbidity and in general are sicker than surgical patients. The diagnosis of sepsis is, therefore, more difficult in this particular population. Excluding medical patients can potentially over-estimate the diagnostic performance of the test.

It is evident from our meta-analysis that PCT is far from fulfilling the role of a 'magic bullet' in sepsis diagnosis. Although PCT may be useful in certain situations, such as reducing antibiotic use in respiratory infection [14], its diagnostic performance has been variable in different clinical settings and among different patient populations. Further studies are, therefore, needed before PCT can be recommended for wider use.

Gene Expression Profiling of Sepsis – Microarrays

The current thinking in sepsis research is based on a conventional biological model, in which a single molecule evokes a series of reproducible and consistent physiological events. Such an approach is too simplistic and restrictive, as no single molecule can recapitulate the complex changes in cellular states that occur in sepsis. If research in sepsis diagnosis is to advance, a change of direction is needed.

A new paradigm shift is now possible with the arrival of the genome era. Microarrays allow one to examine the expression level of a gene by measuring the abundance of mRNA. The procedure involves isolation of mRNA from tissues, generating complementary DNA (cDNA) using the mRNA as a 'template', and hybridization of the cDNA mixture with a slide 'imprinted' with DNA of known genes. By tagging the cDNA with a fluorescent dye, the expressed genes can be identified by the position of the imprinted known DNA on the slide. Using microarrays, investigators can study thousands of genes simultaneously in one single experiment [15]. Microarray is well suited to study sepsis because it offers an unbiased, system biology approach

to examine the expression patterns of many genes. With an ever changing landscape of complex mediators and receptor signalling in sepsis, microarray analysis can capture all these changes in one single genomic 'aerial snapshot'.

Over the past 7 years, more than 160 papers have been published in the area of gene expression in infections using microarray techniques [16]. They were mostly *in vitro* studies examining the transcriptional response of various host cells (macrophage, dendritic cells, lymphocytes, and epithelial cells) when exposed to different micro-organisms (bacteria, viruses, yeast, and protozoa). A few papers also documented *in vivo* studies using animal models of sepsis. Extrapolation of these studies to humans has been difficult, because of the anticipated species-specific genetic make-ups and host-pathogen interactions. These issues are further complicated by the confounding influence of co-morbidities and the many interventions used to treat critically ill patients. To date, there has been only one micorarray study performed in patients with sepsis [17]. The study found that the gene expression profile for some genes in this group of patients was highly homogeneous. The authors found that some of these genes were upregulated while others were downregulated. Although the study suffered from a small sample size and using non-SIRS control patients, the study nevertheless opens the avenue to using gene expression for sepsis diagnosis.

Back in 2001, we started to investigate the possibility of using gene expression to diagnose sepsis. We undertook a gene expression study using microarray with an aim to identify 'signature genes' that could discriminate patients with sepsis from those with non-infectious causes of SIRS. Our preliminary data identified a set of 50 signature genes, most of which are coded for inflammatory response markers, which could discriminate sepsis from SIRS (Fig. 1 and 2). After cross validation, we found that the signature gene could correctly diagnose sepsis with 91 % accuracy. We also

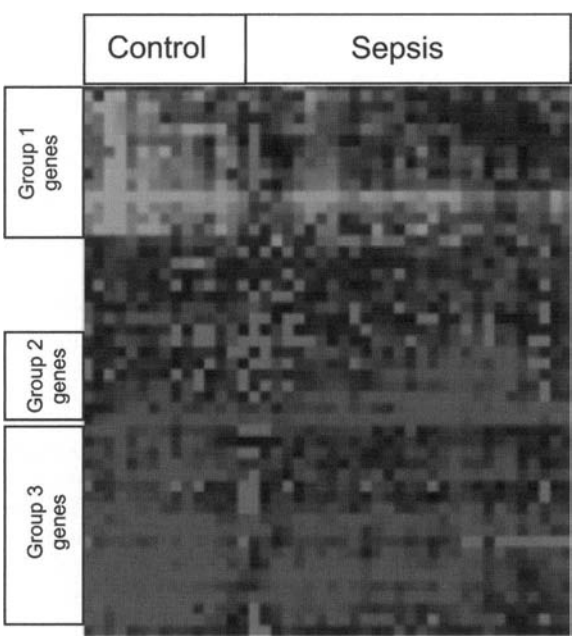

Fig. 1. Gene-expression profiling was performed using oligonucleotide microarrays on peripheral blood samples of 44 critically ill patients. The figure shows the hierarchical clustering of 50 genes which demonstrated significant difference in gene expression levels between control (patient with SIRS) and sepsis patients. Each row represents one gene and each column represents one patient. Three groups of genes can be identified (shown by the differences in the grey tones): Group 1, where the genes were mostly downregulated in the controls; Group 2, where the genes were less expressed in sepsis; and Group 3, where the genes were more upregulated in controls.

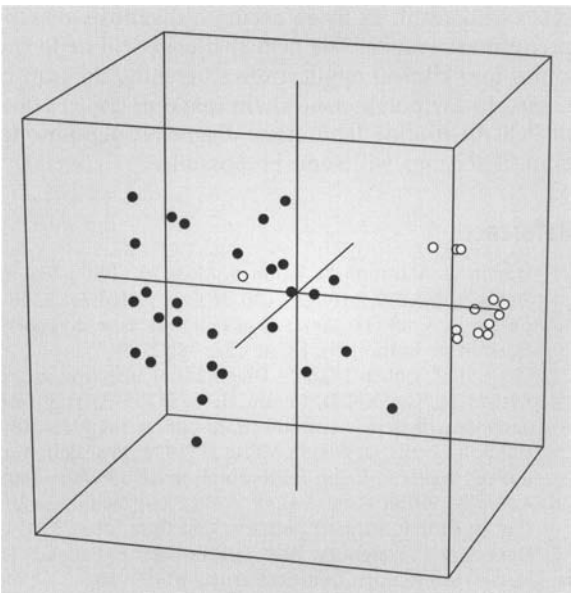

Fig. 2. The discrimination between sepsis and control patients based on gene expression data. Samples are projected into the three dimensional coordinates that capture most of the variability among the 50 predictive genes. White dots represent control patients and black dots represent sepsis patients.

found that, compared to control, certain classes of genes were suppressed in patients with sepsis. These preliminary findings suggest that a genome-wide study can be used to identify multiple diagnostic markers as well as gaining important biological insight into the sepsis signalling network.

Like most evolving technologies and techniques, microarrays suffer from some practical and methodological limitations. Practically, microarray techniques are expensive, require high levels of technical expertise and skills, and are only available in research institutions. Hopefully, rapid advances in technological innovation will make the technique more readily available. Methodologically, gene expressions may change during the course of the disease and in response to treatment. Hence, conditions and times of sample collections may be crucial. In order for the results to be reproducible and meaningful, a standardized protocol has to be developed and adhered to.

Conclusion

Despite technological advances in medicine and our increased understanding of the pathophysiology of infection, sepsis remains a global health problem with mortality remaining persistently high. Conventional methods of diagnosing sepsis, such as clinical evaluation, blood culture, or leukocyte counts have many limitations. Over the past decade, little progress has been made despite the testing of dozens of cytokines and candidate biomarkers. Given the complexity and heterogeneity of the sepsis signalling network, we propose that a genome-wide approach will yield better return as it provides an unbiased, system biology examination of global gene expression profile in sepsis. The adoption of such a data-driven and biology-based approach has been made possible recently by the rapid advance in microarray technology. Using such technology, we believe that the use of multiple signature gene

sets could result in more accurate diagnosis of sepsis patients, as observed in our preliminary results. The next challenge will be to translate the list of these signature genes into clinical application. Currently, this can be done by quantitative reverse-transcriptase polymerase chain reaction (PCR) [18]. With the increasing availability of PCR in routine laboratory diagnosis, genomic testing of our signature genes in clinical settings will soon be possible.

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Procalcitonin: Nice to Know, Need to know, or Needs Further Research?

J.-U. Jensen, J. Løken, and T. Mohr, on behalf of the Procalcitonin and Survival Study (PASS) Group (see Appendix)

Introduction

Sepsis remains a major cause of death in critically ill patients admitted to the intensive care unit (ICU) [1–2]. Infections increase the all-cause mortality during ICU admission from 12.1% in non-infected patients to 43.9% in infected patients [3]. Timely and effective antimicrobial treatment has crucial importance for prognosis. Delayed correct antimicrobial treatment substantially increases mortality [4]. The Surviving Sepsis Campaign has optimized multifactorial sepsis treatment in an evidence-based manner, to the benefit of critically ill patients with sepsis [5].

In order to prevent complications from sepsis, we need to optimize the diagnostics of sepsis at a microbiological level as well as monitoring source control and the effects of antimicrobial chemotherapy.

For the majority of infectious conditions, the present microbiological laboratory does not offer sufficient sensitive and timely diagnostics in a sepsis setting (culture from possible foci). Comparing sepsis to a fast car with bad brakes on a mountain road, this can be compared to "looking out of the rear-window" to control the car – not a very comforting analogy.

Can Classical Methods of Diagnosing and Monitoring Infections be Used in the ICU?

Obviously, in an ICU setting, symptoms are seldom of any value. Classical signs of sepsis observed by the intensive care physician can often be blurred by the inflammatory response and the given treatment, e.g., temperature and dialysis treatment. Specific bacterial infections, often linked to ICU-settings, such as ventilator associated pneumonia (VAP), may be hard to interpret in terms of culture growth, radiology, and clinical findings.

Established inflammatory markers, like C-reactive protein (CRP) and leukocytes, suffer from several drawbacks, including rather slow kinetics in terms of both increase and decrease, and a far from perfect sensitivity and specificity for sepsis. Regarding CRP, we know that nearly all conditions meriting ICU admission increase CRP to pathologic levels. Regarding leukocytes, we know that corticosteroid treatment increases the leukocyte count.

For the reasons listed above, there is a need for a better assessment of sepsis in the ICU. Procalcitonin (PCT) is a promising sepsis marker, which has been proposed as an aid for detecting and assessing bacterial infections. In this chapter, we will try to answer the following questions:

1. What is the nature of the PCT molecule, and in which physiologic and pathophysiologic context should it be placed?
2. Which are the main (claimed) advantages of PCT compared to the already established methods of bacterial infection diagnosis and monitoring in the ICU?
3. How should we use PCT in the ICU? What quantity and frequency of measurements? And how do we interpret PCT levels?
4. Should PCT be implemented in all/any ICUs using the present evidence?
5. How should we expand our evidence base for the use (or non-use) of PCT in the ICU?

What is Procalcitonin?

Production of Calcitonin Precursors in Thyroid Tissue

PCT (~12.6 kDa) is a 114 amino acid polypeptide prohormone of the calcium homeostasis hormone, calcitonin [6]. Calcitonin is produced in the neuro-endocrine medullary C-cells of the thyroid gland, which embryologically derive from the neural crest. The initial investigational move, regarding calcitonin precursors, including PCT, was made when it was demonstrated that medullary thyroid cancer derives from the C-cells [7]. PCT produced in the C-cells undergoes posttranslational processing, leading to release of the mature, bioactive calcitonin hormone of 32 amino acids. A study using a non-selective calcitonin assay, showed increased levels of immunoreactive calcitonin in staphylococcal toxic shock syndrome. Gel-filtration studies showed a large molecule, now known to be approximately the size of PCT [8, 9].

Production of Calcitonin Precursors in Extra-thyroid Tissue

In animal studies, PCT and other precursors of calcitonin have been isolated in the following tissues: Adrenal, spleen, spinal cord, brain, liver, pancreas, colon, lung, fat tissue, testes, and stomach. In bacterial sepsis, the mRNA of the calcitonin (CALC)-1 gene is upregulated more uniformly than the mRNA of other inflammatory cytokines, such as tumor necrosis factor (TNF)-α, interleukin (IL)-1β and IL-6 [10].

The level of mature calcitonin does not increase significantly during sepsis. The explanation for this has been proposed as a 'shift of pathway', from a 'specific pathway' (where calcitonin precursors are released in secretory granules with enzyme activity to process the precursors to mature calcitonin hormone) to a more 'generalized pathway' (where calcitonin precursors are 'bulk released' without secretory granules and thus without enzymatic activity to process peptides). Heat shock proteins (HSPs) may also bind to mature calcitonin under septic conditions, which may change the disposal of the mature calcitonin and/or augment the possibilities of detecting it immunologically [8].

Other calcitonin precursors are also upregulated under septic conditions; of these the mid-region, pro-adrenomedullin, seems to be a promising severity marker in sepsis [11].

Physiologic Role of Procalcitonin

Currently, the physiologic actions of PCT are not well understood at a molecular level, although some researchers claim an effect on intracellular cAMP production [12]. Monneret et al. [13] have shown that PCT modulates regulation of integrin CD

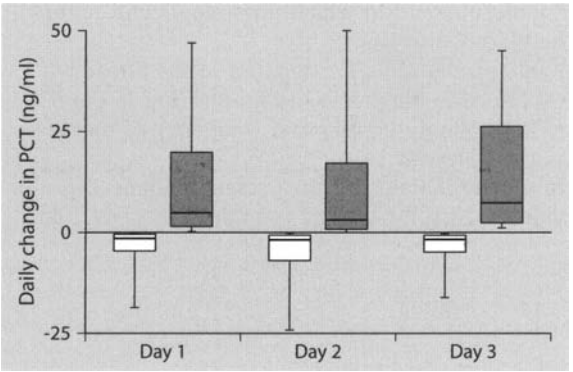

Fig. 1. Daily increases and decreases in PCT after reaching a level of 1.0 ng/ml. Lines are medians, boxes show 25%- and 75% quartiles, and whiskers show 95% CI. Increases are generally higher than decreases. See text for discussion.

11b, and Wiedermann et al. have shown that PCT can modify chemotaxis in monocytes [14].

Interestingly, in light of the latest knowledge on the microcirculation, septic shock, and the growing understanding of the role of nitric oxide (NO) in these processes, the levels of NO increase and the expression of the NO synthase (NOS) gene is amplified when PCT is added to smooth muscle cells of rats, pre-treated with lipopolysaccharide (LPS), TNF-α, and interferon (IFN)γ [15].

Procalcitonin Toxicity and Potentials for Specific Therapy

In healthy hamsters, injection of PCT did not produce any adverse effects, and did not result in increased levels of TNF-α or IL-1β. However, when TNF-α was injected into healthy animals, PCT levels increased many-fold [16]. PCT injection in septic hamsters increased mortality compared to septic hamsters that did not receive PCT injections [17]. Additionally, administration of PCT specific IgG to septic pigs resulted in an improved survival rate [18]. These findings indicate that PCT may itself have a toxic effect, and that immunoneutralization may prove to be a beneficial treatment for humans.

Increases In Procalcitonin Levels Compared to Speed of Elimination: Can we Count on Decreasing Procalcitonin Levels when Bacterial Infection is under Control?

Jensen et al. found that the rate of increase of PCT, when bacterial infection was not yet controlled, was generally much higher than the rate of elimination when infection was under control [19] (see Fig. 1). This is an important feature of PCT: If elimination matched production, we could risk seeing a decreasing level of PCT, when bacterial infection was still not under control.

Procalcitonin Elimination, Kidney Failure and Dialysis

A frequent and therapeutically challenging complication of sepsis is acute oliguric renal failure. This fact raises the question of how PCT is eliminated and, if it is partially or totally eliminated by the kidneys, how would this affect the use and interpretation of PCT in sepsis patients with acute renal failure?

Is Procalcitonin Eliminated by the Kidney, and How is the Rate of Elimination Affected by Renal Dysfunction?

The elimination of PCT is presently not completely understood. Some degree of proteolysis is expected as with other plasma peptides/proteins. Additionally, some renal clearance has been claimed, primarily from the fact that the serum half-life of PCT seems to be increased in patients with renal failure [20]. In this study [20], PCT elimination was measured in 67 patients with a) normal creatinine clearance, b) moderately impaired creatinine clearance, and c) severely impaired creatinine clearance. The plasma half-life of PCT in the three groups was reported as a median of 30.0 hours (a), 35.4 hours (b), and 44.7 hours (c). These findings indicate that, although PCT elimination is probably in part renal, even patients with severe renal impairment eliminate PCT at a substantial rate. This suggests that even in patients with severe renal impairment, PCT elimination kinetics take over when the bacterial infectious condition is under control, although the decrease should be expected to be at a lower rate than in patients with normal renal function.

Procalcitonin Use During Continuous Veno-venous Hemofiltration and Hemodialysis

Level et al. [21] and Sitter et al. [22] have examined PCT levels in patients with and without bacterial infection, who are undergoing hemodialysis. Although both studies rely mainly on single measurements of PCT, the results show seemingly good discrimination among hemodialysis patients with and without bacterial infection. Both studies propose a higher PCT cut-off, of 1.0 ng/ml or 1.5 ng/ml, for bacterial infection in hemodialysis-patients.

Level et al. [23] investigated PCT clearance during continuous veno-venous hemofiltration (CVVH) in patients with septic shock and acute oliguric renal failure. The sieving coefficient of PCT was 0.19 at 6 hours, which is close to the expected for a 13,000 Da polypeptide molecule (for comparison, the sieving coefficient of creatinine with a MW of 113 Da is 1.0; the sieving coefficient of myoglobin, MW of 17,000 Da, is 0.35; and the sieving coefficient of IL-1β, MW 17,000 Da, is 0.18). There was a non-significant tendency towards a decrease in the plasma levels of PCT in these patients. Although this was a small study (n = 13), it indicates that: 1) PCT is not cleared very fast by CVVH, and 2) there is some degree of clearance by CVVH, so PCT levels will decrease when bacterial infection is under control.

In support of these data, we found (unpublished data in an ICU population, described in [19]) that the chance of a decrease in PCT the first day after a limit of 1.0 ng/ml was reached, was similar in a group of patients with acute renal failure compared to patients with normal renal function (25/40 patients [62.5%] vs. 150/233 patients [64.4%]).

In conclusion the present evidence, although limited, suggests that PCT measurements can be used in patients with all degrees of renal failure and during treatment with hemodialysis or CVVH.

Procalcitonin, Trauma, and Surgery

Postoperative infective complications cause a high level of morbidity and mortality. As in other cases of serious bacterial infections, prognosis is highly dependent on

early diagnosis and treatment directed towards the etiologic agent, namely antimicrobial chemotherapy and source control. When treatment is initiated, monitoring the effect of the treatment is important to avoid a delay in change of therapy, when initial therapy is not adequate. Consecutive daily measurements of CRP are widely used for this purpose, but there are serious limitations of this marker in a postoperative setting.

A major disadvantage of CRP is that after surgery and trauma this marker generally increases for several days, reaching a plateau typically on day 2–4 following the event, and, therefore, in most cases not offering the needed guidance for early treatment of bacterial infection [24]. CRP increases consistently in nearly all patients after abdominal surgery and can predict bacterial infectious complications only at a late point in the post-operative course [24–26].

PCT has been proposed as an alternative marker for monitoring patients after surgery and trauma. Several groups have investigated the course of PCT and CRP following abdominal surgery. It has been consistently reported that PCT levels can increase even in surgery without postoperative bacterial infection. However, PCT levels decrease in the majority of patients without complications, as early as the first day after surgery. In contrast, PCT levels increase in the days after surgery in patients with postoperative bacterial infections. Likewise, in a baboon model of trauma, it was found that PCT levels increased shortly after trauma to moderately elevated levels, but decreased within 24 hours [27].

Procalcitonin and Antibiotic Usage in Patients Suspected of Having Lower Respiratory Tract Infection

In a randomized, controlled, single-blinded trial with PCT-guided treatment of patients suspected of having a lower respiratory tract infection admitted to a Swiss emergency department, Christ-Crain et al. demonstrated a relative risk of antibiotic exposure of 0.49 [95% CI 0.44–0.55] in patients receiving PCT-guided treatment [28]. Clinical outcome parameters showed the same result, except for parameters related to antibiotic use, where the PCT-guided group had a significantly reduced use in all subgroups. This result has been reproduced recently [29], also in patients from the emergency department.

These studies demonstrate an advantage in the strategy of PCT-guided treatment compared to clinical judgment in the clinical action of withholding superfluous antibiotics in the emergency department. The strategy of PCT-guided treatment has not yet been investigated in critically ill patients, but a randomized, controlled trial (RCT) is presently ongoing in Denmark.

Sepsis and Assessment of New Diagnostic Markers Against a Gold Standard

Traditionally new diagnostic tools, including new markers of inflammation, are tested against a gold standard for the condition under examination.

Sensitivity/Specificity Assessments and Limitations to this Strategy

Measurements of the percentage of positives in the new test (NEW TEST $_{positive}$) in a population designated positive by the gold standard (sensitivity) and percentage of

negatives in the new test (NEW TEST $_{negative}$) in a population designated to be negative by the gold standard (specificity), are common and in effect, obligatory under the ruling paradigm:

$$\text{Sensitivity} = \text{NEW TEST}_{positive} / \text{GOLD STANDARD}_{positive}$$
$$\text{Specificity} = \text{NEW TEST}_{negative} / \text{GOLD STANDARD}_{negative}$$

A major criticism of this scientific strategy is that very few conditions have a really true gold standard, making assessments of new diagnostic methods more difficult to interpret, when the quality of the gold standard is low. At the same time, the main motivation for assessing new diagnostic methods is often that the gold standard has some serious limitations. This is *par excellence* the situation when testing markers of inflammation and bacterial infection.

Many attempts to standardize diagnosis of infections have been made, and to some degree the latest consensus to standardize sepsis diagnostics [30] has been successful in increasing the number of patients who receive a specialized multifactor/multidisciplinary sepsis treatment. Modern sepsis diagnostics may be very sensitive in finding a group of patients with an increased risk of serious bacterial, viral, parasitic, or fungal infection. However, the nature and pathophysiology of these different infections is, not surprisingly, very diverse. It may be unrealistic to find a single marker of inflammation/infection that increases uniformly (high sensitivity), no matter which microorganism is the cause of infection, and that does not increase when inflammation is caused by non-infectious conditions (high specificity). Finding such a marker would be very surprising, considering the present understanding of immunologic processes during infections with different microorganisms. Additionally, one might ask, what such a "universal marker" could actually contribute to the treatment of patients, since the treatment depends highly on which microorganism we want to treat.

To find several different inflammatory markers, that increase when one or two classes of microorganisms cause infection, may be more realistic from a pathophysiological point of view. This approach may even prove to be of greater clinical value, since specific antimicrobial treatment can be instituted as a consequence of abnormal values of the specific inflammatory marker.

So far, this pathophysiological viewpoint has been used remarkably little in testing inflammatory markers, as many studies have tested the sensitivity and specificity of these markers towards clinical terms like *sepsis* and *infection*, without differentiating between the classes of microorganisms that cause the infection. Some thought should also be given to whether we really just want a marker of infection to find 'clinical sepsis= SIRS + evidence of localized infection'. If we consider that 'clinical sepsis' is a good gold standard, there is no need for change, since this diagnosis is based on microbiological results and simple clinical measures.

Procalcitonin, C-reactive Protein, Leukocytes, and Sensitivity/specificity for Sepsis: The Major Problems

Gold Standard

Many studies have investigated the sensitivity and specificity of PCT measurements for clinical conditions, such as sepsis and infection. As mentioned above, there are some serious methodological limitations in this approach. Additionally, there are limits regarding: a) 'locked bias'; b) the PCT assay used in the laboratory; and c) the chosen strategy of sampling (single vs. consecutive regimens)

'Locked bias'

When an investigation is made, a traditional and widely used method (e.g., CRP) is often tested against an alternative method (e.g., PCT). If a certain infection diagnosis is guided by a marker (e.g., CRP) AND the bacterial infection diagnosis is an endpoint, there will be a positive directed bias towards a coupling of this certain marker and the endpoint. If the new marker is not blinded, this will also be true for the new marker. However, blinding of the new marker is relatively easy: Results of the analyses can simply be concealed until they no longer have an acute relevance. To even out conditions and to avoid an inert advantage of the traditional method, blinding of the analysis of the results of this marker is desirable, but unfortunately, impossible. When CRP is used as guidance for when to look for infection aggressively, infections will more often be found in patients with high CRP blood levels.

The direction of the bias is, however, in favor of conservatism, which to some degree is scientifically sound: If CRP is favored, PCT has to show an even better ability to compete. This, in effect, makes results even more convincing, if they are positive; that is, finding PCT to be better in, for example, discriminating patients with and without bacterial infection. It does not seem reasonable to 'change the world' because of a very small diagnostic advantage.

Assays

The most commonly used assay until ~2003 was the BRAHMS Lumitest®, a sandwich immunoassay. The inter-assay variability for this assay in the measuring interval between 0.0 ng/ml – 1.0 ng/ml was 9–82%. This makes the Lumitest ® assay of little use in this measuring interval [19]. Ironically, this is the interval of most interest regarding localized bacterial infections. Consequently, studies using this assay, and where a majority of the patients suffer from localized bacterial infections, should be interpreted very carefully, not necessarily because of the scientific qualities, but simply because of the quality of the assay. In contrast, the most commonly used assay presently, the KRYPTOR® PCT, has a functional assay sensitivity of 0.06 ng/ml, according to the manufacturer (BRAHMS Diagnostica, Henningsdorf, Germany), which makes this assay secure to use for localized bacterial infections.

Single versus consecutive procalcitonin measurements

Most of the comparisons between CRP and PCT in clinical conditions such as sepsis or infection have been performed with single measurements of both of these markers. It is well documented that trauma and surgery in itself cause an increase in CRP levels for 2–4 days. This makes tight monitoring of patients to discover emerging bacterial infection using CRP after trauma and surgery more or less impossible. PCT levels are increased by other factors than bacterial infection. Some examples are surgery, trauma, and acute left sided heart failure. One possible explanation for this is translocation of endotoxins from the intestine to the blood stream. However, when the initiating event has ended, PCT kinetics will nearly always be rapidly dominated by elimination, and although initial PCT levels may be increased, the level will thereafter decrease in the non-infected patient [24, 27].

By comparing single measurements of PCT (or any other marker) and CRP, one rules out the possibility that PCT may have an advantage in discriminating bacterial infection from non-bacterial conditions if consecutive measurements have been made.

The power of 20 different parameters has been tested in a multivariate Cox-regression model to predict mortality in critically ill patients, including: a) the ini-

tial PCT value ($PCT_{initial}$); the maximum obtained PCT (PCT_{max}); and c) a dynamic parameter regarding if there is an increase in PCT the first day after reaching a cut off of 1.0 ng/ml ($PCT_{increase1.0}$) [19]. Similar parameters regarding CRP and leukocyte levels were investigated. The results were interesting in three aspects: 1) CRP and leukocytes did not show predictive power in any of these analyses; 2) PCT_{max} and $PCT_{increase1.0}$ were both independent predictors of mortality; and 3) $PCT_{initial}$ did not predict mortality. These results support the idea that consecutive measurements of PCT may have a much greater value than single measurements. Hence this strategy should be more dominant in research comparisons.

In spite of the above mentioned limitations, the results from sensitivity/specificity studies are in favor of PCT, compared to CRP, as a sepsis marker (as comprehensively reviewed in the meta-analysis by Simon et al. [31]).

Procalcitonin and Mortality in the ICU: Prognostic Implications

In the attempt to deal with some of the above-mentioned problems and difficulties when comparing PCT with other inflammatory markers, we decided to look at the more solid endpoint, mortality. Additionally, we decided to make daily consecutive measurements of PCT in a mixed population of 472 ICU patients, to investigate if this strategy could increase the value of PCT measurements [19]. The main results are shown in Figure 2 and in Table 1.

These results confirm findings from other investigators that high PCT levels are closely linked to bacterial infection and complications of these infections and, additionally, they demonstrate that a PCT increase ($PCT_{increase1.0}$) is an independent predictor of mortality. The mortality rate was strongly dependent on how many days PCT had been increasing while the patient was in the ICU.

In conclusion, consecutive PCT measurements in the ICU can independently predict potential lethal infections. We believe this information could potentially be very valuable to the intensive care physician, because here we have a marker of inflammation that is better at discriminating bacterial infection from other causes of inflammation, better at monitoring this bacterial infection, and better on a daily basis at stratifying patients into different mortality risk groups, so that treatment can poten-

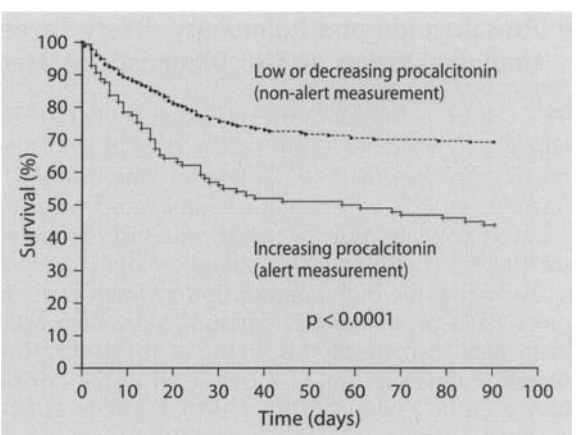

Fig. 2. Survival differences between patients with a decreasing vs. an increasing PCT after a limit of 1.0 ng/ml has been reached for the first time, i.e., PCT decreasing and increasing for just one day.

Table 1. Mortality risks for patients with increasing and decreasing procalcitonin (PCT) levels [19]. p values are estimated with the Chi-square test for equal proportions. Patients with decreasing PCT levels after a limit of 1.0 ng/ml has been reached and patients with constantly low PCT values are counted as "Non-alert PCT". Patients with increasing PCT after 1.0 ng/ml has been reached are "Alert PCT". Patients can be included in several categories: e.g., patients with an increasing PCT trend for three days are also included in categories with increasing PCT trend at one and two days.

Days with delta-PCT after PCT ≥1.0 ng/ml	90-day mortality with PCT day-to-day change: Decreasing ("Non-alert")	Increasing ("Alert")	Relative risk PCT Increasing) (95% CI)	p value for risk difference	Patients (n)
1	30.7%	56.1%	1.8 (1.4–2.4)	<0.0001	336
2	28.7%	62.2%	2.2 (1.6–3.0)	<0.0001	261
3	26.0%	72.4%	2.8 (2.0–3.8)	<0.0001	233

tially be adjusted according to severity of disease, thereby increasing the chances of effective and timely antibacterial treatment.

Is the Problem Solved?

Although consecutive PCT measurements may have the mentioned qualities compared to, e.g., CRP, whether this dynamic strategy can add something really beneficial to the treatment is still unknown. In other words, we do not know if daily consecutive PCT measurements are 'nice to know' or 'need to know'. Are we merely confirming our (already chosen) clinical decisions, or can we actually change diagnostic and therapeutic strategy in a timely manner and thereby reduce complications of bacterial infection and/or reduce mortality in ICU patients?

We need more evidence at a higher level to show this. Therefore, we need RCTs that are statistically powered to investigate mortality differences between patients receiving PCT-guided treatment and the best standard of care.

In the following section we discuss why a lower level of evidence cannot be accepted for this issue.

Procalcitonin and Pulmonary Artery Catheters: A Comparison of the Implementation of Two Diagnostic Strategies in the ICU

The use of pulmonary artery catheters (PACs) in the seventies and eighties has taught us a valuable lesson on the level of evidence needed to introduce new diagnostic strategies in the ICU. Bearing this in mind, we can compare the process of introducing PCT as a diagnostic tool with the historic introduction of the PAC. The point is to determine whether widespread implementation of a new diagnostic method is *actually* or just *seemingly* of significant benefit to the critically ill patient.

Following the initial description by Swan et al. in 1970 [32], it became routine to place PACs in critically ill patients. More than 8000 papers on this technique have been published. In the beginning of the 1990s, it was questioned whether the systematic catheterization of a large part of the critically ill patients was of benefit or may even be harmful [33]. In 1996, Connors et al. [34] published an observational study suggesting a higher mortality in patients monitored with the catheter. The

ICU-world was shaken – had patients been suffering lethal side-effects of this monitoring system? It became obvious that RCTs were needed. Several small-scale and a few large scale randomized trials were conducted, the most recent of which, in patients with acute lung injury (ALI) was published in May 2006 [35]. In this multicenter RCT, no benefit was found in the group monitored with a PAC compared to a central venous catheter. This indicated that PACs should not be inserted routinely.

The same kind of analysis should perhaps be conducted for sepsis markers.

The Need for RCTs in the ICU to Assess whether Procalcitonin-guided Treatment can Reduce Mortality in Critically Ill Patients?

To assess whether it is relevant to conduct a RCT regarding a treatment regime, two main criteria should be fulfilled:

1) The treatment regimen should be effective.
2) It should not be applied too late.

These conditions are presently fulfilled regarding PCT-guided antibiotic treatment, and an investigator initiated RCT, The Procalcitonin And Survival Study (PASS), is presently being conducted in Northern Europe (initiated in Denmark). The primary endpoint is a reduction of 28-day all-cause mortality. If the trial is positive, it will have demonstrated a way of reducing mortality in a broad population of ICU patients at a relatively low cost (approx. 10€ for each analysis kit per day/patient), and worldwide implementation could be suggested, considering the almost absent adverse effects of the strategy. In contrast, if the result is negative regarding all measured primary and secondary endpoints, patients will be spared another useless test, like the PAC.

Conclusion

PCT, a 114 amino acid precursor of calcitonin, is a promising sepsis marker with an advantageous kinetic profile for use on a daily basis in the ICU. Clinical studies have shown a very close relation of this sepsis marker to bacterial infection, and it has been shown that an increasing level of procalcitonin for just one day after a cut-off is reached is an independent predictor of mortality in ICU patients.

The question of whether PCT-guided treatment can reduce mortality or morbidity in ICU patients remains to be answered. We await the results of an RCT to gain evidence either to encourage widespread implementation of PCT measurement in ICUs or alternatively, to stop implementation, if the results of such a trial are negative.

Appendix

The Procalcitonin And Survical Study Group Steering Committee: Dr. Klaus Thornberg, Gentofte ICU, Dr. Thomas Mohr, Glostrup ICU, Dr. Hamid Tousi, Herlev ICU, Dr. Peder Carl, Hvidovre ICU, Dr. Morten Bestle, Hilleroed ICU, Dr. Paul Fjeldborg, Skejby, Aarhus ICU, Dr. Kim Michael Larsen, Aarhus Sygehus ICU, Dr. Niels-Erik Drenck, Roskilde ICU, Dr. Christian Oestergaard Andersen, Dept. of Clinical Microbiology, Herlev, Dr. Gitte Kronborg, Dept. of Infectious Disease, Hvidovre, Dr. Bet-

tina Lundgren, Dept. of Clinical Microbiology, Hvidovre, Dr. Jens Ulrik Jensen, Dept. of Clinical Microbiology, Hvidovre, Professor Jens D. Lundgren, Copenhagen HIV Programme, Hvidovre. Principal Investigators (when not Steering Committee members): Dr. Lars Hein, Glostrup ICU, Dr. Jesper Loeken, Hvidovre ICU. All: Copenhagen University Hospital, Denmark.

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Diagnostic and Prognostic Value of Hormokines as Biomarkers in Severe Infections

M. Christ-Crain and B. Müller

Introduction: What are 'Hormokines'?

The term "hormokine" was proposed to encompass the cytokine-like behavior of hormones, during inflammation and infections [1]. The concept was based on the finding of a ubiquitous expression of calcitonin peptides (i.e., procalcitonin [PCT], calictonin gene related peptides (CGRPs), and adrenomedullin [ADM]) during sepsis. Calcitonin gene products are prototypes of hormokine mediators and can follow either a classical hormonal expression in neuro-endocrine cells or a cytokine-like ubiquitous expression pathway in various cell types [1]. The inflammatory release of hormokines can be induced either directly via microbial toxins (e.g., endotoxin) or indirectly via a humoral or cell-mediated host response (e.g., interleukin [IL]-1β, tumor necrosis factor [TNF]-α, IL-6). Parenchymal cells (including liver, kidney, adipocytes, and muscle) provide the largest tissue mass and principal source of circulating hormokines in sepsis [2]. The greater mRNA induction and peptide release from parenchymal cells in comparison to circulating cells, appears to indicate a tissue-based, rather than a leukocyte-based mechanism of host defense, which is characteristic of hormokines. Lowering of circulating hormokines by specific antibodies improves survival in various animal models of mono- and polymicrobial sepsis. This important finding suggests potential therapeutic use.

Calcitonin Peptides are Prototypes of Hormokines

PCT is a precursor peptide from the hormone calcitonin, and is also referred to as the prototype "hormokine" mediator. After translation from calcitonin-messenger RNA (mRNA), PCT is cleaved enzymatically into smaller peptides, finally to yield the thirty-two amino acid mature calcitonin [3]. In the traditional endocrine view, mature calcitonin is produced mostly in neuro-endocrine C-cells of the thyroid. In the absence of infection, the extra-thyroidal transcription of the calcitonin (CALC)-I gene is suppressed and is restricted to a selective expression in neuro-endocrine cells found mainly in the thyroid and lung. In these neuroendocrine cells, the mature hormone is processed and stored in secretory granules [2, 4].

Interestingly, a microbial infection induces a ubiquitous increase in CALC-I gene-expression and a constitutive release of PCT from all parenchymal tissues and differentiated cell types throughout the body [1]. The induction can be attenuated by cytokines also released during a viral infection (e.g., interferon [IFN]-γ). Thus, under septic circumstances, the entire body could be viewed as being an endocrine gland. Indeed, the transcriptional expression of calcitonin-mRNA is more uniformly

upregulated in sepsis than are the mRNAs of the classical cytokines [5]. Accordingly, PCT levels increase several thousand-fold in severe infections, e.g., sepsis, with a superior diagnostic accuracy as compared to other parameters of microbial infections.

Calcitonin Peptides for the Improved Diagnostic Assessment of Severe Infections

The traditional clinical signs of infection and the routine laboratory tests in sepsis (e.g., C-reactive protein [CRP] or white blood cell [WBC] count) lack diagnostic accuracy and are sometimes misleading. In severe infection, most classical pro-inflammatory cytokines (e.g., TNF-α, IL-1β or IL-6) are increased only briefly or intermittently, if at all. Mortality in sepsis remains high, often due to delayed diagnosis and treatment. In view of this diagnostic and therapeutic dilemma, a more unequivocal test for the differential diagnosis of infection and sepsis is of paramount importance. Recently, in an attempt to improve current definitions of the systemic inflammatory response syndrome (SIRS) and sepsis, it was suggested that PCT be included as an additional diagnostic tool to facilitate and expedite the difficult clinical diagnosis. This suggestion was based on evidence from the literature that, in sepsis, PCT levels increase several-fold until several thousand-fold and on admission this increase often correlates with the severity of the condition and with subsequent mortality [6]. A variety of studies and reviews have shown the superior diagnostic accuracy of PCT as compared to other parameters for the diagnosis of sepsis, independent of the origin of infection (references in [6]). Whereas the increase of other inflammatory markers, such as CRP, is attenuated by immunosuppressive medication (namely steroids), the diagnostic accuracy of PCT remains unaffected [7]. In addition, PCT seems to have a slight advantage over CRP because of its earlier increase upon infection and a better negative predictive value, as for example shown in children with fever of unknown origin [8].

The most frequent source of systemic infection is the lung with respiratory tract infections [6]. A common problem in clinical practice is that in respiratory tract infections, signs and symptoms of bacterial and viral infections overlap considerably. After obtaining the medical history, physical examination, laboratory results, and chest X-ray, the clinician is often left with considerable diagnostic uncertainty. In view of this diagnostic and therapeutic dilemma, a more unequivocal test for the differential diagnosis of respiratory tract infections is clearly needed.

Calcitonin Peptides for Antibiotic Stewardship of Respiratory Tract Infections

The most frequent source of systemic infections is the lung [6]. Lower respiratory tract infections (LRTI), i.e., acute bronchitis, acute exacerbations of chronic obstructive pulmonary disease (COPD) or asthma, and pneumonia, account for almost 10% of the worldwide burden of morbidity and mortality. As many as 75% of all antibiotic doses are prescribed for acute respiratory tract infections in spite of their predominantly viral etiology. This excessive use of antibiotics is the main cause of the spread of antibiotic-resistant bacteria [9, 10]. Thus, decreasing the excess use of antibiotics is essential to combat the increase in antibiotic-resistant microorganisms

[11, 12]. A reduction in antibiotic use results in fewer side effects, lower costs, and, in the long-term, leads to decreasing drug resistance. To limit antibiotic use, a rapid and accurate differentiation of clinically relevant bacterial LRTI from other, mostly viral causes is pivotal [13, 14].

The randomized 'ProRESP' intervention study recently assessed the ability of PCT measurements to identify bacterial LRTIs requiring antimicrobial treatment in the setting of an emergency department [15]. PCT was chosen as the test marker because of its advantages over CRP and other inflammatory markers, namely an earlier increase upon infection, a better negative predictive value, and the unattenuated increase in the presence of immunosuppressive medication (e.g., steroids in patients with COPD) [7]. PCT was measured using a rapid assay with a functional sensitivity of 0.06 μg/l (Kryptor® PCT, Brahms, Hennigsdorf, Germany). The assay time for PCT measurements was less than 20 minutes and results were routinely available within 1 hour (24 hours a day, 7 days per week).

Investigating physicians used an algorithm developed at University Hospital, Basel, Switzerland, to classify patients in the PCT group into four subgroups according to the probability of bacterial infection. The following PCT cut-off ranges were derived by calculating multilevel likelihood ratios and optimized for the setting of an emergency room and hospital:

- A PCT level of <0.1 μg/l suggested the absence of bacterial infection and the initiation or continuation of antibiotics was strongly discouraged. Antibiotic therapy could be considered in critically ill patients. If antibiotics were given, an early discontinuation of antibiotic therapy after 1–3 days was endorsed if PCT levels, checked daily, remained <0.1 μg/l.
- A PCT level between 0.1 and 0.25 μg/l indicated that bacterial infection was unlikely, and the initiation or continuation of antibiotics was discouraged. Antibiotic therapy could be considered in high-risk patients. Again, if antibiotics were given, early termination was endorsed if PCT levels did not increase.
- A PCT level between 0.25 and 0.5 μg/l indicated a possible bacterial infection and the initiation or continuation of antibiotic therapy was encouraged.
- A PCT level of >0.5 μg/l strongly suggested the presence of bacterial infection and antibiotic treatment and continuation was strongly encouraged [15].

The same cut-offs were used regardless of whether or not patients had been pretreated with antibiotics prior to admission to the emergency department. Re-evaluation of the clinical status and measurement of serum PCT levels was recommended after 6–24 hours in all persistently sick and hospitalized patients in whom antibiotics were withheld. The PCT algorithm could be overruled in patients with immediately life-threatening disease (e.g., patients with severe co-morbidity, emerging need for intensive care unit [ICU] admission during the initial follow-up, in patients with hemodynamic or respiratory instability, and in very ill patients with positive antigen test for legionellosis).

Physicians were advised that persistently elevated PCT levels may indicate a complicated course (e.g., empyema or abscess), while PCT levels may remain relatively low in localized infections. PCT levels were reassessed on days 4, 6 and 8 in hospitalized patients with ongoing antibiotic therapy, and in patients showing a worsening or delayed recovery of signs and symptoms, and antibiotics were discontinued using the PCT cut-offs defined above. In patients with very high PCT values on admission (i.e., >10 μg/l), discontinuation of antibiotic therapy was encouraged if levels decreased to below 80–90% of the initial value. In patients with an initial PCT level

>10 µg/l and smaller reductions during follow-up, continuation of antibiotic treatment was encouraged.

For antibiotic stewardship in a medical ICU, modified cut-off ranges may be necessary. Since mean PCT levels are increased in a cohort of critically ill patients as compared to patients in an emergency room or hospital setting, the optimal thresholds of the cut-off ranges are likely to be higher. A suggested algorithm for a medical ICU based on an observational study [6] is shown in Figure 1.

In the ProResp study in the emergency room setting, the percentage of patients with LRTI who received antibiotic therapy in the PCT group was reduced by almost 50%, compared with the standard group [15]. The clinical and laboratory outcome was similar in both groups. Reduced antibiotic use was most striking in acute bronchitis and acute exacerbations of COPD. COPD exacerbations offer a particular challenge in terms of diagnosing an infectious cause. The majority of COPD patients have positive sputum culture results, although these do not necessarily imply an active infection. In the PCT group, positive culture rates were similar in patients in whom antibiotics were given or withheld, as were outcomes, underlining the limited diagnostic usefulness of sputum cultures in COPD. Since patients with COPD have an impaired pulmonary reserve and infection may be locally contained, a PCT cut-off level for withholding antibiotics of <0.1 µg/l is advisable in patients with severe disease. This was validated in the ProCOLD study, which included more than 200 patients [16].

In patients with community-acquired pneumonia (CAP), PCT levels are almost always high. Pneumonia is defined as inflammation of the pulmonary parenchyma,

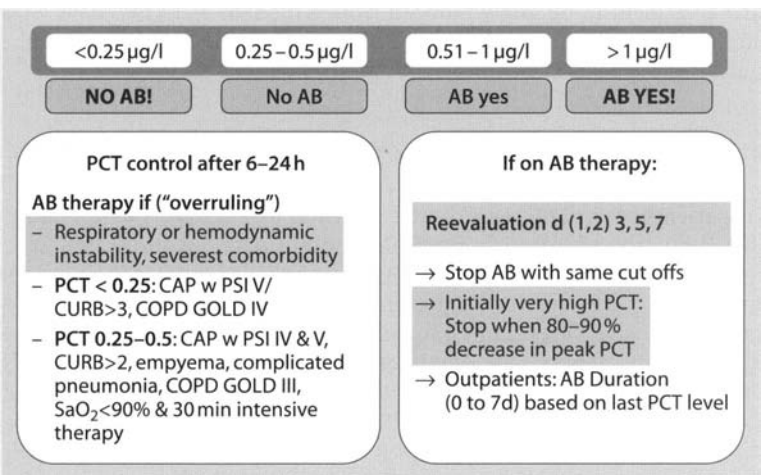

Fig. 1. Procalcitonin-guided antibiotic stewardship.
Procalcitonin (PCT)-guided antibiotic therapy was successfully validated in more than 1200 patients with lower respiratory tract infections in the setting of the emergency room, primary care and hospital. For use in intensive care units, cut-offs have to be adapted to the local setting. Herein, cut-off ranges are proposed based on an observational study performed in the medical intensive care unit [6] and important aspects to be considered in any intensive care unit are highlighted in gray. The cut-off ranges may be even higher in a surgical intensive care unit or if applied in newborns where high PCT levels can be found even in the absence of an infection. Importantly, the course of PCT levels is more relevant than the initial value in critically ill patients. CAP: community-acquired pneumonia; PSI: pneumonia severity index

which is often caused by a bacterial agent, mirrored in markedly elevated PCT levels. In CAP, antimicrobial therapy must be promptly initiated, because a delay in treatment is associated with increased mortality [17]. Thus, the primary value of PCT in CAP is not to reduce antibiotics, but to facilitate the differential diagnosis of new or progressing infiltrates. Accordingly, PCT-guidance could markedly lower the number of antibiotic courses in patients with infiltrates on chest X-ray unrelated to pneumonia.

Importantly, the optimal duration of antimicrobial therapy in CAP is largely unknown [18]. Most likely, it varies from patient to patient and is dependent, among other factors, on the severity of the disease, the adequacy of the host response, and the underlying microorganism. Current guidelines recommend antibiotic courses of 7 to 14 days, depending on illness severity and type of pathogen [19–21]. However, adherence to guidelines is variable [22] and physicians tend to treat longer, especially in elderly patients with co-morbidities and in patients with severe CAP [15, 23]. Optimal duration of antibiotic therapy can be guided by clinical signs such as defervescence, decrease in sputum production and coughing, or improvement in general condition. However, the interpretation of the clinical response lacks standardization and validation and is prone to interobserver variability [24].

The dynamics of PCT levels have prognostic implications, as persistently elevated levels are associated with adverse outcomes [25]. Conversely, decreasing PCT levels suggest a favorable outcome, usually showing a log-linear drop-off and a half life of 20 to 24 hours [26]. We assessed, in a randomized intervention trial, the "ProCAP"-study, the capability of PCT-guidance to shorten antibiotic duration in patients with all severity levels of CAP admitted to the emergency department. We demonstrated in more than 300 patients with CAP that PCT guidance allows the duration of antibiotic treatment to be safely reduced from a median of 12 to 5 days with a similar outcome after a follow-up of 6 weeks. Importantly, measures of clinical and laboratory outcome were similar in both groups [27].

Hormokines as Biomarkers for Prognostic Assessment in CAP

In patients with CAP, improved diagnostic assessment by PCT is important in the differentiation of infection from other, non-infectious infiltrates, and in guiding the duration of antibiotics. In addition, it is pivotal to predict the prognosis of CAP and to estimate CAP severity for guiding therapeutic options such as the need for hospital or intensive care admission, suitability for discharge, and choice and route of antimicrobial agents. The pneumonia severity index (PSI) is a widely accepted and validated severity scoring system that assesses the risk of mortality for pneumonia patients in a two-step algorithm [28]. However, it is complex, which jeopardizes its dissemination and implementation in everyday practice. Therefore, the CURB-65 score has been proposed as a simpler alternative. Additionally, various easy to determine surrogate biomarkers have been proposed to predict disease severity in CAP patients, thus complementing the PSI score [29–31].

Another member of the CALC gene family is adrenomedullin, which is one of the most potent vasodilating agents and has additional immune modulating, metabolic properties [32–35]. Adrenomedullin also has bactericidal activity, which is further enhanced by modulation of complement activity and regulation. Thus, it is not surprising that serum adrenomedullin levels are elevated in sepsis [36]. The reliable measurement of adrenomedullin is challenging, since it is rapidly cleared from the

circulation [32,33]. The more stable mid-regional fragment of pro-adrenomedullin (ProADM) directly reflects levels of the rapidly degraded active peptide adrenomedullin [37].

In our study in over 300 patients with CAP, proADM levels measured on admission emerged as good predictors of severity and outcome of CAP with a similar prognostic accuracy to the PSI and better prognostic accuracy than commonly measured clinical and laboratory parameters. Importantly, proADM levels could improve the prognostic accuracy of the PSI alone, acting as an additional margin of safety [38].

It is advisable to base the difficult task of prognostic assessment and treatment decisions on several and not only one, parameters, each mirroring different pathophysiological aspects. In this context, we also evaluated the prognostic value of atrial natriuretic peptide (ANP), a member of the family of natriuretic peptides. ANP regulates a variety of physiological parameters including diuresis and natriuresis, and reduces systemic blood pressure. Mature ANP is cleaved from carboxyl-terminal amino acids of the prohormone of ANP. The N-terminal portion of the prohormone is secreted in the same molar ratio as ANP [39, 40]. Because of its longer half-life, the N-terminal portion of proANP, particularly the mid-region of this molecule (MR-proANP), has been shown to be a more reliable marker [40]. In CAP, the MR-proANP level may mirror both the inflammatory cytokine response correlated with the severity of pneumonia, and the presence of disease-relevant comorbidities, namely heart failure and renal dysfunction [41–44]. Therefore, we investigated MR-proANP levels in a well defined cohort of 545 consecutive patients with LRTI, to evaluate its prognostic use for severity of disease and outcome. Our study had two main findings. First, plasma MR-proANP levels were increased in LRTI with highest levels in CAP. On admission, MR-proANP levels were a better predictor of severity and outcome of CAP than commonly measured clinical and laboratory parameters and comparable to the PSI [45]. Thus, proADM and MR-proANP are helpful in the risk stratification of patients with CAP. Of course, biomarkers will always oversimplify the interpretation of important variables and proADM and proANP are, therefore, meant to complement, rather than to replace, clinical judgment and/or validated severity scores.

Hormokines as Biomarkers for Prognostic Assessment in Sepsis

A reliable prognostic assessment is crucial, not only in CAP, but equally in sepsis. Sepsis is the leading cause of death in critically ill patients in the United States. It develops in 750,000 people annually, and more than 210,000 of them die [46, 47]. Roughly 9% of patients with sepsis progress to severe sepsis, and 3% to septic shock [48]. Early and adequate diagnosis and risk assessment is pivotal for optimized care of critically ill patients. The APACHE II score was originally suggested as a prognostic scoring system in sepsis and not to be used for individual outcome prediction of sepsis patients [49]. However, despite its inherent limitations, outcome predictors are clearly helpful in identifying those septic patients with a high risk of death, who are more likely to benefit from treatment.

In an attempt to improve current sepsis definitions the use of readily measurable circulating biomarkers is recommended, in the PIRO concept, as an additional tool for the timely assessment and severity classification of septic patients and the prediction of mortality [50].

We showed that proADM has a similar prognostic accuracy to the APACHE II score [51]. This prognostic usefulness is validated by our more recent data in patients with CAP [38]. ProADM may, therefore, prove to be an additional helpful tool for a broader prognostic classification of septic patients. Two main mechanisms may be responsible for the marked increase in circulating MR-proADM and mature adrenomedullin in sepsis. First, as a member of the CALC gene family, adrenomedullin is widely expressed and extensively synthesized during sepsis, similar to other calcitonin peptides, namely PCT and calcitonin-gene related peptides [26]. Bacterial endotoxins and pro-inflammatory cytokines upregulate ADM gene expression in many tissues both *in vitro* and *in vivo* in rodents and humans [52]. In addition, decreased clearance by the kidneys may be responsible in part for the increased levels in sepsis [36].

Hormokines as Mediators in Severe Infections

PCT is a potentially harmful mediator involved in the infection response. The administration of PCT to septic hamsters with peritonitis doubled their death rate. Conversely, immunoneutralizing elevated PCT levels with a specific antiserum greatly increased survival in septic hamsters [53] and pigs [54], even when administered after the animals were moribund. Several characteristics of PCT favor its use as a therapeutic target. In contrast to the transiently increased classical cytokines, for which immunoneutralization trials in humans have been disappointing, the massive increase in circulating PCT levels persists for several days. Furthermore, PCT is frequently increased in overt sepsis, its onset is early (within 3 hours), and the diagnostic accuracy of its measurement should greatly improve patient selection for any study of the therapeutic efficacy of PCT immunoneutralization and antibiotic therapy in humans.

Conclusion

Used in conjunction with optimal clinical assessment, hormokines can improve the diagnostic assessment of sepsis and its precursors (e.g., LRTI, including pneumonia) and can, thereby, guide and reduce antibiotic use. Importantly, hormokines can also improve the prognostic assessment of sepsis and pneumonia compared to other routinely used laboratory parameters or clinical assessment. The therapeutic promise of this approach needs to be explored further.

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Novel Biomarkers and the Outcome from Critical Illness and Major Surgery

D. Fallaha, G. Hillis, and B.H. Cuthbertson

Introduction

As the molecular basis for disease becomes better understood the importance of biomarkers continues to grow. Novel techniques have led to the identification of large numbers of biologically significant compounds that could act as biomarkers. The appeal of these as diagnostic and therapeutic markers is obvious but the choice of marker for everyday clinical practice is not.

The Ideal Biomarker

In the first instance, it is necessary to consider expression in a disease state versus health. Thus levels of an ideal marker should: i) increase pathologically in a reliable fashion depending on the presence of the disease (have a high sensitivity and predictive value with a low coefficient of variation); ii) not increase in the absence of the disease (have a high specificity); iii) change in keeping with the severity and course of the clinical picture; or even better iv) anticipate clinical changes [1]. Few markers have levels at which the risk suddenly rises. Examining sensitivity versus specificity at different marker thresholds gives rise to a receiver-operating-characteristic (ROC) curve. A clinically-useful biomarker will be one with a large area under the curve. Finally, a suitable marker should of course be relatively cheap and easy to assay.

Natriuretic Peptides

Several important cardiac biomarkers have already been recognized and are being developed for the diagnosis, monitoring, and prognostics of cardiac disease in both primary and secondary care. The natriuretic peptides are a family of hormones involved in the regulation of fluid and blood pressure homeostasis where they negatively feed back in response to myocardial overload. Raised plasma concentrations are seen in patients with cardiac disease, particularly those with congestive heart failure. Although levels may vary widely between such individuals, it has been clearly demonstrated that persistently elevated levels strongly correlate to symptoms, cardiac events, and mortality [2]. B-type natriuretic peptide (BNP) is a 32 amino acid peptide mainly secreted from the cardiac ventricles in response to ventricular strain. On secretion, proBNP, the stored form of BNP, is cleaved into an inactive N-terminal fragment (NT-proBNP) and the endocrinologically active BNP.

The active moiety promotes natriuresis, diuresis, and vasodilatation. These actions probably underlie its importance in the homeostasis of compensated asymptomatic heart failure. As might be expected, levels increase with age, and interestingly, levels are higher in females. Despite trending higher in patients with renal impairment, no clear independent relationship has been demonstrated between BNP and renal function [3, 4].

BNP and Chronic Congestive Heart Failure

There are approximately 120,000 clinically suspected cases of chronic congestive heart failure in the UK annually; the reliability of such diagnoses is poor, especially in primary care [2]. Current guidelines state that formal assessment and diagnosis must include echocardiographic screening [5]. There are obvious practical difficulties in meeting this standard of care. Evidence is growing that BNP measurement will provide the ability to markedly reduce the number of patients referred for formal cardiological assessment [2]. BNP may also give useful information to detect the presence of diastolic dysfunction [6]. In particular, it does so even in the presence of echocardiographically-preserved systolic function (ejection fraction >50%) [7]. It has been suggested that BNP could 'become the same to heart failure as thyroid function tests are to hypothyroidism' [8]. It could conceivably be used for screening significant but asymptomatic left ventricular systolic dysfunction in the general population or for the monitoring of response to therapy [8]. BNP is less labile than its sister, atrial natriuretic peptide (ANP), and this confers practical advantages in clinical use. Plasma BNP and NT-proBNP levels can routinely be measured by radioimmunoassay or immunoradiometric assay from an EDTA blood sample. A bedside assay is also now available and approved by the FDA for near-patient testing [9].

BNP and the Critically Ill Cardiac Patient

Acute cardiac failure

BNP and NT-proBNP have demonstrated utility in differentiating the cause of breathlessness in the emergency room. In one seven center, prospective, multinational trial involving over 1,500 patients, BNP was compared against other biochemical values and historical and physical findings [10]. BNP was the single most accurate predictor of the presence or absence of congestive heart failure in the study group; diagnostic accuracy at a cut-off of 100 pg/ml was 83%. The negative predictive value of levels of less than 50 pg/ml was 96%. In regression analysis it additionally appeared an independently valuable adjunct to other clinical variables.

BNP has even been therapeutically applied and examined in the acute setting of heart failure. The largest double-blinded placebo controlled trial, the VMAC (Vasodilation in the Management of Acute Congestive Heart Failure) study (489 patients) was broadly inclusive and did not exclude patients with acute coronary syndrome, preserved systolic function, arrhythmia, or renal insufficiency. BNP infusion demonstrated clear efficacy in cardiovascular offloading as confirmed by invasive monitoring. It appeared at least as effective as glyceryl trinitrate infusion but with subjective benefits (chiefly less headache) and an absence of rebound effect or tachyphylaxis [11]. However, further data analysis suggests detrimental effects. There is a need for larger studies to verify safety and demonstrate benefit based on true clinical endpoints before it can be recommended [12].

Acute coronary syndromes

Troponin-I and creatine-kinase MB fraction (CK-MB) are the mainstay of objective diagnosis in acute coronary syndromes as direct markers of myocardial injury. This is an established role that has been well discussed previously [13].

Following acute myocardial infarction (MI), patients with clinical or invasive evidence of elevated left ventricular (LV) filling pressures have a poorer outcome as measured by mortality and the incidence of chronic congestive heart failure [14]. This may reflect a direct association between elevated filling pressures and infarct size [15] as well as related adverse effects on ventricular remodeling, neuro-hormonal activation, and myocardial excitability [16].

The routine measurement of pulmonary artery occlusion pressure (PAOP) has obvious drawbacks. Elevated LV filling pressure after acute MI may be identified non-invasively using clinical assessment and/or chest radiography. Echocardiographic measures additionally provide quasi-objective non-invasive estimates of LV filling. There is now an increasing literature supporting the use of BNP measurement as a clinical marker in the period after MI. BNP identifies those patients likely to have significant LV systolic dysfunction making it very useful in centers that cannot provide echocardiography for all their infarct patients [8]. It appears at least as good as (and may even be better than) echocardiography at identifying those patients who are at high risk of progressive ventricular dilatation, heart failure or death [8]. Overall results suggest value in an integrated approach to patient work-up combining clinical, radiological and biochemical disease markers.

One such series of 378 patients examined the relative utility of BNP in predicting all-cause mortality in the immediate period following acute MI (24–48 hrs) as compared to the ratio of early transmitral flow to early mitral annulus velocity (E/é) and conventional clinical, radiological, and echocardiological markers (Kruszewski et al., unpublished data). E/é is a novel combined measure of early LV filling and diastolic function which has been shown to be a superior non-invasive marker of LV filling pressures [17]. Both the E/é ratio (hazard ration [HR] 1.04 per unit increase, $p=0.03$) and BNP (HR 1.01 per 10 pg/ml increase, $p<0.001$) were found to be powerful independent predictors of mortality. By receiver operator plot the optimum predictive cut-off for BNP in this cohort was 515 pg/ml, which displayed a sensitivity of 62% and a specificity of 92%. BNP levels >515 pg/ml and E/é ratio >15 added incremental prognostic information to conventional variables. Likewise, both provided additional prognostic data, even when the other was available. Study limitations meant that the sickest patients could not be included. Despite this limitation, the study clearly demonstrated that powerful prognostic information could be obtained in the early period after infarction using methods representative of common clinical practice. In fact, given their more subtle clinical signs, the resulting study group might well represent the patients who would benefit most from prognostication.

While early prognostication obviously remains valuable by allowing for early intervention, the optimal sampling intervals for such parameters are still being defined. Additional evidence suggests that evaluation of serial BNP levels after infarction can refine risk stratification during follow-up. In one such study, patients with elevated baseline BNP levels who returned at four months with levels lower than 80 pg/ml displayed only a moderately increased risk compared to those with low levels throughout [18]. This further contrasted to patients with BNP levels lower than 80 pg/ml immediately post-event but with elevated levels at four months; such individuals showed a four-fold risk of death or new congestive heart failure (HR: 4.5; 95% CI 2.3–8.6), findings that might relate to the effects of adverse remodeling.

Cardiac surgery

In the UK over 30,000 patients per year undergo cardiac surgery, with a hospital mortality of approximately 2.5% for coronary artery bypass grafting (CABG) and 4.1% for cardiac valvular surgery increasing to 12.6% after one year. Cardiac surgery is also associated with major morbidities, such as stroke, myocardial infarction and malignant arrhythmia, with a quoted incidence of up to 22% [19]. The most common methods of risk stratification are clinical scoring systems such as the Parsonnet score and EuroSCORE [20, 21]. These and other existing methods of predicting outcome following cardiac surgery are imprecise. All scoring systems may overestimate mortality in 'high-risk' patients, but underestimate mortality in low to moderate risk groups. Although such scores may be helpful when comparing outcome between cardiac surgical units, none has sufficient predictive accuracy to identify individuals who will die or experience an adverse event with an acceptable level of sensitivity and specificity. They, therefore, function as clinical audit tools and are not widely used in clinical risk prediction and risk modification strategies [22].

Sensitive and specific biochemical tests are, thus, being sought to augment the accuracy of risk assessment and to ultimately improve outcome. At present, both cardiac troponin-I (cTnI) and BNP levels are actively being studied in this respect. There is good reason to explore these markers; existing evidence suggests that both cTnI and BNP levels reflect the state of the myocardium after surgery. Cardiac troponin levels directly reflect myocardial cell necrosis, whereas BNP levels primarily reflect ventricular filling and pressure and rise in response to myocardial ischemia [23]. They, therefore, correlate with aortic cross-clamp time and the duration of cardiopulmonary bypass (CPB). Prolonged ischemia will also result in myocyte necrosis and for this reason levels of BNP and cTnI closely correlate with each other [24].

Troponin-I in CABG

In one large cohort study in Aberdeen (1,356 patients), the significance of cTnI levels measured at 2 and 24 hours following surgery was examined in relation to subsequent short, medium and long-term mortality [22]. Troponin generally appeared a powerful predictive variable. Although significant in univariate analysis, two-hour measurements had no power of prediction once adjusted for operation complexity. However, risk remained highly significant for 24 hour troponin levels even when adjusted for all other variables, suggesting a relationship based on pathological events rather than inherent operative factors. cTnI levels measured at 24 hours were independently predictive of mortality at 30 days (odds ratio [OR] 1.02, 95% CI 1.01–1.02; $p<0.001$), 1 year (OR 1.02, 95% CI 1.01–1.03; $p<0.001$), and 3 years (OR 1.01, 95% CI 1.01–1.02; $p=0.002$). This association was strongly enhanced in the upper quartile, which might suggest a useful cut-off at around 8 ηg/ml (OR adjusted for operation type 3.24, 95% CI 1.55–6.77). These data are backed up by other similar studies which suggest troponin levels do not separate well in relation to mortality until beyond the 12–24 hour mark [25, 26]. One study did in fact show a significant relationship of operating room troponin levels to adverse events, although this was chiefly as a result of subsequent infarction rather than mortality [27].

Attempts to try and establish universally accepted at-risk thresholds for troponin remain challenging. Figure 1 highlights the influence of operation type on interpretation of troponin levels, with complexity of surgery being strongly related to increased troponin release. Thus, a troponin level in the lowest quartile for valve surgery may have very different interpretation if found in a patient receiving one vessel CABG. The complexity of surgery will undoubtedly be related to a host of

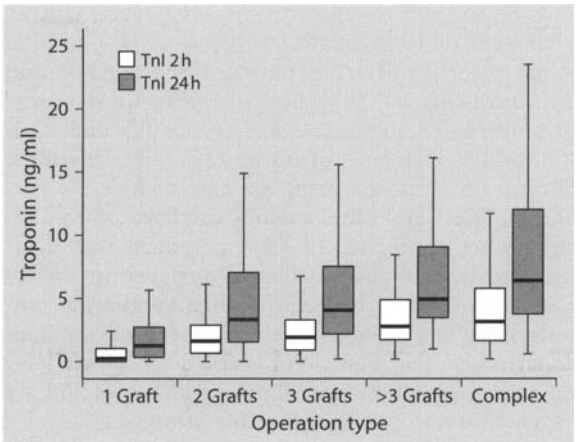

Fig. 1. The relationship between number of coronary grafts and cardiac troponin I (TnI) release at two and 24 hours after surgery [22]

influences on troponin rise, including pre-operative condition and time on bypass. Added to this are more local variables such as surgical technique, patient case-mix, and inter-assay variability; all must be taken into account when trying to interpret troponin levels. At present it seems most appropriate for individual centers to establish their own limits. There is also the need to better examine other adverse events within this general cohort to try and build a fuller picture of the post-operative period.

BNP in CABG

The pattern of release of BNP during CABG has been characterized in several small studies. Very early (one to two hours) after surgery, BNP levels tend to fall, rising thereafter to levels considerably higher than baseline. The two most detailed studies have both found that BNP levels peak around one day following CABG, falling thereafter but remaining significantly above baseline for several days [24, 28]. However, others have found the highest post-operative levels at one week [29]. This group additionally demonstrated significantly higher pre-operative BNP levels in the 14 patients who required inotropic support against the twelve patients who did not (mean 77 vs. 34 pg/ml, respectively, $p=0.03$).

A number of more recent studies continue to suggest value in the pre-operative measurement of BNP. Chello and colleagues studied 31 patients with moderate to severe LV dysfunction undergoing CABG [30]. Mean BNP levels were significantly lower by the time of follow-up (at a mean of ten months) and the extent of these changes also correlated with alterations in systolic function. In the sub-group whose ejection fraction did not improve following surgery (<5% increase) pre-operative levels of BNP were higher, suggesting utility in predicting functional recovery. In another study of 60 patients undergoing elective CABG, elevated pre-operative levels of BNP were associated with an increased mortality after two-year follow-up (mean levels 65 pg/ml in non-survivors ($n=10$) versus 29 pg/ml in survivors ($n=50$) [24]. Likewise, above a threshold of 80 pg/ml, two-year survival was only 66% (eight of 12 patients) versus 87% (42 of 48) with levels below this cut-off. This prognostic utility was independent of pre-operative ejection fraction and modified Cleveland scoring.

Our work on several surgical cohorts has examined both intensity of care and mortality as outcomes in relation to pre- and post-operative NT-proBNP and BNP levels. In the larger unpublished cohort, 255 patients were recruited and subse-

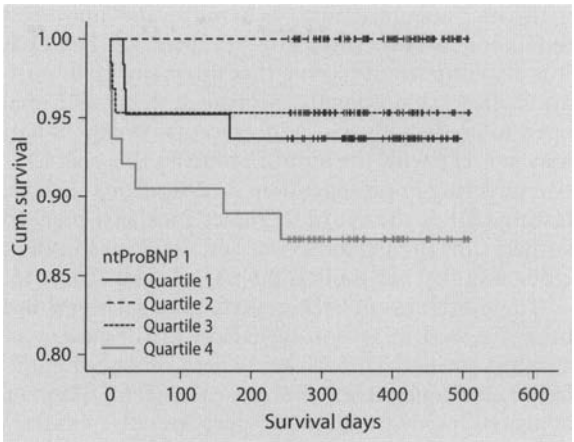

Fig. 2. The Kaplan-Meier curves for the relationship between N terminal pro-B-type natriuretic peptide quartiles and cumulative survival after coronary artery bypass surgery with a median follow-up of one year. The median BNP being 357 pg/ml with a 25th centile level of 127 pg/ml and a 75th centile level of 1359 pg/ml.

quently presented for elective cardiac surgery. At baseline, NT-proBNP levels correlated well to age ($p<0.001$), risk scoring (euroSCORE, Parsonnet [$p<0.001$]) and LV dysfunction ($p<0.003$). Figure 2 shows the Kaplan-Meier survival curves compared by NT-proBNP quartiles. Of note, individuals in the fourth quartile displayed an approximate four-fold relative risk (a threshold of 1359 pg/ml). Requirements for peri- and post-operative inotropic and peri-operative mechanical support all strongly correlated to NT-proBNP levels as did extended length of hospital stay (all $p<0.001$). Work on a smaller cohort (50 patients) that examined post-operative BNP levels at six and 24 hours also predicted most of these events [31]. The only similar previous study also found pre-operative BNP levels to be strongly predictive of mortality in keeping with this body of work. It did not however demonstrate a correlation between twelve-hour post-operative BNP levels and this specific outcome [23].

Overall, current data suggest that pre-operative measurement of BNP has real potential not only to assist in the counseling of patients and relatives but also to provide a strategic benefit. Scheduling of high risk patients for when other demands on intensive care services are likely to be lower (for example on days when other scheduled patients are at low risk) should reduce the need to cancel operations due to the non-availability of intensive care beds. Pre-operative levels might also be used to identify patients who require more intensive pre-operative medical therapy before presentation for surgery. As already discussed, there is evidence that BNP levels can be used to guide the therapy of patients with heart failure and that such an approach reduces adverse cardiac events [32]. The expectation of the need for increased peri-operative support should also allow the elective provision of mechanical cardiovascular support for weaning from CPB and assist in the early recognition of patients requiring intense medical management after surgery.

BNP in the Non-Cardiac Surgical Patient

Elective surgery

Examining major surgery, nearly 30,000 patients die around the time of operation each year in the UK alone [33, 34]. As with cardiac surgery, the majority of these deaths are clearly related to cardiac events together with significant long-term morbidities, such as stroke, non-fatal myocardial events, and malignant arrhythmia. In

high-risk patients, cardiac ischemic events may occur in over 30% of those undergoing major vascular surgical procedures, with an early cardiac death rate of 17% [35]. It is also important to note that the majority of post-operative cardiac complications are in fact asymptomatic. Scoring systems and imaging strategies have been developed to predict adverse cardiovascular events in patients undergoing major non-cardiac surgery with the aim of reducing this risk [36, 37]. This may involve the effective targeting of pre-operative investigations such as non-invasive or invasive cardiac testing [36] or the use of therapies such as β-blockade [38] but again, as with cardiac surgery, low predictive accuracies, variations in the constituent parameters and poor clinical utility has limited the use of such scores in practice.

The usefulness of BNP in predicting such peri-operative cardiac complications has been assessed in several scenarios. In Glasgow, a small high-risk cohort of vascular surgical patients (American Society of Anesthesiologists (ASA) Grade 3/4) was followed post-operatively for evidence of MI [39]. Pre-operative BNP concentrations appeared highly predictive of peri-operative cardiac events irrespective of other variables. Median plasma BNP levels in patients who experienced a fatal or non-fatal MI ($n=11$) were 240 ρg/ml (interquartile range 172–344) versus 39 ρg/ml (interquartile range 15–70) in those who did not ($n=30$). As compared to the Eagle clinical scoring system [36], BNP with a threshold of 100 ρg/ml showed greater predictive value (area under the ROC curve: BNP 0.957 versus Eagle 0.714, $p=0.001$). Sensitivity and negative predictive value were similar (100% for both) but with BNP showing a superior specificity (90% vs. 40%) and a greater positive predictive value (78% vs. 38%). All patients who experienced events had pre-operative BNP levels >120 ρg/ml. Patients with a formal history of ischemic heart disease trended towards an almost two-fold risk of cardiac events but only half of those who experienced events had a previous history. Patients receiving β-blockade showed a trend towards a lower event rate in keeping with the suggested cardioprotective role of these drugs [40].

Two-hundred and four patients undergoing major elective non-cardiac surgery in Aberdeen were studied (unpublished data). Peri-operative death or new MI (cTnI >0.32 ηg/ml) was defined as a combined primary endpoint. Troponin threshold was set as per local assay to provide a coefficient of variation for infarction of less than 10% without pre-operative cTnI elevation or a non-cardiac etiology (such as pulmonary embolism) [37]. Pre-operative BNP levels were raised in patients who died or suffered a peri-operative MI (median 52.2 ρg/ml vs. 22.2 ρg/ml, $p=0.01$). BNP predicted this outcome with an area under the ROC curve of 0.72 (95% CI 0.59–0.86). An optimal cut-off point of 40 ρg/ml for pre-operative BNP differentiated patients with an almost seven-fold increased risk of cardiac events in the early post-operative period (OR 6.8, 95% CI 1.8–25.9, $p=0.003$). A pre-operative BNP above this cut-off point was also associated with an increased postoperative hospital stay.

In a similar cohort in Japan, 190 patients undergoing major or minor surgery under general anesthesia were studied with pre-operative sampling for NT-proBNP in addition to routine pre-operative work-up [41]. Cardiac death together with acute coronary syndrome, heart failure, and sustained cardiac arrhythmias (>30 secs) were defined as 'cardiac complications'. Fifteen of the 190 patients experienced complications; four had acute coronary syndrome and 13, congestive heart failure. NT-proBNP concentration was significantly higher in patients with a cardiac complication; a level greater than 450 ρg/ml was predictive of cardiac complications with a sensitivity of 100% and a specificity of 82.9%. Other factors associated with cardiac complications were a higher ASA grade, age, and clinical cardiac impairment, but in a multivariate analysis NT-proBNP level was the only independent factor. Of note,

the rate of cardiac complications was not affected by whether the operation was major or minor (twelve of 158 [7·6%] versus three of 32 [9%], p=0·733).

As with cardiac surgery, these data already show the usefulness of pre-operative BNP levels in identifying patients who are at risk of peri-operative cardiac complications in this setting.

Emergency surgery

Major emergency surgery has a mortality rate considerably greater than that of elective surgery [33]. In the National Confidential Enquiry into Peri-operative Deaths (NCEPOD) in 2002 [33], 57% of reported deaths were after urgent or emergency operations despite non-elective operations constituting only 16% of total surgical workload. This figure disguises considerable inter-specialty differences. Orthopedics and general surgery were the specialties with the largest number of non-elective cases (25.9% and 21.2% cases, respectively). This is perhaps unsurprising given the greatly increased risk of fractured neck of femur and acute abdominal pathology in the older age groups. The initial disease that requires surgery may be complicated by a number of factors. Tissue hypoperfusion and acidosis can result from vomiting and loss of fluid into the gastrointestinal tract or malignant disease [42]. Chronic co-morbid disease, documented or otherwise, is more common in this group with a decreased physiological reserve. It thus also unsurprising that the Scottish Audit of Surgical Mortality (SASM) indicates that 72% of operative deaths in patients undergoing emergency surgery are in patients aged over 70 years [34]. Despite these varied elements, the single largest cause of morbidity and mortality in patients undergoing major emergency surgery is the development of post-operative cardiac events [35]. Mangano et al. determined that cardiac complications are two to five times more likely to occur with emergency surgical procedures than with elective operations [35]. This finding is not surprising because the necessity for immediate surgical intervention may make it impossible to evaluate and treat such patients optimally.

Consequently, it was suspected that BNP would be an even more powerful predictor of outcome in this patient group. A cohort of 40 patients undergoing major non-cardiac emergency surgery in Aberdeen was studied (unpublished data). Blood samples were taken pre-operatively and on days one and three after surgery for BNP and cTnI analysis. Twelve-lead electrocardiograms (EKGs) were performed at the same time as blood sampling. The patients were followed-up until hospital discharge for the development of cardiac complications. The primary outcome was the predictive power of BNP for the combined end-point of cardiac death or early post-operative cardiac event (defined as *de novo* cTnI $\geq$ 0.10 ηg/ml and/or the development of EKG changes suggestive of significant acute myocardial ischemia/infarction within 72 hours of surgery). Pre-operative BNP levels were significantly higher in patients who experienced a post-operative cardiac event (median 400.1 ρg/ml vs. 89.6 ρg/ml, p=0.011). Pre-operative BNP levels were also shown to be a good predictor of post-operative morbidity, as determined by the Day 3 Post-Operative Morbidity Survey (POMS) [43]. Pre-operative BNP levels were higher in patients classified at greater risk by pre-existing risk assessment indices. The study displayed a number of inherent limitations chiefly related to sample size. A low cTnI threshold (<0.1 ηg/ml) is a poor discriminator between true thrombo-occlusive events and other more benign causes of cTnI elevation. The use of such a broad combined endpoint was necessary to generate significant outcomes in this small pilot study. Study size also precluded a subgroup analysis to compare the effectiveness of measurements across specialties. Patients too unwell to give consent were excluded, thus, creating an inclusion bias

with a likely underestimation of post-operative cardiac events. Limitations notwithstanding, this preliminary study was suggestive of a useful relationship that merits more definitive investigation. Interestingly, a significant number of subjects had elevated pre-operative troponin levels, therefore, indicating pre-existing myocardial injury. cTnI levels are not currently routinely measured in the pre-operative assessment of a patient but in light of these observations further work may wish to examine their utility.

BNP in the General Intensive Care Unit

Sepsis represents one of the major challenges in medicine. Despite the widespread use of intensive care units (ICUs), broad-spectrum antibiotics, surgical interventions, nutritional support, and more modern therapies, its incidence continues to rise, with an unacceptably high mortality (30–70%) [44]. This is, in part, due to a high degree of heterogeneity due to variables such as age, weight, gender, underlying disease, immunological factors, and the severity of infection.

Myocardial dysfunction is a common complication of all critical illness, particularly severe sepsis, and is associated with poor outcome. This may not always be initially apparent due to adaptive responses, but is often followed by overt myocardial dysfunction and failure [45]. Measures of myocardial dysfunction are varied, but lack accuracy and validation in clinical practice. The utility of the pulmonary artery catheter has been questioned lately by research suggesting negative effects on outcome [46]. Two-dimensional echocardiography is a valuable investigation, but the clinical usefulness depends on the experience of the operator and the quality of the images obtained. Measurements of cardiac output or cardiac index may be useful but are highly dependent on load conditions and heart rate [47]. Once again the appeal of a biomarker that would aid management or even potentially predict outcome is obvious. Previous work has already established that levels of BNP (and also ANP) are elevated in septic shock [48]. However, the relationship between BNP and LV filling pressure is an imperfect one, particularly in heterogeneous populations with the potential for rapid fluctuations in hemodynamic status [49]. One reason for this may be the tendency for BNP levels to remain elevated despite reductions in PAOP. This may in turn reflect the half-life of BNP within the circulation and/or the potential for factors other than acute filling pressure to influence its production. Indeed, this may underpin the powerful prognostic utility of BNP which may integrate data both on acute and more chronic filling pressures. These and other data have led to the suggestion that BNP is principally a measure of raised intra-cardiac pressures and, as such, may be an excellent indicator of 'global' myocardial function [50].

McLean and colleagues have examined the ability of BNP to detect myocardial dysfunction in the general ICU [3]. All patients admitted to a combined medical and surgical ICU over a four-week period were included in the study with BNP measured on the point of admission. Cardiac dysfunction was defined as LV systolic or diastolic dysfunction, right ventricular (RV) systolic dysfunction or as a hemodynamically overloaded RV. Diagnosis was based on past history, symptoms, EKG, chest X-ray, echocardiography, blood tests, and physical examination. BNP was a powerful independent predictor of cardiac dysfunction. Such patients displayed significantly higher mean BNP levels as compared to the non-cardiac dysfunction group: 516+/−385 pg/ml ($n=26$) vs. 67+/−89 pg/ml ($n=58$) ($p<0.0001$). At a threshold of 144 pg/ml, BNP exhibited 92% sensitivity, 86% specificity, and 96% negative predictive value with a total area under the ROC curve of 0.96. The sensitivity further improved to 96% when the analysis was confined to patients over 55 years of age.

The actual prognostic utility of BNP in a heterogeneous population of critically ill patients admitted to intensive care, or in those admitted with severe sepsis, had not previously been investigated. The hypothesis that, as a measure of global myocardial health, admission levels of BNP would be predictive of 30-day mortality, was suggested and tested. Our cohort comprised 78 consecutive patients admitted to the general ICU [4]. BNP levels correlated with age, sex, and non-significantly with creatinine clearance as seen in previous work [3]. However, BNP levels measured on ICU admission and at 24 hours were not found to be significantly higher in patients who died within 30 days as compared to survivors (all $p > 0.05$). Despite admission BNP levels being higher in patients with severe sepsis and septic shock, again they were not higher in those who died. Indeed, using a cut-off point of 100 pg/ml for BNP (a value described to be useful in identifying patients with heart failure [10]), there was a trend towards *lower* BNP levels in all non-survivors. Although this trend was not significant in patients who died of severe sepsis and septic shock, their admission BNP values also tended to be lower than in survivors. These results are difficult to explain and require confirmation. It is known that patients with sepsis who fail to exhibit LV dilatation have a reduced ejection fraction and stroke work indices and ultimately a worse prognosis [51]. It could be that a rise in BNP levels is associated with appropriate ventricular dilatation and thus it is a failure of this response that is associated with reduced BNP levels and a greater mortality. Further work will be required to test this interesting hypothesis.

Conclusion

With an aging population and an increasing ability to perform complex interventions, cardiac complications have become the primary cause of death after major surgery and a major contributor to death in the critically ill. Myocardial injury in both these settings, as measured by troponin, now appears to have clear long-term sequelae as well as implications for immediate levels of care. BNP appears to be a powerful integrator of myocardial dysfunction with the ability to assess the likelihood of such injury at the same time as providing the means to monitor disease progression and response to treatment. All of the above is with the aim of more appropriate monitoring and investigation with intervention at the earliest possible stage. This should have positive implications for healthcare efficiency, clinical resource utilization, and ultimately patient outcome. However, these markers still await true verification in interventional studies. In the interim, we suggest they should be considered as valuable adjuncts to current clinical practice.

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Molecular Approaches to Detection of Bacteria in Critical Care Patients

J.L. Flanagan, S.V. Lynch, and J. Wiener-Kronish

Introduction

For over 100 years clinical medicine has relied on culture-based techniques to define infections in patients. However, over the past 30 years, it has become clear that culture-independent methods more completely describe both microbial diversity and community dynamics and the importance of such interactions in states of health and disease has been revealed [1, 2]. Furthermore, the use of culture-based techniques has clouded our understanding of the pathogenesis of human infections. The concept that one species causes infection by entering the host, defeating the host's defense system and multiplying to a threshold that allows it to cause injury is probably only applicable for a small subset of microbes, e.g., bioterror agents. The new emerging paradigm in microbial pathogenesis is that many organisms, such as *Streptococcus pneumoniae*, already exist in bacterial communities of the oro- and nasopharynx of most healthy individuals and that a change in their virulence gene expression and/or an increase in numbers permitting dissemination cause symptoms of infection [3]. The molecular signals that bring about these shifts in pathogen physiology are not fully understood; however, given the importance of bacterial cell-to-cell signaling (quorum sensing) it is possible that shifts in bacterial community composition may lead to emergence and dominance of pre-existing pathogenic species within the community. This hypothesis is supported by the finding that within hours of their admission to the intensive care unit (ICU), critically ill patients exhibit dramatic changes in the bacterial communities colonizing their oro- and nasal pharynx [4, 5]. These shifts in community composition are multifactorial and are significant for the pathogenesis of nosocomial infections, particularly those of the lungs. However, to date, changes in bacterial species composition have largely been described by culture-dependent techniques that both inadequately document the bacterial population composition and insufficiently describe community dynamics.

Bacterial Communities in Humans

Gastrointestinal Microbial Communities

As adults, approximately 90% of the cells present in our bodies are of microbial origin [6] and only a small fraction of these can be detected by traditional culture techniques [7]. It is now apparent that human health depends on the microbial population present in the body; this is most clearly demonstrated by data relating health to intestinal microbial flora. The intestines are colonized rapidly following birth and this microbial community varies in an age-, diet- and health-dependent manner [8].

The gastrointestinal microbiome plays a substantial role in host metabolism by enhancing and maximizing energy production from food, contributing to beneficial biosynthetic pathways (e.g., essential amino acids and vitamins) and, through decontamination, reducing exposure to toxic substances [9]. Interestingly, recent studies suggest that the gut microbiota is altered in obese individuals [10] and in patients with cardiovascular disease [11]. Further, the bacterial communities of infants with allergic diseases appears to be different to that of non-allergic infants and it has been demonstrated that changes in the intestinal microbiota of these infants decrease allergies [12, 13]. The results of these studies are striking and suggest that a similar paradigm may hold true for many other disease states. However, to date there have been no culture-independent investigations into the microbial communities associated with critically ill patients.

Microbial Communities of the Oro- and Naso-Pharynx

In addition to colonization of the gastrointestinal tract, the oral pharynx of neonates becomes colonized (most likely inoculated during birth), and this microbial community also has a significant effect on health. In adults, it has been suggested that ventilator-associated pneumonia (VAP), a subset of hospital-acquired pneumonia, occurs following nosocomial colonization of the oropharnyx, which occurs rapidly in patients hospitalized in ICUs [14–16]. Multiple factors cause such oropharyngeal colonization, including desiccation of the mucosa, decreased salivary secretion, mechanical injury induced by nasogastric and endotracheal tubes, and decreased immunoglobulin A content [17–19]. Dental plaque, which exists on the subgingival and supragingival surfaces of the teeth [20–24], also appears to be a source for microbes that colonize and infect ICU patients [17–19]. Sequential sampling of dental plaque from ICU patients has demonstrated that more than 50% of patients acquiring a respiratory infection exhibit prior colonization by the pathogenic organism at a dentogingival site [25]. In fact, El-Sohl et al. documented that respiratory pathogens isolated from dental plaque were genetically identical to those recovered from bronchoalveolar lavage (BAL) fluid in patients from nursing homes [18]. It appears, therefore, that many patients are not newly colonized in the hospital, but rather bacteria identified in their respiratory samples originate from the oral microbial population particularly from their dental plaque (Fig. 1). Conditions in the ICU, especially oral intubation, may permit specific pathogens to proliferate and dominate the microbial community in addition to providing them with a protected conduit to the lungs.

Several clinical studies have attempted to prevent perioperative pneumonia by perturbing oral flora. Most of the investigations used prophylactic chlorhexidine oral rinse pre- and peri-operatively and demonstrated a significant decrease in nosocomial pneumonia [25–28]. DeRiso et al. [26] documented a significantly decreased incidence of nosocomial lung infections in patients undergoing open-heart surgery who received twice-daily 0.12% chlorhexidine oral rinse as part of a double-blind, placebo-controlled trial. Patients who received the rinse had a 5% rate of nosocomial respiratory infection compared to 14% in the non-treated group. In a separate study, systemic antibiotic use and mortality were also significantly decreased in those patients who received oral chlorhexidine treatment; 1.2% mortality in the treatment group compared to 5.6% in the untreated group [29]. In another trial that was not double blinded, a 52% reduction in the prevalence of nosocomial pneumonia occurred with chlorhexidine rinses in patients undergoing heart surgery [30]. It, therefore, appears that antiseptic rinsing (due to its effect on the oral micro-

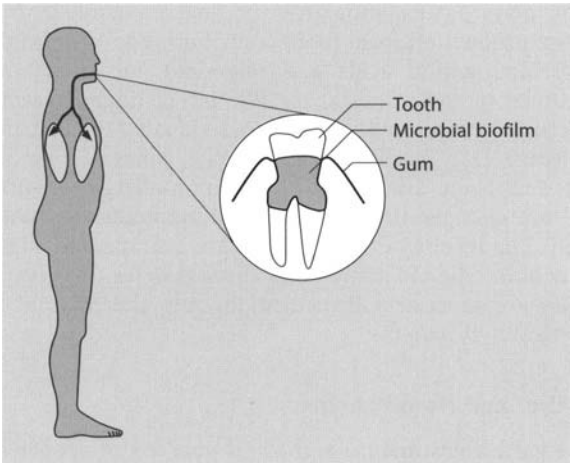

Fig. 1. Species populating sub- and supra-gingival microbial biofilms can act as a source of microbial infection of the respiratory system. Increased incidence of infection is observed in orally intubated, mechanically ventilated patients.

bial community) has been successful in decreasing the incidence of nosocomial infection in cardiac surgical patients.

Most recently, patients requiring mechanical ventilation for 48 hours or more were enrolled in a randomized, double-blind, placebo-controlled trial with three arms: chlorhexidine, chlorhexidine and colistin, or placebo [29]. Trial medication was applied every 6 hours to the buccal cavity. Oropharyngeal swabs were obtained daily and quantitative cultures performed. Endotracheal colonization was monitored twice weekly. The daily risk of VAP was reduced in both treatment groups compared to the placebo treated group. Both treatments led to a significant reduction in Gram-positive organisms. However, only the chlorhexidine and colistin combination treatment led to a significant reduction in both Gram-positive and -negative organisms. In the group that received this treatment, endotracheal colonization was reduced more compared to the group treated with chlorhexidine alone; however, both treatments were equally effective in VAP prevention [29]. This investigation again documented the importance of the oral microbial population in the pathogenesis of VAP. While this investigation reported a decrease in the percent of positive culture results in the treatment groups, it failed to document specifically which bacterial species were affected by each treatment and the oral microbial community dynamics associated with a reduced incidence of VAP. The positive results reported warrant a more comprehensive (culture-independent) evaluation of alterations in microbial diversity affected by such treatments, including assessment of the total microbial community present and how specific treatments may cause shifts in microbial community dynamics that affect patient health.

It is generally accepted that most of the microbes resident in the oral cavity exist as biofilms. Bacterial biofilms are composed of microcolonies of cells non-randomly distributed in a matrix composed of expopolysaccharide [20, 31, 32]. This protective layer provides a permeability barrier against antimicrobials, thus increasing resistance of bacteria within the biofilm. Physiological heterogeneity is known to exist within biofilms; antibiotics may kill actively growing bacteria in the very outer region of a biofilm but slow-growing cells embedded deep within the matrix remain impervious to such treatments [33]. It has also been shown that bacteria in biofilms exhibit differential gene expression compared to those grown as free-swimming or planktonic cultures [34]. An excellent example of this phenomenon is increased

expression of efflux pumps by bacterial biofilms [35]. These pumps normally export cell-cell signaling molecules involved in coordinating the activities of the bacteria in the biofilm; however, they can also export antimicrobials that enter the bacterial cell [36]. This serves to reduce the effective intracellular concentration of antimicrobial and provides an additional resistance mechanism to cells in the biofilm.

Dental plaque located both above and below the gingival margin, represents a mixed-species biofilm in which non-random coaggregation of specific bacterial species is known to occur. It has been established that at least six specific microbial groups or complexes exist within subgingival plaque [37]. Interestingly, several studies have revealed that the strains of bacteria observed in both healthy subjects and those with periodontitis appeared similar, but the absolute numbers and proportions of the periodontal pathogens were significantly higher in diseased individuals. While these studies expand our understanding of the microbial community dynamics that underlie periodontal disease, it remains unclear how these biofilms of mixed microbial populations contribute to VAP or nosocomial pneumonia. To date, investigations into the effects of antiseptic rinses on the oral microbial community have not evaluated biofilms or the majority of the oral floral, but have reported only the small number of bacterial species that can be cultured.

Culture-Independent Assessment of Microbial Communities

Due to fastidious growth requirements of the majority of bacteria, standard culture methods do not adequately document bacterial number or diversity [2, 38]. Even attempts to replicate specific bacterial environments by supplying specific essential nutrients do not significantly increase the number of culturable bacteria; 80% of microbes identified using molecular techniques cannot be cultured [2, 7, 38]. More recently, culture-independent techniques have been used to define the presence of microbial species in a variety of environments. The most commonly used method for members of the bacterial and archael domains makes use of the ubiquitous 16S rRNA gene [39]. Members of both domains possess conserved sequences within their 16S rRNA genes, which flank regions of sequence variability. One approach using this gene is to construct fluorescently labeled probes homologous to the 16S rRNA gene of the species of interest. This technique, termed FISH (Fluorescent *in situ* Hybridization), has been used widely for culture-independent detection of specific bacterial species [40, 41]. However, this approach is limited in the number of species that can be interrogated due to constraints on the number of fluorescent labels that can be employed in any one experiment and novel species cannot be identified using this technique since probe design necessarily anticipates the species present. However, the technique remains a useful method for culture-independent direct interrogation of samples.

A more wide-ranging approach, also based on the 16S rRNA gene, is to construct clone libraries. Initially, a pair of 'universal' primers is designed, based on the sequence of the conserved regions of the 16S gene. This permits amplification of the full length 16S rRNA gene from all microbes in a given sample. This amplicon pool is then cloned into vectors to generate a clone library. Individual clones are subsequently sequenced and analysis of the entire ~1500 bp 16SrRNA gene in each clone permits 16S rRNA sequences to be clustered into groups (where a threshold of sequence similarity is established ~98% identity) and the identification and relative abundance of species present in a microbial community established [2]. One advan-

tage of clone libraries is the ability to identify novel species. Cloning and sequencing of the 16s rRNA gene requires homology in the 'universal' regions of the gene to be used as a priming site for DNA polymerase but unique species can be revealed by sequencing the entire gene. Thus, the presence of novel bacterial and archaeal species can be determined in a culture-independent manner. This technique has been used for a number of years in the field of environmental microbial ecology and has provided insights into the microbial populations and dynamics in a number of environmental samples [42–44]. More recently the approach has also been used to document the microbial communities associated with the periodontal cleft and intestine of human subjects [45, 46]. Additionally, this approach has demonstrated that the number of microbes identified by culture represents a very small fraction of those actually present as determined by culture-independent techniques [2]

However, generation of 16S clone libraries is time-consuming and expensive due to the need for extensive sequencing to detect lower abundance species in samples dominated by a small number of specifies. Recently, a novel microarray-based approach has been developed that permits parallel sampling of all known bacterial species (as of March 2004) in a single assay. This microarray, termed the PhyloChip, uses taxonomic-specific clusters of oligonucleotide probes to detect specific organisms [47]. As with clone library generation, DNA is extracted from a sample and the 16S rRNA gene amplified by polymerase chain reaction (PCR). However, this amplicon pool is fragmented, labeled with biotin and hybridized to the microarray. Arrays are washed and the presence of bacterial species detected by scanning the array for fluorescence (Fig. 2).

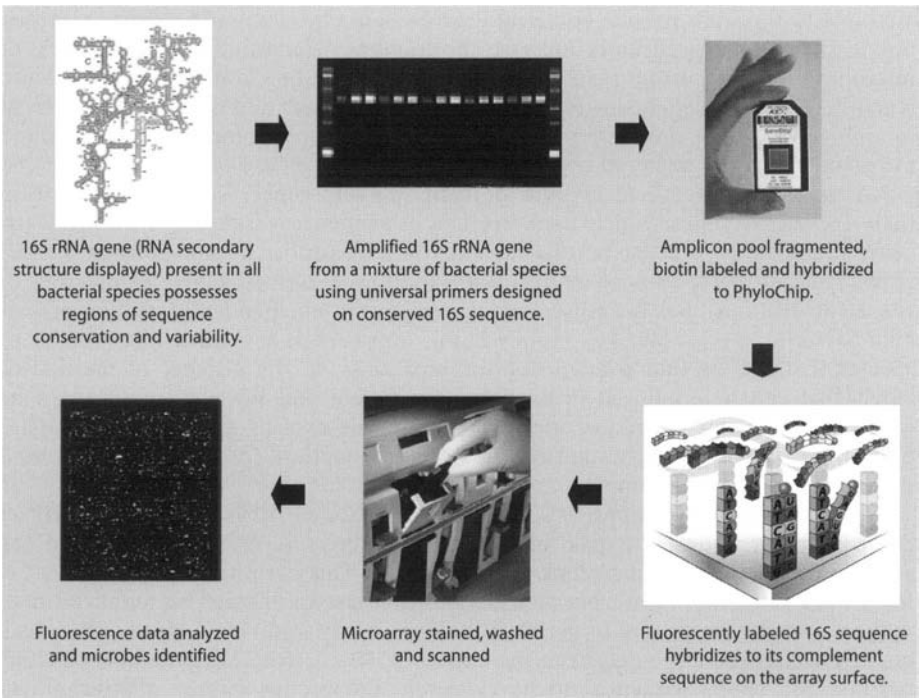

Fig. 2. Schematic of 16S rRNA gene amplification and subsequent microbe identification using the novel PhyloChip.

The PhyloChip is advantageous in that it is rapid, permits massive parallelism, and detects low abundance species even in the presence of dominant organisms in a microbial community.

Culture-independent techniques such as FISH, 16S rRNA clone libraries, and the PhyloChip, represent alternative approaches for microbial detection and diversity determination in a clinical setting [1, 46, 47]. Compared to current clinical culture methods, clone library and PhyloChip techniques provide a more comprehensive picture of microbial diversity and provide tools for increased understanding of how perturbations of microbial communities contribute to states of health and disease.

The Use of 16S rRNA to Detect Novel Organisms

As mentioned above in addition to using 16S rRNA for microbial community determination, this gene has also been useful in detecting the presence of novel organisms. In the 1990s patients with acquired immunodeficiency syndrome (AIDS) were found to have abnormal collections of small blood vessels, bacillary angiomatosis, in their skin and visceral organs. *Rochalimaea henselae*, the organism responsible for bacillary angiomatosis, was found by amplifying part of the 16S rRNA gene from tissue samples obtained from these patients [48]. Similarly, *Ehrlichia chaffeensis*, a new species associated with tick bites, was found using 16S rRNA amplification and sequencing of DNA extracted from leukocytes obtained from infected patients [49]. In 1991, using this technique on a small bowel specimen taken from a patient with Whipple's disease and *Tropheryma whipplei*, the etiologic agent of this disease was discovered [50]. Given that so many idiopathic diseases currently exist, application of culture-independent methods to such disorders appears fundamental to increasing our understanding of these disease processes.

Conclusion

The utility of molecular approaches to bacterial detection and description of bacterial community dynamics includes rapid generation of results, more comprehensive analysis of microbial communities and community dynamics in clinically relevant sites, and the ability to monitor microbial community alterations during antibiotic therapy. Monitoring bacterial communities during therapeutic administration would enable the efficacy of such treatments to be assessed rapidly in patient samples. Indeed, these molecular tools may redefine what truly constitutes 'infection' and provide a much better understanding of the contribution of microbial community structure to pathogenesis. Given the copious use of antibiotics by critical care practitioners, improved understanding of these concepts is central to improved patient care. The continued use of standard culture techniques prevents a more complete understanding of microbial dynamics associated with disease and during therapy that contribute to poor patient outcome. Over the coming years, as culture-independent techniques become more widely used clinically, our comprehension of disease pathogenesis and effective measures for treatment will improve dramatically.

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Sepsis and Infection: Management

Understanding and Optimizing Outcome in Neonates with Sepsis and Septic Shock

K.N. Haque

Introduction

It is estimated that four million newborn infants die every year, of these 35% (1.6 million) die from sepsis [1]. Preterm infants are at greater risk of developing infection (often multiple) between birth and the first month of life compared to term infants. It is estimated that intra-uterine infection, a risk factor for developing severe infection, is present in up to 35% of preterm deliveries [2].

While most of the mortality from sepsis is mainly in the developing world, in UK, USA, and Australia it is around 20% among low birth weight infants. This figure has not declined over the last three decades [3], despite modern perinatal and neonatal intensive care.

The prevalence of sepsis, meningitis, and other confirmed bacterial infections has been estimated to range between 1 to 5/1000 live births. However, for preterm infants this prevalence is much higher at 1/230 preterm births. Thus, it is not surprising that the number of very low birth weight infants evaluated and treated for infections is around 50% of all admissions to neonatal nurseries [4]. In the USA, as many as 600,000 infants are screened to 'rule out' sepsis and an estimated 130,000 to 400,000 are treated with antibiotics every year [5] with less than 20,000 actually having proven infection!

Worryingly, term, and preterm infants in particular, who develop infection have between 30–80% increased risk of neuro-developmental impairment and have a 30–100% increase in odds for poor head growth, a good predictor of long term morbidity [6, 7].

Preterm infants are 20 times more likely to get infection than term infants. This risk is greatest after the first week of life except for Group B streptococcus (GBS) infection, which is more frequent during the first week of life. With near universal use of intra-partum prophylaxis for maternal GBS carriage, the incidence of neonatal infection with GBS is falling. However, there are reports [8] suggesting a gradual increase in the incidence of early onset Gram-negative infection.

Whil the incidence of sepsis (1–5/1000 live births) is nearly universal in the developed world, the incidence of septic shock in neonates has not been well documented though it is not uncommon with Gram-negative, GBS, and herpes simplex infections. With current modalities of management, the progress of sepsis to septic shock can be limited, but if organ systems become dysfunctional then sepsis can rapidly progress to 'severe sepsis' and 'septic shock', which is viewed as a continuum of the same condition [9]. Though the exact figures for septic shock in the neonatal period are difficult to obtain, it is thought to be around 1–5% of all infants with proven severe sepsis [10].

Defining Neonatal Sepsis

The beginning of wisdom is calling things by their right names
Chinese Proverb

Sepsis means 'putrefaction', i.e., the decomposition of organic matter (by bacteria or fungi) resulting from an interaction between germs and host [11]. Until the landmark consensus conference of experts organized by Roger Bone in 1991 [12], clinicians defined infection and/or sepsis as they pleased. The definitions of infection and sepsis suggested by Bone and his colleagues in 1991 were for adults. They were adapted according to age-adjusted values of clinical variables for children by Hayden in 1994 [13], but for a number of reasons clinicians did not find these definitions immediately useful in clinical practice; thus, most pediatricians did not use them. In 2002, an international pediatric consensus conference was specifically organized with the aim of defining sepsis and organ dysfunction in children. Their recommendations were published in 2005 [14]. These definitions related mainly to children and not neonates, hence, a follow-up conference under the sponsorship of the International Sepsis Forum was held in 2004 to resolve this and other issues from the 2002 conference. The definitions that follow have been adapted from the recommendations of the last (2004) conference [9].

Sepsis is a common and complex entity, with marked heterogeneity in the patients affected and with wide variations in outcome [15]. While the ability of a senior clinician to diagnose sepsis in a neonate is high [16] there is still a lack of diagnostic certainty at the cot side. This lack of certainty may be due to a lack of specific and sensitive clinical and laboratory parameters, and differing patho-physiology, exposure, and susceptibility of the newborn to infection according to gestational maturity.

The preterm infant, due to poor host defense mechanisms, responds differently from an adult or even older child to infection with the same pathogen. To add to this complexity, we now know that there is significant genetic variation in how one responds to infection, e.g., some babies have predominantly interleukin (IL-6, IL-8) based response to infection whilst others enlist a predominantly granulocyte colony-stimulating factor (G-CSF) response [17]. Another difficulty in reaching a consensus on the definition of sepsis is that the international consensus definitions that have been adapted for pediatric and neonatal use [13, 14, 18] differ from those being used by large neonatal research networks [19]. This lack of consensus highlights the fact that infection, far from being a homogeneous condition, reflects a continuum from fetal inflammatory response syndrome to sepsis, severe sepsis, septic shock, multi-organ failure, and death (Fig. 1). The difficulty for the clinician is to determine and define precisely the phase in which his/her patient is at any given moment as the patient may move from one phase to another imperceptibly.

Controversy is also rife on how to define septic shock in children and no definition of septic shock exists for neonates. In general terms, shock is defined as a state of inadequate tissue perfusion with insufficient delivery of oxygen and other metabolic substrates necessary to meet metabolic needs. Traditionally, shock has been classified according to its pathophysiology and etiology, i.e., hypovolemic, cardiogenic, or distributive. Hypovolemic and distributive shock are most common in neonates, representing a decrease in circulating intravascular volume that significantly affects tissue perfusion.

Thus, to simplify the matter of definitions, for this chapter we will be using the definitions suggested by ourselves [9].

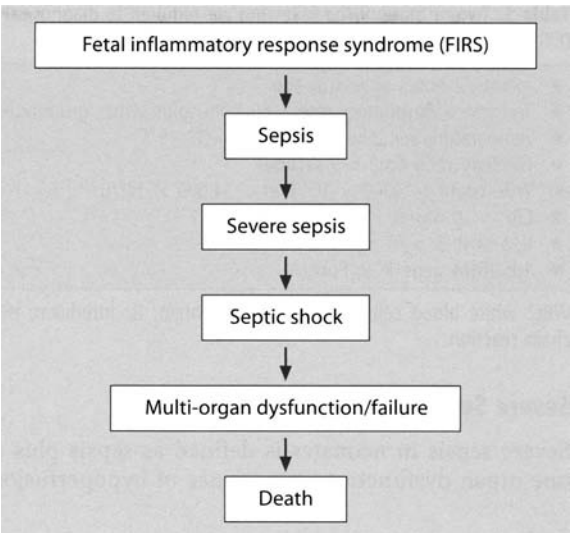

Fig. 1. Neonatal sepsis is a continuum

Specific Definitions

Systemic Inflammatory Response Syndrome

The term, systemic inflammatory response syndrome (SIRS) was proposed by the consensus conference of experts in 1991 [12] to describe a non-specific inflammatory process following trauma, burns, infection, pancreatitis, and other diseases in adults. The definition of SIRS was modified at the pediatric consensus conference in 2002 [14] such that the presence of either temperature or total leukocyte count had to be met. The age-specific values for abnormal vital signs and laboratory parameters used in these definitions were not evidence-based but were based upon expert opinion.

SIRS represents physiological derangements that are non-specific but are frequently present in patients with sepsis. Addition of predisposing factors and or biological markers (such as C-reactive protein [CRP], procalcitonin [PCT], and cytokines) may help to identify sepsis as the cause of SIRS. It is recognized that progression from SIRS to sepsis to severe sepsis and to septic shock is associated with an incrementally greater mortality risk.

Fetal Inflammatory Response Syndrome

A diagnosis of fetal inflammatory response syndrome (FIRS) can be made in an infant of less than 72 hours of age, who manifests two or more of the parameters shown in Table 1, usually secondary to either an ascending infection from the birth canal, or rarely, hematogenous spread from the mother.

Sepsis

Sepsis results from a complex interaction between pathogens and the human host. It is diagnosed when the signs and symptoms of SIRS or FIRS are present and the cause is confirmed as an infectious process.

Table 1. Two or more of the following are required to diagnose the fetal inflammatory response syndrome (FIRS) [9].

- Infant 72 hours or less in age.
- Tachypnea (respiratory rate >60 bpm) plus either grunting/retraction or desaturations.
- Temperature instability (<36 °C or >37–9 °C)
- Capillary refill time >3 seconds
- WBC count (<4000 × 10^9/L or >34,000 × 10^9/l)
- CRP >10 mg/dl
- IL-6 or IL-8 >70 pg/ml
- 16S rRNA gene PCR: Positive.

WBC: white blood cell; CRP: C-reactive protein; IL: interlukin; rRNA: recombinant RNA; PCR: polymerase chain reaction.

Severe Sepsis

Severe sepsis in neonates is defined as sepsis plus respiratory distress plus at least one organ dysfunction or evidence of hypoperfusion or hypotension.

Septic Shock

As alluded to earlier, definition of septic shock is problematic. In the newborn, septic shock is frequently due to significant redistribution and loss (third spacing) of intravascular fluid usually without a fall in blood pressure until late. Shock in neonates is better represented and recognized clinically by tachycardia (heart rate >180 bpm), signs of decreased perfusion (measured differently, e.g., increased capillary refill time >3 seconds, or hypotension ≥2SD below normal range for age), decreased peripheral pulses compared to central pulses, mottled or cool extremities or decreased urine output.

Pathophysiology

It is believed that sepsis represents an uncontrolled inflammatory response initiated by a pathogen. Conventional wisdom has been that the pathogen is responsible for disease and outcome. However, it is more likely that it is the host's own response to the presence of the pathogen that makes the disease and determines the outcome, because our arsenal for fighting off pathogens is so powerful that we are more in danger from them than from the invaders.

Previously, sepsis was viewed primarily as an inflammatory disorder. More recent studies indicate that the mechanism of sepsis include activation of hemopoietic cells, release of mediators, derangement of coagulation and cytokine homeostasis, and endothelial alterations, the latter being responsible for the leak of intravascular fluid, hypoperfusion, and hypotension.

The Sepsis Cascade

Sepsis results from a complex but sequential array of interactions between pathogens and the host; for example, despite similar clinical presentation, the molecular and cellular processes depend on whether the organism is Gram-negative, Gram-positive, fungal, or viral in origin. Gram-negative organisms release lipopolysaccha-

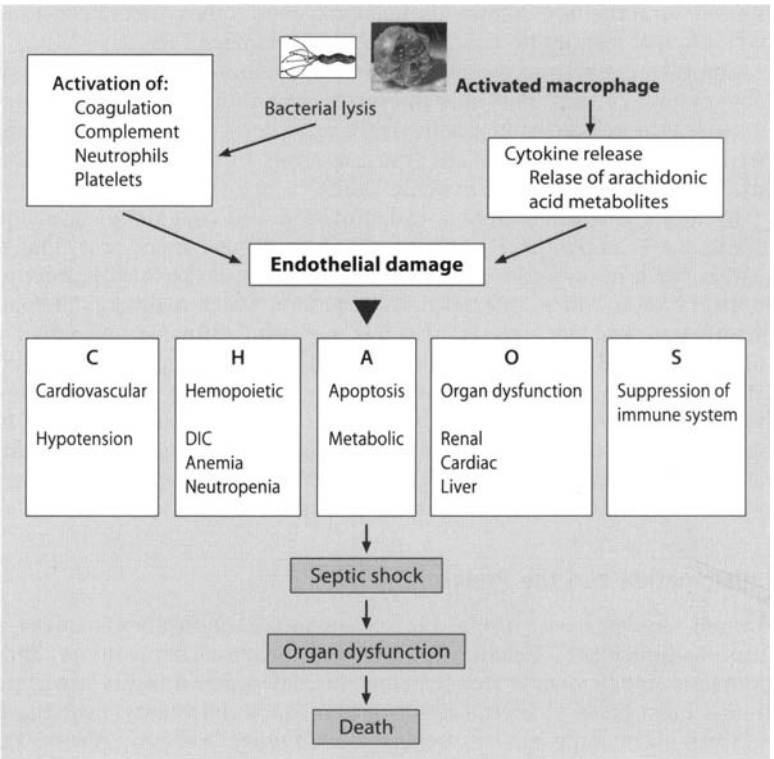

Fig. 2. The sepsis cascade

ride (LPS), an endotoxin from within the cell wall of the bacteria during lysis, while Gram-positive bacteria, fungi, and viruses initiate sepsis response by releasing exotoxins and cellular antigenic components. Both responses initiate the sepsis cascade (Fig. 2) by release of primary inflammatory mediators from activated cells e.g., macrophages. Release of mediators also activates both coagulation/complement and cytokine systems leading to damage of the endothelium resulting in leukocyte migration outside the blood vessel into the parenchyma towards the site of infection, and micro-thrombin formation [20] over the inflamed and activated endothelium.

Normal endothelium is responsible for vascular integrity and permeability. Infection damages the endothelial integrity resulting in vasodilatation and leak of cells and fluids into the tissues, which in turn leads to accumulation of fluid in the extravascular compartment, tissue edema and hypotension. To overcome this leak, the activated endothelium increases the number of thrombin receptors on its surface to localize coagulation factors and platelets at the site of injury.

The Inflammatory Response

The inflammatory response in the newborn is the same as in an adult albeit this response may be deficient quantitatively rather than qualitatively. Activated macrophages produce a range of pro-inflammatory mediators, like tumor necrosis factor (TNF)-α, IL-1, IL-6, IL-8, platelet activating factor (PAF), leukotrienes, and thromboxane-A_2, which activate many other cells leading to endothelial damage [21].

Injured endothelium allows granulocytes and other mediators to leak into the parenchyma, leading to 'CHAOS' and organ damage (Fig. 2).

Complement system essential for innate immunity is activated by pro-inflammatory cytokines and not only increases chemotaxis and phagocytosis, but also increases the release of histamine from mast cells, which further increases capillary permeability and enhances the 'third spacing' of fluid commonly seen in preterm infants with severe sepsis or septic shock.

In adults and the newborn, inflammation and coagulation are closely linked in sepsis; for example, TNF-α, IL-1, and IL-6 activate monocytes that express tissue factor, which in turn stimulates the extrinsic arm of the coagulation pathway leading to formation of fibrin. Interestingly, thrombin, which maintains the balance between coagulation and fibrinolysis, also has a pro-inflammatory effect on macrophages, monocytes, and endothelial cells. In sepsis, thrombin generation becomes unregulated leading to an initial hypercoagulable state. Sepsis impairs the normal fibrinolytic response leaving the body less able to remove micro-thrombins, frequently recognized as disseminated intravascular coagulation (DIC) seen early in sepsis in the newborn. Following the initial phase of DIC, coagulation factors are consumed very rapidly leading to fibrinolysis and bleeding.

Inflammation and the Preterm Brain

Animal studies have shown that endotoxin dramatically sensitizes the immature brain to injury [22]. Relationship between infection, brain injury, and neuro-developmental impairment is slowly being elucidated. Brain injury associated with infection is most likely to be the result of multi-factorial events involving cytoxic injury and vascular compromise associated with hypoxic ischemic events. The presence of inflammatory cytokines in the central nervous system is known to inhibit proliferation of neuronal precursor cells, activate astrogliosis, and stimulate oligodendrocyte death, all of which increase the risk of white matter injury [23]. Oligodendrocytes, which play a central role in the development of periventricular leukomalacia, appear to be particularly vulnerable to damage in sepsis and hypoperfusion.

In a large cohort of 1078 infants born before 32 weeks gestation, and/or weighing less than 1500 grams at birth, Leviton and colleagues [24] have shown that intrauterine infection and FIRS were independent predictors for development of cystic white matter injury.

Clinical Events Leading to Septic Shock

Sudden septic shock in infants with Gram-negative infection is not unusual in newborn infants, particularly in very preterm infants whose host defenses are poor and who frequently undergo invasive procedures or have intravascular devices inserted. One of the most important factors in progression from infection to septic shock is the use of inappropriate or delayed antibiotic therapy [25]. In the neonate, the usual cause of shock secondary to infection is the redistribution and leak of intravascular fluid causing hypovolemic shock and tissue (including cardiac) hypoperfusion.

Herpes simplex virus Type I, an infection that occurs in approximately 1:5000 live births is one infection that may present with intractable 'shock' without any history of maternal infection with herpes virus. Thus, it is advisable to include anti-viral (acyclovir) medication in the management of an infant who either does not respond

to standard therapy or has persistent signs and symptoms of infection with negative bacterial or fungal cultures or an infant who presents in septic shock.

Biological Markers

Investigators have long sought biological marker/s that would serve for early and accurate detection of sepsis. There are an increasing number of such tests, but despite initial enthusiasm most of them can be relegated to the growing heap of biomarkers that have failed to accurately differentiate between sepsis and other non-septic critical illness [26]. Of the new biomarkers, soluble triggering receptor expressed on myeloid cells –1 (sTREM-1) appears to be promising. It has a sensitivity of 96% and specificity of 89% [27]. Using a multiplex bead system we have perfected measurement of an array of cytokines using a drop of blood on blotting paper with a 2-hour turn around time. We find macrophage inflammatory protein (MIP)-1β to be most predictive, sensitive and specific for sepsis in the newborn. The most commonly available and used biomarkers are shown in Table 2.

Table 2. Sensitivity, specificity, and positive (PPV) and negative (NPV) predictive values of commonly used biomarkers of sepsis in the newborn (adapted from [3]).

Test	Sensitivity	Specificity	PPV	NPV
Blood Culture	11–38	68–100	90–100	72–100
WBC <4000, >30,000	17–90	31–100	50–86	60–89
I/T ratio >0.02	81	45	23	92
CRP >10 mg/dl	37	95	63	87
IL-8 >70 pg/ml	77	76	42	94
I/T ratio +CRP	89	41	24	94
IL-8+CRP	91	74	43	98
16 sPCR	96	99	89	99
sTREM-1 >60 ng/ml	96	89	86	96

WBC: white blood cell; I/T ratio: immature to total neutrophil ratio; CRP: C-reactive protein; IL: interleukin; PCR: polymerase chain reaction; sTREM-1: soluble trigger receptor expressed on myeloid cells-1. All values are percentages

Management

Management of neonatal sepsis and septic shock is based on the principles of initial resuscitation, killing the pathogen by early administration of appropriate antibiotics, correction of the consequences of sepsis, and correction of both coagulation and immunological homeostasis along with boosting host defenses [9].

While there are evidence-based recommendations for management of severe sepsis and septic shock in adults and children there is either a total lack or a severe paucity of such evidence-based recommendation for management in the newborn. Recommendations offered here are based on available evidence from literature and clinical practice; they cannot replace the wisdom of an experienced clinician who makes a clinical judgment based on the availability of unique sets of clinical variables for individual patients.

Initial Resuscitation

This should begin as soon as it is recognized that the patient has either severe sepsis or is in septic shock and not delayed until the patient is transferred toneonatal intensive care facility. Early goal-directed therapy has been shown to reduce mortality from septic shock in adults [28] and its principles can be applied to neonates. Most severely septic babies will be tachycardic and hypovolemic before their blood pressure falls; therefore, blood pressure should not be used as a marker of either shock or hypoperfusion. Measurement of oxygen saturation and serum lactate are more acceptable measures of tissue oxygenation and perfusion.

Antibiotics

As soon as the diagnosis of severe sepsis or septic shock is made, appropriate cultures and tests for biomarkers of sepsis should be taken and appropriate broad-spectrum bactericidal antibiotic therapy initiated. Delay in starting or inappropriate therapy has been shown to increase poor outcome [25]. The choice of antibiotic depends on the susceptibility pattern, but should cover all likely pathogens. Initial antibiotic regimen should be altered on the basis of microbiological and clinical data. Once the causative organism has been identified then antibiotics can be targeted only against that organism. It should be remembered, however, that in sepsis and septic shock often there is accompanying renal and hepatic dysfunction leading to abnormal volumes and levels of distribution of drugs; therefore, therapeutic plasma levels should be monitored.

The duration of antibiotic therapy is debatable. A balance should be achieved between adequate duration versus the desire to minimize resistance, super-infection, and other complications from prolonged use of antibiotics. We would recommend monitoring and titrating the duration of antibiotic therapy with serial measurements of CRP or preferably IL-6 or IL-8. It is also important for clinicians to remember that blood cultures are frequently negative in newborns with sepsis and septic shock, thus the decision to continue or stop antibiotic therapy must be made on clinical grounds plus the use of other surrogate biomarkers of sepsis and not only on negative blood culture results (Table 2).

Intravascular access devices are potentially a major source of severe sepsis or septic shock; they should be promptly removed after establishing other vascular access. Prophylactic antibiotic (vancomycin) therapy has been shown to be of some benefit [31] but it increases the development of resistant or insensitive organisms, hence it is not recommended.

Mechanical ventilation

Respiratory failure in severe sepsis and septic shock is common. Due to low functional residual capacity, neonates with severe sepsis may require elective intubation and ventilation [29]. A clear airway does not indicate effective breathing. Failure of gas exchange may be caused by lung parenchyma infection or infiltration with activated neutrophils. Thus it may be beneficial for the newborn to be electively sedated and ventilated. Care should be taken in premature babies to avoid hyperoxemia to prevent retinopathy and free radical lung damage. It is equally important to avoid over distention of alveoli, which is a potent inducer of IL-6 release. IL-6 release is associated with immune paralysis, increased severity of systemic infection, and emergence of secondary lung infection (i.e., ventilator-associated pneumonia).

Newborn babies with septic shock frequently have radiological changes similar to those seen in acute lung injury (ALI) in adults. This is due to consumption of surfactant. This secondary surfactant deficiency induces a respiratory distress syndrome-like clinical picture. It has recently been reported that the use of a surfactant called Calfactant® which has a high concentration of collectin, a protein that collects bacteria and contributes to their killing [30], is beneficial in this situation.

Fluid therapy

Fluid resuscitation is the hallmark of hypovolemic and septic shock reversal. It does not matter whether colloid or a crystalloid solution is used. However, volume distribution is much larger for crystalloids and, therefore, resuscitation with crystalloids requires more volume of fluid than colloid to achieve the same end point. To prevent reperfusion injury it is preferable to increase the total volume and rate of fluid infusion rather than give repeated boluses of fluids. Isolated boluses of 20 ml/kg given over 20 to 30 minutes may occasionally be required to improve heart rate, cardiac, and urine output. Reliance should not be placed on blood pressure as an end point for adequacy of fluid resuscitation. In severe sepsis and septic shock the endothelium is 'leaky', thus the end points to aim for are normalization of heart rate, capillary refill time, oxygen saturation, and acidosis. It is important to remember that those infants who after adequate fluid resuscitation do not self-diurese may need diuretics to prevent fluid overload.

Inotropic and Vasopressor Therapy

Adequate fluid resuscitation is the fundamental 'key' to the hemodynamic management of septic shock and must be achieved before instituting either vasopressor or inotropic agents. Dopamine increases heart rate, cardiac output and mean arterial blood pressure due to its vasoconstrictive effect. Dobutamine improves cardiac contractibility and cardiac output. In neonates, there is usually low cardiac output and low systemic resistance in severe sepsis and shock; therefore, dopamine is usually the first choice. In a systematic review [32] dopamine was found to be marginally more effective in the short term. Clinically it does not significantly alter the outcome which inotrope is used first. Experience with vasopressors in the newborn is limited, with no randomized controlled trials.

Coagulation

In severe sepsis and septic shock, endothelium all over the body becomes prothrombotic and anti-fibrinolytic. Systemic anti-thrombotic factors like protein C are consumed leading initially to predominance of prothrombotic factors causing DIC. When enough prothromotic factors are consumed then spontaneous bleeding occurs. It is important, therefore, to determine early whether the infant is in a prothrombotic or fibrinolytic phase. Appropriate coagulation studies should be undertaken frequently. If the baby has a prolonged prothrombin time/partial thromboplastin time and low fibrinogen then it is likely to be DIC. If, however, fibrinogen levels are normal or high then it is likely to be thrombotic thrombocytopenic purpura. Although routine use of fresh frozen plasma to correct laboratory clotting abnormalities is not recommended, some professional bodies [32] have recommended its use and we also find it useful. There is no consensus or firm guideline on when to give platelet transfusion. Most authorities recommend that platelets

should be transfused when an infant's platelets are anywhere between 5000 and 30,000 $\times$ 10^9/l.

Anemia

Red blood cell transfusion in septic patients improves oxygen delivery to tissues. No optimum level of hemoglobin has been established but it is recommended that hemoglobin be maintained above 10 g/dl in neonates with sepsis.

Glucose Control

Tight glycemic control has become very popular as an approach to goal-directed therapy and has been incorporated into many sepsis 'care bundles'. Hyperglycemia, particularly cortisol-induced hyperglycemia as seen in severe sepsis or septic shock, is immunosuppressive and prothrombotic. Hyperglycemia in severe sepsis is due to insulin resistance, which prevents glucose entering into the Krebs cycle. Muscles, and in particular cardiac muscle, depend on insulin dependent type II and type IV glucose transporters to get glucose into the Krebs cycle. Hence early institution of insulin therapy in hyperglycemic states (as seen in severe sepsis) ensures that glucose is delivered into the Krebs cycle. There is no consensus as to what is the ideal blood glucose level except that it should not be lower than 30 mg/dl. Similarly, there is no agreement as to what is the upper limit of blood sugar when insulin therapy should be initiated. Solutions containing 10% dextrose as maintenance fluid are adequate to provide energy (glucose 4 to 8 mg/kg/minute). Care should be taken to avoid rapid fluctuation in blood glucose levels by giving boluses or high concentration glucose infusion.

Bicarbonate Therapy

There is no evidence to support the use of bicarbonate therapy in the treatment of hypoperfusion induced acidemia associated with sepsis. Bicarbonate solutions are very hyperosmolar even when diluted. Bicarbonate infusion if given rapidly may increase the chances of ventricular hemorrhage in the newborn, particularly the preterm infant.

Nutrition

During severe illness, an infant's metabolic requirements are increased and the infant is catabolic, breaking down his/her own tissues (especially muscle) to use as metabolic fuel. This is worse in preterm infants who have poor muscle mass and energy reserves. This catabolic process can and should be limited by providing appropriate quantities of energy, minerals, and vitamins. Enteral feeding is preferable as it reduces bacterial translocation from the gut mucosa into the circulation and also helps preserve gut mucosal function. If enteral feeding is not possible or an additional energy source is required then it should be provided by the intravenous route remembering that parenteral nutrition is associated with significant complications, which are exaggerated during sepsis.

Strategies to Prevent Organ Function

Organ failure results from inadequate organ oxygenation due to poor perfusion. In developing strategies to maintain or restore organ function the aim should be to improve delivery of oxygen and nutrition to all tissues.

Kidney

Ion channels in tubular epithelium are energy/oxygen dependent thus particularly sensitive to hypotension and hypoxia. Nearly two thirds of infants with severe sepsis or septic shock will develop renal function abnormalities. These should be looked for and urgently addressed with conventional methods. There is no evidence that renal replacement therapy (hemofiltration or hemodialysis) is of any benefit. Standard measures should be taken to correct hyperkalemia, metabolic acidosis, and poor urine output.

Liver

During septic shock, the liver may be damaged by periods of hypotension and redistribution of fluid away from it. This is reflected in a sharp rise in liver enzymes in the blood and worsening coagulation profile. With adequate fluid and oxygen resuscitation this damage is often self-limiting and reversible.

Gastrointestinal tract

An empty gut may worsen sepsis and other organ dysfunction by increased bacterial translocation across inflamed or damaged intestinal mucosa. H_2-antagonists and proton pump inhibitors have been used to reduce mucosal damage in adults. Though no controlled clinical trials are available in the newborn they are frequently used in neonatal units. Use of these drugs has the disadvantage of reducing gastric acidity allowing bacterial overgrowth hence their routine use is not recommended. It is very important to make every effort to provide the septic infant with some enteral feed (trophic feeding) except when there is clear evidence of gut injury, e.g., necrotizing enterocolitis.

Boosting Host Defense by Immunomodulation

Most neonates, preterm infants in particular, have deficiencies both in their innate and adaptive immunity. Their immune deficiency is directly proportional to the degree of prematurity. Immunological immaturity is inversely related to gestational age [9]. Sepsis (endotoxin/exotoxin) induces immune paralysis, which is frequently seen in the newborn and results in further reduction in the ability of their macrophages and neutrophils to kill pathogens. To boost these functions various immunomodulatory therapies have been tried:

Colony Stimulating Factors

Both granulocyte and granulocyte-macrophage colony stimulating factors (G-CSF, GM-CSF) have been used as adjuncts to standard therapy in the treatment of neonatal sepsis. While the use of these factors has been shown to increase the number of circulating white cells, their use has not been shown to reduce mortality from neonatal sepsis or septic shock [34].

Steroids

Although steroid therapy has been found to be useful in adults and children with severe sepsis, there are no studies of their use in neonates with sepsis.

Protein C and activated protein C

Protein C concentrations reach adult levels around three years of age. Sepsis depresses protein C levels, hence it is an attractive idea to provide protein C supplementation in sepsis. To date there are no randomized clinical trials using recombinant activated protein C in neonates with sepsis.

Pentoxifylline

This carbonic anhydrase inhibitor has been shown to improve white cell function. In one randomized controlled trial in premature infants, pentoxifylline, was shown to significantly reduce mortality [35].

Intravenous Immunoglobulin (IVIG)

Polyclonal and IgM-enriched IVIG have been shown to reduce mortality from sepsis in newborn infants [36]. In the most recent Cochrane review [37], of nine studies involving 553 neonates with suspected infection, six (n=318) reported mortality. The use of IVIG was associated with a statistically significant reduction in mortality in infants with proven sepsis (RR 0.63 [95% CI −0.40 − 1.00]). Treatment in seven trials (n = 262) of cases with subsequently proven infection also resulted in a statistically significant reduction in mortality following IVIG therapy (RR 0.55 [95%CI −0.31 − 0.98]). Similarly, reports [36] using IgM-enriched IVIG have shown significant reductions in mortality from sepsis in the newborn (RR 0.35 [95%CI −0.23 − 0.54]).

Conclusion

Neonatal sepsis is common and mortality from sepsis in very low birth weight infants remains high despite the use of broad-spectrum antibiotics and intensive care. To reduce this excessive loss of life, there needs to be a better understanding of pathophysiology of sepsis, severe sepsis, and septic shock in the newborn. It also needs an approach to management which involves a whole package (Table 3), which includes killing the pathogen, correcting the sequel of sepsis caused by pathogens and the body's own activated defense systems, and boosting host defenses.

Table 3. Sepsis management package

- Clinical suspicion of sepsis (risk factors)
- Appropriate sepsis screen (inclusion of cytokines and PCR)
- Start appropriate antibiotic therapy (short duration)
- If perfusion is poor **AND** serum lactate > 5 mmol/l give 20 ml/kg of colloid or crystalloid (earlier the better). If still hypotensive, start inotropes early
- Maintain Hb > 10 g/dl
- Maintain calorie intake > 100 kcl/day or > 80 kcl/day if on TPN
- Maintain oxygen saturation between 90 − 94
- Consider adjuvant IVIG therapy

TPN: total parenteral nutrition; IVIG: intravenous immunoglobulin; Hb: hemoglobin

It is recognized that while this article may be static, the understanding, diagnosis and optimum management of severe sepsis and septic shock is a dynamic process. It is hoped that the currently established interventions will, over a period of time, be based on evidence or abandoned and newer interventions developed and proven.

Wisdom is not what you know
But what you do with what you know.
Anonymous

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Effects of Vasoactive Agents on the Gastrointestinal Microcirculation in Septic Shock

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Introduction

Recent studies have demonstrated the importance of microcirculatory alterations in the early stage of sepsis [1–3]. The gastrointestinal tract, particularly perfusion of the splanchnic bed and the integrity of the gut mucosa, occupies a key position in the pathogenesis of multiple organ failure (MOF) in sepsis.

The mechanisms of alterations in microvascular hemodynamics during sepsis are particularly complex since they involve not only systemic changes such as increase in cardiac output and decrease in systemic pressure but also changes in regional blood flow distribution and alterations in local microvascular regulatory mechanisms due to the effects of inflammatory mediators on endothelial or vascular smooth muscle cells. This complexity is particularly evident regarding changes in the intestinal microcirculation for which the decrease in systemic pressure cannot alone explain the intestinal microvascular disturbances associated with endotoxemia. Indeed, our group has previously demonstrated by intravital videomicroscopy that the hemodynamics in the intestinal microcirculation during endotoxic and hemorrhagic shock were completely different for the same amount of pressure decrease [1]. Factors that can possibly affect the microcirculatory response to shock or the redistribution of blood flow toward the mucosa have been identified by others. The involvement of inflammatory mechanisms mediated by leukocyte activation and cytokine release is possibly more important in sepsis than in hemorrhagic shock (even if also present in the latter situation). Moreover, hemorheological factors, such as alterations of red blood cell (RBC) shape, can also contribute to deleterious changes in villus perfusion during sepsis [4, 5].

In the early phase of septic shock, when fluid administration fails to restore adequate arterial pressure and organ perfusion, it is recommended that therapy with vasopressor agents should be initiated [6]. Vasopressor therapy may also be required transiently, to maintain perfusion in the face of life-threatening hypotension, even when adequate cardiac filling pressures have not yet been reached. Potential agents include dopamine, norepinephrine, phenylephrine, epinephrine, and vasopressin. Martin et al. [7] reported the superiority of norepinephrine over other vasopressors in treating septic patients. Other studies have reported the potential value of the use of vasopressin in septic patients [8, 9], which allowed the withdrawal of other catecholamines and improved systemic circulation.

However, the microvascular effects of these vasopressor agents on the splanchnic circulation are still largely unknown and may play a role in their selection for a given patient. Thus, the ideal vasopressor for the first-line agent in the early phase of septic shock is highly debated. Another question is whether vasodilatory agents

may have a place in the treatment of the microcirculatory dysfunction. Indeed, more and more data in the literature suggest that vasodilator agents are capable of "opening the microcirculation" and restoring it after septic shock [10].

Effects of Vasopressor Agents

In mice submitted to endotoxic shock, we investigated the effects of norepinephrine and vasopressin on intestinal microcirculation by intravital microscopy [11]. We measured RBC flux and velocity in villus tip arterioles and the density of perfused villi. One hour after endotoxin injection, the mean arterial pressure (MAP) decreased significantly to 46 ± 4 mmHg. The density of perfused villi, RBC velocity, and flux in the villus tip arterioles were dramatically decreased in hypotensive sepsis. The doses of vasopressors were titrated to restore MAP to its baseline level. Neither norepinephrine nor vasopressin administered at doses sufficient to restore MAP was able to restore RBC velocity and flux to their preshock baseline values. However, norepinephrine and vasopressin prevented additional decreases in RBC flux and velocity (Fig. 1). These findings are in line with a study by Levy et al. [12], which reported that the mesenteric blood flow was not restored either by norepinephrine or by vasopressin administered at doses that restored MAP. This clearly showed that restoring MAP, which is usually the first end-point during resuscitation in septic

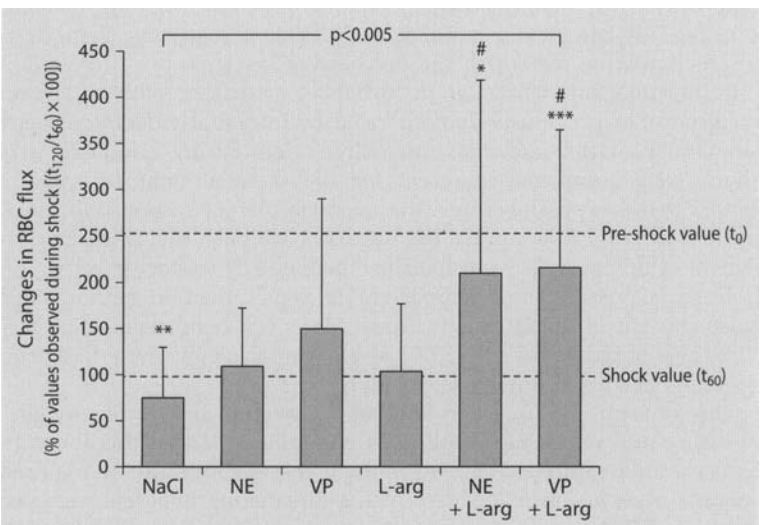

Fig. 1. Changes in erythrocyte (RBC) flux in villus tip arterioles. Anesthetized and ventilated mice received *Escherichia coli* endotoxin (2 mg/kg bolus i.v.) at t_0, which induced after 1 hr (t_{60}) a decrease in mean arterial blood pressure (MAP) to 40–50 mmHg associated with a significant decrease in RBC flux. The mice were randomly allocated to different treatment groups (n=6 in each group): continuous i.v. infusion for 1 hr with saline (NaCl, control group), norepinephrine (NE), vasopressin (VP), L-arginine (L-arg), NE+L-arg, or VP+L-arg. The doses of vasopressors (used alone or combined with L-arginine) were titrated to restore MAP to the baseline level. Changes in RBC flux between pre- and post-treatment were expressed as a percentage of the pre-treatment value and were significantly different among groups ($p<.001$). Values are expressed as mean ± SD. *$p<.05$, **$p<.01$, and ***$p<.001$ for t_{120} vs. T_{60}. #$p<.05$ treatments vs. control at t_{120}. From [11] with permission.

shock, is not sufficient to restore microvascular oxygen supply in intestinal mucosa. As previously suggested by Vallet et al. [13] occult hypoxia may persist despite restoration of normal MAP. However, it is important to note that one important frequent limit of small animal models is that it is difficult to ensure that fluid resuscitation is optimized and the effects of vasopressor agents may differ according to the volemic state. It should also be noted that our study in murine intestinal microcirculation cannot rule out possible differences in metabolic effects of the two drugs studied, which might also interfere with the adequacy of oxygen supply to oxygen demand. Thus, the finding of similar hemodynamic effects of norepinephrine and vasopressin in the intestinal microcirculation does not necessarily contradict the observation made recently by Levy et al. [12] that vasopressin, but not norepinephrine, may attenuate the gut metabolic disturbances provoked by endotoxic shock in rats (lactate or lactate-to-pyruvate ratio changes).

The microvascular effects of V1a agonists are still controversial and it is difficult to have a clear opinion about the splanchnic hemodynamic effects of V1a agonists. One point which is clear is that, like all vasopressors, the effects of V1a agonists vary among different organs and are a function of the administered dose. Concerning the macrocirculation, Malay et al. [14] reported that low doses of vasopressin, typically used in the clinical management of septic shock, do not impair blood flow to carotid, renal, mesenteric, or iliac circulations. However, moderately higher doses of vasopressin may induce ischemia in the mesenteric and renal circulations. These data indicate that the safe dose range for exogenous vasopressin in septic shock is narrow, and support the current practice of fixed low-dose administration, generally 0.04 units/min and not exceeding 0.1 units/min. Concerning the microcirculation, some studies have suggested that vasopressin can impair the mesenteric microcirculation [15, 16]. In a murine septic shock model (cecal ligation and perforation), Westphal et al. [15] reported dramatic hypoperfusion of intestinal villi after arginine vasopressin administration. However, it should be noted that in the model of sepsis used by these authors, the MAP was almost normal. Under this condition, a beneficial effect of a vasoconstrictor is very unlikely. Recently, Knotzer et al. [17] reported that increasing dosages of continuously infused arginie vasopressin in non-endotoxic pigs decreased cardiac output and systemic oxygen delivery with decreases in intestinal oxygen supply and mucosal tissue PO_2 due to a reduction in microvascular blood flow in the jejunal mucosa. Conversely, during endotoxemia, the same group observed that vasopressin did not induce a further decrease in cardiac output despite effective vasoconstriction and did not further compromise mucosal tissue PO_2 or oxygen supply in the acute phase of endotoxic shock in pigs [18]. The volemic state is also essential in the effect produced by a given concentration of vasopressin. A study by Asfar et al. [19] clearly illustrates this point. The authors observed that low-dose terlipressin significantly increased ileal microcirculation in fluid-challenged endotoxic rats but not in hypodynamic endotoxic rats. Clinical studies have also analyzed the effect of V1a agonists on the gastrointestinal microcirculation. In norepinephrine-dependent patients in septic shock, van Haren et al. [20] reported that continuous infusion of low-dose vasopressin (0.06 U/min) resulted in a significant increase in the gastric mucosal-arterial PCO_2 gradient compatible with gastrointestinal hypoperfusion. In addition, when norepinephrine was replaced by vasopressin, in a dose sufficient to keep MAP constant in norepinephrine-dependent septic shock patients, the gastric mucosal-arterial PCO_2 gradient dramatically increased [21]. In contrast, in a randomized, controlled study in 48 patients with septic shock treated with high-dose norepinephrine (0.6 μg/kg/min),

the gastric mucosal-arterial PCO_2 gradient was better preserved with the combined infusion of vasopressin (0.06 U/min) and norepinephrine without any deleterious effects. In addition, Morelli et al. [22] recently observed an increased gastric mucosal flow (laser Doppler) in patients with septic shock treated with high doses of norepinephrine after a 1 mg bolus of terlipressin. However, the same group, using a 1 mg bolus of terlipressin to reverse hypotension episodes in anesthetized patients chronically treated with renin-angiotensin system inhibitors for arterial hypertension, reported a negative effect on gastric mucosal perfusion [23]. This is in line with a clinical case reported by Boerma et al. [16], in which sublingual orthogonal polarization spectral (OPS) imaging was performed after administration of terlipressin in a patient with catecholamine-resistant septic shock. Despite increasing doses of norepinephrine, a MAP of 60 mmHg could not be maintained and the patient became oliguric. At baseline, the overall microcirculatory flow was well preserved, without evidence of heterogeneity. After a single bolus of 1 mg terlipressin, a rise in MAP and urinary output occurred but a dramatic decrease in perfused small-vessel numbers was observed. In contrast, Dubois et al. [24] showed that vasopressin (0.02 U/min) increased MAP and allowed partial weaning of other vasopressors without worsening sublingual microcirculation.

Thus, caution should be taken when considering such a potent vasoconstrictor when correcting blood pressure during shock. Before the results of currently ongoing randomized clinical trials to ensure that vasopressin has beneficial effects on organ function and outcome, are available, it is important to limit its prescription to the recommendations: Low doses in septic shock patients refractory to other vasopressors after adequate fluid resuscitation [6]. Concerning terlipressin, it is difficult to have a clear opinion about its gastrointestinal effects, because only a few studies have evaluated the use of this synthetic long-acting analog of vasopressin in septic patients.

Effects of Vasodilatory Agents

Since inducible nitric oxide synthase (iNOS) has been shown to contribute to systemic hypotension during sepsis, inhibition of NOS has been proposed as a possible strategy for restoring systemic pressure in addition to the use of vasoconstrictors. However, this inhibition can lead to a deleterious decrease in tissue perfusion possibly due to amplification of vasoconstrictive mechanisms [25]. On the other hand, some groups have reported that endotoxemia is associated with low plasma arginine levels, and beneficial circulatory modulation by L-arginine administration has been reported by some authors [26, 27]. These data suggest that vasodilatory agents may have a place to ensure adequate microcirculatory perfusion in sepsis patients. Spronk et al. [28], using OPS imaging to assess the sublingual microcirculation, showed, in a small cohort of patients with septic shock, that infusion of 0.5 mg of nitroglycerin resulted in a marked increase in microvascular flow. In this study, It is important to note that nitroglycerin infusion was started after fluid optimization and that the improvement in the microcirculation was observed despite a drop in MAP (19 mmHg). De Backer et al. [3] reported in patients with septic shock that changes in sublingual microvascular perfusion were independent of changes in cardiac index and changes in blood pressure during dobutamine administration. In addition, these authors showed that the endothelial vasodilatory response was intact in septic patients, since topical application of acetylcholine (endothelium-dependent

vasodilator) totally reversed the microvascular alterations with recruitment of no-flow capillaries. With this background, it seemed to us worthwhile to use our experimental mouse model of intravital microscopy under endotoxemia to answer the following questions: Is L-arginine by itself able to counteract microcirculatory disturbance, and is the combination of vasopressors and L-arginine more efficient in improving intestinal villus microcirculation than vasopressors alone? [11]. Despite the lack of an effect of L-arginine on MAP, we did observe a beneficial effect in preventing the deleterious changes in microvascular hemodynamics (Fig 1). It should be remembered that rodent models of endotoxemia have a large iNOS response, which can amplify the observed effects of L-arginine. In addition, the combination of L-arginine with vasopressors allowed a better restoration of intestinal villus hemodynamics than administration of vasopressors alone (Fig. 1). One possible explanation of this beneficial effect is that despite the restoration of MAP by vasopressors, distal microvessels were still hypoperfused due to local vasoconstrictors or leukocyte/platelet-dependent mechanisms associated with sepsis, which could be inhibited by NO. Another possibility is that NO may interact in a complex way with the effects of the administered vasopressor to modulate locoregional blood flow distribution more in favor of the mucosa. Potent vasoconstrictive and potent vasodilatory agents do not antagonize each other but rather lead to an additive beneficial effect on mucosal perfusion [29]. It is clear that additional studies will be required for a proper demonstration of this concept.

Conclusion

When fluid administration fails to restore an adequate arterial pressure in septic shock, therapy with vasopressor agents is required. Properly used (after performing fluid optimization and in a titrated way) norepinephrine and vasopressin appear to prevent any further decreases in intestinal microcirculatory hemodynamics. Addition of a vasodilatory agent to norepinephrine or vasopressin may be of interest, but additional studies will be required for a proper demonstration of this concept and it is too soon to treat our patients in septic shock with a combination of vasodilators and vasopressors. The fact that monitoring of the microcirculation is increasingly available in our patients must contribute to a better assessment and treatment of sepsis-related microvascular dysfunction.

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Hemodynamic Effects of Activated Protein C in Septic Shock

N. Sennoun, O. Desebbe, and B. Levy

Introduction

Recombinant human activated protein C (rhAPC) has been demonstrated to reduce the mortality rate of adult patients with severe sepsis [1]. This effect is thought to be related to a reduction in coagulation and, to a lesser extent, to a reduction in the inflammatory response to sepsis. Data from the Protein C Worldwide Evaluation in Severe Sepsis (PROWESS) study demonstrated that the use of rhAPC was associated with a quicker reduction in cardiovascular failure.

The present chapter addresses whether rhAPC has specific cardiovascular properties leading to hemodynamic improvement. The putative mechanisms involved in this action are also discussed.

Human Data

Patients with Septic Shock

In a retrospective study of septic shock in humans, Monnet et al. [2] compared 22 patients treated with rhAPC with 22 non-treated patients matched for age, simplified acute physiology score (SAPS) II, mean arterial pressure (MAP), and norepinephrine dose at the theoretical start of rhAPC infusion (H_0). Blood lactate level, number of organ failures, and number of patients treated with corticosteroids and with renal replacement therapy were also similar between the two groups. The therapeutic strategy involved maintaining MAP at 75 mmHg by decreasing or increasing catecholamine levels. MAP remained stable and was similar in the two groups (86 ± 16 vs. 89 ± 9 mmHg at 24 hours [H_{24}]). From H_0 to H_{24}, the required dose of norepinephrine increased in the control group (+38%, from −41 to +38%) but decreased in the treated group (−33%, from −74 to +11%; $p<0.05$) (Fig. 1). There was no difference between groups in dobutamine dose or in the amount of fluid administered during the 24 hour follow-up. Lactate levels tended to decrease in rhAPC patients while they tended to increase in the control group, although the difference in the time course between both groups did not reach statistical significance.

Endotoxemia in Healthy Volunteers

The effects of rhAPC were also studied by Kalil et al. [3] in a classical model of endotoxemia. All subjects received a bolus of 2 ng/kg lipopolysaccharide (LPS) 2 hours after start of infusion of rhAPC or placebo. Physiological measurements were performed 5 min prior to LPS infusion and halted 6 hours after the LPS. At a single

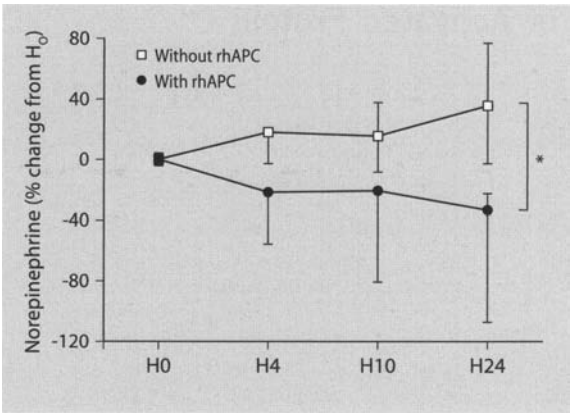

Fig. 1. Time course of the norepinephrine dose (proportional change from the value at H_0; mean and interquartile range) in patients receiving rhAPC and in matched control patients. The norepinephrine dose was measured at the start of rhAPC administration (treated patients) or at the time of its theoretical administration (control patients) (H_0) and 4, 10, and 24 h later (H_4, H_{10}, and H_{24}, respectively). From [2] with permission

time point (3 h after LPS administration), MAP was significantly higher in volunteers receiving rhAPC compared to those receiving placebo (rhAPC 75 mmHg vs. placebo 65 mmHg, $p<0.04$). However, no differences in hemodynamics were observed at other time points. There were no effects of rhAPC on markers of thrombin generation (prothrombin fragment [F1+2], thrombin-antithrombin [TAT]), D-dimer levels, or circulating concentrations of plasminogen activator inhibitor (PAI)-1. There were also no differences in tumor necrosis factor (TNF)-α or interleukin (IL)-6 levels between the rhAPC and placebo groups.

Derhaschnig et al. (4), in a similar model, did not find any differences in MAP evolution. Likewise, rhAPC had no significant effects on LPS-induced changes in hemodynamics, including hypotension or tachycardia, circulating cytokine levels, markers of platelet and endothelial cell activation, or leukocyte counts.

Hence, there is no definite proven effect of APC on hemodynamics from the above human endotoxemia studies.

Animal Data

APC Properties on Vascular Reactivity are Linked to Nitric Oxide Synthase and Tumor Necrosis Factor

In rats [5], endotoxin-induced hypotension, as well as increases in plasma levels of nitrite/nitrate (NO_2-/NO_3-), were significantly prevented by intravenous administration of APC (100 μg/kg). This hypotension was also inhibited when APC was administered 30 min after endotoxin administration. APC inhibited the increase in lung inducible nitric oxide synthase (iNOS) levels by inhibiting expression of iNOS mRNA in endotoxin-treated animals. In order to distinguish the direct antithrombin properties of APC from its anti-inflammatory effects, the authors also investigated the effects of a direct inhibitor of thrombin generation and demonstrated that this inhibitor had no effect on MAP. In a further series of experiments designed to demonstrate the link between APC efficiency and TNF production, the effects of an anti-TNF agent, of leukopenia, and of the selective inhibition of NOS by aminoguanidine were equally assessed, all resulting in the same observed effects on MAP as those noted with APC. Furthermore, APC inhibited the increase in lung iNOS and TNF-α levels by inhibiting iNOS mRNA expression. Thus, APC is able to reduce endotoxin-

induced hypotension in rats by inhibiting TNF-α production. Furthermore, the above study suggests that APC inhibits the endotoxin-induced increase in NOS activity and TNF-α levels by inhibiting their transcription *in vivo* [5].

High Doses of APC Improve Vascular and Cardiac Function in Endotoxic Shock

Using elevated doses of murine APC (240 μg/kg/h), Favory et al. [6] recently tested whether APC treatment improved cardiovascular function in rats infused with *Escherichia coli* endotoxin. More specifically, they tested the hypotheses that APC (a) prevents arterial hypotension, microvascular perfusion deficit, and depressed ventricular systolic performance, and (b) attenuates inflammatory response in terms of leukocyte activation and plasma TNF-α, macrophage migration inhibitory factor (MIF), and nitrite/nitrate levels. Using a non-sedated, continuously infused model of endotoxemia, the study revealed that endotoxin was associated with a drop in arterial pressure that was attenuated by APC. Of further interest, the authors also investigated the effects of APC on myocardial performance evaluated on a modified Langendorff isolated heart preparation. LPS induced a reduction in left ventricular (LV) systolic performance in the absence of changes in coronary perfusion pressure. Reduction in LV systolic performance was also prevented in LPS-treated rats who were treated with APC. To explain their results, Favory et al. demonstrated that APC was associated with a decrease in nitrate/nitrite, TNF-α and MIF. Moreover, the endotoxin-induced increase in cardiac myeloperoxidase (MPO) activity, an index of leukocyte tissue sequestration, was prevented by APC treatment. Lastly, using intravital microscopy, APC was found to be associated with improved microvascular perfusion and reduced leukocyte rolling and adhesion.

Low Doses of APC also Improve Vascular and Cardiac Function in Endotoxic Shock

Based on these previous results, we elected to investigate the effects of rhAPC on global and regional hemodynamics as well as on tissue perfusion [7]. Using a classical model of ventilated sedated rats, endotoxemia was induced by administration of 10 mg/kg of *E. coli* LPS. Monitoring included continuous measurement of MAP, abdominal and mesenteric aortic blood flow, muscle laser Doppler, and muscle PO_2, and muscle lactate by microdialysis. Vascular reactivity was assessed by dose-effect curves using incremental doses of norepinephrine. Cardiac function was assessed by measuring the evolution of cardiac output under incremental doses of norepinephrine. Measurements also included blood lactate, nitrite and nitrate, and TNF levels. Rats were continuously resuscitated with 18 ml/kg/h saline leading to a hypokinetic model. Two groups were investigated: one group treated with a continuous infusion of 33 μg/kg/h rhAPC and the other with saline.

Endotoxin administration was associated with a marked and sustained decrease in MAP. Continuous infusion of APC was associated with a significant improvement in MAP. More surprisingly, associated with the increase in MAP was a better maintenance of cardiac output in the continuously infused rhAPC group. This effect appeared to be delayed comparative to the effects on MAP. No differences were observed in mesenteric blood flow. However, associated with the improvement in the macrocirculation were signs of better tissue perfusion following rhAPC treatment as assessed by: 1) greater muscle blood flow estimated by laser Doppler; 2) greater muscle PO_2; and, finally 3) a decrease in blood lactate in the APC group. We

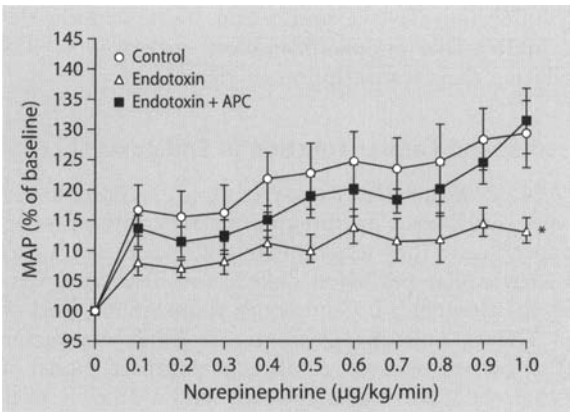

Fig. 2. Using dose response curves with norepinephrine, APC was indeed found to be associated with an improvement in vasoreactivity when compared to endotoxin treated rats [7]. * $p < 0.05$ vs control and endotoxin+APC group.

further hypothesized that the effects of rhAPC on MAP were likely due to an improvement in catecholamine vasoreactivity. Using dose response curves with a pure α-agonist, rhAPC was indeed found to be associated with an improvement in vasoreactivity (Fig. 2). Cardiac effects of rhAPC were confirmed using dose-response curves with epinephrine but not with phenylephrine. In the experimental groups, the decrease in cardiac output due to the increase in myocardial afterload was attenuated by rhAPC suggesting that APC improves the response to β1 stimulation as also suggested by a higher heart rate

Limitations of Animal Studies

Clearly, endotoxin administration is not representative of human sepsis. Endotoxin, when compared to human septic shock, leads to a hyperinflammatory state as assessed by the very elevated levels of TNF. More important is the timing of intervention. In all models used, APC was given either simultaneously or immediately following septic challenge. It is also important to discuss the species specificity of APC's non-anticoagulant activities. For example, in both the Isobe [5] and Favory [6] studies, the dosages used were far more elevated than the 24 μg/kg/h used in humans.

Mechanisms Behind APC-improved Hemodynamics

The Anti-inflammatory Effects of APC

Clearly, and at least in experimental models, APC decreases NOS activity and TNF production. Nevertheless, in the PROWESS trial [1], the investigators were unable to demonstrate a significant effect on any of the five cytokines examined: TNF, IL-1, IL-6, IL-8, and IL-10. In nearly all of the non-clinical studies, APC concentrations were higher than in the PROWESS trial.

In the two placebo-controlled, blinded studies [3, 4] conducted with rhAPC in human endotoxemia, drotrecogin alfa (activated) did not significantly decrease the levels of multiple cytokines (TNF-α, IL-1β, IL-6, IL-8, or IL-10) nor those of leukocyte cell-surface adhesion molecules when compared with the placebo group. In addition, in a placebo-controlled human pulmonary endotoxin model, drotrecogin

alfa (activated) was administered intravenously at 24 μg/kg per hour for 16 hours, starting 2 hours prior to the endotoxin challenge [8, 9]. rhAPC treatment did not have any significant effect on inflammatory cytokine (TNF-α, IL-1β, IL-6, IL-8, 1L-10), or monocyte chemoattractant protein (MCP)-1 levels in the bronchoalveolar lavage fluid compared to the placebo group.

The Effect of APC on Leukocyte–endothelial Cell Interactions

Cellular effects

Recent studies suggest that the anti-inflammatory properties of the protein C pathway may not involve the lowering of inflammatory cytokine levels, but rather involve a lowering of the leukocyte chemotactic response and a modulation of the interaction of leukocytes with the activated endothelium. Intriguingly, the effect of APC on leukocytes appears to be limited to chemotaxis since other leukocytic functions, such as phagocytic and oxidative burst remain unaffected [10–13].

Animal studies

Using intravital microscopy of the dorsal skin fold in a hamster endotoxemia model, Hoffmann et al. [14] demonstrated that intravenous administration of rhAPC at 24 μg/kg/hr significantly reduced endotoxin-induced leukocyte rolling and adhesion in both arterioles and venules. More recently, Favory et al. [6], also using intravital microscopy on the distal ileum, demonstrated that APC partially prevented microvascular perfusion deficit in LPS-administered rats, Likewise, mean red blood cell (RBC) velocity and wall shear rates, which were lower in LPS-administered rats than in controls, were partially restored in the APC-treated LPS-administered group. Leukocyte rolling and adhesion on the mesenteric venule endothelial surface were increased after LPS injection whereas APC treatment reduced leukocyte rolling and adhesion in LPS-administered rats. The authors also observed a decrease in heart leukocyte infiltration.

Thus, the improvement in cardiovascular failure observed in endotoxemic rats may perhaps be related to the properties of APC on leukocyte behavior [15, 16].

Conclusion

Clearly, data strongly suggest that rhAPC improves cardiovascular failure in animal models of sepsis. It is not clear whether this effect is of importance in the observed improvement in patient mortality in the PROWESS study. The mechanisms involved are likely multifactorial. The improvement in vascular reactivity to catecholamines should and could be easily evaluated in clinical practice using dose-response curves.

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Adrenomedullin in the Treatment of Cardiovascular Dysfunction and Sepsis

C. Ertmer, H. Van Aken, and M. Westphal

Introduction

The existence of vasoactive peptide hormones was first reported more than 100 years ago [1]. Since then, several oligo- and polypeptides influencing blood pressure regulation have been identified. Some of these are direct vasoconstrictors, such as vasopressin, angiotensin II, endothelin-1, and neuropeptide Y and are classified as hypertensive agents. Other peptides, e.g., the natriuretic peptides, vasoactive intestinal peptide (VIP), and calcitonin-gene related peptide (CGRP), have direct vasodilatory properties and, thus, lower systemic blood pressure. Adrenomedullin belongs to the vasodilatory peptide hormones and plays a crucial role in the regulation and preservation of cardiovascular, endocrine and immunologic homeostasis [2].

This chapter will discuss the biochemical and pharmacological characteristics of adrenomedullin and focus on its specific role in the pathophysiology and treatment of cardiovascular diseases and sepsis.

Adrenomedullin: Molecular Biology and Physiological Effects

Molecular Structure, Production, and Clearance

Adrenomedullin is a 52 amino acid peptide hormone which was first isolated from pheochromocytoma cells by Kitamura et al. in 1993 [3]. Due to its origin from the adrenal medulla, the molecule was named 'adrenomedullin'. Adrenomedullin is generated from a larger propeptide, preproadrenomedullin, which consists of 185 amino acids and is spliced by posttranslational processing into the 164 amino acid peptide, proadrenomedullin [4]. Splicing of preproadrenomedullin is induced by a C-terminal pair of arginine amino acids, representing a typical processing signal [5]. The proadrenomedullin molecule is then enzymatically converted into adrenomedullin and proadrenomedullin N-terminal 20 peptide (PAMP). A disulfide bond between amino acids 16 and 21 accounts for the typical ring structure of the adrenomedullin molecule (Fig. 1). Due to structural homologies to CGRP within the amino acid sequences, both peptides belong to the calcitonin/CGRP/amylin-peptide family [6].

The messenger ribonucleic acid (mRNA) of adrenomedullin has been identified in several cells and tissues [7]. The most important sites of adrenomedullin production are summarized in Table 1.

Adrenomedullin production and secretion is augmented by several pro-inflammatory and pro-atherogenic factors, such as tumor necrosis factor (TNF)-α, interleukin (IL)-1β, IL-6, lipopolysaccharide (LPS), endothelin-1, angiotensin II (AT II), and aldosterone [8–10]. In healthy human beings, adrenomedullin plasma levels

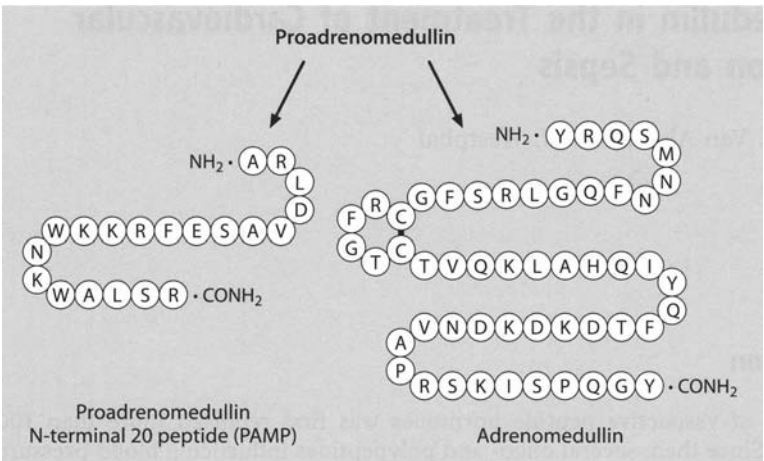

Fig. 1. Amino acid sequences of adrenomedullin and adrenomedullin N-terminal 20 peptide. From [79] with permission

Table 1. Adrenomedullin producing cells and tissues. Modified from [79] with permission

Tissue	AM producing cell type
Cardiovascular system	Vascular endothelial cells Vascular smooth muscle cells Atrial and ventricular myocardial cells
Endocrine system	Cells of the adrenal zona glomerulosa Chromaffin cells Pheochromocytoma cells Cells of the posterior pituitary gland
Respiratory system	Bronchial epithelial cells Alveolar macrophages Smooth muscle cells
Urogenital system	Glomerular and tubular epithelial cells Ovarian granulosa cells Endometrial epithelial cells Endometrial macrophages Prostate epithelial cells
Gastrointestinal tract	Mucosal epithelial cells Pancreatic cells
Blood cells	Macrophages Granulocytes Lymphocytes Monocytes
Central nervous system	Astrocytes
Skin	Keratinocytes

range between 1 and 10 pmol/l (mean concentration 2.27 ± 1.0 pmol/l) [6, 11]. The lung plays the major role in endogenous adrenomedullin clearance [12]. Therefore, impaired pulmonary clearance of adrenomedullin (e.g., in septic shock) accounts for sustained adrenomedullin plasma levels under pathologic conditions [12].

Signal Transduction of Adrenomedullin

To date, three subtypes of G-protein-coupled adrenomedullin receptors have been identified. The receptors consist of an adrenomedullin binding site (calcitonin-receptor-like receptor, CRLR) and a receptor activity-modifying protein (RAMP). The RAMPs (1–3) determine the subtype of the adrenomedullin receptor and are essential for the translocation of the CRLR into the cell membrane [13]. Whereas the CRLR/RAMP-1 complex serves as a receptor for adrenomedullin and CGRP, the remaining two subtypes (CRLR/RAMP-2 and -3) represent specific adrenomedullin receptors [6]. Binding of adrenomedullin to the CRLR results in a G-protein-dependent activation of adenylate cyclase. The subsequent elevation in intracellular cyclic adenosine monophosphate (cAMP) concentration mediates relaxation of vascular smooth muscle cells via activation of protein kinase A, and thus contributes to vasodilation.

Conversely, several adrenomedullin-related effects are not mediated by CRLRs and appear to be independent of the cAMP system [14]. In vascular endothelium cells, adrenomedullin activates phospholipase C, which subsequently increases intracellular calcium (Ca^{2+}) via the inositol-1,4,5-trisphosphate cascade [15]. Elevations in Ca^{2+} concentrations in endothelial cells, in turn, directly stimulate nitric oxide (NO) production via the endothelial NO synthase (eNOS). Endothelium-derived NO activates the cyclic guanosyl monophosphate (cGMP) system in vascular smooth muscle cells, thereby contributing to vasodilation. Table 2 gives an overview of specific adrenomedullin effects within different vascular cell types.

Table 2. Signal transduction and intracellular effects of adrenomedullin in different vascular cell types. Modified from [5]

Vascular cell types	Intracellular effects
Vascular endothelial cells	Activation of adenylate cyclase Elevation of cAMP Increase in intracellular calcium Stimulation of NO synthesis Elevation of cGMP Reduction of reactive oxygen species Inhibition of apoptosis
Vascular smooth muscle cells	Activation of adenylate cyclase Elevation of cAMP Reduction of reactive oxygen species Modulation of migration and proliferation
Vascular adventitial fibroblasts	Activation of adenylate cyclase Elevation of cAMP Modulation of inflammatory mediators Inhibition of proliferation

cAMP: cyclic adenosine monophosphate; cGMP: cyclic guanosyl monophosphate; NO: nitric oxide.

Cardiovascular Effects of Adrenomedullin

Impact of endogenous adrenomedullin in healthy animals

Endogenous adrenomedullin has no effect on blood pressure in healthy subjects. Thus, adrenomedullin$^{+/-}$ knockout mice do not have a higher mean arterial pressure (MAP) compared to adrenomedullin$^{+/+}$ mice [16]. However, gene deficient mice are more vulnerable to endothelial injury. In injured arterial intima of adrenomedullin$^{+/-}$ mice, eNOS expression is significantly reduced while the concentration of reactive oxygen species (ROS) is increased versus adrenomedullin$^{+/+}$ mice. Therefore, adrenomedullin$^{+/-}$ knockout mice have a much higher risk of developing arteriosclerotic lesions [16].

In addition, endogenous adrenomedullin exerts antioxidative, angiogenic, and anti-apoptotic activities via activation of Akt and/or mitogen-activated protein kinases (MAPKs) [17, 18]. These findings suggest that endogenous adrenomedullin may act not only as a vasodilator but also as a vasoprotective agent. Notably, the above mentioned effects of endogenous adrenomedullin cannot be studied in completely in adrenomedullin deficient subjects (adrenomedullin$^{-/-}$), since this genotype is embryonically lethal [16]. Endogenous adrenomedullin thus represents a vital peptide hormone involved in the regulation and preservation of cardiovascular homeostasis.

Exogenous adrenomedullin infusion under physiologic conditions

Westphal et al. reported that exogenous infusion of adrenomedullin in sheep results in a decrease in systemic and pulmonary arterial blood pressure, mainly due to a direct reduction in systemic and pulmonary vascular resistance [19]. Low doses of 10 ng/kg/min (1.7 pmol/kg/min) contributed to a moderate decrease in MAP in healthy sheep [19]. Infusion of 50 and 100 ng/kg/min (8.3 and 16.7 pmol/kg/min, respectively) was associated with marked elevations in heart rate and cardiac index that were accompanied by reductions in MAP and systemic vascular resistance index (SVRI). Notably, adrenomedullin infusion did not result in a critical decrease in MAP below 65 mmHg [19].

Adrenomedullin induces vasodilation by both endothelium-dependent and endothelium-independent mechanisms. Endothelium-dependent vasodilation is mainly mediated by a NO-related increase in cGMP. cGMP in turn inhibits vascular smooth muscle myosin light chain kinase and mediates an efflux of Ca^{2+} ions [15]. On the other hand, the direct increase in vascular smooth muscle cAMP levels is linked to endothelium-independent vasodilation [20]. The mode of action via two different pathways explains the marked vasodilatory potential of adrenomedullin and a significant potency that is well-maintained even in the presence of impaired endothelium-dependent vasorelaxation (e.g., pulmonary hypertension or sepsis) [6, 21, 22].

The effects of exogenous adrenomedullin have been investigated in several regional vascular beds in the rat [23]. It has been reported that adrenomedullin lowers vascular resistance in the lung, heart, renal, and adrenal vasculature, thereby, improving regional blood flow despite a decrease in perfusion pressure. In addition, adrenomedullin increases glomerular blood flow and urine output by dilating glomerular vessels. Previous experiments also revealed that adrenomedullin exerts a specific natriuretic effect [24]. Taken together, experimental studies in healthy subjects provide evidence that adrenomedullin is a potent vasodilatory peptide hormone that is critically involved in the regulation of tissue perfusion.

In healthy human volunteers, exogenous adrenomedullin induces vasodilation and is associated with an increase in heart rate and cardiac index [19, 25]. These effects reflect sympathetic activation as well as a direct inotropic effect of adrenomedullin [26, 27]. Furthermore, adrenomedullin (1 to 3 pmol/kg/min) causes an increase in left ventricular ejection fraction (LVEF) and cardiac index, and, therefore, decreases LV end-systolic diameter [25]. No adverse cardiac events have yet been reported during or following the adrenomedullin infusion period in any of the above-mentioned studies.

Immunologic Effects of Adrenomedullin

The expression of adrenomedullin on surface epithelial cells is associated with mucosal host defence [28]. Antimicrobial potential against commensals and pathogenic strains of bacteria has been reported for adrenomedullin. Gram-negative and Gram-positive bacteria appear to be equally sensitive against adrenomedullin, whereas no activity against *Candida albicans* has been shown [28]. The upregulation of adrenomedullin expression in epithelial cells following LPS exposure implies a central role of adrenomedullin in the innate immune system of epithelial surface layers [28].

In circulating blood, adrenomedullin is mainly bound to adrenomedullin binding protein-1 (AMBP-1), which is also known as complement factor H [29]. AMBP-1 inhibits complement activation by binding factor C3b and by serving as a co-factor for complement factor I [30]. In addition, AMBP-1 augments the adrenomedullin-mediated induction of cAMP in fibroblasts and increases its potency as a growth factor [29]. The antimicrobial, immunomodulatory and hemostatic activity suggests a central role of adrenomedullin and AMBP-1 in the physiologic network of immunologic homeostasis.

Endocrine Effects of Adrenomedullin

In vitro and *in vivo* investigation demonstrated a central inhibition of adrenocorticotropic hormone (ACTH) release from the pituitary gland following adrenomedullin exposure. Adrenomedullin infusion in sheep results in a decrease in endogenous ACTH levels [31]. Samson and colleagues reported that the reduction in ACTH secretion is reversed by AT II, thereby suggesting antagonistic effects of adrenomedullin and AT II on pituitary hormone release [32].

In rat adrenal cells, secretion of aldosterone is markedly reduced in the presence of adrenomedullin [33]. The underlying mechanisms include a central reduction of AT II and ACTH stimulated aldosterone release as well as a direct cAMP-mediated effect of adrenomedullin. In the adrenal medulla, sympathetic nerve stimulation results in co-secretion of adrenomedullin and catecholamines *in vitro*. However, adrenomedullin does not impact on basal catecholamine release [6]. Moreover, the secretion of vasopressin, insulin, and AT II, as well as salt and water uptake, is inhibited by adrenomedullin *in vitro* [6, 34].

In summary, adrenomedullin-related cardiovascular effects are not restricted to vasodilatory effects but include also the inhibition of stress hormones from the pituitary and adrenal gland. The net effects of the cardiovascular and endocrinologic actions are vasodilation, vasoprotection, and reduction in total body volume.

Role of Adrenomedullin in the Pathophysiology and Treatment of Acute and Chronic Heart Failure, Pulmonary Hypertension, and Sepsis

Pulmonary Hypertension

Preclinical studies

The pulmonary vasculature expresses a high density of adrenomedullin receptors [35]. Experimental studies in rats and cats demonstrated that endogenous as well as exogenous adrenomedullin dilates pulmonary vessels and increases pulmonary arterial blood flow [36, 37]. The vasodilatory activity of adrenomedullin in the rat pulmonary vasculature is mainly mediated by increased endothelial NO synthesis [36]. Short-term infusion of adrenomedullin has been reported to ameliorate pulmonary hypertension resulting from LV failure in rats [37]. Interestingly, long-term infusion of adrenomedullin in monocrotaline-induced pulmonary hypertensive rats not only reduced pulmonary artery pressure, but also attenuated right ventricular (RV) hypertrophy and medial thickening [38]. These findings suggest that beyond its vasodilatory effect within the pulmonary circulation, adrenomedullin also has a significant antiproliferative potential

The above-mentioned studies support the assumption that upregulation of adrenomedullin expression represents a protective mechanism in the pathophysiology of pulmonary hypertension. In conditions where endogenous adrenomedullin synthesis becomes insufficient (e.g., prolonged diseases), exogenous adrenomedullin substitution appears to be a causative treatment strategy. When comparing adrenomedullin with alternative clinical approaches to treat pulmonary hypertension, such as inhalation of NO or aerosolization of prostaglandins, adrenomedullin may be advantageous due to its longer lasting and pronounced inotropic effects [39].

Clinical studies

Clinical studies have confirmed that adrenomedullin plays also an important role in the regulation of pulmonary vascular tone in humans under physiological conditions and in the presence of pulmonary hypertension [18]. In this context it is noteworthy that adrenomedullin plasma levels are typically elevated in proportion to the severity of pulmonary arterial hypertension. In such patients, both intravenous and aerosolized adrenomedullin has been shown to effectively reduce pulmonary arterial pressure, thereby improving systemic and pulmonary hemodynamics as well as gas exchange [21, 40, 41].

Nagaya et al. studied the effects of intravenous adrenomedullin (8.3 pmol/kg/min) in a small, randomized, controlled clinical trial in patients suffering from pulmonary hypertension (n = 13) [41]. Notably, exogenous adrenomedullin contributed to a 44% increase in cardiac index, a 32% decrease in pulmonary vascular resistance index, and a decent reduction in mean pulmonary arterial pressure (4%). These authors also compared the efficacy of intrapulmonary infusions of adrenomedullin, acetylcholine (ACh), and adenosine trisphosphate (ATP) on pulmonary vascular tone in five patients with pulmonary hypertension [21]. Adrenomedullin infusion into a segmental pulmonary artery resulted in intrapulmonary concentrations between 10^{-8} and 10^{-9} mmol/l. At 10^{-8} mmol/l, the increase in relative blood flow velocity was equivalent ($\approx$ 40%) to 10^{-4} mmol/l of ACh and 10^{-5} mmol/l of ATP. This finding reflects a much stronger pulmonary vasodilative effect of adrenomedullin as compared to equimolar concentrations of either ACh or ATP. In this context, it is

important that ACh and ATP mediate pulmonary vasodilation in humans mainly by increasing endothelial NO release and thus cGMP formation [22, 42]. Nagaya et al., however, demonstrated that the adrenomedullin-mediated improvement in systemic and pulmonary hemodynamics in patients suffering from pulmonary hypertension is associated with increased cAMP, but not cGMP levels [21]. Therefore, it can be concluded that very small amounts of intravenous or intrapulmonary adrenomedullin dilate pulmonary vessels even in patients with impaired endothelium-dependent vasorelaxation, as is the case in severe pulmonary hypertension [22].

The effects of nebulized adrenomedullin (1.7 nmol/kg) have been investigated during cardiac catheterization and exercise testing in patients with idiopathic pulmonary hypertension (n=11) [40]. Aerosolized adrenomedullin significantly increased symptom-limited maximum work rate (+15 W) and cardiac index (+12%) and decreased mean pulmonary arterial pressure (−13%) as well as pulmonary vascular resistance (−22%). In these patients, the peak adrenomedullin plasma levels following adrenomedullin inhalation averaged 22.9 ± 2.1 pmol/l. Notably, neither heart rate nor MAP were significantly altered by this approach. Therefore, the authors concluded 1) that aerosolized adrenomedullin is an effective alternative in the treatment of idiopathic pulmonary hypertension, and 2) that aerosolized adrenomedullin has a more positive dose/response ratio as compared to the intravenous route of application.

In summary, intravenous and aerosolized adrenomedullin administration represent effective and safe options in the treatment of pulmonary hypertension. Due to its significant endothelium-independent vasodilatory potential, adrenomedullin is even effective in very severe cases [21, 22]. Since no adverse effects were noticed in any of the investigated patients, exogenous adrenomedullin appears to possess an excellent benefit/risk ratio in the treatment of pulmonary hypertension.

Congestive Heart Failure and Myocardial Infarction

Endogenous adrenomedullin plasma levels

Patients with congestive heart failure are characterized by elevated adrenomedullin plasma levels that closely correlate with the severity of disease (New York Heart Association [NYHA] functional class I, 4.1 ± 1.0; II, 5.6 ± 1.6; III, 6.4 ± 0.8; IV, 13.2 ± 6.8 pmol/l) [43]. In addition, an increase in plasma levels of adrenomedullin has been identified as an independent risk factor for mortality in patients with chronic ischemic heart failure [44].

Circulating adrenomedullin levels are also elevated in acute cardiac injury. In a cohort of patients with acute myocardial infarction (n=121), endogenous adrenomedullin concentrations were markedly increased (17 ± 1 pmol/l) from day 2 to day 4 post-infarction [45]. Notably, initial adrenomedullin plasma concentrations above 14 pmol/l were clearly associated with an increased 2-year mortality as compared to patients with lower adrenomedullin plasma levels. In addition, the adrenomedullin concentration was positively correlated with clinically relevant markers of cardiac decompensation and mortality, such as brain-type natriuretic peptide (BNP). The strong relationship of adrenomedullin plasma levels with hemodynamic dysfunction and survival suggests a key role of adrenomedullin in the pathophysiology of acute and chronic heart failure. Although not (yet) routinely performed in clinical practice, determination of endogenous adrenomedullin concentrations represents a simple and useful approach to predict outcome in these patients.

Exogenous adrenomedullin infusion in experimental heart failure

In sheep with pacing-induced congestive heart failure, adrenomedullin infusion (1.7–16.7 pmol/kg/min) was associated with decreases in mean arterial and left atrial pressure and increases in cardiac index, cAMP plasma levels, creatinine clearance, and natriuresis [46, 47]. These effects were associated with a decrease in aldosterone and an increase in renin plasma levels. The marked increase in cardiac index following adrenomedullin infusion can be explained by both a reduction in biventricular afterload and a direct inotropic effect [25, 27].

Another important finding is that adrenomedullin reduces blood pressure more effectively in sheep with heart failure than under physiologic conditions [46, 47]. Likewise, sodium excretion, creatinine clearance, and urine output were increased to a higher extent in sheep with heart failure as compared to healthy subjects [46, 47]. It, therefore, appears that exogenous adrenomedullin does not impair renal function despite a moderate lowering of renal perfusion pressure.

In summary, preclinical studies have clearly shown that adrenomedullin reduces biventricular afterload and intravascular volume, improves renal function, mediates sodium excretion, and exerts a direct inotropic activity. Therefore, adrenomedullin appears a useful agent for the treatment of congestive heart failure.

Exogenous adrenomedullin infusion in patients with congestive heart failure and myocardial infarction

Nagaya and colleagues investigated the clinical effects of exogenous adrenomedullin (8.3 pmol/kg/min) in patients with congestive heart failure and healthy controls (n=7 each) [48]. Whereas adrenomedullin decreased MAP and increased heart rate in patients with congestive heart failure to a lesser extent than in healthy patients, the increase in cardiac index and the reduction of endogenous aldosterone concentrations was more pronounced in patients with heart failure. The hemodynamic and renal effects of adrenomedullin are similar to those of atrial natriuretic peptide (ANP), which is already clinically used in the treatment in congestive heart failure. Oya et al. have also performed experiments in this area and compared the effects of equimolar amounts (16 pmol/kg/min) of adrenomedullin and ANP in this indication [49]. Whereas adrenomedullin predominantly increased cAMP plasma levels, ANP only elevated cGMP concentrations. Thus, the vasodilatory effects of adrenomedullin appear to be predominantly endothelium-independent, whereas ANP acts primarily through in increase in endothelial NO release. Treatment with adrenomedullin resulted in a greater increase in cardiac index and a more sustained decrease in MAP and SVR as compared to patients treated with ANP. Nonetheless, ANP was more effective in improving renal function, as evidenced by increased urinary output and sodium excretion. From the above mentioned studies, it can, therefore, be concluded that both adrenomedullin and ANP are effective in the treatment of congestive heart failure, but act in a different manner (cAMP vs. cGMP). In equimolar amounts, adrenomedullin is more effective than ANP in improving systemic hemodynamics and may be favorable in patients with low cardiac output [49]. In this regard, administration of adrenomedullin proved advantageous over ANP in 14 patients following myocardial infarction who were treated with equimolar amounts (8.3 pmol/kg/min) of either of the two drugs [50]. In this study [50], only adrenomedullin improved myocardial contractility and increased coronary sinus blood flow. In contrast, ANP had no impact on these variables. In addition, adrenomedullin improved LV relaxation without increasing myocardial oxygen consumption.

Although no outcome studies have yet evaluated the value of adrenomedullin in the treatment of congestive heart failure and myocardial infarction, preclinical and clinical data strongly suggest that adrenomedullin improves cardiovascular performance and renal function in these patients. Adrenomedullin may, therefore, represent a safe and effective treatment option in patients with acute or chronic heart failure. However, since adrenomedullin increases heart rate and decreases diastolic perfusion pressure, patients should be carefully monitored for potential myocardial ischemia.

Sepsis and Septic Shock

Sepsis, septic shock, and sepsis-associated multiple organ failure (MOF) belong to the most common causes of morbidity and mortality in intensive care unit (ICU) patients and are associated with high costs [51]. The fact that approximately 30% of the patients with severe sepsis and septic shock die, underlines the socio-economic need for effective treatment strategies [51].

Septic shock is associated with sustained arterial and venous vasodilation resulting in profound arterial hypotension. Since cardiac index is typically increased in volume-treated patients, this condition is often referred to as a 'hyperdynamic circulation' and associated with increased oxygen delivery and tissue perfusion and decreased SVR [52]. When sepsis progresses, the hyperdynamic shock may convert into a moribund hypodynamic circulation that is characterized by a reduction in cardiac output and oxygen delivery, as well as arterial hypotension and compromised tissue perfusion [53]. In this context, Shoemaker et al. demonstrated that about 16% of septic shock patients suffer from a hypodynamic circulation (defined as cardiac index <2.5 l/m^2/min) [54] and that this situation is associated with increased mortality. However, whether the conversion of a hypodynamic into a hyperdynamic circulation improves survival is unclear and remains to be investigated [55].

Endogenous adrenomedullin plasma levels in septic shock

Besides the typical 'early cytokines', such as TNF-α, IL-1β, and IL-6, endogenous adrenomedullin plasma levels are also markedly increased in early experimental septic shock [56]. Interestingly, the increase in endogenous adrenomedullin concentrations is positively correlated with changes in cardiac index. Antibodies specifically blocking the biological activity of adrenomedullin prevented the occurrence of a hyperdynamic circulation in rats that underwent cecal ligation and puncture (CLP) [57]. Therefore, endogenous adrenomedullin appears to be an essential mediator of the hyperdynamic circulation in sepsis. On the contrary, other mediators, such as inducible NOS (iNOS)-derived NO, prostaglandins, CGRP, catecholamines, and endothelins do not play an essential role in the pathophysiology of hyperdynamic circulation in experimental septic shock [52].

In addition to experimental evidence, there are several clinical observational studies reporting a marked increase in plasma levels of endogenous adrenomedullin in sepsis (107 ± 139 pmol/l) [58] and septic shock (226.1 ± 66.4 pmol/l) [59]. Moreover, increased adrenomedullin levels are negatively correlated with survival in septic shock patients [60]. Adrenomedullin may, therefore, represent an interesting adjunctive marker to judge the severity and prognosis of septic patients [60].

Exogenous adrenomedullin infusion in sepsis and septic shock

In transgenic mice overexpressing endogenous adrenomedullin, endotoxin infusion attenuated the drop in blood pressure and significantly increased survival [61]. The increase in endogenous adrenomedullin may, therefore, represent a beneficial counter-reaction against sepsis-associated cardiovascular collapse. In this context, Wang et al. reported that during the course of CLP-induced polymicrobial sepsis in rats, the hyperdynamic circulation converted into a hypodynamic state [62]. At the same time, endogenous adrenomedullin plasma levels and vascular responsiveness to exogenous adrenomedullin diminished. Likewise, AMBP-1 synthesis was reduced in these rats [63]. The reduction in endogenous AMBP-1 levels was mainly linked to increased LPS concentrations during sepsis, since LPS blockade by polymyxin B prevented the drop in AMBP-1. Due to these imperative findings, Yang et al. investigated whether an exogenous infusion of AMBP-1 was sufficient to re-establish vascular sensitivity against exogenous adrenomedullin in hypodynamic septic rats [64]. These authors reported 1) that exogenous AMBP-1 increased the vascular effects of endogenous adrenomedullin, and 2) that a combined infusion of adrenomedullin and AMBP-1 was suitable to treat and prevent hypodynamic septic shock in rats. Our own study group evaluated the effects of sole adrenomedullin infusion in a clinically relevant model of hypodynamic ovine endotoxemia. In this study, adrenomedullin (8.3 pmol/kg/min) was sufficient in both the treatment and prophylaxis of hypodynamic septic shock. In addition, exogenous adrenomedullin attenuated the degree of pulmonary hypertension and improved global oxygen transport (unpublished data). In a recent study, adrenomedullin infusion reduced the four-day mortality of endotoxemic and septic mice from 100% in untreated subjects to 40% [65]. In the latter study, adrenomedullin treatment also prevented the infiltration of inflammatory cells into the lung, liver, and intestine. Furthermore, adrenomedullin treatment consistently reduced the mortality of septic shock resulting from CLP or endotoxemia.

At first glance, an additional reduction in SVR and MAP may limit the therapeutic use of adrenomedullin in this indication. However, our study group has recently shown that even relatively high doses of intravenous adrenomedullin (16.7 pmol/kg/min) do not result in a critical drop in blood pressure [19]. In this regard, it is conceivable that the substantial increase in tissue perfusion may outweigh the vasodilatiory effect. Since reduced vascular responsiveness to exogenous adrenomedullin has been described in late septic shock, a simultaneous infusion of AMBP-1 might be useful in this specific situation [66].

Anti-inflammatory effects of adrenomedullin in sepsis an septic shock

The anti-inflammatory potential of adrenomedullin represents another beneficial effect in septic shock. Adrenomedullin inhibits inflammation by three different mechanisms: 1) modulation of pro-inflammatory cytokines (e.g., TNF-α, IL-1β, and IL-6); 2) induction and activation of eNOS-derived NO synthesis; and 3) anti-apoptotic activity [52].

Modulation of pro-inflammatory cytokines

Pro-inflammatory mediators, such as LPS, TNF-α, IL-1β, and IL-6, are markedly increased in septic shock and induce adrenomedullin synthesis [60, 67]. Notably, Kubo et al. reported that adrenomedullin inhibits TNF-α and IL-6 production in endotoxin-activated RAW 264.7 cells [68]. Likewise, adrenomedullin has been shown to suppress IL-1β production in fibroblasts [69]. On the contrary, Wong and col-

leagues demonstrated that adrenomedullin exerts pro- and anti-inflammatory activities at the same time [70]. These authors [70] reported that exogenous adrenomedullin inhibits the *in vitro* secretion of TNF-α and increases the release of IL-1β, IL-6, and macrophage migration inhibitory factor (MIF) in parallel. These findings suggest that adrenomedullin plays a central role in the complex network of pro- and anti-inflammatory cytokines that is often referred to as compensatory anti-inflammatory response syndrome (CARS) [71]

In addition to the above mentioned *in vitro* studies, Yang and colleagues recently reported that adrenomedullin and AMBP-1 infusion downregulated the expression of TNF-α, IL-1β, and IL-6 20 hours after induction of septic shock following CLP in rats [72]. Notably, the inhibition of these pro-inflammatory cytokones was associated with a marked reduction in hepatocellular injury, lactic acidosis, and mortality.

Activation of eNOS-derived NO synthesis

In early sepsis, eNOS-derived NO synthesis is typically increased and may represent a useful mechanism to augment blood flow to areas of compromised tissue perfusion [73]. *Vice versa*, late septic shock is characterized by a substantially impaired eNOS-mediated vasodilation, which subsequently may compromise loco-regional tissue perfusion [74]. Reduced eNOS expression by endothelial cells may, therefore, be a significant co-factor of 1) reduced endothelium-mediated vasorelaxation, and 2) impaired tissue perfusion in late septic shock.

In this context, Wang and colleagues demonstrated that a combined infusion of adrenomedullin and AMBP-1 preserved ACh-induced vasodilation, a mechanism that is mediated via eNOS activation. In addition, the latter group reported that the CLP-induced decrease in eNOS induction is directly prevented by a combined infusion of adrenomedullin and AMBP-1 (unpublished observations cited from [52]). Adrenomedullin may, therefore, be critically involved in the regulation of eNOS induction and appears to preserve tissue perfusion and oxygenation by activating this pathway.

Anti-apoptotic effects

Apoptosis is a physiologic process of cell death that is kept in balance with cell regeneration. In inflammatory states, such as septic shock, however, this balance is shifted towards increased apoptosis [75]. Inhibition of apoptosis has been shown to improve survival in septic mice [76]. Exogenous adrenomedullin inhibits endothelial cell apoptosis *in vitro* and, thereby, prevents thrombosis, tissue damage, blood flow abnormalities, and increased vascular permeability [77]. Thus, the anti-apoptotic effects may also account for the beneficial effects of adrenomedullin in septic shock.

In summary, pre-clinical studies suggest that endogenous adrenomedullin represents a valuable diagnostic marker to judge the severity and prognosis of severe sepsis and septic shock. Infusion of exogenous adrenomedullin with or without AMBP-1 is effective in the prevention and treatment of hypodynamic septic shock. In this context, adrenomedullin attenuates tissue damage, systemic inflammation, apoptosis, and organ injury and, thereby, decreases mortality. Since pre-clinical studies have repeatedly reported the safety and efficacy of exogenous adrenomedullin, clinical studies are now needed to confirm these beneficial effects in human septic shock.

Potentially Unwanted Side Effects of Adrenomedullin

Patients treated with intravenous adrenomedullin frequently report facial flushing and conjunctival injection during infusion [78]. These unwanted side effects are

caused by increased facial blood flow and immediately resolve after cessation of adrenomedullin infusion. Headache is another common adverse effect of adrenomedullin therapy [78].

Since adrenomedullin reduces LV afterload and, thereby, increases heart rate, it bears the potential risk of increasing myocardial oxygen demand and reducing coronary perfusion pressure. However, to our knowledge, no adverse cardiac effects of exogenous adrenomedullin have yet been reported. In the setting of septic shock or other conditions associated with arterial hypotension, the additional reduction in systemic blood pressure and LV afterload may limit the therapeutic use of adrenomedullin in these indications. Future research is needed to investigate the usefulness of combining adrenomedullin with a vasopressor in the treatment of shock states.

Conclusion

Adrenomedullin plays a pivotal role in the pathophysiology of several cardiopulmonary diseases, which include, but may not be restricted to, pulmonary hypertension [18], congestive heart failure [45], and septic shock [57]. Experimental and clinical research has demonstrated that adrenomedullin is a safe and beneficial adjunct in the clinical management of pulmonary hypertension [21, 40, 41] and congestive heart failure [48]. Due to its endothelium-independent mechanism of action and its direct inotropic effect, adrenomedullin may even be superior to alternative clinical approaches, such as natriuretic peptides or NO [39, 49]. In addition, pre-clinical studies have shown that adrenomedullin infusion is suitable to convert the moribund hypodynamic circulation into the more stable hyperdynamic sate in septic shock [64]. In the experimental setting, exogenous adrenomedullin is associated with an improvement in oxygen supply, a reduction in pro-inflammatory cytokines, and an improved survival [65]. Clinical studies evaluating the effects of exogenous adrenomedullin in the common setting of sepsis and systemic inflammatory response (SIRS) are eagerly awaited.

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Antioxidants for the Treatment of Endothelial Dysfunction in Critical Illness

B. Mullan, M. Duffy, and D. McAuley

Introduction

Before 1980, the endothelium was considered to be an inert hemostatic barrier. Furchgott and Zawadzki were the first to demonstrate the necessity of the vascular endothelium for vasodilatation to acetylcholine [1]. If the vascular endothelium was removed, the blood vessel failed to relax in response to acetylcholine, but still responded to glyceryl trinitrate. This endothelium-dependent vasodilatation is mediated by an endogenous mediator, initially named endothelium-derived relaxing factor (EDRF), but which was subsequently identified as nitric oxide (NO). From this discovery a new era of endothelial research developed. The endothelium is now known to have a key role in the maintenance of vascular homeostasis. It actively regulates vascular tone, platelet aggregation, coagulation, fibrinolysis, and leukocyte activation [2]. Endothelial function is impaired in critical illness and may be important in the pathophysiology of multiple organ failure (MOF) [3].

A number of metabolic derangements may potentially affect the endothelium. Many of these derangements are related to increased oxidative stress [4]. Oxidative stress may result from increased free radical generation and/or antioxidant depletion. Critical illness is associated with increased oxidative stress [5]. Tissue damage, whether from trauma, ischemia or infection, can generate reactive oxygen species (ROS) via a number of mechanisms. In critically ill patients there are reduced stores of antioxidants, reduced plasma or intracellular concentrations of free electron scavengers and decreased activities of enzymatic systems involved in the detoxification of ROS [6]. Circulating antioxidant levels decrease rapidly after insult, trauma, or surgery and remain low for several days or even weeks.

Antioxidants have been reported to improve endothelial function in various conditions, such as atherosclerosis, hypertension, diabetes, and dyslipidemia [7]. A recent meta-analysis has suggested that trace elements and vitamins that support antioxidant function, either alone or in combination with other antioxidants are safe and may be associated with a reduction in mortality in critically ill patients [8]. This chapter will discuss the effects of oxidative stress on endothelial function. In addition, the therapeutic potential of antioxidants to reverse endothelial dysfunction in critical illness will be explored.

The Endothelium

Based on morphological characteristics, the anatomy of the arterial vessel wall is divided into three components: tunica intima, tunica media and tunica adventitia.

The tunica intima consists of a single layer of endothelial cells that line the vessel lumen and the internal elastic lamina membrane. Far from being inert, the endothelium is metabolically active, and should be thought of as a complex organ with a multitude of autocrine, paracrine, and endocrine properties [9]. Various endothelial-derived mediators help to regulate vascular homeostasis (Table 1). Of these molecules, NO is essential (Fig. 1). NO, originally termed "endothelium-derived relaxing factor" by Furchgott and Zawadzki, is a potent vasodilator [10]. The balance between NO, various endothelial-derived vasoconstrictors, and the sympathetic nervous system, modulates blood vessel tone. Changes in this balance affect peripheral vascular resistance and hence blood pressure. In addition, NO suppresses platelet

Table 1. Regulatory functions of the vascular endothelium

Function	Mediators
Platelet aggregation	Prostacyclin (–) Nitric oxide (–)
Coagulation	Von Willebrand factor (+) Thrombomodulin (–) Heparin-like proteoglycans (–)
Fibrinolysis	Tissue plasminogen activator (+) Urokinase (+) Plasminogen activator inhibitor-1 (–)
Vascular smooth muscle tone	Prostacyclin (–) Nitric oxide (–) Endothelium-derived hyperpolarizing factor (–) Endothelin (+)
Vascular smooth muscle proliferation	Nitric oxide (–) Transforming growth factor β (–) Platelet-derived growth factor (+)
Leukocyte adhesion	Nitric oxide (–) E-selectin (+) Intercellular adhesion molecule-1 (+) Vascular cell adhesion molecule-1 (+)

+, stimulates or increases; –, inhibits or decreases

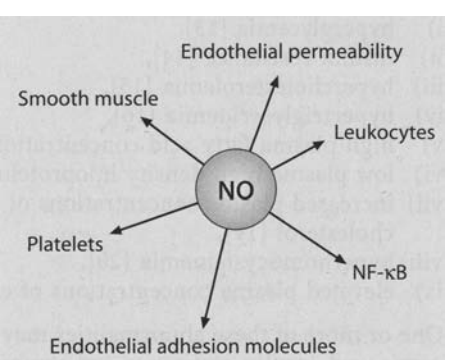

Fig. 1. Endothelial-derived nitric oxide (NO)-mediated biological effects: control of endothelial permeability; vasodilatation and inhibition of vascular smooth muscle cell proliferation; inhibition of platelet aggregation; inhibition of cell adhesion molecule expression; inhibition of leukocyte adhesion to the endothelium; inhibition of nuclear factor kappa-B (NF-κB).

aggregation, controls endothelial permeability, inhibits leukocyte adhesion to the endothelium, attenuates vascular smooth muscle cell proliferation, and may have an important role in the regulation of myocardial contractility [10]. Furthermore, NO can inhibit the activation and expression of certain endothelial cell adhesion molecules, and influence the activity of nuclear factor kappa-B (NF-κB) [11].

Endothelial-derived NO is synthesized from the amino acid L-arginine by the endothelial isoform of NO synthase, eNOS. Several co-factors are required for NO biosynthesis. These include nicotinamide adenine dinucleotide phosphate (NADPH), flavin mononucleotide (FMN), flavin adenine dinucleotide (FAD), tetrahydrobiopterin (BH_4) and calmodulin. Once synthesized, the NO diffuses across the endothelial cell membrane and enters the vascular smooth muscle cells where it activates soluble guanylate cyclase (sGC), leading to an increase in intracellular cyclic guanosine-3',5-monophosphate (cGMP) [10]. cGMP mediates many of the biological effects of NO including the control of vascular tone and platelet function. NO also interacts with heme, DNA and thiols, to alter the function of other key enzymes and ion channels.

There is a continuous basal synthesis of NO from the vascular endothelium. A number of chemical and physical stimuli may activate eNOS and lead to increased NO production. Chemical agonists include acetylcholine, bradykinin, serotonin and substance P. Hemodynamic shear stress exerted by the viscous drag of flowing blood is an important physical stimulus. The mechanisms of shear stress-induced NO release are complex, involving extremely rapid initiation via ion channel activation, and subsequent events related to signaling pathway activation, such as phosphorylation of eNOS protein and increased expression of eNOS mRNA and protein.

Endothelial Dysfunction

Dysfunction of the endothelium can be considered to be present when its properties, either in the basal state or after stimulation, have changed in a way that is inappropriate with regard to the preservation of organ function. Vascular tone and permeability may change, and the endothelium may lose its anti-thrombotic, anti-inflammatory, and pro-fibrinolytic properties. Endothelial dysfunction characterized by loss of NO-mediated endothelium dependent vasodilatation has been shown to occur early in a number of disease states, including acute systemic inflammation [12]. Physiological derangements which have been associated with reduced endothelial NO bioavailability include:

i) hyperglycemia [13],
ii) insulin resistance [14],
iii) hypercholesterolemia [15],
iv) hypertriglyceridemia [16],
v) high plasma fatty acid concentration [17],
vi) low plasma high density lipoprotein (HDL)-cholesterol concentration [18],
vii) increased plasma concentrations of small, dense low density lipoprotein (LDL)-cholesterol [19],
viii) hyperhomocysteinemia [20],
ix) elevated plasma concentrations of endogenous eNOS inhibitors [21].

One or more of these abnormalities may coexist in critical illness (Fig. 2). Hyperglycemia and insulin resistance are common in critically ill patients [22]. They may

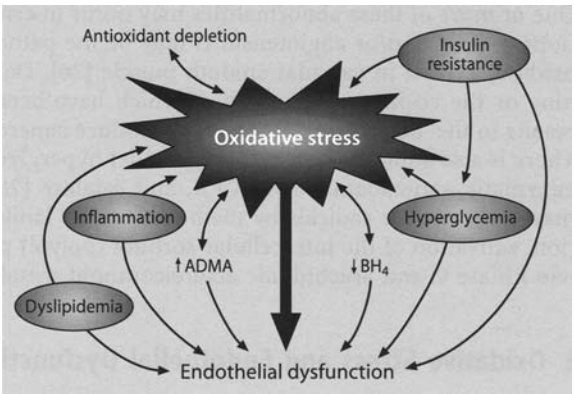

Fig. 2. Factors contributing to endothelial dysfunction in critical illness.
ADMA: asymmetrical dimethylarginine. BH_4: tetrahydrobiopterin.

represent a manifestation of the neuroendocrine stress response, but they may also be side-effects of treatment. Lipid and lipoprotein metabolism can be modified by the inflammatory response [23]. Inflammation results in a dyslipidemia characterized by hypertriglyceridemia, decreased plasma HDL-cholesterol concentration, and increased plasma concentration of small, dense LDL-cholesterol. Asymmetric dimethylarginine (ADMA) is an endogenously produced inhibitor of eNOS. Plasma ADMA concentrations are elevated in critically ill patients [24]. ADMA accumulates by the combination of increased proteolysis and decreased eliminatory pathways. Hyperglycemia, insulin resistance, dyslipidemia, and high plasma concentrations of ADMA each have specific inhibitory actions on the L-arginine-NO pathway. However they are all also associated with increased oxidative stress and their adverse endothelial effects may be predominantly mediated by this mechanism [25].

Oxidative Stress

The term oxidative stress refers to a condition in which cells are subjected to excessive levels of free radicals, or ROS. Under normal circumstances, around 98% of the oxygen consumed by mammalian cells can be accounted for by the catalytic reduction of oxygen to water by mitochondrial cytochrome C, without the release of radicals. The other 2% undergoes sequential one electron reductions to form superoxide anion and hydrogen peroxide. Cytosolic and mitochondrial superoxide dismutase (SOD), cytosolic glutathione peroxidase (CPX), and peroxisomal catalase contribute to the maintenance of minimal intracellular concentrations of superoxide anion and hydrogen peroxide, along with a number of non-enzymatic antioxidants. Other oxidants which have relevance to vascular biology include peroxynitrite, hydrochlorous acid, the hydroxyl radical, reactive aldehydes, lipid peroxides, and nitrogen oxides.

Increased oxidative stress may arise as a result of several potential mechanisms:

- increased ROS generation by eNOS, NAD(P)H oxidase, or other metabolic pathways
- impaired expression/activity of SOD, CPX, or catalase
- reduced levels of non-enzymatic antioxidants, such as glutathione, α-tocopherol, or ascorbate

One or more of these abnormalities may occur in critical illness [8]. High concentrations of lipid and/or angiotensin II may be the pathological stimulants of NAD(P)H oxidase, at least in vascular smooth muscle [26]. Deficiency of the substrate L-arginine or the co-factor BH_4, both of which have been described in the critically ill, results in the 'uncoupling' of eNOS to produce superoxide anions instead of NO [27]. There is also some evidence to suggest that hyperglycaemia lowers the activity of the enzymatic antioxidants, SOD, CPX, and catalase [28]. Furthermore, hyperglycemia may generate free radicals by methods such as labile glycation, glucose auto-oxidation, activation of the intracellular sorbitol (polyol) pathway, and stimulation of protein kinase C and arachidonic acid/eicosanoid metabolism [29].

Oxidative Stress and Endothelial Dysfunction

Endothelial function may be impaired during oxidative stress secondary to the direct damaging effects of free radicals on lipid, protein and DNA. Cell membrane structure and function may thus become adversely modified, and the expression and activity of important metabolic enzymes may be reduced. Oxidative stress may also affect the L-arginine-NO pathway. The superoxide radical can directly inactivate endothelial-derived NO [30]. NO reacts with superoxide at a diffusion limited rate to form peroxynitrite. NO is one of the few molecules produced at sufficiently high physiological concentrations to compete with SOD for superoxide. Not only does superoxide directly inactivate NO via the formation of peroxynitrite (which is also damaging to cells), but in the presence of redox active transition metals, superoxide can initiate oxidation of LDL [31]. This, in turn, may cause vascular dysfunction. The inhibitory effect of oxidized LDL on endothelial function is thought to be the result of specific lipid oxidation products, of which lysophosphatidylcholine appears to be the most potent. A number of studies have shown that both native and oxidized LDL can inhibit the synthesis and release of NO by endothelial cells, or attenuate its biological activity [32].

The expression/activity of eNOS may be affected by the redox state. Oxidation of sensitive regulatory thiol groups on eNOS have been shown to impair enzyme function [33]. Oxidative stress may also deplete NADPH and BH_4, both essential cofactors for NO synthesis. In addition, depletion of BH_4 causes eNOS to increase the production of superoxide anion, further exacerbating oxidative stress and endothelial dysfunction [27]. NOS-mediated superoxide production may originate from either the flavin domain of the enzyme, the heme domain, or both. The shift in eNOS production from NO to superoxide results in decreased NO bioavailability.

Oxidative stress may reduce the sensitivity of guanylate cyclase to NO [34]. NO binds to the sixth co-ordinate site in the heme cofactor of guanylate cyclase. NO-dependent enzyme activation occurs only when the heme iron is in the Fe^{2+} state. When the heme iron is in the Fe^{3+} state, NO does not activate the enzyme.

Recent research has suggested that, as well as producing free radicals, the inflammatory cascade may be stimulated by them. NF-κB is a ubiquitous rapid response transcription factor involved in the inflammatory activation of endothelial cells. ROS have been shown to increase NF-κB activation [35]. Further inflammatory stimulation of the endothelium by NF-κB may potentiate endothelial dysfunction.

ADMA, the endogenous inhibitor of eNOS, is metabolized by the enzyme dimethylarginine dimethylaminohydrolase (DDAH). This enzyme can be inhibited by oxidation [24]. As a consequence, ADMA can accumulate in situations of increased oxidative stress. In the presence of high concentrations of ADMA, eNOS uncouples pro-

ducing superoxide instead of NO, which leads to further oxidation of DDAH, further accumulation of ADMA and endothelial dysfunction.

Finally, experimental evidence also suggests that oxidative stress may regulate endothelial cell apoptosis, i.e., programmed cell death. Free radicals such as the hydroxyl radical can induce endothelial cell apoptosis [36]. Enhancement of apoptosis may be another mechanism whereby oxidative stress can contribute to impaired endothelial function.

Antioxidants

Antioxidants are substances whose presence in relatively low concentrations can significantly inhibit the rate of oxidation. Natural antioxidants can be classified as enzymatic and non-enzymatic. The enzymatic antioxidants are SOD, catalase, and GPX. A number of trace elements are necessary co-factors for these enzymes (e.g., selenium for GPX, zinc for cytosolic SOD, and manganese for mitochondrial SOD).

The non-enzymatic antioxidants can be subdivided into scavengers and transition metal chelators. Scavenging antioxidants react with free radicals to prevent tissue damage. In scavenging free radicals these molecules are themselves oxidized. The scavenging antioxidants may be water-soluble (ascorbate, glutathione, urate) or lipid-soluble (α-tocopherol, carotenoids).

α-tocopherol (vitamin E) is the principle antioxidant in the lipid phase of cell membranes. Glutathione, a tripeptide containing a sulfhydryl group, is present in millimolar concentrations intracellularly and acts as both a substrate for CPX and as a direct scavenger of radicals and their metabolites. Ascorbate (vitamin C) is the most effective aqueous phase antioxidant in human plasma. It readily scavenges superoxide, peroxyl and hydroxyl radicals. Ascorbate can also act as a co-antioxidant by regenerating α-tocopherol from the α-tocopheroxyl radical, produced via scavenging of lipid-soluble radicals. This is a potentially important function as *in vitro* experiments have shown that α-tocopherol can act as a pro-oxidant in the absence of co-antioxidants such as ascorbate. Ascorbate has also been shown to regenerate urate, glutathione, and β-carotene *in vitro* from their respective one-electron products.

Other potential non-endogenous antioxidants include the xanthine oxidase inhibitors and the lazaroids. Xanthine oxidase has been implicated in superoxide formation during the reperfusion of ischemic tissues. There is some experimental evidence to show that allopurinol, a xanthine oxidase inhibitor, can act as an antioxidant in this situation [37]. The lazaroids are 21-aminosteroids which inhibit iron-dependent lipid peroxidation. Their clinical antioxidant role remains uncertain.

In vivo Assessment of Endothelial Function

Endothelial function cannot be measured directly in humans *in vivo*. Estimates may be obtained indirectly by measuring endothelium-dependent vasodilatation (to physical or pharmacological stimuli), plasma levels of endothelium-derived regulatory proteins, and microalbuminuria (Table 2). In addition, assays for urine NO_3^- and urine cGMP can be used to estimate whole body NO activity/production. However, abnormalities in the plasma levels of endothelial-derived proteins may signify endothelial stimulation rather than endothelial damage, and assays for NO activity in the urine are heavily affected by diet.

Table 2. Markers of endothelial dysfunction

Marker	Interpretation
Impaired endothelial-dependent vasodilatation	Decreased bioactivity of endothelial-derived vasodilators e.g., nitric oxide
Von Willebrand factor	Increased thrombotic activity
Plasminogen activator inhibitor-1	Decreased fibrinolytic activity
sE-selectin, sICAM-1, sVCAM-1	Increased leukocyte adhesion to endothelium
Microalbuminuria	Increased endothelial permeability

ICAM: intercellular adhesion molecule; **VCAM**: vascular cell adhesion molecule

There is evidence to suggest that markers of endothelial dysfunction may be prognostic for clinical outcome. Impaired endothelium-dependent vasodilatation predicts future cardiovascular events in patients with ischemic heart disease [38]. Impairment of endothelium-dependent vasodilatation has also been reported to independently predict postoperative outcome in high-risk vascular surgery patients [39]. Microalbuminuria has been found to be predictive of outcome in a wide variety of acute conditions including trauma, surgery, ischemia-reperfusion, acute pancreatitis and meningitis. In a recent prospective observational study in critical care, microalbuminuria within 15 minutes of ICU admission was as good a predictor of death as APACHE II or simplified acute physiology score (SAPS) II probabilities after 24 hours [40].

Antioxidants and Endothelial Dysfunction

A number of *in vivo* studies have shown that antioxidants may reverse NO-mediated endothelial dysfunction in patients with cardiovascular disease, or risk factors for cardiovascular disease [7, 41]. Antioxidants may improve endothelial NO bioavailability by increasing NO synthesis, by decreasing NO destruction, or by enhancing end-organ sensitivity to NO. In addition, antioxidants may attenuate the inflammatory activation of endothelial cells, and may even suppress endothelial cell apoptosis.

Pre-treatment with the antioxidant, ascorbic acid, can attenuate the adverse hemodynamic effects of experimentally-induced acute hyperglycemia [42]. High-dose ascorbic acid can also prevent hyperglycemia-induced endothelial dysfunction in healthy human volunteers [43]. Many studies have noted an association between stress-induced hyperglycemia and poor clinical outcome during acute illness. Strict glycemic control has been reported to improve morbidity and mortality in critically ill patients [22]. Unfortunately, tight control of blood sugars in intensive care is difficult and not without risk. Even with intensive insulin therapy, it may take up to 24 hours to achieve constant normoglycemia in such patients [44]. As endothelial dysfunction may be important in mediating the adverse effects of acute hyperglycemia, systemic supplementation with ascorbic acid may have a protective role during the period of time between the detection and the correction of hyperglycemia. Clinical studies are required to test this novel hypothesis.

In experimental models of sepsis, both prophylactic and delayed administration of ascorbic acid may protect microvascular function [45]. Intra-arterial infusion of

ascorbic acid has been observed to counteract impairment of endothelium-dependent vasodilatation during *Escherichia coli* endotoxemia [46]. N-acetylcysteine has also been shown to significantly attenuate endotoxin-induced alterations in leukocyte-endothelial cell adhesion and macromolecular leakage in rats [47].

A meta-analysis of clinical studies in critical illness has suggested that trace elements and vitamins that support antioxidant function, particularly selenium, either alone or in combination with other antioxidants are safe and may be associated with a reduction in mortality [8]. The high-dose parenteral route appeared to have a stronger impact on outcome than the enteral route. There is limited clinical research on the use of antioxidants as prophylaxis against endothelial dysfunction in critical illness. A study in 37 severely burned patients, reported that adjuvant administration of high-dose ascorbic acid (66 mg/kg/h) during the first 24 hours after injury significantly reduced resuscitation fluid volume requirements, body weight gain, and wound edema formation [48]. A reduction in the severity of respiratory dysfunction was also apparent. The study did not, however, show a reduction in mortality. A randomized, single center, prospective trial of antioxidant supplementation (α-tocopherol and ascorbic acid) in critically ill surgical patients revealed a significant reduction in the incidence of organ failure and a shortened length of ICU stay [49]. A large multicenter randomized trial to confirm these benefits is now warranted.

Conclusion

Impaired endothelial function in critical illness may be due in part to increased oxidative stress. Endothelial dysfunction is predictive of clinical outcome. Targeting protective therapies at the endothelium may help to prevent the occurrence of infective complications or the progression to MOF. Clinical studies in patients with endothelial dysfunction secondary to atherosclerosis, hypertension, dyslipidemia, and diabetes, have shown that antioxidants can improve endothelial function. Antioxidants may exert their beneficial vascular effects via a number of direct and indirect mechanisms. Antioxidant modification may be a simple and inexpensive adjunct therapy in critical illness. Further clinical investigation into the potential benefits of antioxidant therapy in critical care is urgently required.

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Plasma Cortisol: Time to Look Deeper?

J. Cohen, J. Prins, and B. Venkatesh

Introduction

The importance of the stress response in survival from critical illness is not in dispute. Adrenalectomized animals or patients with documented adrenal insufficiency have a high mortality when exposed to physiological stress [1, 2]. However, in the setting of critical illness, attempting to determine what constitutes an appropriate stress response is not straightforward.

Evidence of activation of the hypothalamo-pituitary axis is a frequently used surrogate for induction of the stress response. In the normal patient population, investigation of the hypothalamo-pituitary-adrenal (HPA) axis centers around measurements of plasma cortisol, often before and following stimulation with high doses of synthetic adrenocorticotropic hormone (ACTH). Criteria have been developed based on hormonal profiles and responses to stimulation tests to define inadequate stress responses or a condition which is loosely termed relative adrenal insufficiency. Whilst there is acceptance of the concept of relative adrenal insufficiency, the diagnostic criteria for it, based on plasma cortisol and the response to ACTH, continue to generate controversy. These issues have been reviewed at length in a recent publication by Venkatesh et al. [3].

Interest has begun to turn to free cortisol estimation, and early work in this area is encouraging [4, 5]. An area which has received little attention in critical illness, but is gaining widespread consideration in endocrine practice, is the extent of alteration in cortisol metabolism, which in turn will determine the exposure of tissues to adequate concentrations of cortisol.

Normal Cortisol Metabolism

Cortisol, the major glucocorticoid synthesized by the adrenal cortex plays a pivotal role in normal metabolism. Its secretion is under the control of the hypothalamic pituitary axis. There are a variety of stimuli to secretion, including stress, tissue damage, cytokine release, hypoxia, hypotension and hypoglycemia. These factors act upon the hypothalamus to favor the release of corticotropin releasing hormone (CRH) and vasopressin. These in turn stimulate the secretion of ACTH, which stimulates the release of cortisol, mineralocorticoids (principally aldosterone), and androgens from the adrenal cortex. During periods of stress, trauma or infection, there is an increase in CRH and ACTH secretion and a reduction in the negative feedback effect, resulting in increased cortisol levels, in amounts roughly proportional to the severity of the illness [6–8].

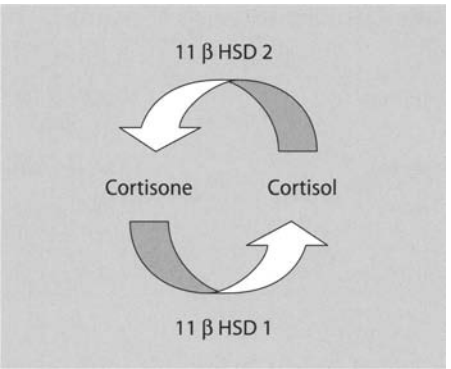

Fig. 1. The cortisol–cortisone shuttle

The majority of circulating cortisol is bound to an alpha-globulin called transcortin (corticosteroid-binding globulin, CBG). At normal concentrations of total plasma cortisol (e.g., 375 nmol/l or 13.5 μg/dl) less than 5% exists as free cortisol in the plasma; however, it is this free fraction that is biologically active. In normal subjects CBG can bind approximately 700 nmol/l (i.e., 25 μg/dl) [9]. At levels greater than this, the increase in plasma cortisol is largely in the unbound fraction. CBG is a substrate for elastase, a polymorphonuclear enzyme that cleaves CBG, markedly decreasing its affinity for cortisol [10]. This enzymatic cleavage results in the liberation of free cortisol at sites of inflammation. CBG levels have been documented to fall during critical illness [4, 11, 12], and these changes are postulated to increase the amount of circulating free cortisol.

Until recently, it was believed that the major determinant of cortisol activity *in vivo* was the plasma free cortisol concentration. However, it has become apparent that cortisol activity inside cells is modulated by the actions of an enzyme system, the 11 β hydroxysteroid dehydrogenases, type 1 (11β-HSD1) and type 2 (11β-HSD2). These enzymes are responsible for the interconversion of active cortisol and inactive cortisone (Fig. 1) [13]. The concentration of cortisol at the receptor site is critical in determining its action and is a balance between synthesis and metabolism. Whilst a number of enzymes play a role in the metabolism of cortisol (11β-HSD, 5 alpha and beta reductases, and 6 beta hydroxylase), quantitatively the activity of 11β-HSD is the most important pathway (Table 1). 11β-HSD modulates the selectivity, specificity, and intensity of glucocorticoid dependent processes and regulates intracellular concentrations of cortisone (inactive) and active cortisol. The enzyme was discovered in 1953 by Amelung and colleagues who described the interconversion of cortisol and cortisone [14]. The 11β-HSD1 isoform has a widespread expression throughout the body, being found primarily in liver, lung, adipose tissue, vascular tissue, ovary, and central nervous system (CNS). Its primary action *in vivo* appears to be reductase, catalyzing the formation of active cortisol from inactive cortisone. Previously it was thought the enzyme was bi-directional in action, but it now appears that it only exhibits dehydrogenase activity in disrupted cells. The reason for this is not clear, but it may be related to the specific intracellular localization of the enzyme [15]. Conversely 11β-HSD2 functions physiologically only in the dehydrogenase mode, catalyzing the formation of cortisone from cortisol. 11β-HSD2 has its major site of action in the kidney, where it functions to inactivate cortisol prior to its binding and activation of the mineralocorticoid receptor. The tissue distribution of these

Table 1. Structure and activity of the 11β-HSD enzymatic system

	11β-HSD1	11β-HSD2
Structure	Bidirectional, but mainly reductase, NADPH cofactor	Dehydrogenase, NAD cofactor
Function	Converts cortisone to cortisol	Converts cortisol to cortisone
Tissue	Liver, lung, gonads, pituitary, brain	Kidney, colon, salivary glands, placenta
Inhibition by carbenoxolone	Moderate	Strong

enzymes plays a major role in the regulation of glucocorticoid and mineralocorticoid receptor activation. For example, the higher concentration of 11β-HSD2 in the kidney prevent excess mineralocorticoid effects in the renal tubules from circulating cortisol. Similarly, in the placenta, 11β-HSD2 protects the fetus from the deleterious effects of maternal glucocorticoids.

The set point of total body 11β-HSD1 vs. 11β-HSD2 activity may be estimated by measuring the serum ratio of total cortisol to total cortisone (F:E ratio) [16–18]. The normal plasma F:E ratio ranges between 4–7 [19]. Abnormalities of the HSD enzyme system and, therefore, altered metabolism of cortisol have been implicated in the pathogenesis of hypertension, obesity, vascular disease, and the metabolic syndrome [20–23].

Role of 11β-HSD Activity in Human Disease

Syndrome of Apparent Mineralocorticoid Excess

Defects in the gene encoding for 11β-HSD have been described in patients suffering from apparent mineralocorticoid excess, an extremely rare inherited hypertensive disorder [24]. The condition is characterized by low levels of renin and aldosterone, severe hypertension and hypokalemia. This syndrome is thought to be caused by defective peripheral conversion of cortisol to cortisone. Patients thus appear to be suffering from excess mineralocorticoid activity, despite measured levels being reduced. The lack of 11β-HSD2 activity in these individuals allows cortisol to activate the mineralocorticoid receptor and function as a potent mineralocorticoid. Management by blocking the mineralocorticoid receptor with spironolactone appears to be effective.

Obesity and Metabolic Syndrome

The role of 11-βHSD1 appears to be to increase glucocorticoid activity in tissues in which it is expressed. Its potential importance in the pathogenesis of a wide variety of common clinical conditions is now becoming apparent. Mice with an over expression of 11β-HSD1 in adipose tissue have been shown to have higher levels of glucocorticoids in the portal vein, and exhibit obesity, hyperglycemia, and hypertension [25]. This observation, coupled with studies in human obese subjects, has led to the suggestion that 11β-HSD1 activity may be associated with the development of the metabolic syndrome, a group of cardiovascular risk factors including insulin resistance, hypertension, and obesity. Early work on selective 11β-HSD1 inhibitors in

animal models has demonstrated decreases in blood glucose, triglycerides, and body weight [26].

Miscellaneous

11β-HSD1 activity has also been associated with the development of osteoporosis [27], the pathogenesis of polycystic ovary syndrome [28], and in the regulation of vascular tone [29].

Activation of 11β-HSD in Stress and Critical Illness

While there has been a great deal of interest in the local tissue activity of the 11β-HSD system, there has been relatively little work into its systemic role. Relative changes in the activity of the isoenzymes could influence glucocorticoid availability at a tissue level. This would be of particular relevance in patients subjected to a significant stress response.

There are some limited human data to support the notion that an upregulation of 11β-HSD-1 occurs in response to stress. Vogeser et al. examined the serum of 15 unselected hospitalized patients having C-reactive protein (CRP) estimations. Using the serum F:E ratio as a marker of the set point of total body HSD activity they demonstrated a significant correlation between elevated CRP levels and the F:E ratio ($r=0.56$, $p<0.001$); multivariate regression analysis showed that this association was independent of serum cortisol [30]. The same group used a similar methodology to determine HSD activity in cardiac surgical patients. Postoperatively, they were able to demonstrate a significant increase in the F: E ratio as compared to the preoperative level (11.3 vs. 5.4, $p<0.001$), which persisted throughout the four days of the study [31]. In contrast, the serum cortisol level doubled on the first postoperative day, but then declined. The authors hypothesized that these data suggested that after surgical stress increased activity of 11β-HSD1 would be a more chronic response, acting to increase glucocorticoid action at a tissue level, despite declining plasma cortisol concentrations.

To our knowledge there are currently no published data examining 11β-HSD activity in the critically ill. However, our group has produced some data (unpublished) in patients with severe sepsis, trauma, and burns which support the previous findings. Persistent elevations in F:E ratios in patients suffering stress suggest that the set point of 11β-HSD activity is shifted towards reductase activity; this implies that the tissue activity of cortisol is increased, despite steady or even declining serum cortisol concentrations.

Mechanisms Behind Activation of 11β-HSD in Critical Illness

Multiple mechanisms might come into play to account for the differential regulation of the 11β-HSD enzyme systems. The observed increase in the F:E ratio could be explained by substrate overload of 11β-HSD2; however, if this were the case, plasma cortisol concentrations and the F:E ratio would be expected to change in parallel, which was not observed [31]. Direct mediation by ACTH is also unlikely given that F:E ratios have been shown to increase in non-ACTH-dependent hypercortisolism [32].

More likely mechanisms would be enhancement by inflammatory mediators, and transcriptional regulation from other hormones. Pro-inflammatory cytokines have been demonstrated *in vitro* to upregulate 11β-HSD1 activity. Studies on cell cultures have demonstrated that tumor necrosis factor (TNF)-α enhances cortisol availability to the cell by enhancing the activity of 11β-HSD1 and suppressing that of 11β-HSD2 [33, 34]. Such effects have been demonstrated in a variety of cell types including smooth muscle [35], lung epithelium [36], and adipose tissue [37].

Hormones acting on 11β-HSD1 expression include glucocorticoids, growth hormone, sex steroids, insulin, and thyroid hormone. Glucocorticoids increase expression, whilst growth hormone decreases it; the effect of the other hormones appears to vary from tissue to tissue and among species [38].

Other potential mechanisms for altered 11β-HSD1 activity include changes in redox potential within the cell. The oxo-reductase activity seen in intact cells requires NADPH and leads to the activation of glucocorticoids. Thus, concentrations of NADPH (which in turn are determined by cytosolic redox) influence the activity of the enzyme [39]. Critically ill septic patients frequently demonstrate alterations in cellular redox potential mediated largely by endotoxin [40]. Patients with trauma and burns may also demonstrate changes in redox resulting from altered tissue perfusion [41].

Hypotheses for Future Research

Clearly the investigation of glucocorticoid activity at a tissue level in critically ill patients is at a very early stage. However, the recognition that a significant level of control is being exerted that is not directly observable from plasma cortisol measurements is of interest. An intriguing possibility is that measurements of 11β-HSD activity, either by F:E ratio or direct tissue estimation, may serve as valid markers for HPA axis activity in septic patients. It could be hypothesized that downregulation of 11β-HSD1 activity or an increase in 11β-HSD2 activity would lead to tissue hypocortisolism, manifesting as hypotension and inotrope dependence. This group of patients may benefit from steroid administration. Conversely, an upregulation of 11β-HSD1 activity, and/or downregulation of 11β-HSD2, would lead to an excess of tissue cortisol activity, manifesting as hyperglycemia and insulin resistance. In these patients, selective 11β-HSD1 inhibitors, such as benzothiazole derivatives, may have a role [42]. Further studies examining the tissue activity of each 11β-HSD isoenzyme, and of a size large enough to detect an association with outcome would be required to investigate this possibility.

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Glucose Control and Monitoring in the ICU

C. De Block and P. Rogiers

Introduction

Recently, stress hyperglycemia, occurring in the vast majority of critically ill patients, has become a major therapeutic target in the intensive care unit (ICU). Stress associated with critical illness induces the release of counter-regulatory hormones. In addition, several clinical interventions, such as administration of corticosteroids, enteral or parenteral nutrition, or dialysis, further predispose patients to hyperglycemia. Moreover, in critical illness, changes in carbohydrate metabolism occur resulting in insulin resistance and relative insulin deficiency.

Hyperglycemia is associated with adverse outcomes, not only after myocardial infarction, cardiothoracic surgery, and stroke, but also in the ICU. Achieving normoglycemia appears crucial to obtaining the benefits of insulin therapy, which include a reduced incidence of acute renal failure, accelerated weaning from mechanical ventilation, and accelerated discharge from the ICU and hospital. In addition, it is a cost-effective intervention. However, the advantages of normoglycemia must be weighed against the increased risk of hypoglycemia.

Obtaining normoglycemia requires considerable nursing effort, including frequent glucose monitoring and adjustment of insulin dose. Moreover, the inherent clinical perturbations of critically ill patients (fluctuating severity of illness, changes in nutritional delivery, off-unit visits to diagnostic imaging) produce frequent changes in insulin requirements. Current insulin titration is based on discontinuous glucose measurements, which may miss fast changes in glycemia. In a pilot study using continuous glucose monitoring, we observed that insulin therapy based on discontinuous glucose measurements failed to maintain normoglycemia in most subjects [1]. Similar to the continuous, online display of blood pressure and cardiac output for optimal titration of inotropes and vasopressors, continuous glucose monitoring, using a well-tolerated and accurate device, may help to signal changes in glycemia and to optimize titration of insulin therapy in the ICU.

Prevalence of Stress Hyperglycemia

Stress-induced hyperglycemia is very common in the ICU, being present in 50–85% of critically ill patients (Table 1) [1–22]. However, true prevalence of stress-induced hyperglycemia is difficult to assess because there are discrepancies in definitions, particularly regarding the cut-off level by which one defines hyperglycemia, in the homogeneity of study populations with in/exclusion of diabetic patients, in severity of illness, and in the timing of blood glucose sampling. In general, up to 25–30% of

Table 1. Prevalence of stress hyperglycemia in the ICU

	ICU type	number of patients	definition of hyperglycemia (mg/dl)	hyperglycemic patients (%)
Van den Berghe et al. [2]	surgical ICU	1548	>110	75
Egi et al. [3]	surgical ICU	783	>110	81
Van den Berghe et al. [4]	medical ICU	1200	>110	>85
Ligtenberg et al. [5]	medical ICU	1085	>180	28
Cely et al. [6]	medical ICU	100	>110	64
De Block et al. [1]	medical ICU	50	>110	74
Finney et al. [7]	mixed ICU	523	>110	>85
Freire et al. [8]	mixed ICU	1185	>110	59
Christiansen et al. [9]	mixed ICU	135	>110	100
Krinsley et al. [10]	mixed ICU	1826	>120	42
Umpierrez et al. [11]	mixed ICU	239	>126	56
Whitcomb et al. [12]	mixed ICU	2713	>200	27
Zimmerman et al. [13]	cardiothoracic ICU	342	>150	50
Latham et al. [14]	cardiothoracic ICU	984	>200	29
Swenne et al. [15]	cardiothoracic ICU	374	>120	95
Yendamuri et al. [16]	trauma ICU	738	>135	25
Laird et al. [17]	trauma ICU	516	>110	94
Sung et al. [18]	trauma ICU	1003	>200	25
Wintergerst et al. [19]	pediatric ICU	980	>110	87
Faustino and Apkon [20]	pediatric ICU	942	>120	75
Srinivasan et al. [21]	pediatric ICU	152	>126	86

patients admitted to the ICU have diabetes, and up to one third of critically ill patients present with previously unrecognized diabetes or glucose intolerance [23].

Etiology of Stress Hyperglycemia (Fig. 1)

The onset of stress hyperglycemia in critical illness is driven by excessive release of counter-regulatory hormones (glucagon, growth hormone, catecholamines, glucocorticoids) and cytokines (interleukin [IL]-1, IL-6 and tumor necrosis factor [TNF]-α) [24–26]. Counter-regulatory hormones inhibit hepatic glycogenesis and peripheral glycolysis while promoting gluconeogenesis, hepatic and muscle glycogenolysis, and peripheral lipolysis. Pro-inflammatory cytokines such as TNF-α, IL-1, and IL-6 may induce a state of peripheral and hepatic insulin resistance, and stimulate the hypothalamic-pituitary-adrenal axis.

In addition, several conditions may promote hyperglycemia during stress. These include diabetes, obesity, cirrhosis (which impairs glycogen storage), pancreatitis (insulin deficiency), increasing severity of illness, hypokalemia (impairs insulin secretion), bed rest and advancing age. Bed rest leads to peripheral insulin resistance via impaired skeletal muscle glucose uptake combined with increased fasting plasma insulin concentrations. In addition, several clinical interventions can worsen this picture, including administration of dextrose, enteral or parenteral nutrition, or drugs (corticosteroids, thiazide diuretics, phenytoin, phenothiazines, vasopressors), and dialysis [26, 27]. Moreover, alterations in carbohydrate metabolism contribute to the development of stress hyperglycemia [25, 28]. Hepatic glucose output is augmented more than two-fold in critical illness via increased gluconeogenesis and gly-

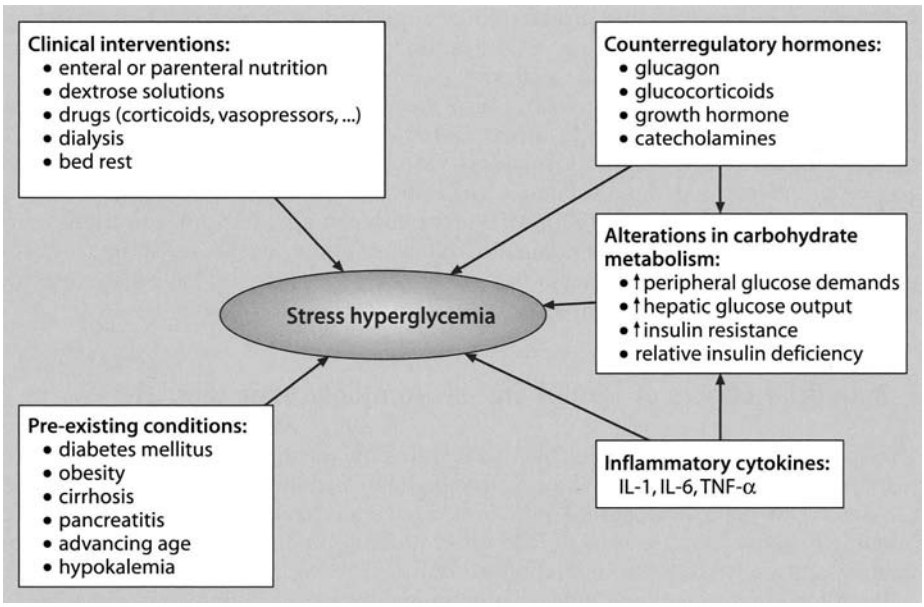

Fig. 1. Etiology of stress hyperglycemia – IL: interleukin; TNF: tumor necrosis factor

cogenolysis. Insulin resistance is characterized by increased hepatic glucose output, less insulin action in muscle (reduction of glucose uptake, glucose oxidation, glycogen synthesis and protein anabolism) and in adipocytes (increased lipolysis rate with consequently higher availability of free fatty acids and glycerol) and impaired insulin secretion.

Adverse Effects of Hyperglycemia

Manifest hyperglycemia promotes osmotic diuresis with hypovolemia and electrolyte abnormalities including hypokalemia, hypomagnesemia, and hypophosphatemia. Hyperglycemia may also worsen catabolism in skeletal muscle. Other mechanisms to explain the relationship between stress hyperglycemia and morbidity include an attenuated host defence, increased inflammatory cytokines, increased coagulability, endothelial dysfunction, increased oxidative stress, and changes in myocardial metabolism due to altered substrate availability [26–30].

Hyperglycemia adversely affects immune function and increases susceptibility to infection [31]. Hyperglycemia may also impair fibrinolysis and platelet function, which lead to hypercoagulability and an increased risk of thrombotic events [28, 29]. Moreover, glucose causes abnormalities in vascular reactivity and endothelial dysfunction. Endothelial dysfunction may result in a compromised microcirculation. Subsequent cellular hypoxia contributes to the risk of organ failure and death in critically ill patients [32].

In addition to cellular glucose overload, vulnerability to glucose toxicity may be due to increased generation and deficient scavenging of reactive oxygen species (ROS) produced by glycolysis and oxidative phosphorylation. Hyperglycemia-

induced mithochondrial overproduction of superoxide activates the four pathways (polyol pathway, protein kinase C activation, production of advanced glycation products, increased hexosamine pathway) involved in the pathogenesis of diabetic complications [33]. Hyperglycemia is also associated with increased levels of free fatty acids (FFA) which may 1) affect endothelial nitric oxide (NO) production, thereby impairing endothelium-dependent vasodilation; 2) increase myocardial oxygen requirements and thus ischemia; 3) decrease myocardial contractility; and 4) induce cardiac arrhythmias [30, 34]. Furthermore high FFA concentrations may increase ROS generation in mononuclear cells and induce insulin resistance in myocytes and hepatocytes. FFA excess has numerous consequences, called lipotoxicity, which is a critical feature of multi-organ failure (MOF) [35].

Beneficial Effects of Insulin and of Normoglycemia (Fig. 2)

The multiple potential benefits of insulin infusion during acute illness include a reduction in hyperglycemia via enhanced insulin-mediated glucose transport and via decreased hepatic glucose production, anabolic effects, positive influences on immune function, suppression of ROS generation, and positive effects on the endothelium and on hepatocytic mitochondrial ultrastructure and function [23].

First, insulin lowers blood glucose predominantly by increasing glucose uptake in insulin-sensitive tissues, particularly skeletal muscle [28]. Insulin also decreases hepatic glucose production by stimulating glycogen synthesis and by suppressing gluconeogenesis [25, 29]. Second, insulin has anabolic actions; it promotes muscle protein synthesis and inhibits lipolysis. Insulin may also provide myocardial protec-

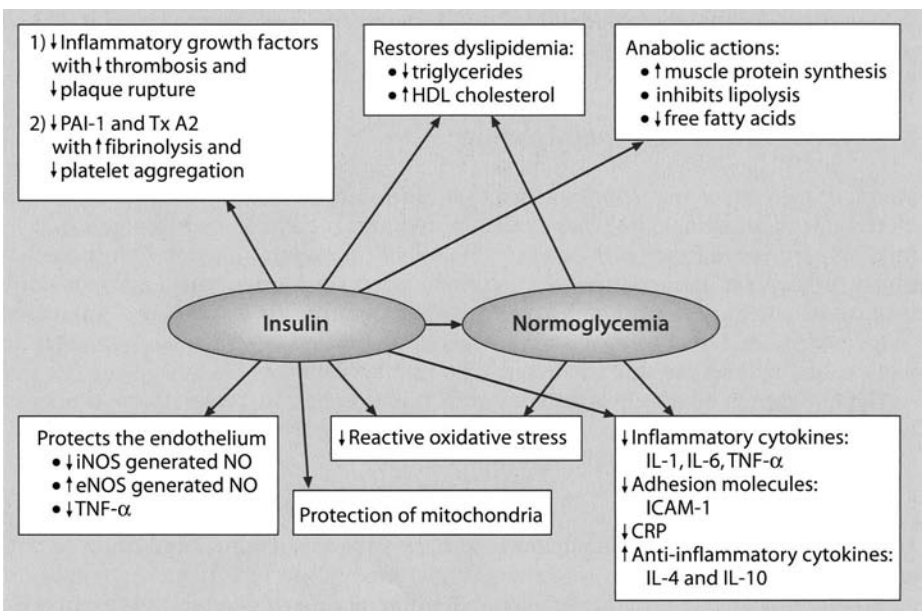

Fig. 2 Beneficial effects of insulin. Tx: thromboxane; HDL: high density lipoprotein; IL: interleukin; TNF: tumor necrosis factor; NOS: nitric oxide synthase; CRP: c-reactive protein; PAI: plasminogen activator inhibitor

tion during ischemia by suppressing FFAs and increasing availability of glucose as a myocardial substrate. In addition, insulin itself has direct cardioprotective effects during reperfusion, mainly via anti-apoptotic properties [28]. Third, intensive insulin therapy partially restores the dyslipidemia present in critically ill patients, which explains part of the beneficial effect on mortality and organ failure [35]. Fourth, insulin has a key inhibitory role in the regulation of inflammatory growth factors, which are central to atherogenesis, plaque rupture, and thrombosis, the final events which precipitate acute myocardial or cerebral ischemia and infarction. Insulin also reduces thromboxane A_2 production and plasminogen activator inhibitor-1 (PAI-1) activity, thereby decreasing platelet aggregation and increasing fibrinolysis [28, 29]. Fifth, insulin protects the endothelium via inhibition of excessive inducible NO synthase (iNOS)-generated release of NO [32]. Low NO concentrations generated by endothelial NOS are beneficial for the endothelium and organ function, whereas high NO levels, generated via iNOS may lead to endothelial dysfunction and tissue injury. On platelets, insulin exerts an anti-aggregatory action via induction of NO. Sixth, in euglycemic conditions, insulin appears to inhibit pro-inflammatory cytokines (TNF-α, IL-1, IL-6,) and adhesion molecules (soluble intercellular adhesion molecule-1), in addition to C-reactive protein [25]. TNF-α causes endothelial dysfunction and apoptosis, triggers procoagulant activity and fibrin deposition, and enhances NO synthesis in a variety of cells. Alternatively, prevention of hyperglycemia may contribute. Insulin also enhances the production of the anti-inflammatory cytokines, IL-10 and IL-4. Seventh, insulin suppresses ROS generation [25]. Finally, strict glycemic control with intensive insulin therapy prevents or reverses ultrastructural and functional abnormalities of hepatocytic mitochondria [36]. Mitochondrial dysfunction and the associated bioenergetic failure are regarded as factors contributing to MOF, the most common cause of death in the ICU.

Whether achieving strict normoglycemia or the administration of insulin is the decisive factor explaining the wide range of clinical benefits is still open to discussion. Strict control of hyperglycemia seems to be of paramount importance [2, 4, 7, 10]. A post hoc analysis of the Leuven study [2] revealed a linear correlation between the degree of hyperglycemia and the risk of death, which persisted after correction for insulin dose and severity of illness [27]. Patients in the conventional insulin treatment group who showed only moderate hyperglycemia (110–150 mg/dl or 6.1–8.3 mmol/l) had a lower risk of death than those with frank hyperglycemia (150–200 mg/dl) but a higher risk of death than those who were intensively treated with insulin to restore blood glucose levels to below 110 mg/dl. Similarly, for the prevention of morbidity (bacteremia, anemia, and particularly critical illness polyneuropathy), it appeared crucial to reduce glycemia to < 110 mg/dl. For the prevention of acute renal failure, insulin dose was an independent determinant. From all these data it is clear that the clinical benefits seen in critically ill patients are not just due to one single phenomenon. Many pathways may play a role; some of them being more dependent on achieving normoglycemia, whereas others are likely to be affected by non-glycemic, and even non-metabolic, effects of insulin.

Clinical Evidence for Achieving Normoglycemia in the ICU

In a variety of clinical settings, stress hyperglycemia has been shown to negatively affect patient morbidity and mortality. Even without a prior diagnosis of diabetes mellitus, hyperglycemia independently predicted poor outcome for patients sustain-

ing myocardial infarction [37–39], cardiothoracic surgery [2, 13, 22, 31, 40], stroke [41–43], or trauma [16–18].

Patients undergoing Cardiovascular and Cardiothoracic Surgery

Following acute myocardial infarction, hyperglycemia predicted increased rates of congestive heart failure, cardiogenic shock, and death [37–39]. A meta-analysis of 15 studies including over 6,000 patients, showed that among critically ill non-diabetic patients sustaining myocardial infarction, those with glucose levels in the range of 110–140 mg/dl had an almost 4-fold higher risk of death than patients who had lower glucose values [37].

Patients undergoing cardiothoracic surgery with concurrent perioperative hyperglycemia have increased morbidity rates including wound and sternal infection, pneumonia and urinary tract infection, and perioperative mortality rates [2, 13–15, 22, 31]. Insulin therapy to maintain blood glucose <150–200 mg/dl halved the rate of deep surgical site infections (mediastinitis, deep sternal, vein donor site) [22, 31]. Continuous insulin infusion therapy reduced absolute mortality by 57% [22]. In another study, tight glycemic control in diabetic patients undergoing coronary artery bypass grafting (CABG) lowered the incidence of atrial fibrillation, decreased recurrent ischemic events, and shortened postoperative length of stay [40].

Stroke Patients

Hyperglycemia has been reported to increase infarct size, worsen functional outcome, lengthen in-hospital stay and increase hospital charges [41–43]. A meta-analysis of 32 observational studies found that after stroke of either subtype (ischemic or hemorrhagic), admission glycemia of 110–144 mg/dl (6.1–8.0 mmol/l) was associated with a 3-fold increased risk of in-hospital 30-day mortality in non-diabetic patients and a 1.3-fold increased risk in diabetic patients [41]. Acute and final infarct volume change and outcome were negatively affected in patients with mean blood glucose levels ≥ 126 mg/dl (7 mmol/l) as measured by conventional and continuous glucose monitoring [42].

Trauma Patients

In trauma patients, hyperglycemia proved to be an independent predictor of mortality and of in-hospital and ICU length of stay, when controlling for age, injury severity score, and gender [16–18, 44]. In addition, infectious complications, including pneumonia, urinary tract infections, wound infections, and bacteremia, were significantly increased in hyperglycemic patients [16, 18, 44]. Ventilator days were also higher in patients with hyperglycemia [44].

Critically Ill Patients Admitted to the Intensive Care Unit

The landmark study of Van den Berghe et al. [2] in a surgical ICU ($n=1,548$), mainly composed of cardiothoracic surgery patients, showed that intensive insulin therapy aimed at maintaining glycemia between 80–110 mg/dl reduced the overall in-hospital mortality by 34%, blood stream infections by 46%, acute renal failure requiring dialysis or hemofiltration by 41%, critical illness polyneuropathy by 44%, and transfusion requirements by 50%. It also reduced the need for prolonged

mechanical ventilatory support, and the length of ICU stay. The benefit of intensive insulin therapy was particularly apparent among patients requiring intensive care for more than 5 days [2].

In the medical ICU study by the same group, comprising 1,200 patients, intensive insulin therapy significantly reduced the incidence of newly acquired renal failure, accelerated weaning from mechanical ventilation, and accelerated discharge from the ICU and the hospital [4]. In contrast to patients in the surgical ICU, those in the medical ICU had no significant reduction in bacteremia, which may be explained by the fact that among medical ICU patients sepsis often triggers admission to the ICU. In addition, in-hospital mortality was only reduced among patients staying in the ICU for ≥ 3 days. Most likely, the beneficial effects of intensive insulin therapy require time to be realized. Indeed, the intervention is not aimed at curing disease, but at preventing complications. In addition, the potential benefit of glucose regulation may be small because of the high mortality caused by the underlying diseases.

In a retrospective review of 1,826 critically ill medical and surgical patients, the lowest hospital mortality occurred in patients with mean glycemia between 80–99 mg/dl [10]. Importantly, there was no difference in mortality based on the presence or absence of diabetes. Independent predictors of mortality were APACHE II score and glycemia. In an extension study including 1,600 patients, Krinsley noted a 75% reduction in newly acquired renal insufficiency, a 19% reduction in the number of patients undergoing transfusion of packed red blood cells, a 11% decreased length of stay in the ICU and a 29% reduction in mortality in patients treated with intensive insulin therapy [45]. Insulin therapy in this study aimed to reach glucose values <140 mg/dl. However, no mortality benefit of intensive insulin therapy was apparent in patients with APACHE II scores ≥ 35 [45]. In patients with acute respiratory distress syndrome (ARDS), hyperglycemia was associated with critical illness polyneuropathy and myopathy, causing prolonged mechanical ventilation and ICU stay [46].

In another prospective ICU single center study including 531, mainly cardiothoracic, patients, Finney et al. observed a mortality benefit with a speculative upper limit of 145 mg/dl for the target blood glucose level [7]. In a retrospective study of 7,049 critically ill patients, not only mean glycemia, but also the variability of blood glucose concentration, were independent predictors of ICU and in-hospital mortality [47]. The authors, therefore, suggested that reducing the variability of glycemia might be an important aspect of glucose management.

It is not clear whether the relation between acute hyperglycemia and increased mortality risk is consistent for all critically ill patients. In the study by Umpierrez et al. the mortality rate for newly hyperglycemic patients in the ICU approached one in three [11]. Freire et al., studying 1,185 medical ICU patients, did not find admission hyperglycemia to independently predict in-hospital mortality [8]. Ligtenberg et al., retrospectively studying 1,085 consecutive patients admitted to a mixed ICU, suggested that higher glucose levels reflect disease severity, but are not an independent risk factor for mortality [5]. Whitcomb et al., reviewing records from 2,713 ICU patients, concluded that the association between admission hyperglycemia and in-hospital mortality was not uniform. Hyperglycemia was an independent risk factor only in patients without a history of diabetes in the cardiac, cardiothoracic, and neurosurgical ICUs [12]. In a retrospective study of 783 surgical ICU patients, Egi et al. calculated that the number needed to treat to prevent an ICU death varied between 38 and 125, at the cost of approximately 9 cases of hypoglycemia. This wide variation in number needed to treat depended on baseline mortality and case selection [3].

At the latest Scientific Sessions of the American Diabetes Association, Falciglia et al. presented data on 216,775 critically ill patients and confirmed that hyperglycemia was an independent predictor of mortality in the medical, surgical and cardiac ICU, starting at 1 mg/dl above normal glucose levels (111 mg/dl). The impact of hyperglycemia on mortality was variable but was most pronounced in stroke patients (relative risk 3.4–15.1), followed by acute myocardial infarction patients (relative risk 1.6–5.0). A weaker impact was seen in sepsis, pneumonia, and pulmonary embolism. However, in some conditions such as chronic obstructive pulmonary disease and liver failure, glycemia seemed not to affect mortality. The effects seen were also greatest in patients without diagnosed diabetes.

In contrast to the above-mentioned trials, the multicenter German study (the VISEP trial), designed to randomize 600 subjects with medical or surgical severe sepsis to conventional or intensive insulin therapy, was stopped after recruitment of 488 subjects because of no difference in mortality and frequent hypoglycemia in the intensive insulin therapy arm (12.1 vs 2.1%) [48]. However, the experimental design failed to exclude confounding variables by not controlling for conventional aspects of sepsis care (antibiotics, resuscitation, mechanical ventilation). The results of ongoing multicenter studies (Normoglycemia in Intensive Care Evaluation and Survival Using Glucose Algorithm Regulation [NICE-SUGAR] enrolling 3,500 patients in Europe, and the Comparing the Effects of Two Glucose Control Regimens by Insulin in Intensive Care Unit Patients [GLUCONTROL] enrolling 1,500 pateints in Australia, New Zealand and Canada) are anticipated in 2007.

Aggressive treatment of hyperglycemia with insulin may, however, be limited by an increased risk of hypoglycemia. Recognition of hypoglycemia in a patient who is receiving sedatives and analgesics with or without neuromuscular blocking agents in the ICU is problematic, potentially leaving the hypoglycemic state unappreciated for a critical period before treatment. In addition, the response to hypoglycemia may be blunted in critical illness. The reported rates of hypoglycemia vary between 0–30%, but differences as to its precise definition make comparisons difficult. The VISEP study was stopped prematurely because of this increased hypoglycemia risk [48]. Interestingly, despite the obvious increase in hypoglycemic events, no adverse clinical outcomes associated with hypoglycemia have been reported in any of the studies. Hemodynamic deterioration, convulsions, or other events were not noted during hypoglycemic episodes. Independent risk factors for hypoglycemia, aside from intensive insulin therapy, include a prolonged ICU stay (>3 days), renal failure requiring dialysis, and liver failure [4]. In addition, there is always the possibility of the occasional human error. Insufficient frequency of glucose monitoring may also contribute.

Pediatric ICU

In the retrospective study of Wintergerst et al. including 980 non-diabetic pediatric ICU patients, 87% of subjects had blood glucose levels >110 mg/dl. In their study, not only hyperglycemia, but also increased glucose variability and hypoglycemia were associated with increased length of stay and mortality [19]. Faustino and Apkon, studying 942 non-diabetic PICU patients, found a correlation between mortality risk, length of stay and hyperglycemia [20]. Srinivasan et al. showed that peak blood glucose and duration of hyperglycemia were independent predictors of mortality in a group of pediatric ICU patients receiving vasoactive infusions or mechanical ventilation [21].

Management of Hyperglycemia

The preponderance of stress-hyperglycemia has encouraged intensivists to apply early, tight glycemic control without a complete understanding of when (threshold), in whom (population), and how early (timing), this intervention should be started. Also the optimal level of glycemic control is not known. The first step in the management of stress hyperglycemia is to identify and treat the most common precipitating causes. Second, the patient population in which insulin therapy might benefit, should be clearly defined. Third, consensus should be obtained regarding the target level of glycemia. Fourth, glycemic excursions should be carefully monitored, preferably on a continuous base, and a comprehensive, validated, easily implementable insulin infusion protocol should be provided.

In which Patients should Intensive Insulin Therapy be Applied and What is the Target Glycemic Level?

The risk/benefit ratio for intensive insulin therapy may change according to baseline mortality, patient selection, and ICU type (e.g., post-cardiac surgery ICU, neurologic ICU, trauma ICU, medical ICU) as shown by Whitcomb et al. [12]. Thus, different ICUs should carefully consider formal decision analysis of the possible benefits and risks of intensive insulin therapy before implementing such a protocol.

The Surviving Sepsis Campaign guidelines recommend maintaining a blood glucose level of <150 mg/dl in patients with severe sepsis [49]. Finney et al. observed the best survival when mean glycemia was between 110–145 mg/dl [7], whereas Krinsley observed the lowest hospital mortality in patients with mean glycemia between 80–99 mg/dl [10]. The target glycemia in the Leuven studies was 80–110 mg/dl [2, 4]. The American Diabetes Association and the American College of Endocrinology have issued guidelines recommending in-hospital intensive insulin therapy to maintain preprandial blood glucose levels at ≤ 110 mg/dl and postprandial glycemia <180 mg/dl in critical care patients [50]. The preferred method of insulin administration in critical illness is continuous insulin infusion using a dynamic scale protocol with frequent blood glucose measurements. Data are difficult to interpret because of the diverse clinical settings, the varying methods of insulin administration, and the different targets and timing of glycemic control. While any single cut-off value by definition is arbitrary, we and others believe that we should aim for a blood glucose that is as near to normal as is safe and practical. The potential for improvement in ICU patient outcomes, combined with a low-cost drug, make intensive insulin therapy an attractive option.

Glucose Control and Monitoring in the ICU

Insulin requirements vary widely in patients depending on insulin production reserves, insulin sensitivity, caloric intake in the ICU, the nature and fluctuating severity of the underlying illness and the administration of medications. The need for a protocol to guide the prescribing and monitoring of insulin infusions is evident due to the significant heterogeneity and dissatisfaction with current insulin infusions. The analysis of the correct amount of insulin to be administered requires a relatively high degree of skill, and this expert assessment will need frequent revision as the clinical situation changes. Therefore, the physician who may be most knowledgeable about the optimal methods of administration of insulin (the endocrinolo-

gist) should be a member of the team caring for a critically ill patient. Goldberg et al. proposed an insulin infusion protocol that was based primarily on the velocity of glycemic change rather than on absolute blood glucose levels, and on the current insulin infusion rate [51]. The complexity of an insulin infusion protocol requires at least a 2-to-1 patient-to-nurse ratio.

There are many obstacles to implementing insulin infusion protocols in an ICU. Insulin infusion protocols add significantly to the work of managing ICU patients. Every hour, the nurse must perform a glucose measurement, document the results, and make the necessary adjustments to the insulin drip. This process may take up to 3–5 minutes every hour (2 hours per day). Moreover, a prevalent fear of hypoglycemia may hinder the widespread acceptance of intensive insulin infusion protocols. Training, education and continuing feedback is necessary to motivate ICU nurses. Kanji et al. showed that standardization of i.v. insulin therapy improved the efficiency and safety of glycemic control in critically ill adults, improved nursing acceptance, but also increased the workload as 35% more glucose measurements were required with the intensive insulin protocol [52].

In the future, the development of a closed-loop control system that automatically regulates the dose of insulin based on glucose measurements could permit tight glycemic control without increasing the workload of the nursing staff. An accurate continuous glucose monitoring system combined with an algorithm for calculation of the appropriate insulin infusion rate are pre-requisites for the establishment of such an automated glycemic control system. Plank et al. observed that compared with routine protocols, treatment according to a fully automated model predictive control algorithm resulted in a significantly higher percentage of time within the target glycemic range (80–110 mg/dl) [53].

How to Evaluate Glycemic Control in the ICU?

An objective measure of hyperglycemia for assessing glucose control in acutely ill patients should reflect the magnitude and duration of hyperglycemia. In studies of acutely ill patients, regular indices of glucose regulation that have been used are admission glucose, maximum glucose, and mean glucose. However, they are based on either a single measurement or on a subset of measurements, and, therefore, they are not indicative of overall glycemia. Just as we prefer continuous, online display of blood pressure and/or cardiac output for optimal titration of inotropes and vasopressors, a continuous display of blood glucose levels seems mandatory for optimal titration of insulin therapy in the ICU [54, 55].

Continuous Glucose Monitoring in the ICU

Strict glycemic control improves clinical outcomes in critically ill patients. In addition, reducing variability in blood glucose concentrations might be an important aspect of glucose management [47]. Implementation of strict glycemic control in daily ICU practice may be facilitated by a continuous glucose monitor.

Current continuous glucose monitoring systems measure interstitial glucose concentrations. However, previously published data on the reliability of continuous glucose monitoring systems in diabetic patients cannot be automatically transferred to a different situation like intensive care, where many variables can interfere with performance of such systems (e.g., subcutaneous edema, hypotension, vasoactive

drugs). A precise evaluation of the accuracy of the system and the quality of sensor performance in the ICU setting is necessary, and must represent the premise of every clinical or research utilization of these devices. The following requirements have to be met by continuous glucose monitoring systems: 1) immediate availability of the measurement result, 2) high frequency of measurements, 3) fast sensor signal stability after application and over time [56].

Current continuous glucose monitoring systems measure glucose in the interstitial fluid. Under physiological conditions there is a free and rapid exchange of glucose molecules between blood plasma and interstitial fluid and, for this reason, changes in blood glucose and interstitial fluid glucose are strongly correlated [56]. Nevertheless, changes of glucose concentrations in interstitial fluid lag behind those in the blood. The lag time seems to be consistent, irrespective of increments/decrements in glycemia and insulin levels. In the ICU setting, the hemodynamic alterations encountered (hypotension, shock, vasopressor or inotropic need) did not affect accuracy [1, 57]. Such variables would rather affect the process of subcutaneous glucose recovery, resulting in a calibration issue, rather than in a sensor performance issue. This could be solved by frequent calibration [1]. Calibration should be performed in times of glucose stability [56]. In any case, a lag time of < 10 min is clinically acceptable since online adjustment of insulin dose should be based on immediate detection of unacceptable rates of change (> 25 mg/dl/h).

The Continuous Glucose Monitoring System® (CGMS, Medtronic Minimed, Northridge, CA, USA) is currently approved by the U.S. Food and Drug Administration (FDA) as a 'retrospective' Holter-style glucose monitor. It is a percutaneous 'needle-type' sensor, measuring glucose in the interstitial fluid every 5 minutes for up to 72 hours. The GlucoDay® device (A. Menarini Diagnostics, Florence, Italy) is based on the microdialysis technique that measures glucose concentrations in the dialysate from subcutaneous interstitial fluid. It is approved by the European Community (CE). Glucose concentrations are measured every 3 min by the glucose sensor over a 48-h period [1].

Only a few studies have used continuous glucose monitoring systems in critically ill patients [1, 42, 57, 58]. In a pilot study, we investigated the accuracy and applicability of the GlucoDay® continuous glucose monitoring device in the medical ICU [1]. Fast changes in glycemia were noted immediately (Fig. 3), whereas this was noted much later (~1–3 hours) when only using intermittent blood glucose measurements. Hyperglycemia was present in 74% of MICU patients and target glycemia (80–110 mg/dl) was reached only 22% of the time, revealing the inadequacy of current insulin protocols and the potential of an accurate continuous glucose monitoring system in this setting. Similar results were reported by Goldberg et al. investigating the use of the CGMS® device in the medical ICU [57]. No adverse events were noted in either study [1, 57]. Vriesendorp et al. investigated the use of the GlucoDay® device during and after surgery and encountered a high technical failure rate [58], which was mainly attributed to breaking of the microdialysis fiber during transfer from the surgical bench to the ICU bed. In our study, only one fiber broke. Baird et al. using the GlucoDay®, observed that acute and final infarct volume change and outcome were negatively affected in patients with mean blood glucose levels ≥ 126 mg/dl (7 mmol/l) [42].

Javid et al. have tested the Extracorporeal Glucose Monitoring System (EGMS®, Medtronic Minimed, Northridge, CA) in patients on extracorporeal bypass. This pilot study suggested that the EGMS is a reliable tool for continuous blood glucose monitoring in critically ill patients on extracorporeal life support, cardiopulmonary bypass, and renal replacement therapy [59].

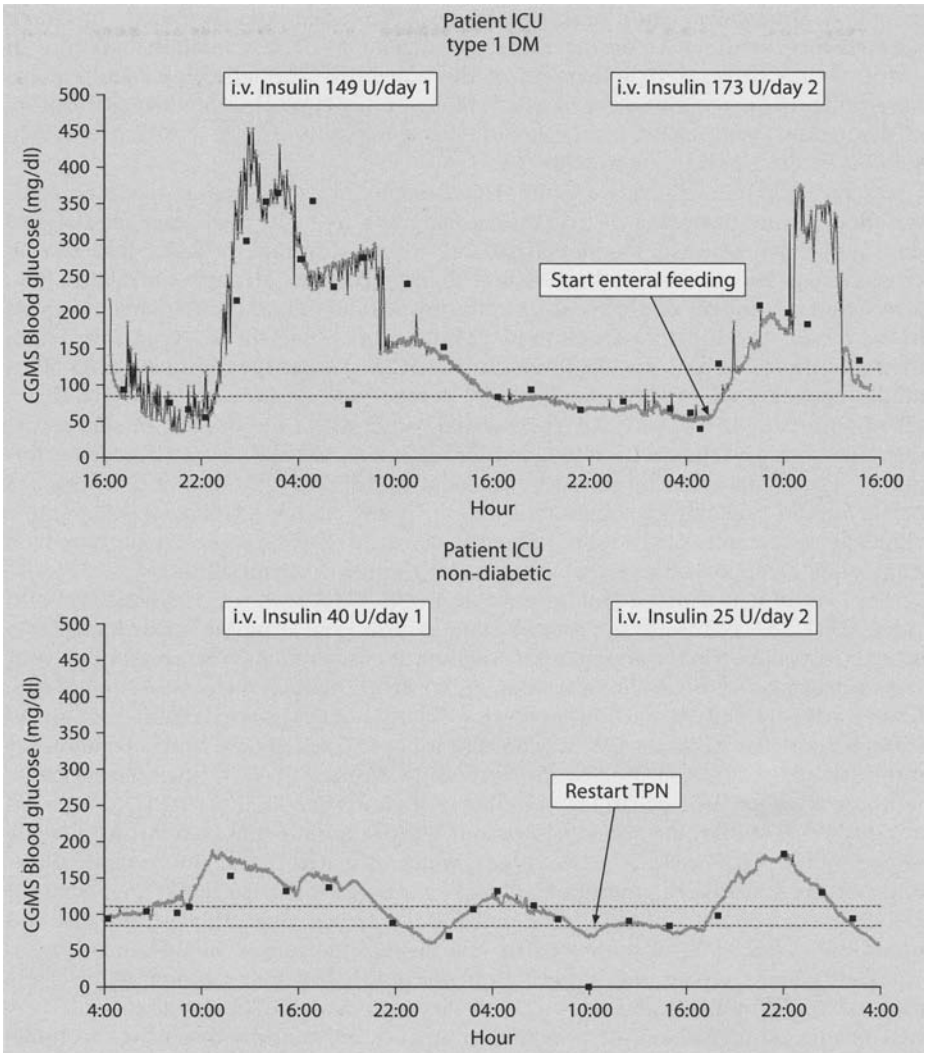

Fig. 3. Examples of continuous glucose monitoring profiles in ICU patients. Top panel: A patient with brittle type 1 diabetes mellitus in cardiogenic shock; enteral feeding was started after 36 h; lower panel: A stable non-diabetic patient admitted due to respiratory insufficiency; total parenteral nutrition (TPN) was started after 30 h. Little squares are arterial blood glucose readings.

Chee et al. conducted a study to determine if continuous subcutaneous glucose monitoring using the CGMS® could be used in real-time to control glycemia in five critically ill patients [55]. They concluded that the automatic sliding scale approach of closed-loop glycemic control is feasible in patients in ICU, but more work is needed in the refinement of the algorithm and the improvement of real-time sensor accuracy.

How to Use Data Obtained with Continuous Glucose Monitoring?

The presentation of the vast amount of data collected during continuous glucose monitoring must be made in an easy to understand fashion so that the physician can interpret it adequately. First, the continuous glucose monitoring system should display the actual glucose measurement and in the future a warning alarm should be available if the actual glucose value is below or above a predefined target value. Second, continuous glucose monitoring provides trend information. By presenting the direction of glucose changes, this trend analysis may provide additional information to take preventative actions in time. It might be possible in the future, using complex mathematical trend analysis, to predict the course of glucose changes for longer time periods ahead. Third, continuous glucose monitoring data provide an accurate impression of the blood glucose profile over 24 hours a day, thereby detecting many glucose fluctuations. Fourth, the profiles of several days can be superimposed to detect specific glucose patterns in specific time periods. Thus, continuous glucose monitoring provides information about the direction, magnitude, duration, and frequency of glycemic fluctuations. Continuous glucose monitoring will permit smoother, timelier adjustments in insulin infusions to more quickly achieve target glycemia and it will provide early warning about incipient hypoglycemia.

In conclusion, our data and those of Goldberg et al. suggest that using continuous glucose monitoring in critically ill patients looks promising [1, 57]. If further developed as a 'real-time' glucose sensor, continuous glucose monitoring technology could ultimately prove clinically useful in the ICU, by providing alarm signals for impending glycemic excursions, rendering intensive insulin therapy easier and safer. Closed loop systems, with computer-assisted titration of insulin dose, will go a step further and will reduce nursing workload and lower the risk of hypoglycemia. The European community-funded CLINICIP (Closed Loop Insulin Infusion for Critically Ill Patients) project aims to develop a low-risk monitoring and control system that allows health care providers to maintain strict glycemic control in ICUs using a SC-IV closed loop system.

Cost-effectiveness of Achieving Normoglycemia in the ICU

Controlling hyperglycemia in patients with either known diabetes or newly discovered hyperglycemia in the hospital has been shown to be cost-effective in many settings. Van den Berghe et al. showed that in her surgical ICU, the extra costs of intensive insulin therapy, which were nearly double the cost of the conventional treatment, were more than offset by a 25% reduction in the total hospitalization costs [60]. Intensive insulin therapy resulted in improved medical outcomes, and a reduced length of stay in the ICU and in the hospital, thereby resulting in an estimated annual cost savings of $ 40,000 (31,400 €) per ICU bed. Intensive insulin therapy proved to be cost-effective, saving $ 3,360 (2,638 €) per patient [60]. The cost savings occurred because of reductions in ICU length of stay and several morbid events such as renal failure, sepsis, blood transfusions, and mechanical ventilation dependency. Krinsley et al. also found intensive insulin therapy to be cost-effective in their mixed medical-surgical ICU, with a net annualized decrease in costs of $ 1,580 (1,240 €) per patient [61]. The savings associated with the intensive glucose management program were, however, not shared equally among the different patient groups. The largest net savings occurred among surgical, cardiac, and gastrointesti-

nal patients. Due to a reduction in hospital length of stay, intensive glycemic control allowed the hospital to serve more patients per bed and generated further income from new patient groups. Thus optimizing glycemic management is not only medically effective, saving lives and reducing morbidity, but also cost-effective to health care systems.

Conclusion

Recently, stress hyperglycemia has become a major therapeutic target in the ICU. Stress hyperglycemia affects the vast majority of critically ill patients and is associated with adverse outcome, including increased mortality. Intensive insulin therapy to achieve normoglycemia may reduce mortality and morbidity, with a reduced incidence of acute renal failure, accelerated weaning from mechanical ventilation, and accelerated discharge from the ICU and hospital. Optimal benefits appear to be achieved with a maintenance of glycemia <110 mg/dl. In addition, achieving normoglycemia is cost-effective. However, reaching and maintaining normoglycemia requires extensive efforts from the medical staff, including frequent glucose monitoring and adjustment of insulin dose. Current insulin titration is based upon intermittent glucose measurements, which may miss fast rises or falls in glycemia. Recent evidence suggests that continuous monitoring of glucose levels may help to signal glycemic excursions and eventually to optimize titration of insulin therapy in the ICU.

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Severe Lung Infections

Current Concepts of Severe Pneumococcal Community-acquired Pneumonia

M. Luján, C. Muñoz-Almagro, and J. Rello

Introduction

Community-acquired pneumonia (CAP) is a major health problem, even in developed countries, being the leading cause of death due to infectious diseases in the USA [1]. CAP has a wide clinical spectrum of severity: up to 80% of patients are successfully managed in primary care, but 1% of patients with CAP are classified as having severe disease, needing intensive care unit (ICU) admission, with 20–50% dying despite all available support and treatment options being utilized. *Streptococcus pneumoniae* is the most common cause of CAP, enclosing the subset group of patients having severe disease [2]. Moreover, bacteremia is not uncommon in pneumococcal CAP (20%) and has been associated with increased severity and mortality compared with non-bacteremic pneumonia [3].

The morbidity and mortality of severe pneumococcal CAP have remained essentially unchanged despite emergence of new antimicrobial options and improvements in critical care medicine. Our failure to improve the outcome from pneumococcal CAP may in part be due to the aging population and the increased burden of comorbid illnesses including larger numbers of immunosuppressed patients due to chemotherapy or human immunodeficiency virus (HIV); however our basic understanding of why people die from this disease is still poor. Several prognostic factors are known to be associated with adverse outcome, however morbidity and mortality are more likely determined by the result of complex interactions between the host's defenses, the virulence of the infecting strain and possibly the timing and choice of

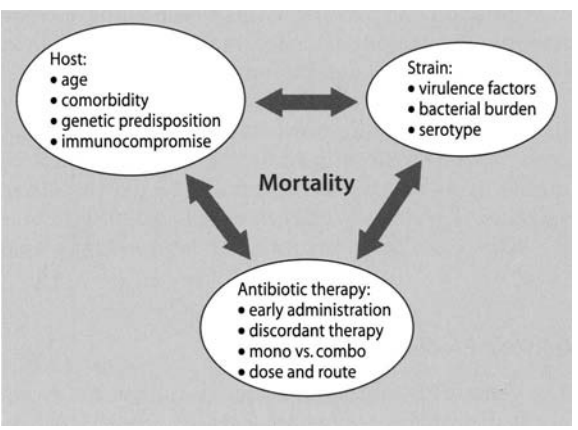

Fig. 1. Interaction of factors influencing survival. From [58] with permission

antibiotic therapy, than the presence or absence of one or more risk factors. The knowledge of these interactions could also explain the variability in the clinical presentation of pneumococcal CAP (Fig. 1).

Microorganism-host Interactions

Age and Comorbidity

Although there is a great variability in studies reporting the influence of age on mortality, there is no doubt that it is one of the main prognostic factors in CAP. Even in the pre-antibiotic era, age was a significant predictor of mortality [4], and the Applied Physiology And Chronic Health Evaluation (APACHE) scores have a significant weighting for age [5]. A meta-analysis suggested that each 10-year age increment represents an increase of 5% in the likelihood of death (OR = 1.05 95% CI 1.01 – 1.09) [6]. Moreover, demographic variables (mainly age) constitute the first step of one of the more commonly used outcome prediction rules in CAP [7].

Nevertheless, it is noteworthy that advanced age (>65 years old) was an independent and protective factor (OR = 0.35) associated with early failure in a cohort of 1383 patients with CAP [8]. This finding supports the hypothesis that the main cause of death in old patients is related more to decompensation of underlying comorbidities or to their deteriorated baseline status than to the severity of the acute inflammatory process. This study [8] also demonstrated that patients at risk of early clinical failure have more severe pneumonia at admission (multilobar pneumonia, pneumonia severity index [PSI] >90 at admission) suggesting that in those early failures despite adequate antibiotic coverage, a genetic mediated predisposition could play an important role (see below).

Nearly all studies in CAP have reported increased mortality in patients with baseline comorbidities, although the influence of each specific comorbidity varies from study to study. Neurologic and neoplastic comorbidities were the most powerful predictors of increased mortality in one meta-analysis [6]. The influence of chronic obstructive pulmonary disease (COPD) is particularly controversial, with a recent study suggesting it may be a protective factor [9], whereas in others it constitutes a risk factor for mortality [10]. Further studies are needed to address this issue further.

Another controversial issue is the impact of vaccination status. The 23-valent pneumococcal polysaccharide vaccine (Pneumovax, Merck) is not 100% efficacious in preventing invasive pneumococcal disease and did not prevent non-bacteremic pneumonia in a recent study. [11], but in another study, focused on elderly people, the 23-valent vaccine prevented pneumococcal pneumonia (with or without bacteremia) and decreased the rates of overall pneumonia and of mortality due to pneumonia in this subset of patients [12]. On the other hand, pneumococcal conjugate vaccines (7-valent, 9-valent and 11-valent) provide adequate coverage for children <2 years old against serotypes most frequently associated with penicillin-resistance [13].

Genetic Predisposition

The genetic risk for severe pneumonia is usually underestimated in clinical practice, but it is probably the major factor in unexpected mortality in young and previously

healthy patients and in the variability in clinical presentation in patients with similar baseline status and same infecting strain.

Briefly, when the host recognizes the presence of foreign antigens through specific antigen recognition pathways (predominantly the innate but also the acquired immune system), a pro-inflammatory reaction is initiated, in order to eradicate the infecting strain. At the same time, an anti-inflammatory reaction is mandatory to counteract potential deleterious effects of pro-inflammatory mediators. An imbalance between these two reactions can lead to a deficient response to infection. Thus, an excessive pro-inflammatory response, or a deficient anti-inflammatory response, could lead to septic shock or secondary organ damage, and conversely, a deficient pro-inflammatory or enhanced anti-inflammatory reaction could lead to persistent infection [14]. The main pro-inflammatory cytokines so far identified as being important in CAP are tumor necrosis factor-alpha (TNF-α), lymphotoxin-α (LTA), interleukin (IL)-1 and 6, and the principal anti-inflammatory mediators appear to be IL-10 and IL-1 receptor antagonist (IL-1ra).

The host response against pulmonary infection is represented by the innate and adaptive components of the immune system. The innate immune system is considered as the first line of defense against invading microorganisms. The classical and alternate complement pathways play a key role in the innate response, but there are other pathways that contribute to the opsonization of microorganisms. One of these is the lectin pathway, mannose-binding lectin (MBL) being one of the opsonins. Several genetic polymorphisms (mutant alleles with a frequency in the general population of $>1\%$) are associated with a low plasma concentraion of MBL. In a case-control study, Roy and co-workers [15] found that homozygosity for several of these alleles is associated with very low levels of serum MBL, and indeed, with inadequate opsonic function. Consistent with the reduction in function being clinically relevant, the low-function associated mutations of MBL were over represented (OR= 2.59) in patients with invasive pneumococcal disease. Moreover, polymorphisms associated with deficiency of MBL in serum have been associated with an increased risk of developing systemic inflammatory response syndrome (SIRS) and progression of infection to sepsis and septic shock [16].

Toll-like receptors (TLRs) are also part of the innate immune defense and recognize structurally conserved pathogen-associated molecular patterns (PAMPs). TLR-2, and probably TLR-4, are involved in the host innate immune response to pneumococcal infection in mice models [17]. In humans, deficient TLR-mediated cytokine production was associated with recurrent pneumococcal bacteremia in a child [18].

Surfactant proteins are one of the most important molecules in the early stage of pulmonary infection. Surfactant-D (SP-D) seems to have a special ability to interact with several serotypes of pneumococci, clearing the microorganism from lungs and upper respiratory airways and delaying its appearance in bloodstream. Recently, Quasney and co-workers demonstrated that carriage of the C allele at the SP-B + 1580 locus was associated with acute respiratory distress syndrome (ARDS), septic shock, and the need for mechanical ventilation in a cohort of 402 adults with CAP [19].

TNF-α is one of the most extensively studied mediators, due to its critical role in the pro-inflammatory response against infection and its high degree of polymorphism, especially within the promoter region of the gene. The main problem in establishing a causative relationship between individual(s) genotypes and a TNF-α secretor pattern, and indeed outcome, is that there are several loci in linkage associ-

ation in the same region (chromosome 6) that could act as an etiologic factor or merely be a marker for other polymorphisms not just in TNF-α but in one of the many key inflammatory proteins located nearby. One of the best studied polymorphisms is TNF-308. This polymorphism is associated with two patterns of secretion. Carriage of A allele is associated with high TNF-α levels and is associated with septic shock [20], even in CAP [21], whereas polymorphisms associated with low TNF-α levels (G alleles in locus TNF-α-308) have a protective effect against the development of septic shock, but a trend to a greater risk of developing respiratory failure has been documented [21]. As mentioned earlier, another polymorphism in linkage disequilibrium with TNF-α-308 is the LTA +250 polymorphism. Thus, carriage of A alleles of LTA 250 is also associated with high-TNF levels, and inversely, carriage of G alleles is associated with low blood TNF levels, and both loci (LTA +250 and TNF-α-308) are in linkage disequilibrium. Similarly, the same authors reported more recently an association of carriage of heat shock protein (HSP) 70-2+1267 AA genotype and risk of septic shock in patients with CAP, with a stronger association than the LTA 250 genotype described earlier [22].

Anti-inflammatory cytokines are able to counterbalance harmful effects of the inflammatory response. In pneumococcal disease, IL-10 is one of the best studied anti-inflammatory mediators. In humans, the IL-10-1082 gene promoter polymorphism is associated with high IL-10 inducibility (G genotype) [23]. Recently Schaaf and colleagues [24] found that IL-10-1082 G>C was associated with an increased risk of pneumococcal septic shock.

The Virulence of the Infecting Pneumococcal Strain

The pathogenicity of pneumococci has been attributed to several components. The polysaccharide capsule was considered the primary virulence factor of *S. pneumoniae* because although pneumococcus exists in encapsulated and unencapsulated forms, only encapsulated strains have been recovered from clinical specimens. Classical studies carried out by Avery and Dubos [25] demonstrated that loss of the capsule is accompanied by a 100,000-fold reduction in the virulence of pneumoccoci. On the basis of differences in capsular polysaccharide structure, pneumococci can be divided into 90 serotypes, but less than 30 serotypes account for up to 90% of invasive disease in humans. Recent epidemiological studies report the dominant role of individual serotype in determining invasiveness. Brueggemann et al. [26] compared the distribution of invasive isolates with carriage isolates and showed that the potential of pneumococci to cause invasive disease differs by serotype. These authors observed that serotypes 6B, 19F, and 23F are commonly carried but have low invasiveness. Other serotypes, such as serotype 1, 5 or 7F, are rarely carried but they are common causes of invasive disease in some countries due to their high invasiveness. Other authors [27] have reported an association between capsular serotype and mortality; serotypes 3, 6B, and 19F were associated with higher mortality (25% vs 0%) when compared with serotypes 1 and 7F. A recent study has demonstrated that pneumococcal clones of serotype 1 and 7F, primarily infect previously healthy individuals and behave as 'primary pathogens' [28]. In contrast, other clones belonging to serotypes with a lower potential for causing invasive disease, behaved more like 'opportunistic pathogens'. In the majority of cases, infections with such clones cause disease in patients with underlying disease, and are associated with more severe disease.

Current studies suggest that certain pneumococcal proteins contribute significantly to the virulence of individual strains. These proteins are involved in direct interactions with host defense mechanisms. These proteins include hyaluronate lyase (Hyl), pneumolysin, neuraminidase A (NanA), neuraminidase B (NanB), major autolysin (LytA), choline binding protein A (CbpA), pneumococcal surface antigen A (PsaA), and pneumococcal surface protein A (PspA). Development of antibodies against some of these proteins may be a promising approach for use in a future vaccine and have recently been investigated [29]. The antigens that reduce virulence are the best candidates for vaccine development, such as pneumolysin, PspA, and LytA.

- Pneumolysin is a 53 kDa protein produced by all *S. pneumoniae* isolates. The virulence properties of pneumolysin are directly dependent on the action of autolysin. The role of pneumolysin is crucial particularly in the early stages of pneumococcal infection. Pmeumolysin has the ability to lyse cell membranes, activate complement, and stimulate the production of inflammatory cytokines, such as TNF-α and IL-1β [30]. Other studies have reported that low concentrations of pneumolysin are able to inhibit neutrophil and monocyte respiratory response, chemotaxis, bactericidal activity, and production of lymphokines and inmunoglobulins [31]. The cytolytic properties of pneumolysin cause damage to ciliated bronchial epithelial cells, alveolar epithelial cells, and pulmonary endothelium. The result is that the ability of ciliated bronchial cells to clear mucus and particles from the lower respiratory tract is reduced, facilitating the bloodstream spread of infection [32]. In a recent study in a mouse model, Witzenrath and coworkers demonstrated that pneumolysin may play a central role in early acute lung injury (ALI) in pneumococcal pneumonia by causing impairment of pulmonary microvascular barrier function and severe pulmonary hypertension [33].
- Pneumococcal surface protein A (PspA) exhibits structural and antigenic variability between different pneumococcal strains, but is present in most pneumococcal clinical isolates [34]. Hammerschmidt et al. [35] identified PspA as a lactoferrin-binding protein and demonstrated that PspA plays an important role in enabling iron acquisition by pneumococci. The function of PspA appears to be protection against the host's complement system [36]. Briles et al. [37] observed biological evidence of the anti-complementary properties and have shown that PspA reduces the complement-mediated clearance and phagocytosis of *S. pneumoniae.*
- Autolysin is responsible for cell wall lysis [38]. Cell wall degradation has significant physiological consequences. Autolysin activity generates cell-wall breakdown products, which are highly inflammatory and allows the release of intracellular toxins (e.g., pneumolysin).

Pneumococci contain many other proteins that could contribute to pathogenicity, but their role has not yet been completely established. Potential key proteins include hyaluronidase adhesins, and, especially, neuraminidase A and B. Their role in the development of upper and lower infection and sepsis has been emphasized in a recent study [39].

Penicillin-binding proteins (PBPs) are another group of virulence determinants. PBPs are a group of proteins located in the cell wall of pneumococci, and are the key proteins in the determination of penicillin-resistance. It has been hypothesized that pneumococci with reduced susceptibility to penicillin are less invasive than wild strains. In an experimental study in a mouse model, Magnusdottir and coworkers demonstrated that resistant strains had a significantly reduced ability to persist at

the infectious site, and to some extent also to induce infections, compared with fully susceptible strains [40]. In the clinical setting, there are several studies showing that invasive isolates are much more likely to be penicillin sensitive that non-invasive isolates [41]. It seems that the acquisition of resistance is counterbalanced by a 'biological cost' in terms of virulence.

Antibiotic Therapy

None of the conditions described above can be modified in patients admitted with severe pneumococcal pneumonia. Until immunomodulatory therapies appear as a true alternative treatment, genetic markers are interesting adverse prognostic factors but have no current role in clinical management. Currently, the efforts of the physician are directed towards optimizing supportive therapies and choosing the appropriate empiric antibiotic treatment.

In the antibiotic treatment of pneumococcal pneumonia, there are currently two important unresolved questions: The impact of discordant antibiotic treatment on mortality and the hypothesis that dual therapy could improve prognosis.

The Impact of Discordant Therapy on Mortality

The discovery in South Africa of penicillin resistant pneumococci [42] posed the question whether this acquisition of resistance would worsen the prognosis of patients with pneumococcal disease treated with beta-lactams. The answer to this question is not trivial, because it reflects the degree of correlation between the cut-off points used in the laboratory to define categories of susceptibility for pneumococcal strains and the clinical course in patients receiving those antibiotics considered sensitive or resistant *in vitro*.

The impact of penicillin resistance on outcome has been extensively investigated by a great number of experts. The most frequently employed definition of discordant therapy in these earlier studies included intermediate and resistant infecting strains. Early studies seem to suggest that the level of resistance had little effect on the outcome of pneumococcal pneumonia [43]. Surprisingly, few studies have recorded the administered empiric antibiotic therapy, dose, route, and interval of administration, and the definition of discordant therapy has been based only on the minimum inhibitory concentration (MIC). Furthermore, given the complexity of the pharmacokinetics and pharmacodynamics of antibiotic therapy (peak serum concentrations, time above MIC, mechanisms of resistance of different antibiotics) categorical classification based on an arbitrary MIC cut-off for the administered antibiotic seems simplistic [44]

One of the most important studies about this point analyzed the factors affecting mortality in a cohort of patients with pneumococcal pneumonia during the period 1995–1997. More than 5800 patients were included in the study and the authors found that mortality was significantly associated with strains with a penicillin MIC of 4 μg/ml or greater or a cefotaxime MIC of 2 μg/ml or greater, when controlled for underlying conditions and when deaths within the first 4 hospital days were excluded. The reason early deaths were excluded was for the known lack of any impact of antibiotic therapy early in the disease course [45]. Given the results of this and other studies, and following the recommendations of a panel of Centers for Disease Control and Prevention (CDC) experts, in 2002 the National Committee for

Table 1. MIC interpretive standards (in μg/mL) for non-meningeal pneumococcal infections according to the 2002 breakpoints [46]

Group	Antimicrobial agent	MIC (μg/ml) Interpretive standards		
		Susceptible	Intermediate	Resistant
Penicillins	Penicillin	≤ 0.06	0.12–1	≥ 2
	Amoxicillin	≤ 2	4	≥ 8
	Amoxicillin-clavulanate	≤ 2/1	4/2	≥ 8/4
Cephalosporins (parenteral)	Cefuroxime	≤ 0.5	1	≥ 2
	Cefotaxime or ceftriaxone	≤ 1	2	≥ 4
	Cefepime	≤ 1	2	≥ 4
Cephalosporins (oral)	Cefuroxime axetil.	≤ 1	2	≥ 4
	Cefaclor	≤ 1	2	≥ 4
Carbapenems	Imipenem	≤ 0.12	0.25–0.5	≥ 1
	Meropenem	≤ 0.25	0.5	≥ 1
Glycopeptides	Vancomycin	≤ 1	–	–
Macrolides	Erythromycin/Clarithromycin	≤ 0.25	0.5	≥ 1
	Azithromycin	≤ 0.5	1	≥ 2
Fluoroquinolones	Levofloxacin	≤ 2	4	≥ 8
	Moxifloxacin/Gatifloxacin	≤ 1	2	≥ 4
	Grepafloxacin/Sparfloxacin	≤ 0.5	1	≥ 2
Lincosamides	Clindamycin	≤ 0.25	0.5	≥ 1

Clinical Laboratory Standards (NCCLS) raised the breakpoints for susceptibility for pneumococcal non-meningeal infections (Table 1) [46].

Once the breakpoints had been changed, several studies tried to address whether this change led to a better correlation between susceptibility of strains and mortality. In a multicenter study, Yu and co-workers [47] included 844 patients with bloodstream pneumococcal infection from various sites, and found that neither the resistance to penicillin, nor initial discordant therapy were associated with mortality when non-meningeal infection was analyzed. Only discordant therapy with cefuroxime was related to mortality, and the authors argued that the most commonly employed dose (750 mg every 12 hours) was suboptimal to maintain serum levels above MIC for more than 50% of the dosing interval. Of note, in this study patients with several sources of infection were included, and only patients receiving monotherapy were included in the analysis of discordant therapy related to mortality. Interestingly, in a study including 100 patients with bacteremic pneumococcal pneumonia an excess mortality for those receiving discordant therapy was found [10].

What are the reasons for these discrepancies? Probably, most studies are underpowered because less than 15% of patients received discordant therapy, and we expect mortality to be less than 2% in Pneumonia Outcome Research Team severity index (PORT) I to III classes, which represents the vast majority of patients. It is likely that discordant therapy will have a stronger effect in PORT IV/V classes. Moreover, although the resistance and implications of discordant therapy with beta-lactams have been the most extensively studied, discordant therapy including macrolides has been demonstrated as being associated with therapeutic failure [48], and

therapeutic failure with non-pseudomonal fluoroquinolones [49] has also been reported. Are the implications of discordant therapy the same with all groups of antibiotics? The answer to this question is crucial to determine whether the current breakpoints correlate with clinical outcome.

Monotherapy or Dual-therapy for Bacteremic Pneumococcal Pneumonia

The current guidelines of several societies recommend the use of a combination of a beta-lactam plus a macrolide or monotherapy with a respiratory fluoroquinolone (levofloxacin, moxifloxacin) to treat hospitalized patients with CAP. In patients with severe CAP admitted to an ICU, combination therapy (beta-lactam/macrolide) is more usual, in part due to the lack of ventilated patients in randomized control trials evaluating fluoroquinolones as monotherapy. Some investigators have evaluated the outcome of patients receiving combination versus monotherapy in pneumococcal CAP in particular. Combination therapy has been suggested to have a favorable influence on outcome, but prospective, randomized controlled studies are lacking.

Mufson and Stanek [50] performed a retrospective study including 423 patients with bacteremic pneumococcal CAP over a study period of 20 years. The main results were that combination therapy including macrolides was associated with lower case-fatality rates. Nevertheless, their conclusions are weakened by important limitations, because information about sensitivity was not provided and adjustment for severity was not performed.

Waterer and co-workers [51] also retrospectively studied 235 patients with pneumococcal bacteremic CAP. Their hypothesis was that combination therapy with more than one effective antibiotic would be superior to monotherapy in bacteremic pneumococcal pneumonia. To exclude a potential influence of discordant therapy, patients with immunocompromise or strains resistant to prescribed therapies were excluded. Moreover, the group of patients receiving three or more antibiotic agents was excluded from analysis, due to substantially greater severity of disease. Dual empiric therapy was associated with higher survival rates when PSI >90, and the benefit was confirmed in a multivariate analysis (adjusted OR=6.4; 95% CI 1.9–21.7). Nevertheless, possibly due to the wide range of antibiotic regimens prescribed, it was not possible to demonstrate whether one concrete regimen could improve outcome.

More recently, Martinez et al. [52] retrospectively analyzed 409 patients with pneumococcal bacteremic CAP, during a study period of 10 years; 238 patients received a regimen containing a macrolide, whereas 171 did not. In the stepwise logistic regression analysis, lack of prescription of a macrolide in the initial antibiotic empiric therapy was associated with mortality (when adjusted for shock). Conclusions should be interpreted with caution due to the retrospective design of the study and the differences in the groups Thus, patients in the macrolide group were more likely to experience shock, whereas patients in the non-macrolide group were more likely to have a poorer baseline status: more comorbidities, more ultimately or rapidly fatal underlying disease, higher prior antibiotic exposure, steroid use, antineoplastic therapy and more likely to be infected with resistant strains.

Baddour et al. [53] analyzed the influence of combination antibiotic therapy in patients with pneumococcal bacteremia. Although prospectively conducted, the study was not a randomized controlled trial. Eight hundred and fourty-four patients with pneumococcal bacteremia from several sites (793 with pneumonia) were included, and 592 were evaluable for analysis of monotherapy versus combination

therapy. The 14-day mortality was not significantly different for all patients pooled together, but among critically ill patients, defined according to a Pitt bacteremia score >4, combination therapy was associated with lower mortality (23.4 versus 55.3%, $p<0.01$).

Conversely, Harbarth et al. [54] reported the lack of influence of mono versus combination empiric therapy at admission in a subset of 107 patients with monobacterial pneumococcal sepsis. Nevertheless, this study presents certain differences with respect to the others mentioned: first, immunocompromised patients were excluded; second, only six patients received fluoroquinolone-containing regimens; and most importantly, only a proportion of these patients had bacteremia. When only bacteremic patients (n=75) were analyzed, no statistical differences in short-term mortality were found, but the small sample size is clearly underpowered.

More recently, Dwyer et al. [55] found no effect of the addition of a macrolide to a beta-lactam based empiric regimen in case fatality-rate in a cohort of 340 patients with bacteremic pneumococcal pneumonia studied retrospectively. Finally, Rodriguez et al. (unpublished data), using a Cox proportional hazard model, adjusted for severity-of-illness, have recently documented that 28-day ICU mortality rate is significantly reduced in patients admitted to the ICU by CAP with shock, if they receive initial combination therapy.

Prospective controlled trials to address this question have been conducted, but the results have not yet clarified whether combination therapy is superior. Finch et al. [56] compared treatment with moxifloxacin versus amoxicillin-clavulanate with or without clarithromycin in patients with CAP. Mortality in both groups was equivalent but overall mortality was only 4.8%, meaning that the study was underpowered to identify differences in mortality. Similarly, the study of Frank et al. [57] compared levofloxacin versus ceftriaxone plus a macrolide in a cohort of 236 patients with CAP. No differences in mortality were found, but overall mortality was <2% in both arms.

In summary, several retrospective studies have suggested a superiority of combination therapy in comparison with monotherapy in severe pneumococcal pneumonia, but these results have not yet been supported by randomized controlled trials focused on severe pneumonia. Whether these findings can be extrapolated to non-bacteremic patients or in a full cohort of etiologies remains uncertain. Clearly, further studies are needed that focus on PORT IV-V class pneumonia, if endpoints are related to survival. Future studies should compare conventional dual therapy (such as beta-lactam plus macrolide) to monotherapy with newer fluoroquinolones.

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Respiratory Syncytial Virus (RSV) in the Pediatric Intensive Care Unit

M.C.J. Kneyber and F.B. Plötz

Introduction

It is more than half a century ago that Robert Chanock and co-workers recovered a cytopathogenic agent from lung secretions of young infants with lower respiratory tract disease that was similar to an agent that had been identified in an outbreak of infection resembling the common cold in chimpanzees [1, 2]. Because of its characteristic cytopathologic findings in tissue culture where it forms syncytia in epithelial cells, the virus was named respiratory syncytial virus (RSV) [1]. From serological studies, it was observed that almost all children have been infected by RSV by the age of two years [3]. Epidemiological research carried out since its discovery has designated RSV as the most important causative agent of viral lower respiratory tract disease [4]. Approximately 100,000 infants are admitted annually with RSV-induced bronchiolitis in the United States, and the number of hospitalizations is increasing [5]. Because of this, RSV-associated disease imposes a major burden on health care resources [6]. More recently, RSV is increasingly being recognized as an important pathogen causing severe lower respiratory tract disease in elderly and immunocompromised patients [7].

Each winter, pediatric intensivists are challenged by infants with lower respiratory tract disease due to RSV. This chapter summarizes the current knowledge regarding the role of RSV in the pediatric intensive care unit (PICU) and its possible therapeutic options.

Epidemiologic Aspects

RSV is classified within the genus *pneumoviridae* which is a member of the family of *paramyxoviridae*. It is a single stranded enveloped RNA virus. The RSV genome codes for 10 major proteins [8]. Of these proteins, the F (fusion) and the G (attachment) glycoprotein are the major surface antigenic determinants. Two antigenic strains of RSV, group A and group B, can be identified. Both groups co-circulate together but also independently from each other during annual epidemics [9]. The clinical spectrum of RSV-associated disease extends from mild upper respiratory tract infection to severe lower respiratory tract infection including bronchiolitis and pneumonia [4]. Re-infections occur frequently, although they tend to be mild [10].

Severe RSV infection necessitating mechanical ventilation occurs in 2–16% of previously healthy infants [11]. This percentage may increase among so-called 'high-risk' patients. Wang et al. enrolled 689 children younger than two years of age into the Pediatric Investigators Collaborative Network on Infections in Canada (PICNIC)

prospective cohort study [12]. They observed a higher need for mechanical ventilation among infants with congenital heart disease (19.3%), chronic lung disease (25.3%), compromised immune function (14.3%), prematurity (defined by a gestational age less than 37 weeks) (18.2%), and postnatal age below 6 weeks (16.8%) compared to infants with no risk factor (3.2%) [12]. The mean duration of mechanical ventilation may be as long as 10 days [13]. However, not unusually, alternative modes of ventilation, such as high-frequency oscillatory ventilation (HFOV) or extra-corporeal membrane oxygenation (ECMO), are required when severe impaired oxygenation or ventilation persists [11].

RSV is also a neurotrophic virus. We have observed that it causes apnea (defined by a cessation of respiration or a bradycardia with accompanying cyanosis for a period of 20 seconds or longer) in approximately one of every five patients with RSV [14]. Apnea may be the presenting symptom. The odds for mechanical ventilation are increased 6.5-fold in infants who present with RSV-associated apnea. The exact mechanism underlying RSV-associated apnea is unknown, although it is observed that the apnea is of central origin [15].

Usually mortality rates are less than 1% for previously healthy infants, although these percentages may increase up to 10% among high-risk infants [16].

Clinical Phenotype

Lower respiratory tract disease due to RSV is often referred to as 'bronchiolitis'. In fact, an increase in total respiratory system resistance compatible with obstructive disease has been demonstrated [17–20]. However, it is increasingly appreciated that RSV-associated lower respiratory tract disease is a heterogeneous disease, implicating that it is incorrect to label all RSV-associated lower respiratory tract disease as bronchiolitis [21]. It is being argued that RSV can present as an obstructive disease (defined by increased respiratory system resistance with subsequent decreased airflow on expiration, responsible for the audible wheezing) or a restrictive disease [22]. This discrimination is supported by work performed by Hammer et al. in 37 mechanically ventilated infants [19]. These infants were categorized as having obstructive or restrictive disease based upon the findings from pulmonary function testing. Ten infants had decreased respiratory system compliance (Crs) compatible with restrictive disease in conjunction with four-quadrant alveolar consolidation on chest radiograph compared to healthy controls. The remaining 27 children had increased respiratory system resistance (Rrs), compatible with obstructive disease. Infants with restrictive disease required prolonged ventilation compared to infants with obstructive disease. It should be noted, however, that infants with underlying diseases such as prematurity and/or chronic lung disease were also included for analysis. Among the infants with restrictive disease there were three infants with chronic lung disease; it cannot be excluded that these infants had pre-existing lung abnormalities that might have contributed to the altered respiratory system mechanics. For instance, prematurely born infants with chronic lung disease have higher Rrs that predispose them to symptomatic RSV-associated lower respiratory tract disease compared to controls [23].

In most PICUs however, pulmonary function testing is not done routinely. Yet, identification of the type of RSV-associated lower respiratory tract disease is clinically relevant because of the proposed differences in ventilatory strategies needed to treat obstructive or restrictive disease [22]. In addition, identification of the clinical

phenotype aids in targeting a specific population of infants for a therapeutic modality. An alternative for pulmonary function testing could be the use of ventilatory indices that characterize gas exchange. These include the oxygenation index and the alveolar-arterial oxygen gradient ($Aa\text{-}DO_2$). These indices could serve as an easy bedside tool to characterize the patient's pulmonary condition. Tasker and co-workers found an $Aa\text{-}DO_2 > 400$ mmHg during the first 24 hours of mechanical ventilation and a mean airway pressure > 10 cmH_2O associated with radiographic appearances suggestive of RSV restrictive disease [24]. All infants were previously healthy, and had four-quadrant alveolar consolidation on their chest radiograph on PICU admission. However, the definition of severe RSV-associated lower respiratory tract disease in this study was based upon a chest radiograph scoring system developed for prematurely born infants with infant respiratory distress syndrome (IRDS) and has not been validated for patients with RSV to our knowledge [25].

The findings of Hammer et al. [19] as well as those of Tasker et al. [24] are not conclusive. We have retrospectively studied parameters for gas exchange in 53 mechanically ventilated infants with RSV-associated lower respiratory tract disease admitted between 1995 and 2005, and were unable to detect significant differences in the oxygenation index or in the $Aa\text{-}DO_2$ between infants with radiological classified restrictive disease and obstructive disease (unpublished data). We further observed a comparable duration of mechanical ventilation between infants with obstructive and restrictive disease. Our findings suggest that RSV-associated lower respiratory tract disease is a heterogeneous disease that cannot be strictly dichotomized into restrictive and obstructive forms.

Importantly, there are no data on the short-term and long-term airway morbidity in mechanically ventilated infants with RSV-associated lower respiratory tract disease. It is known that post-RSV wheezing (i.e., recurrent wheezing during early childhood) is seen frequently among infants who were hospitalized with mild to moderate RSV-associated lower respiratory tract disease [26]. The exact mechanisms underlying post-bronchiolitis wheezing are unknown. For mechanically ventilated infants it can be hypothesized that the virus causes structural damage to the airways that might be exaggerated by mechanical ventilation. This paucity of data requires further investigations.

Concurrent Bacterial Infection

The majority of infants who are admitted to the PICU with RSV-associated lower respiratory tract disease will have antibiotics prescribed. Clinicians often assume that concurrent bacterial pulmonary infection is probably (partially) accountable for the development of respiratory failure due to RSV. Randolph et al. retrospectively studied the number of positive cultures from blood, urine, cerebrospinal fluid (CSF), and endotracheal aspirates on PICU admission among 63 mechanically ventilated previously healthy infants [27]. All of these infants were treated with antibiotics. They observed a low percentage ($< 2\%$) of concurrent bacterial blood stream infection. In addition, 24 of the children (38.1%) had positive cultures from endotracheal aspirates that could be linked to either possible or probable bacterial pneumonia. These observations were supported by the findings of Bloomfield et al. [28]. These authors observed bacteremia in 6 children out of 208 PICU admissions. Four infants were mechanically ventilated. All of them had been born prematurely or had congenital heart disease.

We have also studied, retrospectively, the occurrence of concurrent bacterial infection in 65 mechanically ventilated infants during 1996–2001 [29]. In 38 of these infants microbiological investigations were performed. All had antibiotics on PICU admission. We found only one positive culture from blood, and 37.5% positive cultures from endotracheal aspirates compatible with bacterial pneumonia. Infants with concurrent bacterial infection had similar C-reactive proteins (CRP) concentrations and white blood cell (WBC) counts compared to infants with negative cultures. In addition, the presence of bacterial pulmonary infection upon PICU admission was undetectable by the oxygenation index as this was equal in infants with and without positive cultures. There were two additional remarkable findings from our study. First, concurrent bacterial infection occurred almost exclusively in previously healthy, term born infants. Second, infants with positive concurrent bacterial infection required prolonged ventilatory support (14.3 ± 2.4 versus 10.6 ± 1.0 days).

All these retrospective observations were confirmed by a study by Thorburn et al. [30]. Their group prospectively collected endotracheal aspirates in 165 mechanically ventilated infants during three consecutive RSV seasons. They observed that 21.8% of the children had concurrent bacterial pneumonia upon PICU admission. Strikingly, these infants also required prolonged ventilatory support compared to infants without bacterial pneumonia. Only 36% of these infants were receiving antibiotics on PICU admission. The majority of bacterial pneumonias occurred in infants with pre-existing morbidity.

All of these results suggest that, at least in some of the children, bacterial pneumonia (partially) contributes to the development of respiratory failure (Fig. 1). Whereas others report such findings among children with pre-existing morbidity, we were unable to confirm this. We advocate refraining from the routine use of antibiotics in children admitted to the PICU. Immediate investigation of the endotracheal aspirate may identify those children in whom antibiotics are justified, although this hypothesis calls for further study, such as a randomized, controlled trial.

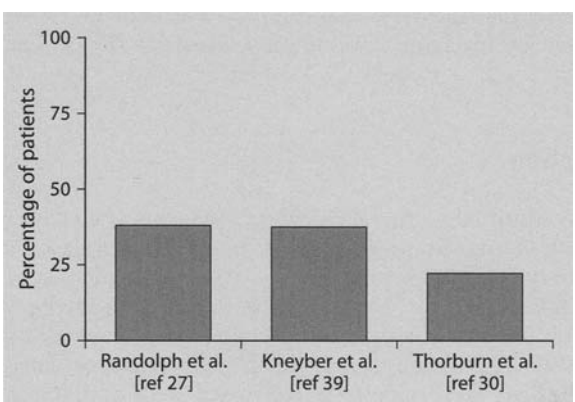

Fig. 1. Percentage of patients with positive microbiologic investigations suggestive for bacterial pneumonia in mechanically ventilated infants with respiratory syncytial virus (RSV) lower respiratory tract disease

Pathophysiology and Disease Severity

The pathophysiological mechanisms underlying RSV-induced respiratory failure with subsequent need for mechanical ventilation are unknown. It seems rational to hypothesize that disease severity can at least in part be related to viral strain or viral

load. Viral strain seems not to be an important factor. We observed that the need for PICU admission and mechanical ventilation is equally distributed among infants with RSV group A and B [31]. The effect of viral load on disease severity is less clear. Conflicting data have been reported on differences in viral load between ventilated and non-ventilated infants. DeVincenzo et al. were unable to find significant differences in viral load obtained from nasal washes between previously healthy ventilated (n=22) and non-ventilated (n=119) infants (5.185 versus 4.963 log pfu/ml) [32]. Others, however, observed significantly higher nasal viral load among ventilated (n=15) versus non-ventilated (n=24) previously healthy infants (5.06 ± 0.34 vs. 3.91 ± 0.35 log pfu/ml, $p=0.022$) [33]. There is one report on differences in viral load among ventilated infants. Van Woensel et al. found a higher viral load in tracheal aspirates of infants (n=14) who met criteria for "severe RSV lower respiratory tract disease" (PaO_2/FiO_2 ratio ≤ 200 mmHg and a mean airway pressure >10 cmH_2O (72.0 ± 28.0 RNA copies) compared to infants (n=8) with "mild" disease (21.1 ± 9.2 RNA copies, $p=0.20$) [34]. Unfortunately, there are no reports on differences in viral load among various categories of mechanically ventilated, high-risk infants.

Since viral strain and viral load are not fully accountable for disease severity, it can be argued that pre-existing structural abnormalities of the respiratory system predispose prematurely born children and children with chronic lung disease or congenital heart disease to a severe disease course. For instance, prematurely born but otherwise healthy infants have an underdeveloped respiratory system that is easily compromised by the direct toxic effects of an infectious agent, such as epithelial necrosis due to invading virus [35]. Young children with chronic lung disease have structurally abnormal airways that tend to collapse easily. In addition, there are structural abnormalities of the lung as a result of pulmonary immaturity at premature birth, that (partially) predisposes them to severe disease necessitating mechanical ventilation [35]. For infants with congenital heart disease with pulmonary hypertension it can be argued that the pre-existing hypoxia is further aggravated during the RSV-associated lower respiratory tract disease.

However, these pre-existing conditions do not fully account for the severity of RSV-associated lower respiratory tract disease since the majority of ventilated infants were previously healthy. Various groups have postulated that the immune response against RSV plays an important role in determining disease severity. This is probably especially valid for healthy term and pre-term infants since they have normal airways [36]. However, it is subject to debate whether or not the immune response against RSV is protective or disease-enhancing. Results from animal studies have led to the assumption that an overshoot of the T-cell response towards a T-helper 2 (Th2) profile may be responsible for severe disease [37]. However, there is much debate on the Th1/Th2 skewing in infants with RSV-associated lower respiratory tract disease. The observation of a Th2 skewed immunological response associated with severe disease in humans has not been universally confirmed [36].

In contrast to the hypothesis that the immune responses against RSV are disease-enhancing, there are strong arguments that both humoral and cellular immune responses against RSV actually protect against severe disease. Low titers of neutralizing antibodies were associated with severe RSV-associated lower respiratory tract disease, although there are no data on the relationship between the titer of neutralizing antibodies and the need for mechanical ventilation [38, 39]. It is well known that, in general, prematurely born infants lack sufficient titers of protective immunoglobulin G neutralizing antibodies because placental transport of IgG occurs late

in gestation, near the end of the third trimester. In addition, early post-natal life is also associated with a physiological immune deficiency defined by hyporesponsiveness of mononuclear phagocytes to stimuli and a diminished T-cell response [40, 41]. This low level of immune response could render very young infants susceptible to severe disease.

The suggestion that cellular immunity protects against severe RSV-associated lower respiratory tract disease has originated from various human studies. Low numbers of T-cells are found in peripheral blood samples of ventilated infants compared to non-ventilated infants, although this may also suggest recruitment of activated T-cells to the lungs [42]. More importantly, low levels of interferon (IFN)γ (a Th1 cell cytokine) were found in nasopharyngeal aspirates of mechanically ventilated children compared to non-ventilated children [43]. In addition, monocyte-derived interleukin (IL)-12 was observed to be inversely related to the duration of mechanical ventilation. IL-12 promotes the differentiation of naive CD4-positive T cells into Th1 cells [44]. Finally, mononuclear cells of ventilated infants exhibited diminished *ex vivo* lymphoproliferative responses and the capacity to produce IFNγ and IL-4 compared to non-ventilated infants. It seems thus likely that severe RSV-associated lower respiratory tract disease in healthy term and pre-term born infants originates from an immature immune system so that they cannot neutralize the virus sufficiently. For children with pre-existing abnormalities of the respiratory system it is probably a combination of both. In addition, it cannot be ruled out that genetic polymorphisms also play an important role in the host susceptibility for severe RSV-associated lower respiratory tract disease.

Therapeutic Options

Four different therapeutic approaches have been the subject of investigation [45]. These include the virostatic drug, ribavirin, corticosteroids, the use of bronchodilators, and exogenous surfactant. Whereas ribavirin and corticosteroids could be curative, bronchodilators and exogenous surfactant remain supportive therapies.

Three reports on the efficacy of ribavirin, comprising 104 mechanically ventilated infants, were reviewed systematically in a Cochrane review [46]. The use of ribavirin was associated with a significant decrease in the duration of mechanical ventilation (mean difference 1.2 days [95% confidence interval −0.2 to −3.4, $p=0.03$]). Normal saline was used as placebo in two studies, whereas sterile water was used in the third study. Because of the serious potential side-effects of sterile water (i.e., induction of bronchospasm), this study was excluded in an additional analysis. Subsequently, the difference in duration of mechanical ventilation became insignificant. In addition, ribavirin is not easy to administer and is associated with teratogenic side-effects. Ribavirin is, therefore, currently seldom used.

The efficacy of corticosteroids has been studied in three investigations [47–49]. These studies cannot be easily compared because of the different dosing and duration of treatment. Van Woensel et al. performed a post-hoc analysis of 14 mechanically ventilated infants in their original trial of prednisolone 1 mg/kg for seven days versus placebo in hospitalized children with RSV [48]. These authors observed a non-significant difference in mean duration of mechanical ventilation (4.7 ± 2.91 versus 6.3 ± 4.23 days). Based upon this post-hoc analysis, they designed a randomized clinical trial in mechanically ventilated infants [49]. Dexamethasone, 15 mg/kg/day every 6 hours for 48 hours, was compared with placebo in 85 patients. Again, no

significant difference in mean duration of mechanical ventilation was found between the two treatment arms. Similar results were obtained in a study including 41 mechanically ventilated infants [47]. Currently, Van Woensel et al. are performing a third randomized, controlled trial (the so-called Steroid Treatment in Artificially ventilated children with Respiratory syncytial virus infection [STAR] trial). A post-hoc analysis from their second randomized trial suggested that corticosteroids might be beneficial among ventilated infants who met criteria for mild disease as defined by Tasker et al. [24].

Depending on the results from the randomized controlled STAR trial, there is at present no rationale to use corticosteroids in mechanically ventilated infants with RSV-associated lower respiratory tract disease. Is it possible to explain why corticosteroids do not improve the disease course? One explanation would be that severe RSV disease does not result from a vigorous immune response (as discussed earlier in this chapter). On the other hand, if it is assumed that the pathophysiology of RSV bronchiolitis resembles that of childhood asthma, then (similar to asthma) corticosteroids would seem to be beneficial [50]. This suggests that correct identification of the clinical phenotype of RSV-assocated lower respiratory tract disease would identify those patients who might benefit from a certain therapeutic modality.

Three studies have evaluated the efficacy of bronchodilators for ventilated infants with RSV-associated lower respiratory tract disease [18, 20–51]. Mallory and coworkers observed a 30% improvement in maximum expiratory flow at 25% (MEF_{25}) of functional residual capacity (FRC) in 13 of 14 mechanically ventilated children with RSV-associated lower respiratory tract disease [20]. Pulmonary function was assessed by deflation flow-volume curve analysis. Hammer et al. performed a more elegant study by excluding infants with restrictive disease [18]. However, only 10 out of 20 infants with obstructive RSV-associated lower respiratory tract disease responded to nebulized albuterol (defined by a $\geq$2-fold improvement of intra-individual coefficient of variation for MEF_{25}). Derish et al. included 25 infants and observed a significant increase in MEF at FRC and a decrease in Rrs in some patients [51]. Do these studies justify the use of bronchodilators in mechanically ventilated children with RSV-associated lower respiratory tract disease? All three studies incorporated infants with pre-existing morbidity, and none were designed to detect an effect on the duration of mechanical ventilation and/or PICU stay. The routine use of bronchodilators, therefore, seems unjustified.

The use of exogenous surfactant seems highly rational, as low levels of surfactant phospholipids and proteins as well as a diminished function of surfactant (lowering surface tension at the alveolar-capillary level) have been described and recently summarized by us [52]. Three randomized, controlled trials on the efficacy of exogenous surfactant have been published [53–55]. The group of Luchetti et al. performed two studies investigating porcine surfactant versus no placebo [53, 54]. In their first study, 20 children with bronchiolitis (only 20% were RSV positive) were randomized to receive either 50 mg/kg porcine surfactant once, or nothing [53]. Methodologic flaws from their first study were corrected in a second study [54]. In this study, 40 children with RSV-associated lower respiratory tract disease were randomized. Oxygenation improved in both studies, and the mean duration of mechanical ventilation was also significantly different between the surfactant and the placebo group ($4.4 \pm .4$ vs 8.9 ± 1.0 days in the first study and $4.6 \pm .8$ vs $5.8 \pm .7$ days in the second study). These findings were confirmed by a study by Tibby et al., randomizing 19 infants to receive either 100 mg/kg bovine surfactant twice or air placebo [55]. These investigators observed no further decrease in oxygenation (oxygen-

ation index and Aa-DO_2) after administration of surfactant. In addition, a trend towards a reduced duration of mechanical ventilation was observed (126 hours vs 170 hours in the control group). Taken together, the findings of these three studies strongly call for a randomized, controlled trial of this strategy with duration of mechanical ventilation as primary endpoint.

Mechanical ventilation remains the mainstay of supportive therapy for infants with RSV-induced respiratory failure. Interestingly, there have been no randomized controlled trials on, for instance, various ventilatory strategies (volume controlled versus pressure controlled) or the level of positive end-expiratory pressure (PEEP) [11]. Mechanical ventilation with heliox has been scantily studied among infants with RSV-associated lower respiratory tract disease. From a pathophysiological point of view, mechanical ventilation with heliox seems rational. Heliox has a density that is one-seventh that of air, resulting in a decreased resistance to gas flow [56]. There is one trial investigating the effect of mechanical ventilation with various concentrations of heliox in ten infants with RSV-associated lower respiratory tract disease [57]. No beneficial effect could be demonstrated. As the study has methodological flaws, and no attempt was made to discriminate the clinical phenotype of the RSV-associated lower respiratory tract disease, the role of mechanical ventilation with heliox requires further study.

Until now, the mainstay of therapy for mechanically ventilated infants with RSV-associated lower respiratory tract disease has been symptomatic. Judging from the outcomes of the various randomized controlled trials, it is highly unlikely that this will change in the near future. The only promising therapeutic modality seems to be exogenous surfactant, although this has no direct curative effect.

Prevention

Vaccination against RSV will not readily be available, but passive immunization can be applied [58]. Passive immunization can be achieved through palivizumab, which is a monoclonal antibody directed against the F-glycoprotein. Presently, its use is advised for: (a) infants not older than 12 months without chronic lung disease born after a gestation of 28 weeks; (b) infants not older than six months without chronic lung disease born after a gestation of 29 to 32 weeks; and (c) infants born after a gestation of 32 to 35 weeks with at least two of the following risk factors: attending child care, with school-aged siblings, exposed to environmental air pollutants, with congenital abnormalities of the airways, or diagnosed with severe neuromuscular disease. Palivizumab is also advised for children younger than two years of age with chronic lung disease or a hemodynamically significant congenital heart disease [59].

Figure 2 summarizes the results of passive immunization with palivizumab and the effect on the number of PICU admissions and mechanical ventilation. Although in the first study on the efficacy of palivizumab (the Impact study), an overall reduction in hospitalizations of 55% was reported in palivizumab recipients (n=1002 vs 500 controls), this was not observed for the number of PICU admissions (1.3% vs 3%) or the number of mechanically ventilated children (0.2% vs 0.7%) [60]. Children with congenital heart disease were studied separately (639 palivizumab recipients vs 648 controls) [61]. Again, the overall reduction in hospitalizations of 45% was not observed for the number of PICU admissions (2% vs 3.7%) or number of mechanically ventilated children (1.3% vs 2.2%).

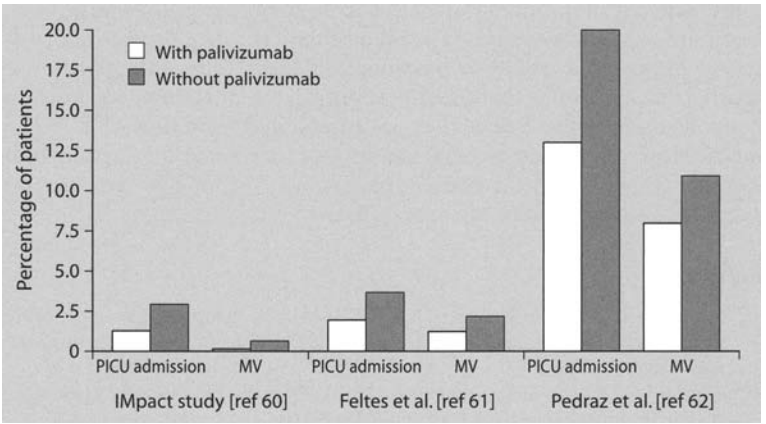

Fig. 2. Percentage of patients in whom mechanical ventilation (MV) and pediatric intensive care unit (PICU) admission was warranted before and after the introduction of the monoclonal antibody palivizumab.

There have been two post-licensure studies reported after the introduction of palivizumab. Pedraz and co-workers studied the efficacy of palivizumab in four consecutive RSV seasons (children without prophylaxis (n = 1583) admitted between 1998 – 2000, and children with prophylaxis (n = 1919) admitted between 2000 – 2002) in Spain [62]. Although they observed a 70% decrease in overall hospitalizations, the numbers of children admitted to the PICU (13% vs 20%) or requiring mechanical ventilation (8% vs 11%) were comparable. Similar observations were made in a national survey performed in Israel during two consecutive RSV seasons (2000 – 2002), including 296 children [63]. After the first season (2000 – 2001), the Israel Ministry of Health issued guidelines stipulating the use of palivizumab in children < 2 years with chronic lung disease and infants born at a gestation of 28 weeks or less. The number of children admitted to the PICU or mechanically ventilated was similar before and after the introduction of palivizumab. They also found that the majority of children admitted to their PICU did not meet the American Academy of Pediatrics criteria, concluding that monthly prophylaxis with palivizumab would be very unlikely to influence the number of PICU admissions.

It thus seems that monthly prophylaxis with palivizumab does not have an effect on the occurrence of severe RSV-associated lower respiratory tract disease necessitating PICU admission and/or mechanical ventilation. This suggests that not only virological and/or immunological factors are (partially) responsible for the development of severe RSV-associated lower respiratory tract disease.

Conclusion

RSV-associated lower respiratory tract disease remains an annual recurring challenge for pediatric intensivists. Despite intensive research over the past decades, the mainstay of therapy for infants with RSV-associated lower respiratory tract disease is still symptomatic. But there are a number of scientific challenges that must be pursued. The role of exogenous surfactant requires further study, as also does mechanical ventilation with heliox. In addition, future studies should focus on the question of why an infant clinically deteriorates and needs mechanical ventilation.

These studies should integrate not only epidemiological aspects, but also virological and immunological aspects, as well as characteristics of respiratory system mechanics. By doing so, it would be possible to identify those infants who might benefit the most from a specific therapeutic approach. Importantly, we also must know what happens to the infants once they are discharged from our ICUs. What is the effect of mechanical ventilation on short-term and long-term airway morbidity? Is there an association with clinical phenotype? As we continue to care for these infants, we eagerly await the results of such studies.

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Pneumocystis Pneumonia in Non-AIDS Immunocompromised Patients

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Introduction

Pneumocystis pneumonia remains one of the leading causes of morbidity and mortality among patients with acquired immunodeficiency syndrome (AIDS) all over the world [1]. However, pneumocystis pneumonia is also a life-threatening opportunistic infection that can occur in other immunocompromised patients, mainly in solid organ transplant recipients, in patients with cancer, and those treated for autoimmune or inflammatory diseases [1].

Pneumocystis pneumonia is caused by *Pneumocystis jiroveci*, a pathogen first identified in the early 20th century by Chagas, and subsequently by Carinii in rat lungs. These authors believed wrongly that they had found a new form of trypanosome. However, several years later, it was established that the organism was an original species, called *Pneumocystis carinii.* For many decades, pneumocystis was misclassified as a protozoan, until new phylogenetic studies demonstrated that it was related to fungi. Pneumocystis organisms have been identified in several mammalians, but different pneumocystis species with distinct genetic characteristics exist, each species being host-specific. Thus, the pneumocystis infecting humans was named *Pneumocystis jiroveci* [1].

In recent years, advances in cancer treatments (including new drugs, high dose and intensive chemotherapy with bone marrow or stem cell transplantation) and immunomodulation have resulted in an increased number of patients with impaired immunity and at risk for pneumocystis pneumonia. This chapter focuses on practical aspects relevant to risk factors, diagnosis, and treatment of pneumocystis pneumonia in non-AIDS patients.

Transmission

For a long time, pneumocystis pneumonia was thought to result from reactivation of latent infection acquired during childhood. This theory was supported by the seroprevalence of anti-*Pneumocystis* antibodies at a young age, the high rate of pneumocystis pneumonia among infants with AIDS, the presence of *Pneumocystis jiroveci* in respiratory specimens from patients without immunosuppression and without clinical signs of pneumocystis pneumonia, and by the characteristics of the organism itself, consistent with long term carriage [2]. However, several lines of evidence subsequently ruled out the possibility of reactivation of latent infection as the sole mechanism for pneumocystis pneumonia, and suggested that infection results more likely from environmental exposure or person to person transmission [2, 3]. In this

way, it was shown that *Pneumocystis* DNA was present in the environment, including pond water and various air samples, and that the incidence of pneumocystis pneumonia was increased in certain geographic areas. Additional data suggest that *Pneumocystis jiroveci* acquisition may occur as a result of airborne transmission from asymptomatic carriers or from patients with pneumocystis pneumonia [2]. Nevertheless, isolation of patients with pneumocystis pneumonia is not currently recommended or applied [2].

Patients at Risk of Pneumocystis Pneumonia

Several retrospective studies have identified clinical conditions associated with the occurrence of pneumocystis pneumonia in human immunodeficiency virus (HIV)-negative immunocompromised patients (Tables 1 to 3). Fifty years ago cases of pneumocystis pneumonia were reported in premature and malnourished infants [4]. Nowadays, in developed countries, patients who are the most likely to develop pneumocystis pneumonia are those suffering from hematological malignancy, solid cancer, inflammatory or autoimmune disease, and solid organ transplant recipients

Table 1. Underlying conditions in three series of non-AIDS patients with pneumocystis pneumonia

	Zahar et al [40] n = 39 (%)	Yale and Limper [16] n = 116 (%)	Roblot et al [39] n = 130 (%)
Hematological malignancies	28 (71.8)	35 (30.2)	75 (57.7)
Solid tumors	7 (17.9)	15 (12.9)	18 (13.8)
Solid organ transplantation	0	29 (25)	9 (6.9)
Inflammatory diseases	0	26 (22.4)	27 (20.8)
Miscellaneous	4 (10.3)	11 (9.5)	1 (0.8)

Table 2. Incidence of pneumocystis pneumonia in various types of malignancies (adapted from [6])

	Number of cases of pneumocystis pneumonia	Patients	Rates, %
Lymphomas	34	9907	0.34
Acute lymphocytic leukemia	5	2929	0.17
Other leukemia	16	5023	0.32
Solid tumors	30	26085	0.11
Cerebral tumors	21	3098	0.68
Bone marrow transplantation	22	1348	1.63

Table 3. Risk factors for pneumocystis pneumonia in patients with systemic diseases (adapted from [52])

	Incidence (number/10000 hospitalizations/year)	OR
Wegener's granulomatosis	89	7.81
Polyarteritis nodosa	65	10.2
Polymyositis	25	4.44
Lupus erythematosus	12	2.52
Scleroderma	8	1.48
Rheumatoid arthritis	2	1

[5–8]. Awareness of patients at risk for pneumocystis pneumonia is of great importance for clinicians, who have to identify patients who require prophylaxis and cases where a diagnosis of pneumocystis pneumonia must be considered.

Hematological Malignancies

It has long been established that children with acute lymphoblastic leukemia receiving chemotherapy have a high risk for pneumocystis pneumonia. In a study by Hughes et al., the infection rate reached 4.1% in this population [9]. Subsequently, cases of pneumocystis pneumonia were reported in patients with almost any hematological malignancy [10]. Although the exact incidence rate of pneumocystis pneumonia for each hematological malignancy is unknown, the highest incidence seems to occur in leukemia and non-Hodgkin lymphoma, attack rates being around 0.2 to 0.5% [6, 10]. Cancer patients who develop pneumocystis pneumonia have often received chemotherapy. However, in our experience, pneumocystis pneumonia may also be inaugural of the underlying disease, occurring before any immunosuppressive therapy (Bollée et al, unpublished data).

Patients undergoing autologous, and especially allogenic hematopoietic stem cell transplantation (HSCT), are also likely to develop pneumocystis pneumonia. Although early studies showed that pneumocystis pneumonia occurred in most cases within the first six months after allogenic HSCT, this notion has been ruled out by more recent reports [11]. In a recent retrospective study, pneumocystis pneumonia occurred with a median of 14.5 months after allogenic HSCT, the infection rate being 2.5%. Moreover, in most cases, patients were receiving immunosuppressive therapy for chronic graft versus host disease or had a relapse of their hematological malignancy [11].

Solid Cancers

Pneumocystis pneumonia occurs less frequently in solid tumors than in hematological malignancies. However, cases have also been often described in a wide variety of solid cancers. The highest risk is reached in patients with primary or metastatic brain cancer, probably reflecting the widespread use of corticosteroids in these patients. An infection rate of 0.1% for overall cancer and 0.7% for brain cancers has been reported [7].

Inflammatory and Autoimmune Diseases

Although rare, pneumocystis pneumonia can also occur in patients with autoimmune or inflammatory disease receiving cytotoxic agents and corticosteroids, especially if they have lymphocytopenia. Among such patients, those with Wegener's granulomatosis appear to have the greatest risk for pneumocystis pneumonia [12].

Solid Organ Transplantation

Solid organ transplant recipients are also likely to develop pneumocystis pneumonia. Incidence rates differ between types of transplantation and are greatest in lung transplantation [13]. It is well established that the incidence of pneumocystis pneumonia among solid organ transplant recipients is highest during the first year following transplantation, especially during the first six months. Rejection treatments,

use of anti-lymphocyte antibodies, and occurrence of immunomodulative infections, such as tuberculosis, cytomegalovirus infection, or hepatitis C, have been demonstrated as being associated with an increased risk of pneumocystis pneumonia [14].

Others

Rarely, pneumocystis pneumonia may occur in other immunocompromised patients, such as those with idiopathic CD4+ lymphocytopenia [15]. Very rarely, cases of pneumocystis pneumonia have been reported in patients without detectable immunodeficiency or who did not develop other opportunistic infections [3].

Immunosuppressive Drugs and Risk of Pneumocystis Pneumonia

In HIV-negative patients who develope pneumocystis pneumonia, the immunocompromised state is partly linked to factors related to the underlying disease causing specific immunosuppression, but is also a consequence of treatments, such as steroids, cytotoxic drugs, and other immunomodulative drugs.

Regardless of the associated underlying disease, steroids have been widely indicated as a major predisposing factor for pneumocystis pneumonia in HIV-negative patients. A retrospective analysis of 116 cases of pneumocystis pneumonia in HIV-negative patients showed that 90.5% were receiving steroids when pneumocystis pneumonia occurred. The median daily dose and median duration of steroids were equivalent to 30 mg and 12 weeks, respectively [16]. However, patients who are not receiving corticosteroids may also develop pneumocystis pneumonia. Indeed, in a recent retrospective study by our group, of 56 HIV-negative patients with pneumocystis pneumonia, we observed that 24 patients (42.8%) were not receiving steroids when pneumocystis pneumonia occurred (Bollée et al, unpublished data). This observation is a crucial issue, stressing that clinicians have to consider the possibility of pneumocystis pneumonia not only in patients receiving corticosteroids.

The risk of pneumocystis pneumonia in cancer patients has been shown to be associated with the intensity of chemotherapy [17]. The precise role of a particular cytotoxic drug in the occurrence of pneumocystis pneumonia is often difficult to establish, due to the association of several drugs in most patients. Certain drugs, such as fludarabine, cladribine, cyclophosphamide, or methotrexate, have been suspected to be associated with a particularly high risk of pneumocystis pneumonia; however, data remain elusive.

Among immunosuppressive therapies used after solid organ transplantation, anti-lymphocyte antibodies clearly increase risk of pneumocystis pneumonia [14]. The risk of pneumocystis pneumonia associated with azathioprine and cyclosporine therapy appears low, whereas it seems higher with tacrolimus [18]. Uncertainties persist regarding the risk associated with mycophenolate-mofenil and sirolimus. Despite ancecdotal reports, the actual risk of pneumocystis pneumonia related to newer drugs, such as anti-tumor necrosis factor (TNF)-α or rituximab, is not yet established.

CD4+ Lymphocyte Counts in HIV-negative Patients Developing Pneumocystis Pneumonia

Pneumocystis pneumonia has been largely described in HIV infected patients with a circulating CD4+ T lymphocyte count less than 200/mm^3. In other immunocompromised patients, the CD4+ count has also been shown to be related to the risk of pneumocystis pneumonia. Pneumocystis pneumonia seems more likely to occur in patients with CD4+ count lower than 300/mm^3 [19]. This suggests that CD4+ counts could be of value to detect patients at risk of pneumocystis pneumonia and determine who should receive prophylaxis against pneumocystis pneumonia. However, the real value of CD4+ count monitoring in HIV-negative patients remains to be established.

Differences in Pathophysiology in Patients With and Without AIDS

In a study by Limper et al., bronchoalveolar lavage (BAL) fluid analysis of patients with pneumocystis pneumonia revealed important differences between patients with and without AIDS. Compared with AIDS patients, each other immunocompromised category (hematological malignancy, solid cancer, solid organ transplantation and steroids therapy) had a significantly lower organism number and more inflammation, estimated by neutrophil count. Furthermore, evidence of inflammation in BAL fluid was inversely correlated with oxygenation and survival [5]. These data explain, at least partly, the differences observed in the clinical presentation of pneumocystis pneumonia in patients with and those without AIDS.

Clinical and Radiological Presentation of Pneumocystis Pneumonia in HIV-negative Patients

Clinical Presentation

As described by several authors [10, 20], HIV-negative patients with pneumocystis pneumonia typically present with fever in about 60 to 90% of cases, dyspnea in 75 to 95%, and cough in 50 to 80%. Delay from onset of symptoms to diagnosis is rather short, varying from 1 to 14 days in most cases. Clinical examination commonly reveals tachypnea and diffuse crackles at lung auscultation. PaO_2 in room air is usually decreased considerably, at around 50 to 70 mmHg. Thus, clinical presentation is typically severe, with rapidly evolving bilateral lung involvement and marked hypoxemia, although, in some cases, evolution is more insidious, with fever and respiratory symptoms developing over weeks (Bollée et al, unpublished data). In comparison with AIDS patients, pneumocystis pneumonia presents as a more acute and severe disease among HIV negative patients, causing acute respiratory failure and often the need for mechanical ventilation [21, 22].

Radiological Presentation

The radiographic features of pneumocystis pneumonia are similar in patients with and without AIDS [22]. Typically, bilateral, or less frequently unilateral, interstitial infiltrates are observed. Rarely, chest radiography may be normal at an early stage. Few data specific to non-AIDS patients are available to date regarding findings from

high resolution computed tomography (CT)-scans. A pattern of extensive ground glass attenuation, which is often distributed in a patchy fashion, with a predilection for perihilar regions of lungs, is the most common feature [23]. In addition to the infiltrates, atypical high resolution CT features, such as nodules, nodular components, or cavities, may be seen. The presence of these atypical images may be related to granuloma formation in response to pneumocystis pneumonia, but are more often indicative of a concomitant cancer or infectious disease process affecting the lungs [23]. In contrast to patients with AIDS, cysts related to pneumocystis pneumonia have not been described in HIV-negative patients with pneumocystis pneumonia.

Diagnostic Strategy

Respiratory Sample Collecting Techniques

Owing to the lack of specificity of clinical and radiological presentation, diagnosis of pneumocystis pneumonia requires microbiological examination from a relevant sample to identify *Pneumocystis jiroveci*. Differences in organism number between patients with and without AIDS have to be remembered in considering the appropriate diagnostic procedure in non-AIDS immunocompromised patients.

Several procedures are available for obtaining respiratory samples. Induced sputum, a technique consisting of collecting a sputum specimen after inhalation of nebulized saline for 20 minutes, has proved to be a useful technique in the diagnosis of pneumocystis pneumonia. Whereas sensitivity of direct examination of induced sputum from AIDS patients is up to 90% in certain centers [24], the few available data concerning HIV-negative patients suggest lower sensitivity of around 50% [25]. Bronchoscopy with BAL represents a more efficient technique for the diagnosis of pneumocystis pneumonia; the sensitivity of direct examination reaches 80 to 100% in patients with AIDS. Once again, the sensitivity of direct examination in HIV-negative patients is not well defined, but appears lower, around 60 to 70% [26]. An important inconvenience of the BAL procedure is the risk of aggravating respiratory state and need for mechanical ventilation, associated with high mortality rates [27]. Interestingly, a concomitant pulmonary infection due to viruses, especially cytomegalovirus, bacteria, or fungi, is frequently detected by BAL in patients with pneumocystis pneumonia [5]. However, no comparative strategy has demonstrated a benefit on mortality or morbidity of avoiding BAL, which remains the procedure to perform when the induced sputum technique has failed to identify pneumocystis. Rarely, when less invasive procedures have failed to detect pneumocystis pneumonia, surgical lung biopsy should be considered [10, 28].

Laboratory Diagnosis

A major obstacle to studying and detecting pneumocystis is the difficulty in culturing this organism, despite many attempts [1].

Direct Examination

Initially, detection of *Pneumocystis jiroveci* in respiratory samples depended on direct examination, which is based on tinctorial stains and immunofluorescence. Several staining methods, such as calcofluor white, Gomori-Grocott, or toluidine blue only allow detection of cysts, and also mark other fungi. Other stains, such as

Wright-Giemsa, Dif-Quik, and Gram-Weigert, can detect both cysts and trophic forms, but their interpretation may be difficult as they also color other organisms. Development of an indirect immunofluorescent stain using monoclonal antibodies was a significant advance, providing a new, rapid, and more sensitive method than conventional stains for detecting *Pneumocystis jiroveci* in respiratory samples [24].

Polymerase Chain Reaction

The detection of *Pneumocystis jiroveci* in clinical specimens is greatly improved by the use of polymerase chain reaction (PCR). In 1990, Wakefield et al., developed a new technique for DNA amplification specific to pneumocystis, using primer sequences for the mitochondrial 5S rRNA gene of *P. carinii* [29]. Subsequent studies highlighted the efficiency of this technique. Principally in AIDS patients, sensitivity was evaluated at 80–100% and 70–85% on BAL and induced sputum specimens, respectively. Specificity also appeared excellent, around 80–100% for BAL fluid and 90–95% for induced sputum specimens [26, 30]. In a study by Sing et al., PCR was investigated in different immunocompromised groups. For combined BAL fluid and induced sputum specimens, sensitivity and specificity were, respectively, calculated at 80 and 100% in AIDS patients, 86 and 97% in transplant recipients, 100 and 95% in cancer patients, 100 and 98% in other immunocompromised patients [26]. Thus, PCR appears to be a significant advance for diagnosing pneumocystis pneumonia, particularly in HIV-negative patients. Compared with AIDS patients, direct examination of BAL fluid or induced sputum specimens in HIV-negative patients is more likely not to be sensitive enough, due to the lower organism burden [5]. In another study, *Pneumocystis jiroveci* was undetected by direct examination of respiratory samples in 7 of 37 HIV-negative immunocompromised patients with pneumocystis pneumonia, and PCR was the only microbiological indication of *Pneumocystis jiroveci* in these patients [30]. These data emphasize that routine microbiological evaluation for pneumocystis pneumonia must involve PCR techniques in all respiratory samples from HIV-negative immunocompromised patients.

PCR and colonization

One limit of the PCR technique is the detection of a very low pneumocystis burden due to colonization in asymptomatic patients. Although lung colonization by *Pneumocystis jiroveci* has also been reported in immunocompetent patients with various bronchopulmonary diseases [31], immunocompromised patients appear much more likely to be asymptomatic pneumocystis carriers [32]. Long-term steroids [33] and low CD4+ counts [34] have been shown to be associated with higher colonization rates. In a study by Leigh et al., induced sputum specimens obtained from 10 immunocompetent individuals and 20 solid organ transplant recipients, all of whom were asymptomatic, were screened for *Pneumocystis jiroveci* using PCR and immunofluorescence. All 30 specimens were negative for *Pneumocystis jiroveci* by immunofluorescence, and 5 transplant patients but no immunocompetent individuals were positive for *Pneumocystis jiroveci* by PCR alone. One of the 5 transplant patients developed pneumocystis pneumonia 6 weeks after sputum induction [35]. However, the risk of clinical pneumocystis pneumonia in an asymptomatic patient with colonization remains largely uncertain to date. Thus, PCR positivity alone on a respiratory sample may reflect true infection, but also colonization, corresponding to a 'clinical false positive' PCR result.

Quantitative PCR

Larsen et al. developed a rapid, sensitive, and quantitative Touchdown-PCR (TD-PCR) assay, which has constituted another significant progress for diagnosing pneumocystis pneumonia and has resolved, at least partly, the problem of distinction between true infection and colonization. In a blind, retrospective study of respiratory samples obtained in HIV-negative immunocompromised patients, the concentration of pneumocystis DNA measured by using quantitative TD-PCR was significantly higher in patients with pneumocystis pneumonia than in those with other respiratory disorders. Moreover, application of a cut-off value allowed a distinction to be made between infection and colonization [36].

Development of Non-invasive Methods for Diagnosing Pneumocystis Pneumonia

BAL remains the most efficient technique for pneumocystis detection, providing better sensitivity than induced sputum specimens. However, BAL is invasive, uncomfortable, and likely to aggravate the respiratory state. The induced sputum technique represents an alternative to BAL, but requires patient cooperation and a qualified therapist to collect good quality samples. For these reasons, development of non-invasive methods for diagnosing pneumocystis pneumonia constitutes an interesting area of research.

Oral washes

Oral wash collected after gargling and rinsing the mouth with sterile saline appears to provide a highly desirable diagnostic specimen, as it can be obtained easily and quickly in most patients, including those unable to sustain invasive procedures. Oral washes do not contain enough organisms to be detected by direct microscopic examination, but PCR may detect pneumocystis DNA in such specimens. Moreover, Larsen et al. demonstrated that quantitative TD-PCR could be used to distinguish between colonization and infection on oral washes, as on BAL specimens [36]. PCR on oral washes has also been shown to be a useful technique for diagnosing pneumocystis pneumonia in HIV-negative immunocompromised patients. Helweg-Larsen et al. reported 100% sensitivity and specificity of TD-PCR on 26 oral washes collected in patients with hematological malignancies, 8 of whom had pneumocystis pneumonia [37]. Thus, examination of oral washes by PCR (especially quantitative TD-PCR) is a promising technique to diagnose pneumocystis pneumonia.

Plasma concentration of S-adenosylmethionine

Pneumocystis is the only known cell that is unable to synthesize S-adenosylmethionine, a key intermediary metabolite for all cells. Thus, pneumocystis must extract this compound from its host, resulting in depletion of S-adenosylmethionine in the infected individual. Plasma S-adenosylmethionine levels were shown to be decreased in AIDS patients with pneumocystis pneumonia, and they rapidly increased after recovery [38] suggesting that measurement of plasma S-adenosylmethionine could be of great value in the diagnosis of pneumocystis pneumonia. However, these promising results will have to be confirmed in the future before this technique can be used routinely.

Prognosis of Pneumocystis Pneumonia in HIV-negative Patients

Mortality

The prognosis of pneumocystis pneumonia is clearly worse in HIV-negative than in AIDS patients, the mortality rate reported as around 30 to 40%. Mortality is even higher in patients requiring mechanical ventilation, reaching 60 to 75% [10, 39].

Prognostic Factors

Many factors associated with mortality in HIV-negative patients with pneumocystis pneumonia have been reported. Unsurprisingly, high respiratory and pulse rate, hypoxemia, involvement of four lung lobes, need for mechanical ventilation or vasopressors, high C-reactive protein and lactate dehydrogenase levels, high severity scores, such as SAPS II (simplified acute physiology score II) or Organ System Failure score, and concomitant pulmonary infection have been associated with higher mortality [10, 20, 39, 40]. Cancer chemotherapy [20, 40] and long-term steroid administration [10, 40] are also related to an increased risk of death. Interestingly, a high neutrophil count in BAL specimens is associated with more severe hypoxemia and a higher mortality [5, 40], which may support the hypothesis of a beneficial effect of adjuvant high-dose steroids in non-AIDS patients, as previously reported in AIDS patients [41, 42].

Curative Treatment of Pneumocystis Pneumonia

Antimicrobial Therapy

Medications used to treat pneumocystis pneumonia do not differ among patients with and without AIDS. As the most effective treatment, trimethoprim-sulfamethoxazole (TMP-SMX) given orally or intravenously for three weeks, must be used as first line treatment unless contraindicated. TMP-SMX acts by interfering with folate metabolism: TMP and SMX inhibit, respectively, dihydrofolate reductase (DHFR) and dihydropteroate synthase (DHPS), which are two integral enzymes for folate synthesis. Emergence of mutations in the DHPS gene related to use of prophylaxis has been a concern for several years. However, whether these mutations confer resistance to TMP-SMX remains uncertain and controversial. Adverse effects, including fever, rash, cytopenia, hyperkalemia, hyponatremia, hepatitis, and interstitial nephritis occur less commonly (around 15%) in HIV-negative compared with AIDS patients [43]. Adjunction of folinic acid may lower the risk of neutropenia, but increases the risk of therapeutic failure [44]. Due to the lack of synergy and the risk of adverse effects, association with other antimicrobial molecules is not recommended for treating pneumocystis pneumonia.

In case of intolerance to TMP-SMX, pentamidine should be used as an alternative. Pentamidine can be administrated intravenously, or by aerosol in mild forms of pneumocystis pneumonia. The most common adverse drug reaction to pentamidine is renal toxicity, which usually occurs after 2 weeks of treatment and can be prevented by adequate hydration. Other adverse effects, including hypotension (especially in case of rapid infusion), heart arrhythmias, hypo or hyperglycemia, hypercalcemia, hyperkalemia, pancreatitis, and metallic taste may also occur. Atovaquone, dapsone, and clindamycin-primaquine represent other options in the treatment of mild pneumocystis pneumonia in AIDS patients. However, due to lack of data, these

drugs cannot be recommended for current use in HIV-negative patients with pneumocystis pneumonia.

Adjuvant Steroids

Steroids are recommended in AIDS patients with pneumocystis pneumonia who have severe hypoxemia (PaO_2 less than 70 mmHg in room air). Indeed, adjuvant steroids have been shown to result in a significant survival improvement by reducing the risk of respiratory failure and mortality [45].

In HIV-negative patients, only a few retrospective studies, including a small number of patients, have investigated the effect of adjuvant steroids. In a study by Delclaux et al, adjuvant steroids were not found to influence requirement for mechanical ventilation or mortality rate [46]. In another study by Pareja et al., adjuvant steroids were associated with a shorter duration of mechanical ventilation, oxygen therapy, and stay in the ICU, without an effect on mortality [47]. In our recent study of 56 HIV-negative patients with pneumocystis pneumonia, we observed a trend toward a decreased mortality in patients receiving adjuvant steroids (18.2% versus 42.2%, NS) (Bollée et al, unpublished data). Thus, a clear benefit of adjuvant steroids in HIV-negative patients with pneumocystis pneumonia has not yet been proved. However, considering the results and the limitations of these studies, and on the basis of our personal experience, we believe that adjuvant steroids should be recommended in HIV-negative patients with severe pneumocystis pneumonia.

Prophylaxis of Pneumocystis Pneumonia

Antimicrobial Drugs Available

Thirty years ago, TMP-SMX was demonstrated to be highly effective and well tolerated in the prevention of pneumocystis pneumonia among cancer patients [48]. Nowadays, TMP-SMX given orally remains the gold standard for pneumocystis pneumonia prophylaxis, similar to curative treatment. Aerosolized pentamidine repeated every month may be an alternative for patients not tolerating TMP-SMX, although, this prophylactic regimen was demonstrated to be less effective than TMP-SMX, as aerosolized pentamidine was associated with an increased risk for pneumocystis pneumonia and other infections and a higher mortality in bone marrow transplant recipients [49]. Dapsone is another available drug for preventing pneumocystis pneumonia. Similar to SMX, dapsone inhibits DHPS. However, dapsone, alone or associated with pyrimethamine, is inferior to TMP-SMX. Moreover, TMP-SMX intolerance often predicts dapsone intolerance. Hence, replacing TMP-SMX by dapsone in case of intolerance is not recommended [50]. Finally, atovaquone constitutes an effective and well tolerated alternative choice, including in bone marrow transplant recipients [51].

Indications for Prophylaxis

In contrast to AIDS patients, there is no consensus on prophylaxis against pneumocystis pneumonia among HIV-negative patients. From our own experience and in view of the risk factors for pneumocystis pneumonia, we believe that patients who should receive prophylaxis are:

- patients with any immunological disorder, including cancer, hematological disease, autoimmune or inflammatory disorders, who receive more than 15 mg of prednisone daily (or equivalent) for more than four weeks
- patients with acute leukemia or non-Hodgkin's lymphoma not in remission or receiving chemotherapy
- Patients undergoing allogenic HSCT; prophylaxis should be maintained for at least one year and continued in patients receiving immunosuppressive drugs, and in those with relapse of their hematological malignancy or with longstanding graft-versus-host disease.
- Patients undergoing autologous HSCT, for at least six months; duration should be extended in patients receiving additional immunosuppressive drugs.
- solid organ transplant recipients should receive prophylaxis for 6 months after transplantation, except lung transplant recipients, who should have lifelong prophylaxis.

TMP-SMX should be stopped during neutropenia, and in patients receiving methotrexate. It remains unclear whether CD4+ monitoring should be used to identify patients who require prophylaxis. However, a CD4+ count less than 200/mm^3 should lead to prescription of prophylaxis, especially in patients with malignancies.

Conclusion

In summary, pneumocystis pneumonia is a severe opportunistic infection, which may complicate the course of a wide variety of diseases leading to immunosuppression. For clinicians caring for immunocompromised patients, knowledge of the conditions associated with pneumocystis pneumonia is crucial for identifying who should receive prophylaxis and a high index of suspicion for pneumocystis pneumonia is important for early adequate diagnosis and therapy. Long term steroids are an important, but not the only, risk factor for pneumocystis pneumonia. In the era of more intensive treatment regimens, new molecules used in cancer chemotherapy or immunomodulation, and extended survival of cancer patients clinicians need to maintain a high level of suspicion regarding pneumocystis pneumonia. Development of PCR and non-invasive methods of detection, such as oral washes, constitute important advances for diagnosing pneumocystis pneumonia, which should be available in an increasing number of centers, to allow optimal management of these patients.

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Mechanisms of Organ Dysfunction

The Role of Neutrophil-Derived Myeloperoxidase in Organ Dysfunction and Sepsis

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Introduction: The Role of the Neutrophil in Sepsis

Neutrophils are the first cells to be activated in the host immune response to infection or injury and are critical cellular effectors in both humoral and innate immunity, central to the pathogenesis of sepsis and multi-organ dysfunction [1]. However, the neutrophil capacity for bacterial killing lacks selectivity, despite stringent regulation, and thereby carries the potential to inflict collateral damage to, and destruction of host tissue [2]. Host tissue damage characterizes both autoimmune and inflammatory conditions and may arise via a variety of mechanisms including premature neutrophil activation during migration, extracellular release of cytotoxic molecules during microbial killing, removal of infected or damaged host cells or debris during host tissue remodeling, and failure to terminate acute inflammatory responses [3]. Sepsis-induced neutrophil mediated tissue injury has been demonstrated in a variety of organs including the lungs [4, 5], kidneys [6], and liver [7].

Innate Immunity

The principle function of the innate immune system is to effect a rapid response to microbial invasion. This is achieved via the complement system and anti-microbial peptides, as well as by phagocytes and antigen presenting cells. Initial microbial contact precipitates a series of simultaneous events including non-specific bacterial recognition via complement receptors (e.g., β2-integrins, Fc-receptors interacting with 'opsonizing' serum components) and phagocytic sensing of pathogen-associated molecular patterns (PAMPs), which are evolutionarily conserved molecules unique to microorganisms. Sensing occurs through a variety of pattern recognition receptors, of which Toll-like receptors (TLR) are the best defined within this context [8].

Neutrophil Biology

Neutrophils are terminally differentiated cells, released into the blood stream following maturation from bone marrow precursors under the influence of granulocyte-colony stimulating factor (G-CSF). Neutrophils have the shortest lifespan of all human cells, existing for only 6–10 hours *in vivo*. Prolongation of survival is an active process requiring new gene expression and protein synthesis. It is achieved following exposure to a variety of inflammatory mediators and is accompanied by markers of

increased neutrophil activity. Resolution of the inflammatory process involves activation of a constitutive program of apoptosis, or programmed cell death, with subsequent phagocytosis by hepatic cells and the reticulo-endothelial system [2].

Neutrophil Recruitment

Neutrophils are mobile cells, which interact with the microenvironment, migrating down a chemotactic gradient to inflammatory loci in response to signals released by infection and tissue injury [9]. Neutrophil recruitment is an active process involving distinct stages of neutrophil rolling, tight adhesion, and diapedesis [10]. Circulating blood neutrophils contact, and transiently interact, with endothelial cell molecules, resulting in slowing and a rolling/release-like motion. Rolling is the initial step in neutrophil tissue recruitment to inflammatory sites, is a prerequisite for imminent tight interactions with the endothelium, and is mediated by selectins and type I membrane glycoproteins. L-selectins on the neutrophil surface mediate interactions with endothelial cells and other neutrophils, facilitating leukocyte transient adhesive contacts with high tensile strength to permit rolling under shear stress. The L-selectin molecule is localized to the neutrophil microvilli and is cleaved during neutrophil activation. Selectins, in addition to cell-cell adhesion may have a contributory role in cell signaling [10].

Chemo-attractants originating from tissues trigger neutrophil responses, replacing neutrophil rolling with a state of tight adhesion [10]. The β2-integrins are cell surface molecules that are primarily responsible for tight adhesion to both the endothelium and extracellular matrix. They interact with the cytoskeleton allowing stabilization of cell adhesion, providing a framework for signaling proteins. Intercellular adhesion molecule (ICAM)-1 on the endothelium interacts with β2-integrins on the neutrophil, producing changes in ligand binding. β2-integrins are also necessary in facilitating neutrophil clearance following apoptosis [11].

In order to reach extravascular sites of infection, neutrophils must transmigrate between or directly through endothelial cells. Neutrophil derived granular enzymes including elastase and gelatinase facilitate the process of diapedesis.

Neutrophil Defenses

Neutrophil defenses incorporate indiscrete processes which may be classified as oxygen dependent and independent. Theses are activated simultaneously upon neutrophil initiation of phagocytosis. Reactive oxygen species (ROS) are generated by means of the respiratory burst. Hypochlorous acid (HOCl), the most bactericidal oxidant produced by the neutrophil, is generated by the unique ability of myeloperoxidase (MPO), the main constituent of azurophilic granules, to oxidize chloride ions at physiological pH. HOCl is the major end product of the neutrophil respiratory burst. Neutrophil degranulation releases a plethora of preformed enzymes and antimicrobial factors from neutrophil granules into phagosomes leading to the formation of phagolysosomes, in which bacteria are inhibited or killed.

Oxygen dependent mechanisms

NADPH oxidase generation of superoxide

The nicotinamide adenine dinucleotide phosphate (NAPDH) oxidase enzyme complex is assembled on neutrophil activation. Once active, this enzyme complex con-

sumes oxygen to generate superoxide ($O_2^{\cdot-}$), metabolites of which play a part in antimicrobial defense strategy. NAPDH oxidase generation of $O_2^{\cdot-}$ controls the rate of production of these metabolites. The sequence of events which leads to the assembly of active NADPH oxidase is described in brief below.

NADPH oxidase is composed of at least six components: $p47^{phox}$, $p67^{phox}$, and $p40^{phox}$ found in resting neutrophils as a cytosolic complex, rac-2 a cytosolic ras-related protein, and membrane bound components $p22^{phox}$ and $gp91^{phox}$, comprising cytochrome b_{558} (cyt b_{558}). Translocation of cytosolic components to membrane and association with cyt b_{558} renders the complex functional. Cyt b_{558} then transfers electrons from NADPH to molecular oxygen generating $O_2^{\cdot-}$ (Equation I).

$$2O_2 + NADPH \rightarrow 2\,O_2^{\cdot-} + NADP^+ + H^+ \qquad \text{(Eqn 1)}$$

Cyt b_{558} is a membrane bound flavohemoprotein located predominantly in neutrophil specific granules and secretory vesicles, and partially in the plasma membrane. The membrane bound cytochrome components, $p22^{phox}$ and $gp91^{phox}$, closely interact with cyt b_{558}. $p47^{phox}$ which has a vital role in oxidase function, chaperones $p67^{phox}$ to the membrane. Membrane bound $p22^{phox}$ binds complexed $p47^{phox}/p67^{phox}$ following translocation and phosphorylation by protein kinase C of the latter. Rac-2 performs a critical role in the activation of NADPH oxidase; activation frees it from its complex with rho-GDI and allows subsequent interaction with $p67^{phox}$ [10].

Although it is well established that $O_2^{\cdot-}$ is not a damaging oxidant and therefore ineffective as a direct bacterial cell killing agent (reviewed in [12, 13]) it nevertheless has an important antimicrobial role. This is aptly demonstrated in chronic granulomatous disease, a group of inherited conditions, in which genetic deficiencies of NADPH oxidase components lead to diminished $O_2^{\cdot-}$ production, thereby providing an ineffectual inflammatory response to infection, contributing to other aspects of disease pathogenesis [10].

How then may $O_2^{\cdot-}$ help combat microbial infection? There are two dominant theories. First, $O_2^{\cdot-}$ may be converted via enzymatic and chemical reaction steps into an array of directly damaging oxidants. This can, for instance, be achieved by superoxide dismutase (SOD) conversion of $O_2^{\cdot-}$ to hydrogen peroxide (H_2O_2). H_2O_2 is in turn, either reduced to O_2 and water by catalase (Equations 2 and 3) or converted to HOCl by the action of MPO. Phagosomal MPO release occurs concomitantly with NADPH oxidase/SOD generation of H_2O_2, therefore, providing substrate for this enzyme and hence the ability to generate HOCl, which is a powerful oxidant with known anti-microbial function. In addition $O_2^{\cdot-}$ generated by NADPH oxidase can potentially react with HOCl to produce the hydroxyl radical ($^{\cdot}OH$) the most aggressive ROS known) and/or with nitric oxide (NO) to produce peroxynitrite ($ONOO^-$) another very reactive oxidant *(vide infra)*. Thus, the end-products of these reactions have the ability to cause damage to and kill microbes (Fig. 1).

$$2\,O_2^{\cdot-} + 2H^+ \rightarrow O_2 + H_2O_2 \qquad SOD \qquad \text{(Eqn 2)}$$

$$2H_2O_2 \rightarrow 2H_2O + O_2 \qquad catalase \qquad \text{(Eqn 3)}$$

Second, release of $O_2^{\cdot-}$ into the phagosome of the neutrophil consumes H^+ ions during dismutation (Equation 2); the subsequent rise in pH within the phagosome is compensated for by a net influx of K^+ ions. The increased phagosomal ionic content signals the release of proteases, which together with the high pH provides ideal conditions for protease action (reviewed in [13]). There is evidence to support both oxidant, and pH mediated antimicrobial actions of $O_2^{\cdot-}$ (reviewed in [12]).

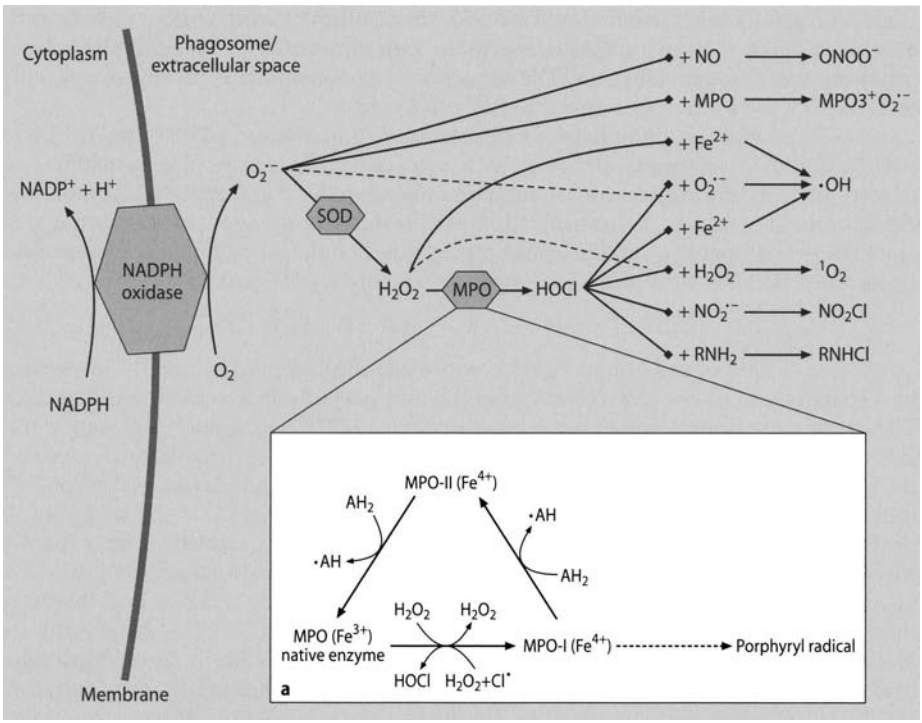

Fig. 1. Generation of oxidants by activated neutrophils, via NADPH oxidase and myeloperoxidase (MPO). **a** Peroxidation cycle of MPO, generation of hypochlorous acid (HOCl) and radicals. Key reactive oxygen species (ROS) and free radicals marked in gray.

Myeloperoxidase generation of HOCl and secondary oxidants

MPO utilizes H_2O_2 to oxidize chloride (Cl^-) to Cl^+ at physiological pH, thereby generating HOCl (Equation 4). The bactericidal properties of HOCl are attributable to its reactive nature and hence its ability to oxidize a variety of molecules including amino acids, nucleotides, lipids, and hemoproteins in much the same way as household bleach (sodium hypochlorite, NaClO) does. However, in addition to bacterial destruction, the non-specific nature of HOCl-mediated reactions also leads to associated host mediated tissue damage *(vide infra)*, which may have both pro or anti-inflammatory consequences.

$$H_2O_2 + 2Cl^- \rightarrow 2HOCl \qquad MPO \quad \text{(Eqn 4)}$$

The enzyme follows the normal peroxidase cycle, in which a single two-electron oxidation of native enzyme to compound I is followed by two successive one-electron reductions to native enzyme via compound II (Fig. 1a). Chloride, as well as other halides and pseudohalides (thiocyanate), are able to reduce compound I directly to native MPO by a two electron process (Equation 5) [14], this process yields HOCl and other hypohalous acids.

$$\begin{aligned} &MPO + H_2O_2 \rightarrow MPO\text{-}I + H_2O \\ &MPO\text{-}I + AH_2 \rightarrow MPO\text{-}II + AH^{\cdot} \\ &MPO\text{-}II + AH_2 \rightarrow MPO + AH^{\cdot} + H_2O \end{aligned} \qquad \text{(Eqn 5)}$$

Table 1. Oxidant products generated as a result of neutrophil activation (see Figure 1) [14, 15, 46].

Oxidant Product	Generated by	Reaction
$O_2^{\cdot-}$ (superoxide)	NADPH oxidase	$2O_2 + NADPH \rightarrow 2\ O_2^{\cdot-} + NADP^+ + H^+$
H_2O_2 (hydrogen peroxide)	SOD	$2\ O_2^{\cdot-} + 2H^+ \rightarrow O_2 + H_2O_2$
HOCl (hypochlorous acid)	MPO	$H_2O_2 + 2Cl^- \rightarrow 2HOCl$
$^1O_2$ (singlet oxygen)	HOCl	$HOCl + H_2O_2 \rightarrow {}^1O_2 + H_2O + H^+ + Cl^-$
$^{\cdot}OH$ (hydroxyl radical)	$O_2^{\cdot-}$ / HOCl	$HOCl + O_2^{\cdot-} \rightarrow {}^{\cdot}OH + O_2 + Cl^-$
$^{\cdot}OH$ (hydroxyl radical)	$Fe2^+$ / HOCl	$HOCl + Fe^{2+} \rightarrow {}^{\cdot}OH + Fe^{3+} + Cl^-$
NO_2Cl (nitryl chloride)	HOCl	$HOCl + NO_2^- \rightarrow NO_2Cl + {}^-OH$ (chlorinating / nitrating compound)
$ONOO^-$ (peroxynitrite)	$O_2^{\cdot-}$	$O_2^{\cdot-} + NO \rightarrow ONOO^-$
RNHCl (chloramines)	HOCL	$RNH_2 + HOCl \rightarrow RNHCl + H_2O$
$AH^{\cdot}$ (radical product)	MPO	$MPO\text{-}I + AH_2 \rightarrow MPO\text{-}II + AH^{\cdot}$ $MPO\text{-}II + AH_2 \rightarrow MPO + AH^{\cdot} + H_2O$ (compound I & II form radical products by a one-electron subtraction)
$MPO^{3+}O_2^{\cdot-}$ (compound III)	$O_2^{\cdot-}$	$MPO^{3+} + O_2^{\cdot-} \rightarrow MPO^{3+}O_2^{\cdot-}$ $MPO^{3+}O_2^{\cdot-} + O_2^{\cdot-} \rightarrow MPO^{2+}O_2^{\cdot-} + O_2$ (involved in oxidation of aromatic substances, via $MPO^{2+}O_2^{\cdot-}$, an active hydroxylating intermediate)

In addition, the activated states of the enzyme, predominantly compound I, are also able to oxidize an array of different substrates which may have further implications for pro and anti-inflammatory responses. Indeed, compound I, and to a lesser extent compound II, form radical products by a one-electron subtraction ($AH^{\cdot}$, Equation 5). Target molecules include tyrosine, tryptophan, sulfhydryls, phenol and indole derivatives, nitrite, H_2O_2, and xenobiotics [15]. Such reactions are favored as the couple compound I/II has one of the highest reduction potentials found in cellular systems [16].

As one of the most potent oxidants produced by neutrophils, HOCl is directly damaging to bacteria as evidenced by chlorination of bacterial components within the phagosome [17]. However, further ROS with the potential for bacterial killing, as well as tissue damage may be produced by additional reactions involving HOCl. Oxidant products generated as a result of neutrophil activation are shown in Table 1.

Oxygen independent mechanisms

Oxygen independent mechanisms of neutrophil-mediated antimicrobial activity are centered on neutrophil granule enzymes and proteins, which form the foundation of the innate immune system. Neutrophil stimulation initiates membrane remodeling by means of exocytosis and phagocytosis. During exocytosis, cytoplasmic granules translocate and fuse to the plasma membrane, either expressing their contents on the cell surface (e.g., adhesion molecule) or discharging them into the extracellular space. Furthermore, exocytosis supplies stores of membrane for the purposes of phagoscytosis, which involves the internalization of receptor-target complexes by calcium triggered cytoskeletal remodeling. Fusion of azurophilic and specific gran-

Table 2. Neutrophil proteins with antibacterial properties.

Protein	Functions
Bactericidal permeability increasing protein (BPI)	allows binding to lipopolysaccharide, has opsonic effects and mediates bacterial attachment to, and phagocytosis by, neutrophils [47] binding causes an increase in the permeability of the outer membrane of Gram-negative bacteria, hydrolysis of bacterial phospholipase and interruption of cell division [48]
Defensins	major components of azurophilic granules disrupt target cell membranes [49] act synergistically with BPI against Gram-negative bacteria
Proteinase 3	found in azurophilic and secretory granules role in the amplification of the inflammatory response.
Elastase	a potent serine protease degrades outer membrane protein (which is highly conserved among Gram-negative bacteria) [50]
PLA_2 Cathelicidins	PLA_2 has potent bactericidal activity against *Staphylococcus aureus* bacterial killing is mediated by phospholipolysis of cell walls cathelicidins, found in the mobile specific granules role in extracellular killing (upon release into inflammatory fluids), by means of bacterial phospholipid hydrolysis both proteins synergize with BPI for bacterial killing [10].
Metalloproteinases (collagenase, gelatinase)	released in an inactive pro-enzyme form, require calcium, function at neutral pH aid migration of neutrophils through basement membrane collagenase, whose enzymatic activity depends upon oxidation by HOCl, has a preference for native collagen gelatinase degrades denatured and native collagen types IV and V activation of pro-gelatinase occurs by both oxidative and non-oxidative mechanisms [10]

ules with phagocytic vacuoles leads to the formation of phagolysosomes. These events culminate in the production of a highly toxic intravacuolar milieu, subject to oxidant metabolites formed by MPO and NADPH oxidase, cationic proteases interacting and disrupting negatively charged bacterial cell wall components, and other antimicrobial proteins.

Proteins with specific antibacterial properties are found primarily in azurophilic granules; these include bactericidal permeability increasing protein (BPI) and defensins (see Table 2). Further proteins have a supporting role in antibacterial defense; these include lactoferrin, whose capacity to bind iron deprives bacteria of a co-factor for proliferation.

Myeloperoxidase

MPO was first isolated from tuberculous empyema fluid by Agner in the first part of the 20th century [18]. Initially named verdoperoxidase due to its green color, it was subsequently renamed, as its distribution was limited to myeloid cell lines. MPO (donor: hydrogen peroxide oxidoreductase, EC 1.11.1.7) [19] is a lysosomal heme

protein unique to neutrophils and monocytes. Monocytes contain one third of the MPO found in neutrophils.

MPO Structure

Among all the mammalian peroxidases, the three-dimensional structure is known only for MPO. It has been refined to 1.8Å resolution by x-ray crystallography [20]. MPO is a strongly cationic glycosylated protein with a molecular weight of ~146 kDa. The homodimer consists of two symmetrically related halves linked by a single disulfide bridge, each consisting of a central structure of 5 α-helixes covalently attached to a central heme group, one from the 14.5 kDa light polypeptide chain and four from the large 58.5 kDa heavy chain, composed of 106 and 467 amino acids, respectively [21].

Each heme group is covalently attached via two ester and one sulfonium linkage. The heme group is a derivative of protoporphyrin IX, modifications on pyrrole rings allowing for formation of two ester linkages. The sulfonium linkage between the sulfur atom of Met^{243} and the β-carbon of the vinyl group on pyrrole ring A, is a unique feature of MPO; other mammalian peroxidase lack methionine at this position [20]. The sulfonium ion linkage serves as an electron withdrawing substituent, and appears to be responsible for the lower symmetry of the heme group and distortion from planar conformation. The latter contributes to the unusual spectral properties of MPO, with a red shift in the Soret band (428 nm) and strong absorption bands in the visible spectrum being responsible for its green color. In addition, the redox properties of MPO, which differ from other mammalian peroxidases, seem to have their origin in the electron withdrawing sulfonium linkage and heme distortion [21]. Selective cleavage along the disulfide bridge linking the two halves of MPO yields hemi-enzyme, which exhibits spectral and catalytic properties indistinguishable from the intact enzyme [20].

MPO Biosynthesis: Genetics

MPO is encoded in a single 14 kb gene on the long arm chromosome 17 (17q23.1) [22]. MPO synthesis is restricted to late myeloblasts, and promyelocytes in the bone marrow, terminating as myeloid progenitors, enter the myelocyte stage of differentiation into neutrophil and related cell types. Monocyte precursors also synthesize MPO during bone marrow maturation, with normal expression limited to this stage of myeloid development. MPO is, therefore, present, but not actively synthesized, in circulating monocytes, Furthermore differentiation into macrophages is accompanied by downregulation of MPO synthesis [23]. However, MPO gene expression can potentially be reinitiated in certain disease states within a suitable tissue setting; e.g., *de novo* macrophage synthesis of MPO in Alzheimer's disease [24], peripheral mature neutrophil and monocyte MPO synthesis in anti-neutrophil cytoplasm antibodies (ANCA)-associated glomerulonephritis [25].

Transcription of MPO is regulated in a tissue and differentiation specific manner. Progressive demethylation in the 5' flanking region of the MPO gene is pre-requisite, providing the open chromatin structure required for transcription. Expression of the gene is regulated by the cell specific transcription factor, AML-1; integrity of the AML-1 binding site is essential for activity of MPO proximal enhancer [26]. Numerous allelic polymorphisms have been identified for MPO, the most studied of which is the promoter region polymorphism, -463G/A. This site contains an Alu receptor

response element (AluRRE), which is recognized by a variety of nuclear receptors, including Sp1 [27], MPO transcription being enhanced by an intact Sp1 binding site (i.e., -463G). The functional significance related to this single nucleotide polymorphism is unclear due in part to the strong gender influence and varied results from studies of different inflammatory diseases. In addition, peroxisome proliferator-activated receptors also regulate MPO gene expression via AluRRE [28]. Furthermore, AluRRE has been implicated in the incidence of a variety of inflammatory disorders [23], the pathogenesis of which may in part be due to regulation of MPO expression.

At the phenotypic level in normal human bone marrow, cytokines such as tumor necrosis factor-α (TNF-α) can decrease MPO transcription, whereas G-CSF induced differentiation of multi-potential progenitor cells results in activation and nuclear recruitment of proteins (Pu1 and C-EBP family) that bind to a distal MPO enhancer, which regulates MPO gene expression via the *proximal* enhancer and AML-1 [26].

A variety of MPO genetic mutations have been identified. Inherited MPO deficiency is more common in the USA and Europe (1 in 2000–4000) than in Japan (1 in 55,000). Deficiency is associated with an increased susceptibility to infection and incidence of malignancy. However, unlike NADPH oxidase deficiencies, reduced or absent MPO function often has a modest clinical phenotype [23]. So whilst MPO does have a role to play in terms of antimicrobial activity in humans, under many circumstances this does not seem to be an essential function.

MPO Biosynthesis: Assembly

During the initial stages of MPO biosynthesis, the 80 kDa primary translational product, *pre*proMPO is converted into 90kDa *apopro*MPO, following co-translational cleavage of a signal peptide, N-linked glycosylation and limited deglucosylation of high mannose oligosaccharide side chains in the endoplasmic reticulum. *Apopro*MPO has no peroxidase activity, as it lacks a prosthetic heme group. During its long half life within the endoplasmic reticulum, oligosaccharide side chains are added, which contribute to the transient associations with molecular chaperones calreticulin and calnexin. The latter are high capacity, low affinity calcium binding proteins, whose interactions with glycoproteins result in limited deglucosylation and modification during endoplasmic reticulum transit, promoting correct folding and 'quality' control. Calreticulin is a soluble protein within the endoplasmic reticulum; calnexin is a transmembrane protein. Association of *apopro*MPO with calnexin leads to incorporation of heme forming the enzymatically active *pro*MPO, and exit into the Golgi apparatus and downstream secretory pathway. Heme not only initiates peroxidase activity, but is also essential for the conformational change of *pro*MPO required for export from the endoplasmic reticulum. The heme prosthetic group in MPO is derived from Fe^{III} protoporphyrin IX.

Following transport from the endoplasmic reticulum to the Golgi apparatus, MPO precursors must reach their final intracellular destination or exit into the extracellular space. To achieve this, cells separate proteins destined for intracellular compartments or secretion. The conversion of *apopro*MPO to *pro*MPO in the endoplasmic reticulum is extremely slow; processing in the Golgi apparatus, granule targeting, and secretion are rapid. Like many glycoprotein enzymes, MPO precursors undergo a complex series of proteolytic processing to achieve final protein structure. *Pro*MPO is converted to a short lived 74 kDa intermediate (in the Golgi apparatus) following deletion of a 125 amino acid propeptide. In, or en route to, the primary granule this 74 kDa transient intermediate is cleaved into two subunits (59 kDa

α-subunit and 13.5 kDa β-subunit) linked by covalent bonds associated with the heme group. Mature MPO is formed by interaction of two heavy-light chain units forming a symmetrical homodimer linked by disulfide bond between heavy subunits. Mature dimeric MPO is stored in azurophilic granules. It is the only member of the mammalian peroxidase family that is a dimer.

The secretory pathway is constitutive. Cells must tag and retrieve proteins from the secretory pathway and redirect them to the target organelle. This is achieved either by direct transport through the Golgi apparatus via late endosomes to lysosomes, or by indirect targeting of the plasma membrane and later internalization into early and then late endosomes. During the course through the Golgi apparatus, *pro*MPO destined for secretion is exposed to a variety of transferases and glucosidases, extensively modifying the oligosaccharide units forming complex oligosaccharides. The activity of a constitutive pathway is limited by the rate of synthesis of a given protein. Secreted MPO is monomeric, which suggests that dimer formation of mature MPO either takes place in the granule targeting pathway, or after *pro*MPO has been compartmentalized into the granule. MPO species isolated from human plasma include both precursor and mature forms [23].

Neutrophil Granules and MPO Storage

Neutrophils exhibit four classes of granules. Primary or azurophilic granules contain preformed antimicrobial proteins: MPO, serine proteases and lysosyme hydrolases for release into phagosomes; they appear early at the promyelocyte stage. Specific or secondary granules appear later in neutrophil maturation at the metamyelocyte stage, containing proteins with antimicrobial activity, including collagenase, lactoferrin and gelatinase [10]. Tertiary or gelatinase granules resemble specific granules and are enriched with enzymes that are exocytosed, contributing to the degradation of intracellular junctions and extracellular matrix [2]. On reaching maturity, neutrophils develop secretory vesicles which have cyt b_{558} and adhesion molecules on the membrane surface. They contain proteins of endocytic origin and serve as a reservoir of membrane constituents that can be rapidly mobilized for phagocytosis [2, 29].

Neutrophil stimulation causes intracellular granule secretion in the following order: secretory, gelatinase, specific, and azurophilic. Degranulation is triggered by changes in intracellular calcium; moderate increases of <0.25 μM are required for secretory granules, and 0.7 μM for azurophilic and specific granules[15].

Azurophilic granules contain glycosaminoglycans, molecules that may provide an anionic matrix, binding predominantly cationic cytotoxic granule proteins in a conformation or state that renders them inactive [30].

Neutrophil-induced Host Tissue Damage

Host tissue damage occurs by a variety of mechanisms, including premature activation of neutrophils, extracellular release of cytotoxic molecules during microbial killing and native tissue remodeling, failure of cessation of pro-inflammatory responses, and overwhelmed local anti-oxidant and anti-protease protection. Generated ROS and granular antimicrobial proteins or enzymes are predominantly released into phagosomes, creating a controlled environment of extreme toxicity and, thus, preventing release into the extracellular space. However, in certain cir-

cumstances, where targets are too large to be phagocytosed, these substances may be released into the extracellular environment. Indeed, neutrophil granular enzymes and proteins, in addition to end products of ROS damage have been detected in fluid and tissue isolated from inflammatory sites [31], implicating neutrophils in the pathogenesis of sepsis-induced organ dysfunction [5], chronic inflammatory conditions, and ischemia-reperfusion injury. The neutrophil contribution to the pathogenesis of organ dysfunction is perhaps most evident in the acute respiratory distress syndrome (ARDS). Substantial pulmonary recruitment of neutrophils occurs in ARDS, with non-survivors having the highest numbers [32]. Alveolar and circulating neutrophils are activated, evidenced by increased expression of β2-integrins and cytokine profiles. Activation correlates with an increased degree of lung injury [33]. Furthermore, these cells demonstrate reduced rates of apoptosis. In animal models of lung injury, neutrophil depletion or inhibition of localization attenuates histological injury and improves survival (reviewed in [4]).

Moreover, the array of oxidant molecules (Figure 1) produced by MPO are implicated in tissue damage, the outcome of which is dependent on the dose of the oxidant, higher doses causing necrosis and affecting signaling pathways, and apoptosis and growth arrest (endothelial cells) occurring at lower amounts (reviewed in [34]). Neutrophils are able to generate long-lived oxidants, with half lives of up to 18 hours [35].

Pathways of HOCl-induced Tissue Damage

Numerous *in vitro* studies have demonstrated that HOCl can mediate tissue injury (reviewed in [34]), and HOCl has been detected in various pathological disease states [36]. Indeed HOCl can also halogenate cell constituents, chlorinating amines to chloramines (Equation 6). Chloramines have a longer half life than HOCl, retain two oxidizing equivalents allowing similar reactions to those of HOCl, and are able to cross plasma membranes thereby exporting or importing the potential bio-molecule modifications; in addition, said compounds also breakdown into aldehydes which are chemotactic and, at higher concentrations, cytotoxic [34].

$$RNH_2 + HOCl \rightarrow RNHCl + H_2O \qquad \text{(Eqn 6)}$$

In addition, HOCl reacts with nucleotides and DNA. NADH and NH-groups of pyrimidines are particularly susceptible, with DNA double stranded breaks occurring [37].

Moreover, chlorohydrin derivatives arise from HOCl-mediated chlorination of unsaturated fatty acids and cholesterol [38]. This, in conjunction with the cationic properties of MPO facilitating attachment to biological membranes, leads to the susceptibility of lipid components of biological membranes to attack by HOCl.

Cellular proteins are targets of HOCl, producing several different oxidation products. HOCl can act as a one or two-electron oxidizing agent. Thiol-groups, thioethers (mehionine), heme groups, and iron-sulfur centers are the most readily oxidized, at a rate 100 times that of amines [34]. Cysteine and methionine residues are readily oxidized. Amino groups of lysine and N-terminal amines react forming chloramines, which can subsequently react with thiols. Chloramine formation may generate radicals that result in protein fragmentation, lipid peroxidation, and protein carbonyl formation [34]. In addition, cell lysis has been demonstrated as a consequence of irreversible protein crosslink formation in membranes exposed to HOCl [39].

Tyrosine, tryptophan, histidine, arginine, and amide peptide bonds are oxidant targets of HOCl [34], and as such the end product of the reaction of tyrosine with HOCl, 3-chloro-tyrosine, is used as a marker of neutrophil activation [40].

Although there are many potential biological targets for HOCl mediated reactions, low and high molecular mass thiols are among the most susceptible moieties. As thiols, dependent on biological environment and setting, have numerous important functions including as antioxidants, as redox signaling switches, as ligand-binding moieties, and as key determinants of tertiary structure, any perturbation in thiol oxidation state may have profound consequences.

Indeed, HOCl-mediated cellular necrosis, as demonstrated in rodent macrophage cell lines, has demonstrated disruption of plasma membrane ion transport channels that appears to be attributable to oxidation of membrane thiol groups as a key instigating factor. Furthermore, at lower concentrations of HOCl, apoptosis rather than necrosis ensues [41] indicating a thiol mediated cell signaling function. The fact that HOCl, like chloramines, can penetrate the cell membranes is important in this regard as this property enables this oxidant to instigate changes via reaction with intracellular constituents at sites distant from its zone of production [34].

Modulation of Neutrophil Function In Sepsis

Septic shock and multiple organ dysfunction are the most common causes of death in patients with sepsis, with associated mortalities of 25–30 and 40–70%, respectively. The incidence is increasing; approximately one third of critical care admissions meeting the criteria for severe sepsis in the UK [42]. However, despite an observed decrease in mortality, the number of sepsis-related deaths is likely to rise still further due to an aging population, the use of increasingly sophisticated interventions, and the rising incidence of treatment resistant organisms.

Evidence from numerous observational and *in vivo* studies (Table 3) indicates that MPO-derived oxidants do contribute to tissue modification and damage. However, although therapies that target the neutrophil and reduce either its activation or its accumulation in the tissues can reduce tissue injury in animal models of sepsis, anti-neutrophil therapies have not conferred benefit in clinical trials. Conversely, the cytokine G-CSF, which increases neutrophil release from the bone marrow and delays neutrophil apoptosis, has failed to show convincing evidence of either harm or benefit in human sepsis, although either effect can be reproduced in animal models [2]. There are several known inhibitors of NADPH oxidase and MPO, but their use to date has been confined to *in vivo* animal and *in vitro* studies.

Several clinical trials have been conducted, using interventions targeting pathways or mediators of sepsis, aiming to either directly or indirectly affect neutrophil function. These include anti-lipopolysaccharide, anti-TNF-α, interleukin-1-receptor antagonist (IL-1ra), anti-inflammatory drugs (ibuprofen, corticosteroids), bradykinin antagonist, platelet activating factor acetyl hydrolase, elastase, and NO synthase inhibitors (reviewed in [43]). All have proved ineffective in terms of a mortality benefit. New therapeutic targets are being investigated, with particular focus on bacterial products (TLR) and the coagulation pathway.

An alternative approach, which may be acceptable for use in human studies, relates to certain properties of the plasma protein, ceruloplasmin. Ceruloplasmin, an acute phase protein, and the major copper containing protein of plasma, has several antioxidant functions ascribed to it, including the recently discovered ability to

Table 3. Additional functional changes attributed to MPO and HOCl

Cellular function homeostasis	HOCl decreases ATP at sublethal doses in vitro HOCl can react directly with ATP, limiting its availability HOCl causes inhibition of GAPDH, mitochondrial respiration and glucose transport in vitro HOCl decreases NAD at high doses in vitro (reviewed in [34])
Cellular integrity	HOCl mediated increased cell permeability and oxidative damage to cytoskeletal proteins in vitro, via mobilization of zinc and loss cell thiols (reviewed in [34])
Serine protease inhibitor inactivation	HOCl inactivates α1-anti-proteinase, the major circulating inhibitor of serine proteases
Metalloproteinase activation	HOCl mediates the activation of pro-collagenase and pro-gelatinase [10]
Vessel tone: nitric oxide bioavailability	MPO regulates the availability of nitric oxide (NO) in inflammation (rodent model), localising around the endothelium, thereby impairing NO-dependent blood vessel relaxation[51] NO serves as a substrate for MPO, potentially influencing bioavailability [52]
Cardiac myocyte	HOCl impairs contractility in rodent models via oxidation of thiol groups inducing loss of ATPase activity and inhibition of Na^+K^+ATPase (reviewed in [34])
Neutrophil responses	MPO modulates inflammatory responses, by inactivation of granular contents and decreased binding to chemotactic receptors MPO contributes to the physiological feedback by termination of neutrophil recruitment [15]

bind MPO [44, 45], a process that decreases generation of HOCl by MPO. Indeed, a binding deficit between this protein and MPO may contribute to, or perpetuate, pro-inflammatory responses related to HOCl under certain defined circumstances. The ability to manipulate ceruloplasmin levels in plasma may afford some protection against the unwanted side effects of extracellular MPO activity. However, studies in the research area are limited, hence more investigations need to be undertaken before such a therapeutic approach can be considered.

Conclusion

Oxidative modification of bio-molecules including that attributable to MPO-derived oxidants seems to be an inevitable consequence of tissue injury. There seems little doubt that this is also the case in patients with sepsis. Moreover, there is ample evidence that neutrophil MPO-derived HOCl can cause biological damage and alter pro-inflammatory cell signaling responses with the potential to modulate inflammation and, hence, the onset of the sepsis syndromes. There are, however, confounding issues including the failure of antioxidant therapy (in humans) to limit sepsis and critical illness. The possible explanations for this discrepancy include the complex nature of *in vivo* antioxidant function, the timing of administration, limited focus on pro-inflammatory pathways, which may in themselves prove critical to host defense. Finally, as oxidants, including HOCl, are cell-signaling agents that may up-

regulate beneficial and self regulating responses as well as those of pro-inflammatory origin, modulation of such signaling events by antioxidant intervention may actually prove counterproductive. Thus, although oxidants derived from neutrophil MPO may contribute to the onset of the sepsis syndromes via numerous mechanisms the extent to which the production of these species contribute to, or are a consequence of, the disease process remains unclear.

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Are Mitochondria Responsible for Improved Outcomes in Recent Studies?

A. Johnston and T. Whitehouse

Introduction

The Acute Respiratory Distress Syndrome Network (ARDSnet) group compared low tidal volume ventilation with standard ventilatory strategies [1]; early goal directed therapy (EGDT) advocated administering fluids, blood products, and dobutamine to achieve oxygen delivery goals to septic patients on arrival in the emergency department [2]; and intensive insulin therapy was used to maintain tight glucose parameters in surgical patients [3]. These are landmark but disparate trials that have demonstrated major improvements in outcome and feature in the Surviving Sepsis Campaign Guidelines for managing sepsis [4]. In this chapter, we discuss the role mitochondrial dysfunction plays in critical illness and its manifestation as a disruption of cellular energetics. We suggest that the positive outcomes from the above-mentioned trials relate to a reduction of impaired mitochondrial function and a reduction in the subsequent generation of inflammatory signals.

Mitochondria provide a mechanism for eukaryotic cells to generate ATP from energy-rich molecules. They, therefore, may influence glucose and lipid metabolism, but are also involved in calcium signaling, specialized protein assembly, and apoptosis [5]. Mitochondrial dysfunction in critical illness results in an acquired derangement of energy generation and also results in the generation of reactive oxygen species (ROS). Disruption of ATP production is not necessarily related to failure of local oxygen delivery (DO_2) as it may be caused by direct inhibitors such as endotoxin [6] and may also occur due to the inhibitory and damaging effects of nitric oxide (NO) and other free radicals on the mitochondrial respiratory chain complexes [7, 8]. This effect has recently been suggested in human studies [9]. ROS are formed continuously by the respiratory chain complexes at complex I and III and are involved in the control of both respiration and other cellular processes [10]. ROS generation increases during critical illness when a surfeit of electrons accumulates. These are transferred to oxygen to form superoxide, O_2^- [11].

Ventilation with Lower Tidal Volumes as Compared with Traditional Tidal Volumes for Acute Lung Injury and Acute Respiratory Distress Syndrome (The ARDSnet Trial) [1]

The ARDSnet study group [1] randomized 861 patients with ARDS to standard tidal volumes of 12 ml/kg versus low tidal volumes of 6 ml/kg with limitation of the plateau pressures in the treatment group. The trial stopped recruitment early because the intervention group had a mortality of 31% compared with 39.8% in the stan-

dard tidal volume group – a relative reduction in mortality of 22%. In addition the treatment group had fewer ventilator dependent days. Sixty percent of the patients in both groups had either sepsis or pneumonia.

ARDS is a Manifestation of Systemic Inflammation and Perpetuates Inflammation

ARDS is a heterogeneous syndrome consisting of vascular injury, alveolar leak and alveolar inflammatory infiltration. In its later stages, it may progress to fibrosis. High tidal volume ventilation worsens lung injury by subjecting the alveoli to stretch inducing volutrauma, overexpansion of alveoli due to high ventilation pressures; atelectrauma, shearing from repetitive opening and closing of alveoli; and biotrauma, an inflammatory injury that occurs secondary to the tissue damage caused by both volutrauma and atelectotrauma [12]. Together these forces contribute to the syndrome of ventilator-induced lung injury (VILI). Evidence suggests that ARDS also leads to impaired function in distant organs [13]. For example, in one study, patients with ARDS were at greater risk of renal failure [14].

Is there Mitochondrial Dysfunction in ARDS?

Inappropriate ventilation strategies alone are sufficient to cause pathophysiologic changes identical to those found in ARDS [13]. Examination of rabbit alveoli ventilated with plateau pressures of 50 cmH_2O revealed accumulation of lung neutrophils. In a cell model simulating the effects of ventilation, Chapman and coworkers [15] examined the effects of cyclic mechanical strain on pulmonary cells *in vitro*. They subjected cultured human and rat alveolar type II cells and cultured human airway epithelial cells to mechanical strain and examined the production of $O_2^{\cdot-}$. Superoxide production increased significantly following 2 hours of the application of 15–30% elongation at 30 cycles per minute for human epithelial and alveolar cells, and following the same time period of 15% elongation at 15 cycles per minute in rat alveolar type II cells. Blocking the action of mitochondrial complex I using rotenone reduced the amount of $O_2^{\cdot-}$ produced.

In the same paper, NADPH oxidase activity increased following two hours of 20% strain at 30 cycles per minute suggesting non-mitochondrial generation of $O_2^{\cdot-}$. The authors speculated that the mechanism of mitochondrial production of $O_2^{\cdot-}$ might involve distension of the mitochondrial membrane and activation of the mitochondrial ATP-sensitive K^+ channel, which has been linked to ROS generation.

Lung endothelial cells are also affected by cyclic strain. In an elegant series of experiments Ali et al. [16] examined the effects of a 25% cyclic mechanical strain on cultured human endothelial cells. They exposed cells to 3 seconds stretch and 1 second relaxation at a frequency of 15 cycles per minute. The authors felt this strategy replicated lung vascular stretch during normal tidal respiration, although it is worth noting the inverse ratio of the timing of the stretch. Using fluorescent markers, they found a significant increase in ROS generation after 6 hours of cyclic strain. Reduction in ROS formation was also found following the addition of the mitochondrial complex I inhibitor, rotenone, but not by the addition of the NADPH oxidase inhibitor, apocynin. By treating the cells with cytochalasin D to disrupt the cytoskeletal actin, they were able to almost completely abolish ROS generation. Using antimycin A to block mitochondrial activity after complex III gave a similar response to that produced by strain, suggesting ROS generation at or before complex III. They also compared cells with and without mitochondria and were able to display the net con-

tribution of mitochondrial ROS generation. Other work discussed in the paper has demonstrated that blockage of electron transport prior to complex III abrogates ROS generation. The authors also produced strong evidence that the ROS caused an increase in nuclear factor kappa B (NF-κB) and increased expression of vascular cell adhesion molecule (VCAM)-1 mRNA, which may act to propagate inflammation.

Mitochondria and Alveolar Leak

Ichimura et al. [17] used real-time *in situ* fluorescent microscopy to examine the effect of raised pulmonary venular capillary pressure on mitochondrial calcium flux. Using a rodent model, the authors infused various fluorescent dyes with different excitation frequencies, which were specific to mitochondria, calcium ions, and ROS. The authors found that within one minute, an increased venular capillary pressure of 15 cmH_2O resulted in a marked increase in ROS generation. This was blocked by the complex I inhibitor, rotenone. The use of antimycin A to block the respiratory chain after complex III increased the fluorescent signal, while diphenyleneiodonium and allopurinol, selective inhibitors of NADPH oxidase and xanthine oxidase, respectively, did not reduce ROS generation. The authors concluded that the ROS produced in response to increased capillary pressure was of mitochondrial origin.

Ichimura et al. [17] also suggested that the effect of stretch on mitochondria is mediated in part by a calcium signal from the cytosol rather than being due solely to mechanical disruption of the mitochondrial membrane. By selectively blocking the calcium response in cytosol and mitochondria they were able to show that calcium oscillations in the cytosol led to a mitochondrial calcium response, which resulted in the generation of ROS. These findings are in line with those of Ali et al. [16] and are in keeping with mechanical coupling of the stretch to ROS generation via actin elements in the cytoskeleton.

Neutrophil Recruitment Perpetuates Lung Injury

Neutrophil infiltration into alveoli occurs early in ARDS and is a major (though not invariable) contributor to the pathophysiology [18]. Once in the interstitium and alveoli, neutrophils produce cytokines including tumor necrosis factor (TNF)-α, interleukin (IL)-1β, IL-6, IL-8 and IL-10, oxidants, platelet activating factor (PAF), and proteases, all of which worsen the endothelial, epithelial, and alveolar inflammation [18]. Ichimura et al. [17] suggest that lung venular endothelial mitochondria may contribute to neutrophil recruitment. These authors demonstrated an increase in both P-selectin mRNA expression and P-selectin exocytosis and expression on the endothelial cell membrane. They concluded that ROS generation (in particular H_2O_2) in response to pressure was part of a signaling mechanism resulting in increased P-selectin expression [17]. P-selectin facilitates neutrophil margination and translocation through the endothelium and interstitium into the alveoli as well as playing a role in neutrophil activation.

Mitochondrial Involvement in End-organ Dysfunction in ARDS

Apoptosis is mediated to a large extent by a mitochondrial pathway [19]. For example, a study by Imai et al. [20] demonstrated increased pulmonary and plasma levels of monocyte chemoattractant protein (MCP)-1, IL-8, growth regulated oncogene, and transaminase enzymes – chemokines involved with apoptosis – in a group of

animals ventilated at high tidal volumes. They also assessed apoptosis in lung, kidney, and gut cells using a combination of terminal deoxynucleotidyltransferase-mediated dUTP nick-end labeling (TUNEL)/propidium iodide nuclear staining and electron microscopy and found increased apoptosis in all tissues except lung in the group subjected to the high tidal volume and low positive end-expiratory pressure (PEEP) ventilatory strategy.

Consequences of High FiO_2 in ARDS

In a rodent model using hyperoxia, Pagano et al. [21] suggested hyperoxia alone was a stimulus for apoptosis. They studied the intracellular distribution of cytochrome c and the proapoptotic factor, Bax. In the control animals, cytochrome c was found almost exclusively in the mitochondria, whereas in the ARDS animals it was also found at increasing concentrations in the cytosol from 48 hours reaching statistical significance at 72 hours. Bax protein concentrations increased in the mitochondria in animals exposed to hyperoxia. Marked mitochondrial swelling and disrupted mitochondria accompanied changes in this marker. Cyclosporin A, which blocks formation of the mitochondrial permeability transition pore, was shown to reduce mitochondrial structural changes on electron microscopy as well as reducing macroscopic evidence of lung damage and bronchoalveolar lavage (BAL) protein levels.

The effects of mitochondrial dysfunction in ARDS are summarized in Figure 1.

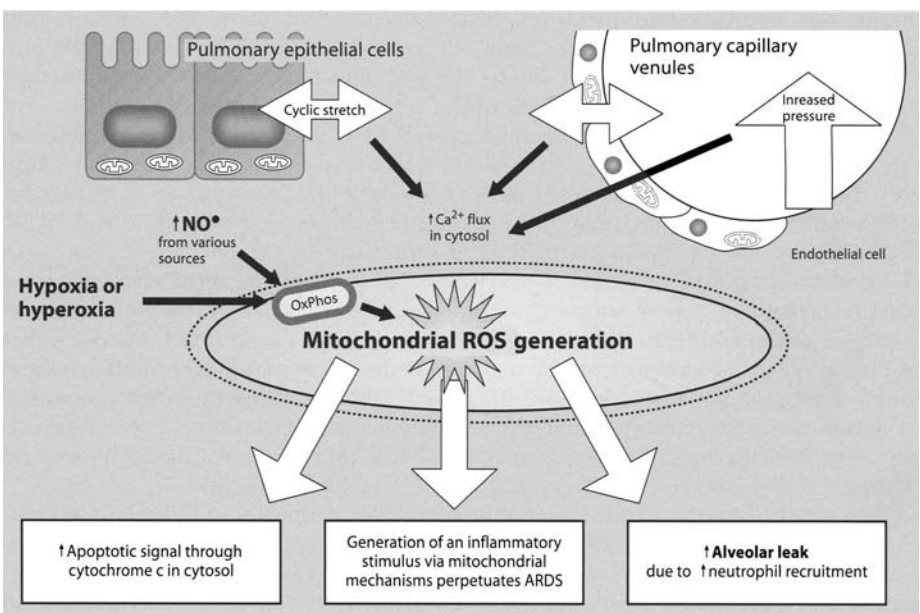

Fig. 1. Mitochondrial mechanisms contributing to lung and distant organ injury in ARDS. The figure summarizes the factors that contribute to mitochondrial dysfunction and generation of reactive oxygen species (ROS). These include stretch of pulmonary epithelial and endothelial cells, hypoxia, hyperoxia, and nitric oxide (NO). The consequences of ROS generation are depicted at the bottom of the figure. OxPhos: mitochondrial respiratory chain complexes.

Early Goal-directed Therapy in the Treatment of Severe Sepsis and Septic Shock [2]

Rivers et al. [2] carried out a randomized controlled trial of resuscitation to predetermined goals for patients attending the emergency room with severe sepsis and septic shock. In-hospital mortality was 30.5% in the intervention group and 46.5% in the standard management group. The authors found improved markers of end organ dysfunction in the treatment group, with significantly lower lactate (suggesting less anerobic respiration), base deficit, and higher pH. Interestingly, the intervention group received the same total amount of fluid over the 72 hours, but the timing of its administration was different as they received 1.5 liters more fluid in the first 6 hours; they also received more blood transfusions and were more often treated with inotropic support.

In contrast, Hayes et al. [22] carried out a trial of elevating systemic DO_2 in 100 patients during which they administered fluids, dopamine, and blood to achieve various goals for cardiac index, DO_2, and oxygen consumption after at least 24 hours of established sepsis. Intensive care unit (ICU) mortality in the treatment group was 50% as compared with 30% in the control group. The nine patients who responded to fluid challenges alone were not randomized and all survived.

Mitochondria are Implicated Early in Critical Illness

Rosser et al. [23] found that tissue oxygen rises in fluid resuscitated rats given endotoxin. This rise occurred even with low doses of endotoxin and occurred rapidly – within one hour of administration. Ninety-five percent of oxygen consumption occurs in the mitochondrion. As tissue oxygen is the balance between supply and oxygen consumption the authors suggest that cellular oxygen use was decreased as oxygen supply was maintained by the use of a resuscitated model [23].

Work in both animals and humans suggests that mitochondria are damaged in sepsis. Damage occurs early in the disease process and various groups have suggested that the mechanism involves NO combining with superoxide to form peroxynitrite which irreversibly damages the respiratory chain complexes I, II, and III [8, 9, 24, 25].

In a baboon model of sepsis, Welty-Wolf et al. [26] administered endotoxin and used intravenous fluids to support the blood pressure. Refractory hypotension was treated with dopamine. In muscle biopsies, these authors found that within 12 hours of the insult, there was electron micrograph evidence of increases in mitochondrial inner membrane surface area; this represented either a scaling up of mitochondrial oxidative phosphorylation capacity in response to increased demand or early evidence of damage to the inner membrane. Subsequent samples at 24 hours and beyond showed changes consistent with damage to the membrane.

Brealey et al. demonstrated that mitochondrial complex I activity was significantly lower at 24 hours in the most severely ill subgroup in skeletal muscle taken from septic rats. This group of rats had undergone a period of more than 6 hours of profound hypotension [25]. In humans, they also showed changes in mitochondrial function within 24 hours of admission to the (ICU) with evidence of more severe mitochondrial dysfunction in patients who did not survive than in those who did [9].

Does Early Mitochondrial Dysfunction also Stem from Hypoxic Injury to the Mitochondria from Local Hypoperfusion?

One well-conducted example is the study by Sakr et al. in patients with septic shock [27]. Using orthogonal polarization spectral (OPS) imaging, the authors examined the pattern of sublingual microvascular perfusion over time. They found that patients who survived had a significant increase in the percentage of small vessels that were perfused. Those patients who died had no significant increase in perfusion. Improved perfusion of small vessels within the first 24 hours was a strong predictor of survival. This has led Ince [28] to comment that red blood cells produce NO in the microcirculation which may then affect mitochondria locally.

Intensive Insulin Therapy in Critically Ill Patients [3]

In 2001, Van den Berghe and co-authors [3] published the results of a prospective randomized controlled study of intensive insulin therapy in 1548 patients in a surgical ICU. The intervention group was treated with insulin to maintain blood glucose between 80 to 110 mg/dl (4.4 and 6.1 mmol/l) and the standard therapy group was given insulin only if their blood glucose increased above over 215 mg/dl (11.9 mmol/l). These authors demonstrated a reduction in 1-year mortality from 8% to 4.6% overall and from 20.2% to 10.6% in patients who required more than five days intensive care. The main benefit was in reducing deaths from multi-organ failure.

Why are Mitochondria Important in Relation to Intensive Insulin Therapy?

The mechanisms underpinning the success of insulin therapy have yet to be clarified. A recent study in patients undergoing coronary artery bypass grafting suggests that intensive insulin therapy does not alter levels of inflammatory cytokines in plasma [29]. Of note, over 60% of the patients in Van den Berghe's trial were post-operative cardiac surgical patients. Subsequent post-hoc analysis of the original intensive insulin paper data [30] suggested that glucose control was more important than insulin dose for key measures including mortality, and for all morbidities except acute renal failure. Van den Berghe's group have also suggested [31] that maintaining normoglycemia rather than an effect of insulin *per se* was protective in endothelium, liver, and kidney in rabbits. Finney et al. [32] also suggested that glucose control rather than insulin dose was the factor responsible for improved outcome in predominantly surgical intensive care patients.

In a subsequent electron microscopy of the subgroup of patients who died during the intensive insulin study [3], Vanhorebeek et al. [33] reported that patients in the intensive insulin group had less evidence of physical disruption of mitochondria despite more severe illness on physiologic scoring scales. They noted abnormalities of the morphology of the inner mitochondrial membrane and swollen liver mitochondria in 78% of patients from the standard therapy group compared with only 9% of the intervention group. Using spectrophotometry they also demonstrated 89% higher activity of complex I and 40% higher activity of complex IV in the intervention group.

Hyperglycemia may cause Mitochondrial Dysfunction

A link between this endothelial cell work and the intensive insulin therapy trial is found in a study in surgical patients by Langouche et al. [34]. These authors studied the effects of intensive insulin therapy on various inflammatory mediators and found significantly lower levels of intercellular adhesion molecule-1 (ICAM-1), E-selectin, and nitrate/nitrite levels in the normoglycemic group. The authors interpreted their results as showing that intensive insulin conferred endothelial protection.

Hyperglycemia-increased superoxide production has been linked with increased monocyte adhesion, apoptotic caspase cleavage, inhibition of eNOS, platelet activation, inhibition of anti-inflammatory enzymes such as prostacyclin synthetase, induction of pro-inflammatory pathways such as NF-κB, and with inhibition of peroxisome proliferator-activated receptor (PPAR)-γ [11, 35], suggesting numerous mechanisms whereby increased mitochondrial ROS generation may produce harmful stimuli.

Conclusion

There are few studies that have unified ICU practice to such an extent as the three studies highlighted above. Mitochondria are integral to healthy cell function and many groups have suggested that mitochondrial dysfunction may be at the root of critical illness.

Stretch in the lung epithelium and endothelium is transduced into ROS, which increase both inflammatory mediators and apoptotic signals. Protective ventilation strategies may reduce mitochondrial dysfunction and consequent inflammatory signaling. Curtailing the septic response by early resuscitation may be of benefit to impaired mitochondria and controlling glucose may also protect the mitochondria from free radical damage. Further work in the critically ill and in other spheres such as patients with diabetes and cardiovascular patients may provide greater insight into mitochondrial dysfunction. Interventions that affect mitochondrial function in the laboratory may one day improve outcomes in our patients.

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The Impact of Chronic Disease on Response to Infection

M.C. Reade, E.B. Milbrandt, and D.C. Angus

Introduction

Most patients with sepsis have underlying co-morbidities. Co-existing disease is typically thought to influence the pathophysiology and outcome of sepsis by reducing physiological reserve. Certainly this is true: A patient with chronic obstructive pulmonary disease (COPD) will tolerate pneumonia less well than a patient with previously healthy lungs. Additionally, many chronic disease states (or their treatments) alter the pre-existing inflammatory and immune milieu. This effect ranges from the obvious (as in the case of patients taking immunosuppressant therapy) to the underappreciated (as in the inflammatory dysregulation associated with obesity). In seeking explanations for differences in the host response to infection, much has been made of the possible effects of genetic variability. However, subtle variations in the underlying state of the immune and inflammatory systems have received little attention.

In this chapter, we begin by presenting evidence that pre-existing inflammation influences the risk of developing infection. Mechanisms of acute and chronic inflammation will be briefly reviewed. Known risk factors and the distinction between altered susceptibility and clinical course will then be described. Knowledge of dysregulated inflammation inherent in many chronic disease states will be summarized. What knowledge there is of the effect of underlying abnormalities of immunity and inflammation on the pathogenesis of sepsis will be presented. The reverse situation, the lingering effect of sepsis on chronic disease, will also be discussed. We suggest that in future, it may be wise to tailor treatments that target inflammation not only to mediators implicated in the 'generic' sepsis patient, but also to abnormalities associated with their co-morbid disease.

Pre-existing Inflammation Influences Risk of Developing Infection

The Health Aging and Body Composition study [1] is an illustrative example of the link between pre-existing inflammation and the development of severe infection. This study identified 3075 'well functioning' patients aged 70–79 years, defined as those who had no difficulty walking one quarter mile, climbing 10 steps, or performing activities of daily living. Levels of tumor necrosis factor (TNF) and interleukin (IL)-6 were measured on entry to the study, and each patient was followed for 6.5 years. Over this period, 161 participants (5.2%) were hospitalized for community acquired pneumonia (CAP). Levels of TNF and IL-6 at baseline were significant predictors of the development of CAP, with odds ratios for the highest tertiles of 1.6

(1.02–2.7) and 1.7 (1.1–2.8), respectively, whereas smoking, coronary heart disease, congestive heart failure, chronic renal failure, and diabetes were not independent predictors for CAP. While this study can be practically applied to identify groups at higher risk of pneumonia, it also suggests an important relationship between chronic inflammation and susceptibility to severe infection.

Distinguishing Acute and Chronic Inflammation

Acute and chronic inflammation are pathologically distinct processes. At a tissue level, inflammation is the response to local injury. When of sufficient magnitude, inflammation has systemic as well as local effects. At the site of injury, acute inflammation involves vasodilation, margination, and extravasation of neutrophils, and increased vascular permeability with the formation of exudates. These effects are coordinated by a variety of soluble mediators, including inflammatory lipid metabolites (platelet activating factor [PAF], prostaglandins and leukotrienes), cascades of soluble proteases and substrates (complement, coagulation, and kinins), nitric oxide (NO), and polypeptide cytokines. Systemically, various acute inflammatory cytokines cause fever, vasodilation, and a shift from anabolic to catabolic metabolism. Acute inflammation usually leads to healing at the site of damage, but if the damaging stimulus persists there can be a shift to chronic inflammation, which is a different pathological process. Chronic inflammation can also develop in the absence of antecedent acute inflammation, as classically occurs in rheumatoid arthritis or 'low toxicity' infections such as tuberculosis, as well as in many of the conditions listed in the next section.

Chronic inflammation is characterized by activation of macrophages, lymphocytes, and plasma cells rather than neutrophils, destruction of tissue, formation of granulation tissue, and fibrosis. Cytokines controlling these processes include IL-1, IL-6, and TNF, which are also involved in acute inflammation. However, in chronic inflammation different cytokines such as IL-10 and transforming growth factor (TGF)-β, which control humoral responses, and IL-2 and interferon (IFN) gamma, which control the cellular response, predominate. Acute and chronic inflammation induce different tissue responses. For example, mice exposed to an inflammatory stimulus for 14 days switch to a different inflammatory mRNA profile in the liver, suggesting different regulatory factors are involved in gene expression when inflammation becomes chronic [2]. Anti-inflammatory cytokines are prominent in both the resolution phase of acute inflammation and in chronic inflammation. Indeed, in the subacute phase of sepsis this may lead to profound immunosuppression [3]. Most importantly, whereas acute inflammation is a constantly changing process, chronic inflammation can become a new steady state.

Known Risk Factors for Altered Prognosis in Sepsis

If chronic disease alters the response to acute infection in a deleterious way, then chronic diseases should be risk factors for susceptibility to infection and the clinical course of sepsis. When chronic diseases are taken together, they do indeed increase mortality [4] (Fig. 1). Unfortunately, it can be difficult to separate the contribution of altered immune and inflammatory function from the effect of decreased physiological reserve. For example, many studies have found a variety of chronic condi-

tions increase risk of CAP: heart disease, lung disease, asthma, immunosuppressive medication, and alcoholism [5]; male gender, congestive cardiac failure, stroke, cancer, and diabetes [6]; smoking and body mass index [7]. These epidemiological association studies have rarely explored the biological mechanisms for this increased susceptibility.

Before proceeding to explore these associations, it is essential to draw a distinction between 'susceptibility to infection' and 'influence on the clinical course once infected'. To effectively defend against infection, a host must mount a pro-inflammatory response. Immunosuppression, and those conditions discussed in the following sections where chronic inflammation blunts the acute pro-inflammatory response, leave the host more prone to developing infection. However, once an infection has become established, effective host defense can be impaired in either of two ways. First, an overwhelming pro-inflammatory response can become detrimental to ultimate survival, worsening hypotension and compromising function in a variety of body systems. Alternatively, a blunted inflammatory response can allow the organism to proliferate to the point of causing organ dysfunction even without robust inflammation. In addition, the immunosuppression that often follows a strong pro-inflammatory response may leave growth of residual initial organisms, or a subsequent superinfection, unchecked. Successful defense against acute infection once it has become established probably relies on a fine balance between pro and anti-inflammatory mediators. Most studies, especially in the clinical context, do not make the distinction between incidence and clinical course. Patients with a putative risk factor either die of pneumonia (for example), or do not. Such studies make it difficult to tease out the likely effects of underlying pro- or anti- inflammatory bias.

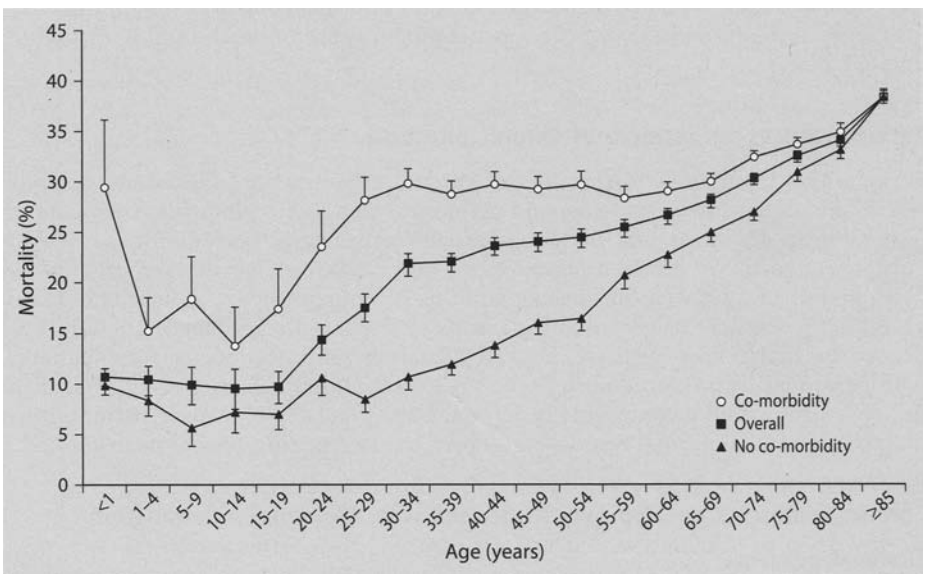

Fig. 1. National age-specific mortality rates for all cases of severe sepsis and for those with and without underlying comorbidity. Comorbidity is defined as a Charlson-Deyo score >0. National estimates are generated from the seven-state cohort using state and national age- and gender-specific population estimates from the National Center for Health Statistics and the U.S. Census. Error bars represent 95% confidence intervals. From [4] with permission

Dysregulated Immunity and Inflammation in a Variety of Chronic Disease States

If chronic disease modulates response to infection by causing abnormal immune and inflammatory function, such abnormal function should be easy to demonstrate in these disease states. Altered susceptibility of such patients to infection may also be seen. This question will be addressed by considering patients with, as examples, autoimmune disease, chronic infection, and chronic morbidities not typically associated with immune function but that appear to influence the susceptibility to and course of sepsis. The effects of treatments that modulate inflammation will also be examined.

Systemic Lupus Erythematosis as an Example of Autoimmune Disease

Patients with systemic lupus erythematosis (SLE) develop and die of infection at an abnormally high rate. While abnormal host immune response is likely to play a part, the effect is often difficult to separate from the immunosuppressant effects of medications (such as steroids) used to treat the disease. However, even before steroids were commonly used for SLE, an abnormally high incidence of infection was observed [8]. Numerous studies (reviewed in [9]) have found increased SLE severity related to increased susceptibility to infection. This effect was independent of treatment. The clinical manifestations of infections are atypical in SLE, and salmonella, pneumococcus, tuberculosis, and viral infections are more common than would be expected. The immune pathogenesis of SLE along with its associated chronic inflammation are presumably at least in part responsible for this abnormal response to infection, although evidence for this is lacking. Abnormal control of inflammation is also likely to be responsible for the most common cause of death in SLE, thromboembolic disease.

Periodontitis as an Example of Chronic Infection

Periodontitis is an archetypal model of chronic inflammation. Elevations of white cell counts, acute phase proteins, and cytokines, along with abnormal coagulation, have all been described, and these markers normalize when periodontitis is treated [10]. The association between periodontitis and cardiovascular disease, atherosclerosis, and diabetes is well established, and this is thought to have an immunological basis [11]. Treatment of periodontitis results in improved endothelial function, evidenced by better flow-mediated arterial dilatation [12]. Monocytes from patients with periodontitis produced more IL-6 in response to lipopolysaccharide (LPS) than did cells from healthy controls [13]. It would be surprising if chronic periodontitis did not cause an abnormal response in sepsis, but to date this has not been studied.

Chronic Conditions not Typically Associated with Abnormal Inflammation

Alcohol dependence

The well known clinical association between chronic alcoholism and sepsis can be explained in multiple ways. Alcoholics tend to be malnourished, are more prone to aspiration, have generally poorer health, and, in acute illness, access health care late. That this association is partly attributable to the immunosuppressant effect of chronic alcoholism was suggested by the observation that alcoholic patients with

septic shock had lower pro-inflammatory cytokine and IL-6 levels than non-alcoholics [14]. Similarly, alcoholic patients had increased anti-inflammatory IL-10 levels, a reduced IL-6/IL-10 ratio, and three times more postoperative wound infections than did non-alcoholic controls [15]. *In vitro* and animal studies support the immunosuppressant effects of alcohol (reviewed by [14]).

Hypertension

Chronic hypertension appears to alter the function of immune cells. For instance, more monocytes from hypertensive patients bound to an endothelial cell layer *in vitro* than did cells from normotensive controls, suggesting hypertension had 'activated' them *in vivo* [16]. The secretion of IL-1β and TNF in response to LPS was greater in mononuclear cells from hypertensive patients [17]. Whether this association between chronic inflammation and hypertension is cause or effect or both is less clear, as recently reviewed [18]. In any case, patients with chronic hypertension might be expected to respond to systemic infection differently than controls. This hypothesis remains untested.

Obesity

Obese patients have a worse prognosis in sepsis [19]. While this is undoubtedly multifactorial, altered inflammation may play a part. Obese experimental animals and humans have higher levels of circulating TNF, angiotensinogen, TGFβ and IL-6, which it now seems are secreted by the adipocytes themselves [20]. In addition, leptin, adiponectin, and resistin, proteins, secreted principally by adipocytes, have immunomodulatory activity [21]. At the other extreme, malnourishment is also associated with increased baseline and stimulated TNF production [22], and malnourishment also predisposes to worse outcome in sepsis.

Smoking and COPD

Smoking worsens mortality in sepsis [7]. The acute effects of cigarette smoke are pro-inflammatory, in part due to oxidative stress and lipid peroxidation. Neutrophils and macrophages are rapidly recruited to the lung, where there is increased expression of TNF and macrophage inflammatory proteins [23]. The effects of chronic smoking, however, are more complex. Chronic smoking causes immunosuppression, which allows the normally sterile lower airways to become chronically colonized with potential respiratory pathogens. Human bronchial epithelial cells exposed to cigarette smoke had reduced granulocyte-macrophage colony-stimulating factor (GM-CSF) and IL-8 production in response to LPS and TNF, at least in part explained by reduced activator protein (AP)-1 activation [24]. Smokers also have a shift from effector to suppressor T cells [25]. COPD eventually develops in 15–20% of smokers. This is characterized by both chronic inflammation in the lung tissue and cytokine mediated systemic effects such as muscle wasting and weight loss [26]. An interesting observation is the protective effect of cigarette smoking on ulcerative colitis, which may in part be due to the chronic immunosuppression smoking causes [27]. The explanation is probably more complex than this, however, as Crohn's disease appears to be worsened by smoking.

Gender differences

It appears that males may be at increased risk for developing sepsis [4] (Fig. 2). However, the differential effects of susceptibility to infection and clinical course once infection is established make this gender effect less clear. Males could either be

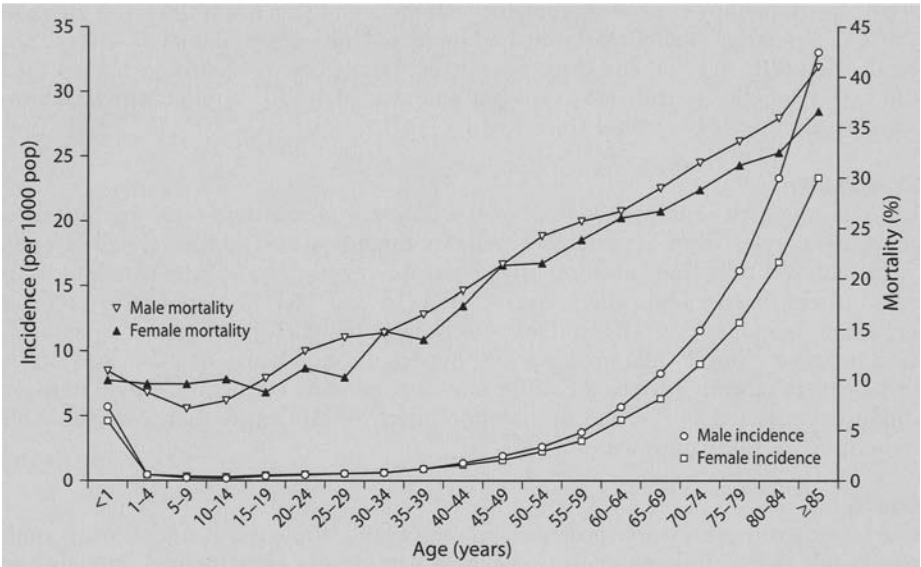

Fig. 2. National age-specific incidence and mortality rates for all cases of severe sepsis by gender, excluding those with human immunodeficiency virus (HIV) disease. National estimates are generated from a seven-state cohort (Florida, Maryland, Massachusetts, New Jersey, New York, Virginia, and Washington) using state and national age-specific population estimates from the National Center for Health Statistics and the U.S. Census. The incidence among women was equivalent to that of men 5 years younger. A similar age-based difference was seen in mortality but, in multivariate regression, this difference was explained by underlying comorbidity and site of infection. Pop: population. From [4] with permission

at greater risk of infection, or at greater risk of developing sepsis once they have an infection. Mortality from sepsis also appears to be influenced by gender. While the above study found age- and comorbidity- adjusted sepsis mortality was the same in men and women [4], another found women with pneumonia were almost twice as likely to die as men [28]. If indeed females are more likely to die once they have pneumonia, this could be explained by hypothesizing that females have a greater acute inflammatory response (and so die of septic shock), or alternatively that they have a lesser inflammatory and immune response which prevents them from effectively clearing the infecting organism. In support of the first hypothesis, cell mediated immunity appears to be more active in females, reflected in stronger responses to immunization and higher rates of autoimmune disease (as reviewed [29]). Supporting the contrary hypothesis, however, are *in vitro* studies, such as that which found that LPS applied to male macrophages stimulated release of more pro-inflammatory cytokines and higher cell surface expression of TLR4 and CD14 than in cells from females [30].

Reconciling these conflicting arguments will require further study. Perhaps men have an 'anti-inflammatory' disposition, making them more susceptible to infection, but with comparatively less risk of the sepsis syndrome if infection occurs because they mount a less robust acute inflammatory response. Just as feasible in the light of the above epidemiological studies is to suggest that men have a 'pro-inflammatory' disposition, and so are less susceptible to infection and have a greater ability to clear infection if it occurs, but that this greater clearance comes at the cost of developing

the acute inflammatory symptoms of sepsis. Further complicating this analysis is the possibility that the ability to mount an inflammatory response has a differential effect on susceptibility to, and clearance of, infection. In any case, the effect of gender (whatever it is) could well have much the same effect on the response to acute infection as the chronic inflammatory conditions discussed here.

Age and frailty

Increasing age is associated with higher levels of circulating inflammatory mediators and acute phase proteins. Contributing factors may include reduced anti-inflammatory estrogen in females, increased fat tissue, and subclinical infections. Many studies suffer from the difficulty of separating co-morbidities from the effects of age alone. However, inflammatory cells from elderly patients produce more pro-inflammatory cytokines than do cells from young subjects (as reviewed [31]). Only a proportion of elderly subjects become 'frail', and the 'frailty syndrome' is associated with increased inflammation and markers of activated coagulation [32]. Inflammatory mediators are strong predictors of mortality in the elderly, independent of other known risk factors [31].

Extreme exercise and chronic sleep deprivation

It is not only pathological conditions that can alter the inflammatory and immune response. Exercise leads to the production and systemic release of acute phase cytokines such as TNF, IL-1, and in particular the counter-regulatory, IL-6, from muscle cells. While the physiological role of cytokines in exercise may be to regulate metabolism, prolonged and exhausting exercise increases susceptibility to acute infection and allergies (reviewed in [33]). Prolonged work shifts in ICU medical personnel also cause a pro-inflammatory state, which correlates with worse endothelial function [34] and, one could speculate, reduced resistance to infection.

Therapy that Might Modulate Inflammation

Therapeutic immunosuppression, such as after organ transplantation, is a well recognized risk factor for severe infection [35]. Chemotherapy for malignancy is similarly immunosuppressant [36]. Red blood cell transfusion depresses immune function, increasing risk of bacterial infection and cancer recurrence, while improving survival of transplanted organs [37]. Immunosuppression associated with therapy is clearly not beneficial for the host response to infection. In contrast, reduction of inflammation appears to confer some benefit. A number of medications not typically associated with inflammation have recently been suggested to improve prognosis in sepsis, such as statins [38] and possibly heparin, which at least in part appears due to an anti-inflammatory effect [39].

Mechanisms Whereby Dysregulated Inflammation Influences Response to Sterile and Infectious Inflammatory Stimuli

Implicit in the above discussion is the sense that many chronic conditions reduce the acute inflammatory response. This effect can be direct, as with alcoholism or after blood transfusion, or indirect, via the immunosuppression associated with the chronic inflammatory state (as with the other conditions listed). The effect of these alterations appears to be different, depending on whether the insult is infectious or sterile.

Attenuation of acute inflammation appears to have a beneficial effect in the face of a sterile insult. Analogous to clinical chronic inflammation is 'endotoxin tolerance' observed in animals and *in vitro*. Prior exposure to a small dose of endotoxin reduces inflammation in response to subsequent exposures. For example, pretreatment of rabbits with endotoxin reduced fever in response to subsequent endotoxin challenge, and reduced mortality [40]. The effect appears to be dependent on IL-10 and TGFβ, as blocking antibodies to these cytokines prevented tolerance [41]. Endotoxin tolerance appears to confer a beneficial effect on the host response to burns, hepatic, renal and cardiac ischemia/reperfusion, and hemorrhagic shock (as reviewed [42]), all of which begin as a sterile inflammatory response.

In contrast, reducing acute inflammation appears to blunt the initial defensive response to infection – with a worsening of outcome if the infection becomes widespread, by which time any benefit from initial anti-inflammatory effects are negated. While there are conflicting opinions [42], the balance of evidence suggests that pre-existing inflammation alters response to acute infection in a harmful way. For example, *Escherichia coli* peritonitis reduced the ability of mice to clear respiratory *Staphylococcus aureus* and pseudomonas infection. This was associated with reduced recruitment of neutrophils into the lungs, and reduced circulating complement levels [43]. Similarly, the immunosuppression generated by cecal ligation and puncture (CLP) in mice resulted in reduced clearance of intratracheal pseudomonas, which was mediated by IL-10. CLP mice exposed to pseudomonas had a 10% survival compared to 95% after sham surgery [44]. It seems that the acute response is impaired in the context of pre-existing inflammation at least in part due to a counter-regulatory anti-inflammatory effect.

There may be additional mechanisms linking pre-existing inflammation to reduced resistance to infection. A high level of circulating cytokines may promote infection by a direct effect on the host/pathogen interaction. TNF, IL-1, and IL-6 all increased the *in vitro* growth of *S. aureus*, acinetobacter, and pseudomonas [45]. Inflammatory cytokines also upregulate bacterial receptors on the host cell surface, increasing the likelihood that bacteria can cause invasive disease. TNF, IL-1, and thrombin increased pneumococcal binding to endothelial cells via the PAF receptor [46].

Long term exposure to TNF uncouples T cell receptor signal transduction pathways. In a study of autoimmune disease, loss of T cell function predominantly involved loss of suppressor T cells, which predisposed to heightened inflammation and autoimmunity [47]. In the context of acute infection, loss of T cell receptor function might worsen inflammation through an effect on suppressor T cells, or reduce bacterial clearance through an effect on effector T cells.

In summary, chronic infection or inflammation appear to reduce the ability to mount an acute inflammatory response. This is beneficial if the acute inflammation is not due to another infection. However, if there is an acute infection, it is more likely to become invasive, and less likely to be cleared. The negative effect of reduced clearance outweighs any transient beneficial effect from reduced inflammation, as the infection overwhelms the host.

The Converse Situation: Resolved Acute Inflammation Accelerates Chronic Disease and Predisposes to Further Infection

While pre-existing chronic inflammation alters the course of acute illness, it also appears that the residual effects of acute inflammation persist long after apparent clinical resolution of disease. For example, higher IL-6 and IL-10 levels prior to discharge were associated with increased 90-day mortality in a cohort of 1808 patients hospitalized with CAP [48]. Mice survivors of intra-abdominal sepsis were at increased risk of subsequent pseudomonas and aspergillus infection [49]. Immune function in patients surviving sepsis is also altered, at least in the short term. These observations may simply reflect a prolongation of the immune system downregulation well described in the later phases of sepsis, as has been reviewed elsewhere [3, 42].

Conclusion

It appears that the impact of pre-existing chronic conditions is much more than to simply reduce physiological reserve. An organism can arrive in a host whose immune system is in a state of normal, suppressed, or heightened activity, and it would be surprising if this host difference did not influence the nature of the response to the insult. While a degree of chronic inflammation may induce a beneficial 'inflammatory tolerance' to sterile inflammatory insults such as ischemia-reperfusion, the adverse effect on the host ability to clear infection appears clear. Furthermore, the negative effects of acute inflammation appear to persist long after the inflammatory stimulus has cleared and the patient appears to have recovered.

Consideration of these phenomena may have implications for the utility of postulated anti-inflammatory therapy in sepsis in various patient sub-populations. Further suppression of inflammation might be the worst strategy in patients with chronically downregulated inflammatory responses. Presumably patients with or without co-morbidities affecting inflammation would form separate subgroups. Perhaps this underlies the finding that only a subset of sepsis patients appears to benefit from suppression of inflammatory cytokines [3].Conversely, this may explain why most studies of immunomodulators fail to find an effect in the overall group. The effect of co-morbidities might be at least as influential in determining host response as are other factors, like genetic variability. At the very least it is important to take into account the effect of pre-existing disease when studying patterns of inflammatory mediators in sepsis.

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Immunomodulatory Effects of General Anesthetics

L.C. Lemaire and T. van der Poll

Introduction

Postoperative patients are prone to develop infectious complications, and the phenomenon of immunoparalysis, defined as a diminished capacity of immunocompetent cells to respond to infectious agents, has been implicated as a major contributing factor. When inflammatory postoperative disorders are already established, intervention is difficult. However, if perioperative modulation of the inflammatory response were possible, this may influence postoperative outcome. General anesthetics exert a variety of effects, including sedation, amnesia, and analgesia. Current research focuses primarily on the effects of these compounds on membrane proteins in the central nervous system (CNS), to elucidate the molecular mechanism of their action. The (side-) effects of general anesthetics on other organ systems have been less extensively investigated. In this chapter, we will discuss the data available on the immunomodulatory effects of general anesthetics and the potential clinical implications of these effects on the development of (postoperative) infections.

We focus on compounds widely used to maintain sedation in the intensive care unit (ICU) and anesthesia during operations. In addition, the effects of ketamine, an anesthetic often used in emergency medicine and commonly used as a general anesthetic in second and third world countries, are described. Effects of local anesthetics on the inflammatory response are beyond the scope of this manuscript; however they have been thoroughly reviewed by Hollmann and Durieux [1].

Clinical Problem

Postoperative patients, trauma patients and patients on the ICU are prone to develop infectious complications, which substantially increase morbidity, hospital stay, and resource consumption. Infections may vary from surgical wound infection to pneumonia or severe sepsis. Even surgical wound infections, categorized as minor complications, can prolong hospitalization up to 7 days and increase the direct median cost of hospitalization by 50% [2]. For example, in patients undergoing gastrointestinal operations, the incidence of surgical wound infection ranges from 10 to 20% which prolongs hospital stay by 2 to 7 days. In addition, postoperative pneumonia or postoperative sepsis in these patients also significantly increases the length of hospital stay and costs.

Conceptually, it is thought that a balanced pro- and anti-inflammatory reaction is necessary for appropriate tissue healing after surgery [3]. An unbalanced systemic pro-inflammatory ('hyperinflammatory') reaction can result in a systemic inflam-

matory response syndrome (SIRS) or even multiple organ failure (MOF). However, a reduced pro-inflammatory reaction may cause a diminished capacity to respond to infectious agents, resulting in increased susceptibility to (postoperative) infections. This phenomenon is termed 'immunoparalysis' [4]. Immunoparalysis was primarily described in response to sepsis and termed 'LPS (lipopolysaccharide) tolerance' [5]. When isolated peripheral blood mononuclear cells of septic patients were exposed to a second inflammatory or infectious stimulus (e.g., LPS), these cells became hyporesponsive or 'anergic': Cells could not be triggered to release pro-inflammatory cytokines. We and others [6, 7] have shown that a comparable immune reaction is found after surgery. In these patients, the first inflammatory stimulus is provided by the surgical trauma itself. It is widely assumed that the relative incapacity of cells like monocytes, lymphocytes, and granulocytes to react to infectious stimuli (the first line of defense against bacteria) renders the host susceptible to infections [3, 4, 8].

What is Causing Immunoparalysis?

The mechanisms underlying immunoparalysis have only been partly elucidated. In septic patients, immunoparalysis is characterized by downregulation of monocytic major histocompatibility complex, HLA-DR, expression and a reduced ability of monocytes to produce LPS-induced tumor necrosis factor (TNF)-α and interleukin (IL)-10 *in vitro* [9, 10]. Downregulation of HLA-DR expression resulted in diminished antigen-presenting activity of monocytes. This caused reduced T-cell proliferation and interferon (IFN)-γ production [11]. Data also showed that IL-10 can induce immunoparalysis by downregulating TNF-α synthesis and self-limiting IL-10 production. When peripheral blood mononuclear cells were treated for 24 h with IL-10 (instead of LPS), washed extensively and restimulated with LPS, synthesis of IL-10 and TNF-α was strongly diminished, similar to the level reached by LPS desensitization. In addition, anti-IL-10 monoclonal antibodies prevented the LPS-mediated induction of LPS tolerance [10]. In the clinical setting it was shown that increased production of IL-10 in the first ten days post-injury correlated significantly with subsequent septic events in trauma and burn patients [12].

In patients undergoing major surgery (e.g., esophagectomy, total gastrectomy), monocytic HLA-DR expression was also decreased [7]. However, deactivation of monocytes did not occur, since TNF-α and IL-10 cytokine production by monocytes was not inhibited. Moreover, to analyze whether the loss of HLA-DR cell surface expression would affect the antigen-presenting capacity of peripheral blood monocytes, unfractionated peripheral blood mononuclear cells were incubated with the bacterial superantigens, staphylococcal enterotoxin A, staphylococcal enterotoxin B, and toxic shock syndrome toxin 1, and antigen presenting capacity-dependent T-cell proliferation was determined. The capacity of monocytes to present antigens and to stimulate T-cell proliferation was not affected. In contrast, major surgery resulted in a predominant intrinsic defect in T-cell function, as revealed after direct activation of T-cells by cross-linking of CD3 and CD28 receptors. IL-2, IFNγ, TNF-α and IL-4 secretion was decreased, while IL-10 secretion was increased.

Toll-like receptors (TLR), a family of transmembrane receptor proteins, are identified as key proteins in humans and mice for the recognition of pathogens [for review see 13]. TLR4 is necessary for LPS signaling [14]. Activation of TLR4 by LPS triggers binding of MyD88 (myeloid differentiation factor 88) to the intracellular portion of the receptor. MyD88 recruits IL-1 receptor-associated kinase (IRAK) 4

Table 1. Potential mechanisms of immunoparalysis

Mechanism	Patients	[References]
Increased IL-10 production and monocyte deactivation	Sepsis	[9–10]
Intrinsic T-cell defects	Surgical patients	[7]
Altered expression of Toll-like receptors	*in vitro* studies	[18–21]
Reduced activation of MyD88, IRAK-1 or increased activity of IRAK-M	Sepsis	[22]

[15]. This results in the phosphorylation of IRAK-1 which in turn interacts with TNF receptor activated factor 6 (TRAF6). TRAF6 forms a complex with transforming growth factor beta-activated kinase (TAK)-1 [13]. TAK-1 acts as the common activator of nuclear factor kappa B (NF-κB), as well as of the p38 (and JNK) mitogen-activated protein kinase (MAPK) pathways. Activation of NF-κB results in transcription of pro-inflammatory cytokines. In parallel, the induced MAPK pathway may generate phosphorylated p38MAPK and activates another transcription factor, activator protein 1 (AP-1). AP-1 also induces the transcription of pro-inflammatory genes [16]. By nature, negative regulators of the TLR signaling pathway exist which prevent strong uncontrolled inflammatory reactions (e.g. IRAK-M, SOCS1, MyD88s [13])

In studies aimed at unraveling the molecular mechanisms of immunoparalysis, downregulation of surface TLR4 expression [17] and dysregulation at different levels of the TLR4-MyD88-IRAK-NFκB signaling pathway have been found [18 - 21]. It has been shown that cells can develop immunoparalysis by degradation of IRAK (not specified whether this is IRAK-1 or IRAK-4 [18–20]) and phosphorylation by protein kinase C (PKC) might be responsible for this [18]. In addition, rapid upregulation of IRAK-M expression, a cytosolic inhibitor of the TLR-pathway, was found following a second endotoxin challenge in human monocytes and in monocytes isolated from septic patients [22].

In summary, the potential mechanisms of immunoparalysis, as described in the literature are depicted in Table 1. Moreover, it should be noted that the (molecular) mechanism underlying the immunoparalyzed state of a patient may be different in septic, trauma, and postoperative patients.

Do General Anesthetics Affect the Immune Response?

General anesthetics exert a variety of effects, including sedation and hypnosis. Immunomodulation by general anesthetics has been proposed based on affected cytokine levels measured *in vitro* [23–25] and *in vivo* [26–31]. Interestingly, a study in patients undergoing transhiatal or transthoracic esophagectomy showed that the occurrence of major postoperative infectious complications was best predicted by increased duration of anesthesia, and not by surgical procedure or operation time [32]. In this section, we review data on the immunomodulatory effects of commonly used general anesthetics. We excluded those *in vitro* studies in which pharmacological (e.g., [34]) instead of clinically relevant concentrations [35] were used.

Propofol

Propofol (2, 6-di-isopropylphenol) is an intravenous sedative-hypnotic agent which is administered to maintain anesthesia peroperatively or to sedate patients in the ICU; this latter use is because treatment effect is more rapid with propofol as com-

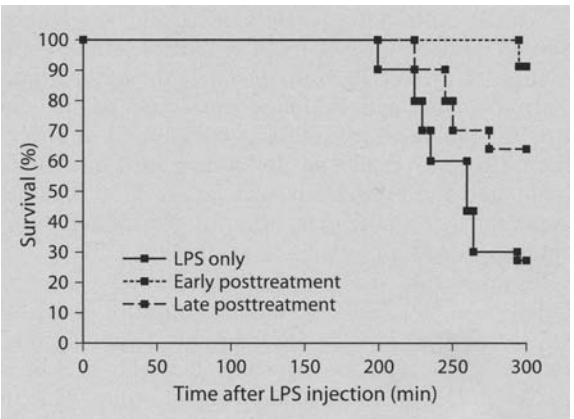

Fig. 1. In rats, endotoxemia was induced by a bolus injection of *Escherichia coli* lipopolysaccharide (LPS) derived from *E. coli* 0111:B4 (20 mg/kg). Animals received either no propofol (LPS only), or propofol was administered intravenously (10 mg/kg bolus followed by infusion at 10 mg/kg/hr) 1 hour after LPS challenge (early posttreatment) or 2 hours after LPS challenge (late posttreatment). Mortality rates were registered. The mortality rate for the early posttreatment group was significantly lower than for the other groups ($p < 0.0001$). Adapted from [31].

pared to continuous infusions of other compounds, such as midazolam or morphine.

Propofol inhibited IL-6 and IL-10 production by LPS-stimulated peripheral blood mononuclear cells *in vitro* [25]. Cytotoxicity and apoptosis of LPS-treated peripheral blood mononuclear cells were unchanged with clinically acceptable concentrations (1–10 μg/ml), while at pharmacological concentrations (50 mg/ml) apoptosis was increased and cytotoxicity decreased [24]. In two studies, rats were challenged with a bolus injection of *Escherichia coli* LPS (*E. coli* 0111:B4, 20 mg/kg over 2 minutes) and, thereafter, either received no propofol, or administration of propofol was started 1 hour (early posttreatment) or 2 hours (late posttreatment) after LPS-challenge (10 mg/kg bolus followed by 10 mg/kg/hr during 5 hours). Posttreatment with propofol in the early stage of endotoxin-induced shock in rats profoundly reduced the mortality rate of rats and attenuated their cytokine response, while treatment at a late stage did not [30, 31]. Mortality rates 5 hours after endotoxin injection were 73%, 9%, and 36% for the endotoxic, early posttreatment, and late posttreatment groups, respectively (Fig. 1). The mortality rate for the early posttreatment group was significantly lower compared to the other groups.

Volatile Anesthetics

Presently, the most commonly used inhalation anesthetics are isoflurane and sevoflurane. These agents have to be delivered by inhalation through an anesthetic system which consists of various components, including an anesthesia machine, a vaporizer, an anesthesia circuit, a ventilator and a scavenging system. Since all these components are not present on ICU-ventilators (mainly the vaporizer and scavenging system), use of volatile anesthetics is limited to peroperative use. *In vitro* studies showed inhibitory effects of volatile anesthetics on the immune responses of different cell types. Isoflurane inhibited IL-6 production by alveolar epithelial cells *in vitro* [23]. Another study showed that LPS-induced NF-κB activation in isolated monocytes was inhibited by clinically relevant concentrations of isoflurane. This was associated with a decreased production of TNF-α and IL-6 [36]. Sevoflurane-mediated suppression of the transcription factor, AP-1, in primary CD3+ lymphocytes from healthy volunteers has been reported, only, however, after 24 hrs incubation of cells at pharmacological concentrations (8 vol%) [37].

Volatile anesthetics (halothane, isoflurane and sevoflurane) also inhibited adhesion of neutrophils to human umbilical vein endothelial cells (HUVECs) upon stimulation of these cells with 10 nM N-formyl-methionyl-leucyl-phenylalanine (fMLP). This coincided with inhibited expression of the adhesion molecule, CD11b, on neutrophils [38]. Comparable data were found in mice, where isoflurane administered 1 hour after LPS challenge (for 30 minutes) inhibited LPS-induced neutrophil recruitment into the bronchoalveolar lavage [39]. Another study revealed that isoflurane augmented the gene expression of pro-inflammatory cytokines in rat alveolar macrophages during mechanical ventilation [29].

In an elegant study by Fuentes and co-workers [40], mice were challenged with a lethal dose of LPS in the absence (control group) or presence of isoflurane (2–2.5 vol%) for 1 hour. Over the 72 hours following the LPS injection, an 85% survival rate was observed for mice injected with LPS in the presence of isoflurane, compared to 23% survival in the control group. This improved survival was associated with decreased TNF-α, IL-6 and IL-10 plasma levels and delayed/inhibited activation of NF-κB. Moreover, the decrease in TNF-α and IL-6 plasma-levels was dependent on the duration of anesthesia, while IL-10 plasma levels were only significantly inhibited after 1 hour of isoflurane anesthesia.

Clinical Studies Comparing Propofol and Isoflurane Anesthesia

Several patient studies have been performed in which isoflurane-anesthesia was compared to propofol-anesthesia. In patients undergoing abdominal surgery, postoperative plasma levels of the anti-inflammatory cytokine, IL-10, were higher in the group anesthetized with propofol compared to the levels in the group anesthetized with isoflurane [26]. Heine et al. showed that in patients undergoing an elective embolization of a cerebral arterio-venous malformation, neutrophil respiratory burst, but not phagocytosis, was reduced significantly more by propofol anesthesia compared to isoflurane-anesthesia [41]. Gene expression of pro-inflammatory cytokines in alveolar macrophages increased during anesthesia and surgery [28], and bactericidal function of these macrophages progressively decreased [27]. Moreover, gene expression of IL-8 and IFNγ in alveolar macrophages was significantly higher during isoflurane anesthesia than propofol anesthesia, while expression of genes for IL-1 and TNF-α were comparable [28].

Ketamine

Ketamine is a phencyclidine derivate with, in contrast to propofol and volatile anesthetics, significant analgesic effects. It usually does not depress the cardiovascular and respiratory systems, but it does possess some adverse psychological effects (hallucinations, delirium) which have to be prevented/treated with, e.g., benzodiazepines. Ketamine is often used in emergency medicine (no depression of ventilation or blood pressure in unstable patients in pain) and widely used as a general anesthetic in second and third world countries (where (anesthetic) health care facilities might be limited).

Recently, a (physiological) anti-inflammatory pathway via the parasympathetic nervous system has been recognized. The neurotransmitter, acetylcholine, prevents activation of the NF-κB pathway and the secretion of high mobility group box 1 (HMGB1) [42]. Consequently, the release of pro-inflammatory mediators, but not the anti-inflammatory cytokine, IL-10, is inhibited [43]. This 'nicotinic anti-inflam-

matory pathway' [44] requires the activation of the α7-nicotinic acetylcholine receptors, which are present on macrophages, microvascular endothelial cells and epithelial cells. Ketamine inhibits the α7-nicotinic acetylcholine receptor in the brain [45], while propofol or isoflurane do not [46]. Although one might expect that inhibition of this receptor by ketamine would consequently lead to attenuation of the nicotinic anti-inflammatory pathway and increased levels of pro-inflammatory cytokines, this has not been found. In contrast, ketamine markedly inhibited TNF-α, IL-6, and IL-10 production in septic mice [47] and in cardiac surgical patients [48]. Interestingly, survival in mice was dependent on the timing of ketamine injection relative to the inoculation of the lethal dose of LPS. Ketamine (10 mg/kg) administered directly before LPS challenge, increased survival rates of mice to 86% compared with 8% in control mice (LPS only) after 5 days. However, when ketamine was injected 2 hours after LPS challenge survival in the ketamine group was comparable to the control group [47]. Unfortunately, this study did not measure cytokine levels in the early or late ketamine treated groups.

Potential Clinical Implications

The general anesthetics described here, volatile anesthetics, propofol and ketamine, have all been shown to affect the immune response. Of note, in animal studies, it has been revealed that the timing of the challenge with an anesthetic is of crucial importance for the effect on survival. Septic animals that were immediately treated with an anesthetic (either isoflurane, propofol or ketamine) after induction of sepsis showed increased survival rates, whereas animals treated at a later stage had survival rates comparable to control animals (sepsis alone). The anesthetics decreased plasma levels of pro-inflammatory cytokines and isoflurane has been shown to inhibit NF-κB. Presumably, early treatment in these studies coincided with the 'hyperinflammatory' phase of sepsis in which decreased pro-inflammatory cytokine levels are preferred. In contrast, in the later phase, one would favor a maintained pro-inflammatory cytokine level in order to prevent immunoparalysis.

Although inter-anesthetic differences on immune responses (and survival rates) have not been investigated in animals, differences between isoflurane-anesthesia and propofol-anesthesia have been studied in surgical patients. Propofol increased IL-10 levels postoperatively. This might indicate that propofol induces immunoparalysis. Isoflurane increased the expression of pro-inflammatory cytokines in lung macrophages. However, it is unknown whether this effect is limited to the lung compartment (isoflurane is administered through inhalation), or will also be found systemically.

The mechanisms underlying the immunomodulatory effects of anesthetics are not known precisely. Propofol can stimulate purified rat brain PKC [49]. Propofol also possibly stimulates PKC in monocytes, downregulates IRAK, and consequently decreases activation of NF-κB and TNF production. In mice, isoflurane has been shown to inhibit NF-κB. In contrast, another study showed that isoflurane was able to activate p38MAPK by itself, and to augment the LPS-induced activation of p38 MAPK [50]. This effect may enhance activation of the transcription factor, AP-1, which could lead to increased pro-inflammatory cytokine release and prevention of immunoparalyis.

Conclusion

General anesthetics are used extensively and for long periods to maintain anesthesia or sedate patients in the operation room or the ICU. It is well known that these patients are prone to develop infectious complications which substantially increase morbidity, hospital stay, and resource consumption. The phenomenon of immunoparalysis, defined as a diminished capacity of immunocompetent cells to respond to infectious agents, has been implicated as a major contributing factor in this process. We have described *in vitro*, animal and patient studies showing that anesthetics can affect immunocompetent cells. Interestingly, the direction of the response (production of pro-inflammatory cytokines, or not) possibly depends on the timing and on the anesthetic used. This observation may be linked to the different effects of the various agents on mechanisms involved in the development of immunoparalysis. Therefore, the choice of anesthetic may be of importance in the occurrence of (postoperative) infections. Although more research is needed to substantiate current knowledge, if modulation of the inflammatory response by anesthetics were possible, this would likely influence outcomes.

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Critical Illness Stress-induced Immune Suppression

J.A. Carcillo

Introduction

Despite the use of Centers for Disease Control and Prevention (CDC) recommended practices to minimize infection risk, nosocomial sepsis and multiple organ failure (MOF) remain a leading cause of morbidity and mortality in critically ill patients. It is well documented that the use of immunosuppressant therapies dramatically increases this risk in patients with cancer, transplantation, and immunologic disease. Although immune monitoring has yet to be universally embraced, withdrawal of immunosuppressant therapies and use of immune restoration therapies is the standard of care when these patients develop sepsis. Critical illness stress can also induce a level of immunosuppression which is as life-threatening as is seen in the purposefully immunosuppressed patient. This chapter reviews the role of critical illness stress-induced immunosuppression in the development of nosocomial sepsis and MOF, and outlines clinical strategies which can be employed to maintain and restore immune function, and reduce morbidity and mortality in critically ill patients.

The Healthy Immune System: How We Recognize and Kill Invasive Microbial Pathogens

The immune system has soluble and cellular components. If one takes soluble serum or plasma and exposes it to microbes there will be a certain amount of microbial killing, also known as serumcidal activity. This is orchestrated by endogenous antimicrobial polypeptides, including defensins, lactoferrin, and bactericidal permeability increasing factor (BPI). The complement system is also a major contributor to serumcidal activiy. In the first 12 hours of infection, circulating mannose binding lectin (MBL) binds to the mannose residues on the microbial surface and activates complement killing. After 12 hours, circulating antibodies and C-reactive protein (CRP) orchestrate a second wave of complement-mediated microbicidal activity.

The cellular component of the immune response also has two waves which are coordinated (Figs. 1 and 2). The first phase is called the innate immune system, which is predominant for 24 to 72 hours. Similar to the MBL pathway, these cells recognize microbial pathogens by a pattern of glycoproteins on their cell surface, which are not seen in human or eukaryote cells. These cells include polymorphonuclear leukocytes (PMN), monocyte/macrophages, and macrophage/dendritic cells, all of which engulf and directly kill microbes through the production of oxygen radicals and tumor necrosis factor (TNF) (only macrophage/dendritic cells produce

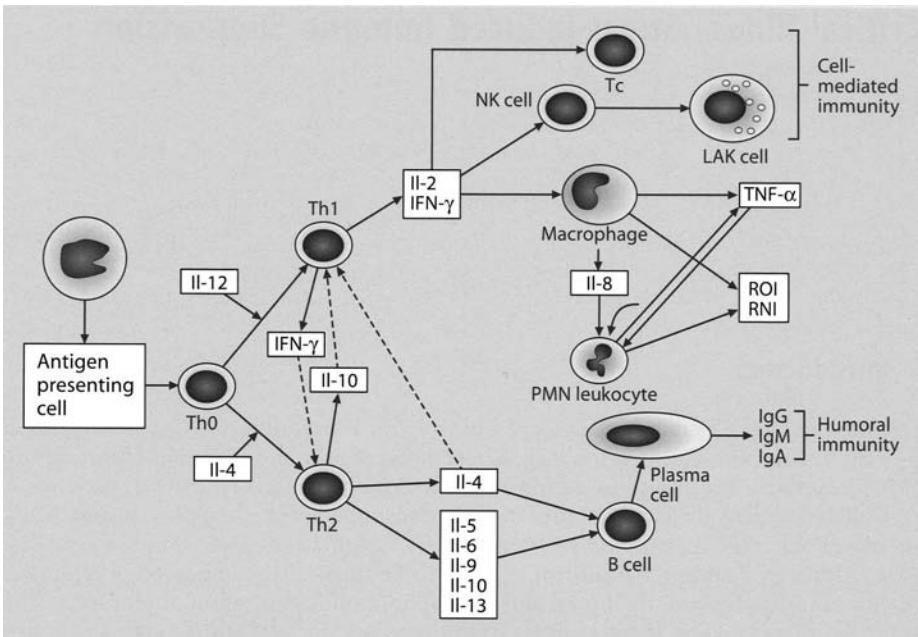

Fig. 1. The normal host immune response. IL: interleukin; IFN: interferon; TNF: tumor necrosis factor; NK: natural killer; ROI: reactive oxygen intermediates; RNI: reactive nitrogen intermediates

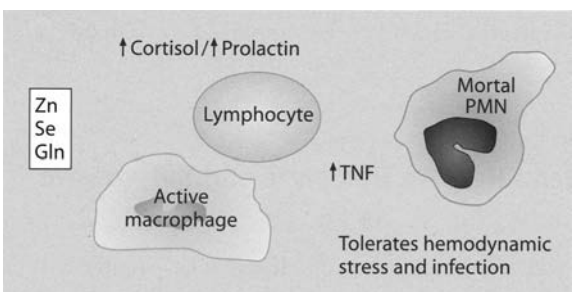

Fig. 2. The normal stress response maintains immune integrity

TNF). Once the bacteria are killed, the adherent PMN undergoes deactivation and apoptosis (programmed cell death) in response to macrophage/dendritic cell generated TNF. However, the macrophage/dendritic cell task is only beginning. These cells then process the antigenic peptides of the killed microbe and present them on the human leukocyte antigen (HLA)-DR antigen on their cell surfaces. Circulating T-lymphocytes now recognize this presented antigen and initiate the adaptive immune response approximately 24 to 72 hours after initial invasion. According to the cytokine milieu at the point of recognition, the adaptive immune response can go in one of two directions: the so called Th1 response or the Th2 response with the most effective host immune response being one that is well balanced between the two.

The Th1 response is more likely in the presence of interferon (IFN)-γ and interleukin (IL)-2. During this process, the adaptive immune response is dedicated to

more efficient killing. In the presence of viral infection, CD8 cytotoxic T cells and natural killer cells are recruited and activated to cell-mediated killing. In the presence of bacterial infection, macrophages call in more PMN leukocytes through secretion of IL-8, and also continue to efficiently kill with greater generation of TNF-α and nitric oxide (NO)/oxygen radicals. In the presence of fungal infection, cytotoxic T cells, natural killer cells, PMNs, and macrophages are all called upon for their fungicidal mission.

The Th2 response is more likely in the presence of IL-4, IL-6, and IL-10 and represents the predominant host response to parasitic infection. During this process, the Th1 response is dampened and B lymphocytes are produced from plasma cells. When Th1 and Th2 are balanced, these B cells participate in antibody production, which both enhances antibody-dependent, complement-mediated killing, and opsonizes encapsulated bacteria so they can be more efficiently phagocytosed by the reticuloendothelial system (macrophage/dendritic cells) in the spleen. However, when the Th2 response is predominant, antibodies are made but are ineffective because dendritic cells and macrophages cannot kill and process even antibody-coated organisms. A balanced Th1-Th2 response is the hallmark of effective and healthy immune function.

How Can the Clinician Manipulate Immunosuppressant Therapies so they do not Prevent the Immune System from Recognizing and Killing Invasive Microbes and Cause Death from Sepsis?

A number of immunosuppressant agents are commonly used with the purpose to kill or inactivate immune cells (Fig. 3). These include, among others, dexamethasone and other steroids, chemotherapy agents, irradiation, monoclonal and polyclonal antibodies, calcineurin inhibitors, and nucleoside analogs. Dexamethasone is among the most potent of all immunosuppressants. It is popular because it induces apoptosis (programmed cell death) in lymphocytes and malignant cells, rapidly reducing tumor size. It is also used widely for its profound anti-inflammatory and anti-TNF effects in the short term treatment of croup. However, although short term use is therapeutic, long term use promotes pseudomonas sepsis. Indeed, the experimental model of fatal pseudomonas pneumonia requires long term treatment with dexamethasone followed by pseudomonas inoculation [1]. Other steroids, including prednisone, methylprednisone, and hydrocortisone, do not share this degree of immunosuppressive effect [2, 3]. Chemotherapy is generally directed to killing of rapidly dividing cells, and since immune cells are rapidly dividing, they are inadvertently killed. Chemotherapy for leukemias directly targets killing of either lymphocytes for lymphocytic leukemia, or PMN cells for acute myelogenous leukemia. These agents induce life-threatening neutropenia. Irradiation is commonly used to kill tumors and is also the mainstay for ablation of recipient bone marrow prior to bone marrow transplantation. Irradiation induces lymphocyte apoptosis. When resident macrophages/dendritic cells ingest these apoptotic lymphocytes they are deactivated and no longer able to recognize, phagocytose, or kill microbial invaders [4]. They are also unable to present antigenic peptides on HLA-DR molecules resulting in incapacitation of both the innate and adaptive immune systems. Monoclonal antibodies to lymphocytes are commonly used in solid organ transplant patients. T-cell antibodies, such as OK T3 or ATGAM, result in T cell lysis and depletion. However, unlike radiation or dexamethasone which cause death by apotosis, the mode of

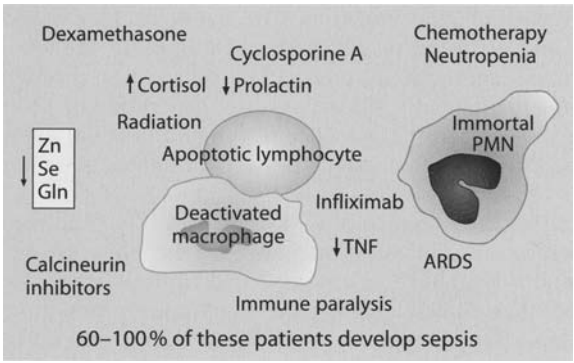

Fig. 3. Immunosuppressants can inhibit the ability to kill infection.

Table 1. Rational immune phenotype-directed therapeutic strategies in patients with critical illness stress-induced immunosuppression

Immunophenotype thresholds	Therapeutic approach
Absolute neutrophil count <500 cells/mm^3	a) Stop chemotherapy b) Administer empiric antimicrobial therapy for neutropenic fever; c) Administer G-CSF, GM-CSF, or WBC infusion for neutropenic sepsis
Absolute lymphocyte count $<1{,}000$ cells/mm^3	a) Stop dexamethasone, dopamine, cyclosporine A b) Administer prophylactic/empiric anti-viral, anti fungal therapies c) Replenish zinc, selenium, glutamine
Hypogammaglobulinemia (IgG <500 mg/dl)	a) Give IVIG q three weeks
Monocyte deactivation – HLA-DR $<30\%$ or 8,000 to 12,000 molecules/cell; Whole blood TNF-α response to LPS <200 pg/ml	a) Stop dopamine, dexamethasone, calcineurin inhibitors b) Replenish zinc, selenium, glutamine d) Apply appropriate antibiotic therapy and remove the nidus of infection e) Give GM-CSF 125 µg/m^2 daily over 12 hours

TNF: tumor necrosis factor; GM-CSF: granulocyte-macrophage colony-stimulating factor; WBC: white blood cell; LPS: lipopolysaccharide; G-CSF: granulocyte colony-stimulating factor

death caused by these lytic therapies includes complement mediated necrosis. When macrophages/dendritic cells ingest these necrotic lymphocytes they remain active. Although the adaptive immune system is impaired, the innate immune system remains intact. B-cell antibodies are used to kill B-cell tumors caused by post-transplant lymphoproliferative disease or lymphoma. Anti-nucleoside analogs, by substituting for functional nucleoside building blocks, prevent DNA synthesis and lymphocyte proliferation in cancer and solid organ transplantation. The calcineurin inhibitors are mainstay immunosupressants in solid organ and bone marrow transplant patients. These agents mediate their effects by inducing a predominant Th2 state which inadvertently prevents Th1-mediated microbial phagocytosis, killing, processing, and presentation to the adaptive immune system.

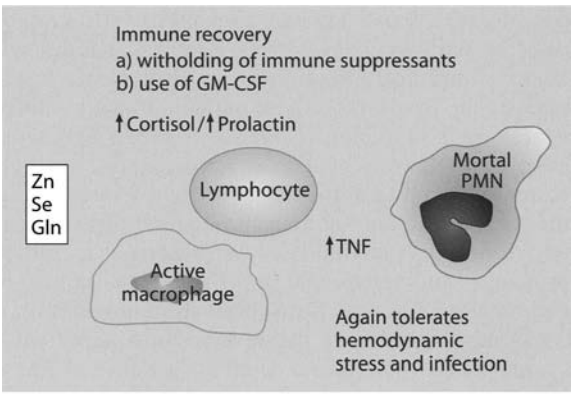

Fig. 4. Immune recovery with immunosuppressant withdrawal

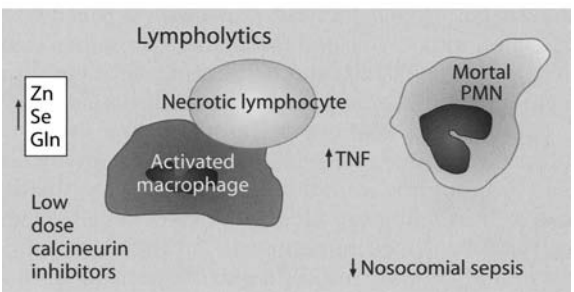

Fig. 5. Lympholytic therapy is a novel strategy to maintain macrophage

Immune phenotyping, although incompletely applied, has identified threshold values below which these immunosuppressants lead to life-threatening immunosuppression-induced nosocomial infection and sepsis (Table 1). An absolute neutrophil count less than 500 cells/mm^3 is associated with the development of sepsis. An absolute lymphocyte count $<1{,}000$ cells/mm^3 for more than three days is associated with a five-fold increased adjusted odds ratio for developing nosocomial sepsis [5]. If present more than seven days, there is a six-fold increased adjusted odds ratio for development of MOF and death. Immunoglobulin G levels <500 mg/dl are also associated with secondary infection and sepsis. Prolonged monocyte deactivation for more than five days, also known as immunoparalysis, is associated with a six-fold increased adjusted odds ratio for nosocomial sepsis, MOF, and death [6]. The threshold for monocyte deactivation is defined by several functional tests including an HLA-DR expression $<30\%$ of normal, or $<8{,}000-12{,}000$ molecules per monocyte cell, or a whole blood TNF-α response of <200 pg/ml to lipopolysaccharide (LPS) stimulation. Specific therapeutic strategies utilized to prevent and treat nosocomial sepsis in special immunosuppressed patient populations are directed to restoration of immune function above these critical thresholds (Figs 4 and 5).

The most common critical threshold crossed in cancer patients is severe neutropenia. The American College of Oncology recommends the prophylactic use of growth factors including granulocyte colony-stimulating factor (G-CSF) or granulocyte/macrophage colony-stimulating factor (GM-CSF) to prevent neutropenia in patients who have previously had a 40% incidence of neutropenic fever after chemotherapy [7]. They also consider the use of G-CSF or GM-CSF reasonable therapy in patients with neutropenic sepsis. White blood cell transfusions are reserved for

patients with sepsis who are unresponsive to growth factors. Other standard care in patients with neutropenic fever/sepsis includes withdrawal of chemotherapy and empiric antibitotic treatment for neutropenic fever. Antifungals are added if fever persists for five days. Cancer patients treated with prolonged courses of dexamethasone have a very high incidence of sepsis and sepsis-related death. Transition to a steroid regimen which uses prednisone, methylprednisone, or hydrocortisone completely reduces this risk likely by preventing lymphocyte apoptosis-related monocyte deactivation and immune paralysis. Bone marrow transplant patients suffer from pancytopenia. Because prolonged lymphopenia is the rule, anti-viral, anti-protozoal, and anti-fungal prophylaxis is administered daily. For patients with hypogammaglobulinemia, intravenous immunoglobulin (IVIG) is also administered on a tri-weekly basis. Bone marrow transplant patients with acute respiratory distress syndrome (ARDS) have a complete absence of Th1 activity in their lungs at autopsy [8]. This state of immune paralysis leads to an inability to kill viral, bacterial, and fungal pathogens. Immune function can be restored in part in these patients by stopping dexamethasone (switching to another steroid for graft versus host disease [GVHD] prophylaxis) and by titrating calcineurin inhibitor therapy such that monocyte function is above the critical threshold.

The field of solid organ transplantation has drastically changed its immunosuppressant regimens since its earliest days. Initially plagued by rejection when steroids and azathioprine were the therapy of choice, the field was boosted by the arrival of calcineurin inhibitors. Grafts no longer suffered acute rejection with implementation of steroid/calcineurin regimens, but patients commonly died from nosocomial sepsis and MOF. Volk and colleagues demonstrated that withholding steroids and calcineurin inhibitors from solid organ transplant patients with HLA-DR expression $<30\%$ and sepsis resulted in near 100% survival without organ rejection, compared to 20% survival if immunosuppression was not withheld [9]. There are now over 300 publications on immunosuppression withdrawal from patients with solid organ transplantation as a strategy to induce immune tolerance. A popular regimen of immunosuppression today uses lytic therapies, such as OK T3 or ATGAM, which deplete lymphocytes through necrosis, not apoptosis, sparing steroid use and maintaining monocyte function above the critical threshold of immune paralysis.

Immunosuppressive/anti-proliferative regimens are also used in patients to shrink hemangiomas. Dexamethasone should not be used long term because of the risk of fatal pseudomonas infection. IFNα has been reported to be effective in this disease. Lymphomas caused by Epstein-Barr virus-mediated transformation with or without transplantation are treated with B cell monoclonal antibodies; however, IVIG must be administered for ensuant hypogammaglobulinemia.

The need for hypogammaglobulinemia treatment is also present in patients with systemic lupus erythematosis treated with B-cell lytic therapies. Macrophage activation syndrome in patients with rheumatologic disease is treated with apoptotic agents, such as dexamethasone, as well as with calcineurin inhibitors. The same principles as above should be applied to these patients. Dexamethasone should be reserved for short term use only and calcineurin inhibitors should be titrated to maintain monocyte/macrophage function above its critical functional threshold.

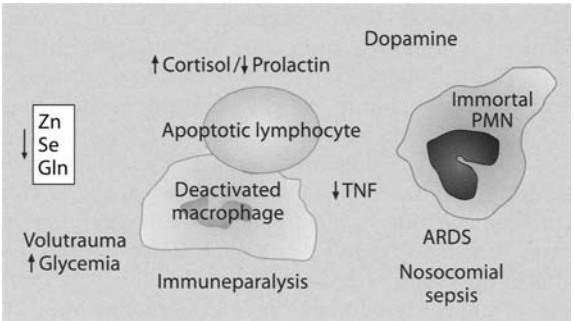

Fig. 6. Critical illness stress-induced immunosuppression (CRISIS)

Critical Illness Stress-induced Immunosuppression

Critical illness stress can induce prolonged lymphopenia and monocyte deactivation even when purposeful use of immunosuppressant therapies is absent. When stress induced immunosuppression is too great for too long the patient develops nosocomial sepsis, MOF, and death. The remainder of this chapter discusses clinical strategies which can be used to prevent and reverse critical illness stress-induced immunosuppression (Fig. 6).

A healthy patient subjected to surgical stress during a dental procedure or uncomplicated surgery will mount a hypothalamic/pituitary gland-mediated stress response with increased corticotropin releasing hormone/adrenocorticotropic hormone (CRH/ACTH) secretion and then adrenal gland cortisol production. This is matched by the increased production of the counter-regulatory hormone, prolactin, from the pituitary gland. The CRH/ACTH and cortisol hormones maintain cardiovascular homeostasis and dampen inflammation. However, if left unopposed, ACTH/CRH/cortisol also induce apoptosis of lymphocytes. Prolactin, through stimulation of Bcl_2, inhibits this lymphocyte apoptotic effect of ACTH and cortisol. Nutrition is also important. Zinc, selenium, and glutamine are all necessary for control of lymphocyte apoptosis.

Critically ill neonates, children, and adults who die from nosocomial sepsis and MOF do so with prolonged lymphopenia (absolute lymphocyte count $<1{,}000>7$ days) and lymphoid organ depletion. At autopsy these patients have apoptosis-mediated depletion of B-cells from lymph nodes and spleen, T cells from thymus, and dendritic cells from spleen [5]. Clinical factors associated with lymphoid depletion in these patients include use of synthetic steroids such as dexamethasone; zinc, glutamine, and/or selenium deficiency; and hypoprolactinemia. Zinc, glutamine, and selenium deficiency commonly occur in critical illness because these nutrients are consumed at higher rates during stress and commonly under dosed during parenteral and enteral nutritional support. Hypoprolactinemia can occur as part of the pituitary dysfunction syndrome of critical illness but can also be induced iatrogenically. Dopamine infusion stimulates the DA_2 receptor, which prevents prolactin secretion by the pituitary gland, and cyclosporine A is a prolactin receptor antagonist. Hotchkiss and Nicholson have shown that caspase inhibitors prevent lymphopenia, reduce bacterial counts, and improve survival in the cecal ligation and puncture (CLP) model of experimental sepsis [10]. Chaudry and colleagues expanded upon this observation showing that treatment with prolactin or metoclopramide (a

DA_2 antagonist which increases prolactin levels) reverses hemorrhagic shock induced lymphocyte apoptosis and susceptibility to CLP sepsis-induced mortality [11]. Clinical therapies which can be used to reverse or prevent lymphopenia and lymphoid depletion in critically ill humans include provision of adequate zinc, glutamine, and selenium; withdrawal of dexamethasone, dopamine, and cyclosporine A; and use of DA_2 antagonists, such as metoclopramide or haloperidol. In this regard, several trials have shown reductions in nosocomial infection in critically ill patients with the addition of zinc, glutamine, or selenium supplements. Norepinephrine has been shown to improve outcomes compared to dopamine [12], and a before and after study showed reduced nosocomial sepsis mortality when dexamethasone was substituted by prednisone or methylprednisone [2]. A single center study demonstrated an associated reduction in mortality in critically ill mechanically ventilated patients treated with haloperidol [13]. Two randomized controlled trials are underway: a single center study of haloperidol in mechanically ventilated adult patients, and a multiple center study of zinc [14], glutamine, selenium [15], and metoclopramide in critically ill pediatric patients.

Critical illness-induced lymphocyte apoptosis does not only result in lymphoid depletion, it also causes monocyte deactivation and immune paralysis. When macrophages are fed lymphocytes killed by irradiation (apoptotic lymphocytes) *in vitro* they become deactivated and have a diminished capacity to produce a TNF-α response to LPS. When macrophages are fed lymphocytes killed by freeze thaw (necrotic lymphocytes) they become activated and are able to produce TNF-α in response to LPS. Hotchkiss and colleagues have shown that adoptive transfer of apoptotic (irradiated) splenocytes sensitizes rats to CLP sepsis-induced mortality, while adoptive transfer of necrotic (freeze thaw) splenocytes protects rats to CLP sepsis induced mortality [16]. The effect is mediated through an IFNγ-Th1 mechanism. IFNγ is decreased in apoptotic splenocytes and increased in necrotic splenocyte-treated subjects, and treatment with IFNγ inhibition ablates the protective effect.

Prolonged monocyte deactivation and immune paralysis induced by lymphocyte apoptosis can also contribute to the development of ARDS. Although TNF-α does not induce apoptosis in circulating or non-adherent PMN leukocytes, it is needed for apoptosis of adherent PMN leukocytes [17]. When monocyte deactivation results after phagocytosis of apoptotic lymphocytes, its production of TNF-α is dramatically reduced. When the Th1 mediated TNF-α response is absent, adherent PMN leukocytes will not apoptose. The hallmark of ARDS is lack of apoptosis of adherent lung PMN leukocytes. To test this possibility, Presneill and colleagues randomized patients with ARDS to GM-CSF therapy. GM-CSF effectively increases the TNF-α Th1 response in deactivated macrophages in the absence of apoptotic lymphocytes, dexamethasone, and calcineurin inhibitors. In this study, no information was given on the presence or absence of these factors. Nevertheless, GM-CSF reduced lung PMN cells, supporting a role for TNF-α in facilitating adherent PMN leukocytes during ARDS [18].

Mechanical stress caused by volutrauma from overzealous mechanical ventilation also leads to a predominant Th2 state with prolonged monocyte deactivation, immunoparalysis, and continued ARDS. Healthy children subjected to large tidal volumes of 12 ml/kg during elective surgery have been documented to have increased circulating levels of IL-6 and IL-10 with reduced monocyte production of TNF-α. Healthy children subjected to physiologic tidal volumes of 6–8 ml/kg have no increase in IL-6 or IL-10 levels and maintain normal monocyte TNF-α produc-

tion [19]. Adults with ARDS receiving larger tidal volumes show similar relationships with increased systemic IL-6 and IL-10, reduced monocyte TNF-α production, and immune paralysis [20]. In this regard, mechanical ventilation with effective tidal volumes of 6–8 ml/kg reduces mortality from ARDS [21].

Endotoxemia and sepsis also lead to prolonged monocyte deactivation and immunoparalysis in critically ill patients. Experimental animals and humans subjected to endotoxin or sepsis challenge, develop a reduced or down regulated monocyte/macrophage derived TNF-α response to LPS. The mechanism of this 'endotoxin tolerance' appears to be driven in part by an IL-10/IL-6 – Th2 milieu which subsequently inhibits the Th1 response. This state of 'endotoxin tolerance', initially thought to be protective, is now known to harmfully increase susceptibility to sepsis-induced death. Experiments show that Th1 dominant mice are more resistant to sepsis than Th2 dominant mice [22]. Treatment with flt–3 ligand, the dendritic cell growth factor, reverses endotoxin tolerance, restores the TNF-α response to LPS, and reduces susceptibility to sepsis mortality [23]; so too does treatment with GM-CSF and IFNγ [24, 25].

The term 'endotoxin tolerance' is not used in humans. Instead patients with sepsis or endotoxemia who develop a TNF-α response to LPS of <200 pg/ml and/or decreased monocyte HLA-DR expression <30% or 8,000–12,000 molecules per cell are said to have monocyte deactivation and immunoparalysis. General surgery patients with immunoparalysis have an 80% mortality with sepsis, compared to only 12% mortality in those who have monocyte function above this threshold [9]. *In vitro* studies show that two drugs, GM-CSF and IFNγ, can reverse endotoxin- or sepsis-induced monocyte deactivation in humans [24]. One case series has demonstrated reversal of immunoparalysis with IFN therapy [26]; however, IFN therapy has greater toxicity than GM-CSF therapy. A randomized controlled trial using a three day course of low-dose GM-CSF demonstrated improved monocyte HLA-DR expression with improved cure rates and resolution of sepsis [27]. Studies designed to evaluate the effectiveness of GM-CSF in patients with sepsis, ARDS, or MOF can provide confusing results if not done with immune phenotyping as well as proper source control. GM-CSF is not effective in reversing immune paralysis if monocytes/macrophages continue to phagocytose apoptotic lymphocytes, or to be immunosuppressed by exogenous immunosuppressant therapy. Hence immunosuppressants, dexamethasone, and dopamine should all be withheld during GM-CSF therapy. GM-CSF will also not be helpful in a patient with sepsis who does not have immune paralysis but instead suffers from failure to remove the nidus of infection due to lack of appropriate antibiotic therapy or surgical drainage. For example, in the activated protein C trial there was no benefit observed in surgical sepsis; however, the mortality rates in those with proper nidus removal and source control was 1% compared to 92% without proper source control [28]. Patients can have monocyte function above critical threshold levels and still die due to lack of removal of the source of sepsis.

Trauma patients also develop stress induced immunosuppression. Lymphopenia is a marker of severity of illness and mortality in trauma and should be addressed with nutritional attention to zinc, selenium, and glutamine status as well as avoidance of dopamine. In patients with severe head injury, the use of pentobarbital, hypothermia, and hypertonic saline all reduce PMN leukocyte function. These therapies should be limited, particularly in patients who develop nosocomial sepsis. Surgical intervention for refractory intracranial hypertension can be preferred to this immunosuppressive regimen in selected patients. Prolonged narcotic use can also decrease PMN leukocyte function and induce monocyte deactivation [29]. Use of

low dose naloxone can be effective in reducing opioid tolerance and potentially reduces these immunosuppressant effects. Hyperglycemia can also impair innate and adaptive immune function. A randomized controlled trial showed that when daily glucose requirements were met (D10 infusion at maintenance fluid dosage) and hyperglycemia was controlled with insulin, surgical patients experienced less nosocomial sepsis, MOF, and mortality [30].

Conclusion

Despite implementation of CDC recommendations for prevention of infection, nosocomial sepsis and MOF remain the most common acquired causes of death and morbidity in critically ill patients. The role of immune dysfunction in this process is evidenced by an increased risk of nosocomial sepsis in patients hospitalized with immune deficiency and/or receiving immunosuppressant therapies [31]. Four iatrogenic immune deficiency thresholds appear important in predicting and reversing risk of nosocomial sepsis: 1) neutropenia, defined as an absolute neutrophil count <500 cells/mm^3; 2) monocyte deactivation or immune paralysis defined by monocyte HLA DR expression $<30\%$ or $<8000-12000$ molecules per cell or whole blood TNF-α response to LPS of <200 pg/ml; 3) lymphopenia, defined as an absolute lymphocyte count $<1{,}000$ cells/mm^3; and 4) hypogammaglobulinemia, defined as an IgG level <500 mg/dl. Therapeutic strategies in these populations include: a) empiric and prophylactic use of antibiotics, anti-virals, anti-protozoals, and anti-fungals; b) withdrawal of immunosuppressant therapy; c) administration of G-CSF, GM-CSF, and IVIG; and d) use of lympholytic rather than lymphocyte apoptotic agents to induce immune tolerance.

Critical illness stress-induced immunosuppression similarly causes patients to reach critical threshold levels of lymphopenia, monocyte deactivation, and immune paralysis and to develop nosocomial sepsis and MOF. Lymphocyte apoptosis and lymphoid depletion occur when the CRH/ACTH/cortisol axis induces programmed cell death in the presence of zinc, selenium, and glutamine deficiency, and/or hypoprolactinemia caused by pituitary dysfunction or dopamine infusion. Immunoparalysis can be caused by macrophage ingestion of apoptotic lymphocytes, or by mechanical ventilation-related volutrauma or endotoxin/sepsis induced Th2-mediated inhibition of macrophage TNF-α production. Surgical and traumatic stress similarly induce lymphopenia and Th2 mediated immunoparalysis. Randomized controlled trials, and before and after studies suggest that nosocomial sepsis can be reduced and survival outcome improved with zinc, selenium, and glutamine supplementation, dexamethasone and dopamine withdrawal, limitation of mechanical ventilator delivered tidal volumes to 6–8 m/kg, effective source control with appropriate antibiotics and nidus removal, and appropriate glucose delivery with insulin therapy directed to glycemic control. Once these tasks have been accomplished, GM-CSF can permit further restoration of the Th1 response, reversing residual immune paralysis, and preventing/resolving nosocomial sepsis.

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Transcription Factors and Nuclear Cofactors in Muscle Wasting

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Introduction

Muscle wasting is commonly seen in patients with sepsis, severe injury, and cancer [1, 2]. The loss of muscle mass in these conditions mainly reflects ubiquitin-proteasome-dependent degradation of myofibrillar proteins although other proteolytic mechanisms may be involved as well [3]. Muscle atrophy is regulated by multiple factors, including glucocorticoids [4], the pro-inflammatory cytokines, interleukin (IL)-1β and tumor necrosis factor (TNF)-α [5, 6], and myostatin [7]. In addition to these catabolic factors, a lack of anabolic signals, such as insulin-like growth factor (IGF)-1 and insulin, is probably also important for the development of muscle wasting in various catabolic conditions.

Although it may be argued that the increased peripheral release of amino acids that is associated with muscle wasting may be beneficial to the organism by providing energy and substrates to various organs and tissues, including the liver, gut mucosa, and cells in the immune system, during prolonged and severe catabolic conditions, the negative effects of muscle atrophy clearly outweigh any potentially beneficial effects. Thus, muscle wasting results in weakness and fatigue that in turn delays ambulation of bed-ridden patients, increasing the risk for thromboembolic and pulmonary complications. The risk for pulmonary complications is further increased when the muscle wasting process affects respiratory muscles [8]. In patients with cancer, the accompanying muscle wasting can significantly impair quality of life and may even reduce the effectiveness of chemotherapy [9]. It has been estimated that approximately 20% of deaths in cancer patients can be attributed to the catabolic response in skeletal muscle [10].

Thus, it is obvious that muscle wasting is a significant clinical problem with sometimes devastating consequences. Despite substantial progress during the past decade in our understanding of mechanisms regulating the development of muscle atrophy, we still do not have any effective treatment by which muscle wasting can be prevented or reversed in critically ill patients. Continued efforts to understand the molecular mechanisms behind the loss of muscle mass in these patients will hopefully help in the development of therapeutic strategies for subjects with this debilitating condition. In this chapter, novel insights into the role of various transcription factors and nuclear cofactors in the development of muscle atrophy are discussed.

Gene Transcription in Muscle Wasting

In previous studies, we and others found evidence that mRNA levels for various genes in the ubiquitin-proteasome pathway are increased in muscle wasting condi-

tions. For example, in research from our laboratory, the gene expression for ubiquitin as well as different components of the 26S proteasome was increased in muscle from septic [11, 12] and burned rats [13] and in muscle from patients with sepsis [14] or cancer [15]. Results in other studies suggested that the increased mRNA levels for various components of the ubiquitin-proteaosme pathway reflected upregulated transcription of the genes rather than increased stability of the mRNA [16].

In more recent studies, Lecker et al. [17] reported that multiple catabolic conditions (uremia, fasting, muscle inactivity and denervation) were characterized by a common set of genes that were significantly upregulated. They called these genes "atrogins" and found that the mRNA levels for two ubiquitin ligases, atrogin-1 and muscle ring finger 1 (MuRF1), were particularly increased [18]. The dramatic increase in the expression of these genes in atrophying muscle has been confirmed by others as well [19] and in studies in our laboratory, atrogin-1 and MuRF1 mRNA levels were increased almost 20-fold in skeletal muscle during sepsis [20]. Other studies provided evidence that the atrogin-1 and MuRF1 gene products regulate the development of muscle atrophy caused by various catabolic conditions and the mRNA levels for atrogin-1 and MuRF1 are frequently used as 'molecular markers' of muscle wasting.

Because the transcription of multiple genes is upregulated in atrophying muscle [17] it is not surprising that much attention has been given to the role of various transcription factors in the regulation of muscle mass. Here, the potential roles of the transcription factors C/EBPβ and δ, nuclear factor-kappa B (NF-κB) and Foxo1 and 3a in the development of muscle wasting are discussed. In addition, recent observations in our laboratory [21, 22] indicating an important role of the nuclear cofactor p300 and its histone actetyl transferase (HAT) activity in the regulation of muscle mass are described.

C/EBP Transcription Factors in Atrophying Muscle

The C/EBP (CCAAT/enhancer binding protein) family of transcription factors consists of at least six members: C/EBPα, β, γ, δ, ε, and C/EBP-homologous protein-10 (CHOP-10), also called Gadd 153 [23]. The different isoforms form homo- or heterodimers that influence the transcription of multiple genes in various organs and tissues. Among the different C/EBP family members, there is evidence that C/EBPβ and δ are particularly important for the inflammatory response [24].

We found that the expression of C/EBPβ and δ was upregulated in the nuclear fraction of muscles from septic rats [25]. This finding was accompanied by increased C/EBPβ and δ DNA binding activity determined by electrophoretic mobility shift assay (EMSA) with supershift analysis. Interestingly, the sepsis-induced increase in the expression and activity of C/EBPβ and δ was inhibited by the glucocorticoid receptor antagonist RU38486, supporting the role of glucocorticoids as an important mediator of sepsis-induced muscle wasting [4, 25]. Although we did not provide direct evidence that C/EBPβ and δ participate in the regulation of sepsis-induced muscle proteolysis, a sequence analysis demonstrated multiple putative binding sites for C/EBP in the promoter regions of genes that are upregulated in atrophying muscle, including genes for calpains and various components of the ubiquitin-proteasome proteolytic pathway [25].

Further support for a role of glucocorticoids in the upregulation of C/EBP transcription factors in atrophying skeletal muscle was found in experiments in which

we treated cultured myotubes *in vitro* or rats *in vivo* with dexamethasone [26]. In those experiments, treatment with dexamethasone resulted in increased protein and mRNA expression and DNA binding activity of C/EBPβ and δ. Because dexamethasone treatment did not influence the expression of C/EBPα, γ, or ε, it is possible that C/EBPβ and δ play a specific role among the C/EBP family members in atrophying muscle. The increase in C/EBPβ and δ mRNA levels in dexamtheasone-treated myotubes was not affected by the protein synthesis inhibitor, cycloheximide, indicating that the expression of the transcription factors was regulated directly by dexamethasone. This differed from the dexamethasone-induced upregulation of atrogin-1 mRNA levels that was blocked by cycloheximide, consistent with a model in which the atrogin-1 gene is activated in skeletal muscle secondary to the upregulation of another gene or genes, possibly C/EBPβ and δ [26].

Thus, our recent observations suggest that C/EBPβ and δ may be involved in the development of sepsis- and glucocorticoid-induced muscle protein breakdown and atrophy. Interestingly, other members of the C/EBP family may regulate lipid metabolism in atrophying muscle. Thus, in a recent study, muscle atrophy caused by denervation was associated with fatty degeneration and upregulated C/EBPα expression in the interstitium of the muscles, suggesting a role of C/EBPα in lipid metabolism in atrophying muscle [27]. This observation supports previous reports that C/EBPα is a transcription factor involved in the regulation of lipid metabolism in various cell types, including muscle cells [28].

NF-κB Plays a Role in Muscle Wasting

NF-κB is probably the most extensively studied transcription factor in the field of inflammation. It is beyond the scope of this chapter to review the mechanisms that regulate NF-κB activation. Recent extensive review articles describe the molecular biology of this important transcription factor, its activation, and the mechanisms by which it upregulates gene transcription [29, 30].

Although the exact mechanisms by which NF-κB regulates muscle protein breakdown are not completely understood at present, there is strong pharmacological and genetic evidence that NF-κB is involved in the development of muscle wasting in various catabolic conditions. We reported that NF-κB DNA binding activity was upregulated in skeletal muscle during early sepsis but was subsequently inhibited during the later course of an experimental model of sepsis in rats [31]. Interestingly, a biphasic response of NF-κB activity was observed in TNF-α/interferon (IFN)γ-treated myotubes as well [32]. Although the mechanisms for this biphasic response of NF-κB, and the biological implications with regards to which genes are activated or repressed, are not known at present, it is possible that the early activation and the late inhibition of NF-κB in septic muscle [31] reflects the role of different mediators involved in muscle wasting. For example, the early increase in NF-κB activity may reflect the influence of pro-inflammatory cytokines (most notably TNF-α) and the subsequent downregulation of NF-κB activity may reflect the effect of glucocorticoids. We and others have reported several lines of evidence that both cytokines and glucocorticoids are important mediators of muscle wasting [4–6]. Other studies have shown that NF-κB activity is upregulated by pro-inflammatory cytokines, including TNF-α [32, 33] and IL-1β [34] in skeletal muscle cells and that NF-κB activity may be inhibited by glucocorticoids in myocytes [35]. This may seem paradoxical because treatment of cultured myotubes with either cytokines or catabolic

concentrations of dexamethasone results in increased protein degradation, activation of the ubiquitin-proteaosme proteolytic pathway, and muscle atrophy [32, 33, 36, 37].

An additional potential mechanism to explain some of these apparently conflicting observations may be that NF-κB influences individual muscle wasting-related genes differentially. For example, there is evidence that NF-κB activates the expression of the ubiquitin ligase, MuRF1, [38] but may act as a repressor of some of the proteaosme subunit genes [39]. An alternative explanation why the role of NF-κB may seem confusing (having a biphasic response; being activated or inhibited by mediators that all induce muscle atrophy) is that different pathways may be involved in the activation of NF-κB. The classic pathway, activated by pro-inflammatory cytokines, involves the activation of a p50/p65(RelA) heterodimer by ubiquitin-proteasome-dependent degradation of NF-κB inhibitor (IκB), triggered by its phosphorylation by the IκB kinase, IKKβ. An alternative pathway for NF-κB activation is the IKKα-mediated phosphorylation and proteolytic processing of p100, resulting in activation of the non-canonical NF-κB pathway involving p52/RelB heterodimer [29]. The exact role of the different pathways activating NF-κB and the influence of cytokines and glucocorticoids on the regulation of these pathways in atrophying muscle are areas for future study.

Although important questions remain to be addressed with regards to the role of NF-κB in muscle wasting, in recent studies we have found strong molecular evidence that NF-κB activation results in muscle wasting [38]. In those experiments, transgenic mice created in the laboratory of Dr Steven Shoelson were used. Mice with a muscle-specific overexpression of activated IKKβ displayed upregulated NF-κB activity, increased expression of MuRF1 (but, interestingly enough, not atrogin-1), atrophy of muscle fibers, and a substantial loss of muscle mass. In other experiments in the same study, muscle-specific expression of an IκBα superrepressor blocked NF-κB activation and prevented denervation- and tumor-induced muscle loss. A similar prevention of muscle atrophy was seen in mice treated with pharmacological inhibitors of NF-κB further supporting a role of NF-κB in the development of muscle wasting. In recent (unpublished) experiments in our laboratory we found that treatment of rats with the NF-κB inhibitor, curcumin, prevented sepsis-induced muscle proteolysis, suggesting that inhibition of NF-κB may prevent muscle wasting in different conditions characterized by muscle cachexia.

It should be noted that although there is evidence that NF-κB is activated in catabolic muscle *in vivo* [31] and in cytokine-treated muscle cells *in vitro* [32–34], and that muscle-specific activation of NF-κB in transgenic mice results in muscle atrophy [38], several important questions remain to be answered. First, it will be important in future studies to determine the mechanisms and biological consequences of the biphasic response of NF-κB in catabolic muscle and in cytokine-treated myotubes. Second, the apparent contradiction between stimulation of protein degradation by both glucocorticoids and cytokines on the one hand, and the inhibition of NF-κB by glucocorticoids and activation of NF-κB by cytokines on the other hand, needs to be resolved. Third, it will be important to determine in greater detail which muscle wasting-related genes are upregulated and inhibited by NF-κB. Finally, the role of an interaction between glucocorticoids and cytokines, both at a systemic and a cellular level, in the regulation of NF-κB activity needs to be determined.

Activation of Foxo Transcription Factors Results in Muscle Atrophy

Recent studies support an important role of some of the Foxo (Forkhead box O) transcription factors in the development of muscle atrophy [40, 41]. The Foxo subfamily of the forkhead transcription factors consists of three members: Foxo1, 3a, and 4. These transcription factors are downstream targets of Akt and are inactivated by Akt-mediated phosphorylation. Phosphorylated Foxo transcription factors are retained in the cytoplasm in their inactive form. Dephosphorylation results in transport into the nucleus of the activated Foxo transcription factors. In recent studies in cultured myotubes, treatment with dexamethasone activated Foxo1 and 3a and upregulated the atrogin-1 and MuRF1 genes, resulting in myotube atrophy [40, 41]. These observations are in line with *in vivo* observations of an association between Foxo transcription factor activation and muscle atrophy in diabetes, fasting, cachexia, and aging [reviewed in 42]. The influence of sepsis, severe injury, and cancer on Foxo transcription factors in skeletal muscle remains to be determined but considering the common response of multiple atrogins in different catabolic conditions, it is probably safe to predict that the Foxo transcription factors are involved in muscle wasting seen in those conditions as well. It should be noted that Foxo transcription factors do not regulate the catabolic response only in skeletal muscle but may also activate an atrogene transcriptional program in cardiomyocytes [43].

p300/HAT Expression and Activity Regulate Protein Degradation in Muscle Cells

In recent years, it has become increasingly clear that in addition to transcription factors, so called nuclear cofactors participate in the regulation of gene activation [44]. Among nuclear cofactors, p300 has attracted much attention [45]. p300 exerts some of its effects through its HAT activity. Although it was initially believed that the major mechanism by which HAT activity regulates gene activation was by increasing the acetylation of histones, disrupting chromatin and enhancing the accessibility of transcription factors to their DNA binding sites, there is evidence that p300 acetylates other proteins as well, including transcription factors and other nuclear cofactors. Another important mechanism by which nuclear cofactors influence transcriptional activity is protein-protein interactions with transcription factors, nuclear cofactors, and other nuclear proteins that are components of the basal transcription machinery. The interaction with other proteins can result in the acetylation of those proteins but may also serve as a mechanism by which other proteins are recruited for regulation of gene transcription.

It should be noted that protein acetylation is determined not only by HAT activity but by histone deacetylase (HDAC) activity as well [46, 47], and the degree of acetylation is the result of the balance between ongoing acetylation and deacetylation of any given protein (analogous to the phosphorylation of proteins that is regulated by the balance between kinase and phosphatase activities).

Recent studies in our laboratory have focused on the potential role of p300 in the development of muscle wasting. In several of those studies we used glucocorticoid-treated cultured L6 myotubes, a rat skeletal muscle cell line. Glucocorticoid-treated myotubes have been used in a large number of studies, in our and other laboratories, as an *in vitro* model of muscle wasting characterized by increased ubiquitin-proteaosme-dependent proteolysis and reflecting the important role of glucocorti-

coids as a mediator of muscle wasting [36, 37, 40, 41]. In initial experiments, we found that p300 protein and mRNA levels were increased in a time- and dose-dependent manner in dexamethasone-treated myotubes [21]. In the same study, co-immunoprecipitation experiments provided evidence for a protein-protein interaction between p300 and C/EBPβ. Because there was no interaction between p300 and C/EBPδ, it is possible that the interaction with C/EBPβ is specific for this transcription factor among the C/EBP family members. In more recent experiments, we have found evidence that C/EBPβ is hyperacetylated in dexamethasone-treated myotubes (unpublished observations), suggesting that the p300-C/EBPβ interaction may influence gene transcription at least in part by hyperacetylation.

In subsequent experiments, we observed that p300-associated HAT activity was increased and HDAC3 and 6 expression and activity were reduced after treatment of the myotubes with dexamethasone [22]. In the same study, treatment of the myotubes with the HDAC inhibitor, trichostatin A (TSA), resulted in increased protein degradation, further supporting the role of hyperacetylation as a regulator of muscle protein breakdown. Interestingly, the increase in protein degradation caused by TSA was similar to the increase caused by dexamethasone and the combined treatment of the myotubes with dexamethasone and TSA gave rise to the same stimulation of proteolysis as was noticed after treatment with either drug alone, suggesting (but not proving) that they share a common mechanism in their actions.

Additional experiments in the same study [22] provided strong genetic evidence for a role of p300/HAT activity in the regulation of glucocorticoid-induced muscle protein degradation. In those experiments, silencing of p300 expression using a small interfering RNA (siRNA) technique blocked the dexamethasone-induced increase in protein degradation and a similar inhibition of dexamethasone-induced proteolysis was seen when muscle cells were transfected with a plasmid expressing p300 that had been mutated in its HAT activity domain and therefore lacked HAT activity.

Thus, our recent studies provide evidence for a role of hyperacetylation, mediated by increased HAT and decreased HDAC3 and 6 activities, in the regulation of glucocorticoid-induced muscle proteolysis. It will be important in future experiments to test whether similar mechanisms are involved in the *in vivo* regulation of muscle wasting seen in catabolic conditions such as sepsis, severe injury, and cancer. It will also be important to determine the exact role of C/EBPβ acetylation in the development of muscle atrophy and to test whether other transcription factors (or other nuclear proteins) are acetylated in catabolic muscle. In that respect, it is interesting to note that studies suggest that the activities of both Foxo transcription factors [48] and NF-κB [30] may be regulated by acetylation/deacetylation. For example, recent studies have provided evidence that the NF-κB p65 subunit is acetylated by p300 after its transport into the nucleus. This acetylation, which occurs on lysine residues 218, 221, and 310, results in increased NF-κB DNA binding and upregulated transcription of NF-κB target genes [30]. p65 is subsequently deacetylated by HDACs, in particular HDAC3, which facilitates the binding of p65 to the inhibitory IκB resulting in nuclear export of p65 and inhibition of NF-κB activity. Thus, the acetylation and deacetylation of p65 seem to act as an important molecular switch regulating NF-κB activity. Ongoing studies in our laboratories are designed to test whether acetylation of p65 (in addition to acetylation of C/EBPβ) plays a role in sepsis- and glucocorticoid-induced muscle wasting. It should be noticed that in other recent experiments we did not find evidence for acetylation of Foxo1 or 3a in dexamethasone-treated myotubes (unpublished observations) suggesting that transcription factors involved in muscle wasting may be differentially regulated by p300/HAT activity.

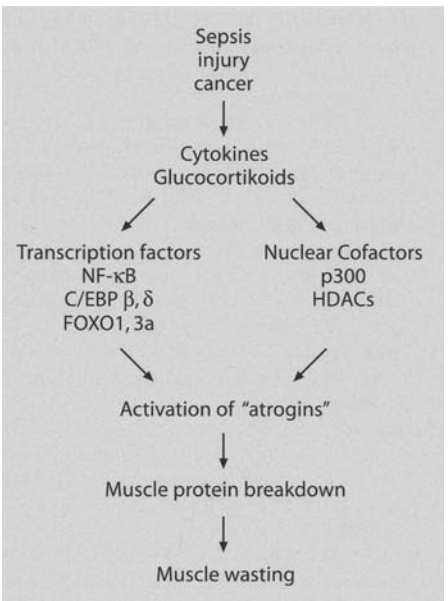

Fig 1. Schematic illustration of the role of transcription factors and nuclear cofactors in the development of glucocorticoid- and cytokine-regulated muscle wasting in sepsis, severe injury and cancer. Although the roles of CCAAT/enhancer binding protein (C/EBP) transcription factors, nuclear factor-kappa B (NF-κB), forkhead box O (Foxo) 1 and 3a, p300, and histone deacetylases (HDACs) are discussed in this chapter, it is likely that other transcription factors and nuclear cofactors also participate in the regulation of protein degradation in atrophying muscle.

Conclusion

Muscle wasting in a number of catabolic conditions, such as sepsis, severe injury, and cancer, is associated with increased transcription of multiple genes, including (but not limited to) genes in the ubiquitin-proteasome proteolyitc pathway. There is increasing evidence that the expression and activity of transcription factors and nuclear cofactors play an important role in the regulation of muscle wasting-associated genes. Among transcription factors, C/EBPβ and δ, NF-κB, and Foxo1 and 3a seem to be particularly important for the development of muscle atrophy. Recent studies suggest that the expression and HAT activity of the nuclear cofactor, p300, are essential for the regulation of muscle protein breakdown, possibly by acetylating C/EBPβ and the NF-κB p65 subunit. The roles of transcription factors and nuclear cofactors in the regulation of muscle proteolysis and the development of muscle wasting are summarized in Fig 1. An increased understanding of the molecular regulation of catabolic events in skeletal muscle is important for the development of novel and targeted therapeutic strategies for the care of patients with muscle wasting.

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Organ Dysfunction in the ICU: A Clinical Perspective

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Introduction

Multiorgan dysfunction is a major cause of mortality in the intensive care unit (ICU) [1–3]. Sequential organ dysfunction syndrome was first described by Tilney et al. [4] in 1973 in a cohort of 18 patients after repair of ruptured abdominal aortic aneurysm and renal failure. The terms multiple organ failure syndrome (MOFS), multiple organ system failure (MOSF), and multiple organ failure (MOF) have since been used to describe this syndrome [5]. Uncontrolled infections were initially thought to be the main cause of multiorgan dysfunction; however, massive activation of inflammatory mediators following other insults, such as severe trauma, may precipitate a similar condition. In 1992, the American College of Chest Physicians/Society of Critical Medicine (ACCP/SCCM) consensus conference [6] recommended definitions of sepsis and the proposed systemic inflammatory response syndrome (SIRS). The term multiple organ dysfunction syndrome was also proposed to describe this syndrome; however, firm definitions of organ dysfunction were not established. Several scoring systems have subsequently been developed to quantify organ dysfunction in ICU patients.

In 2001, several North American and European intensive care societies revisited the definitions for sepsis and related conditions [7]. A staging system for sepsis, PIRO, which stratifies patients on the basis of their Predisposing conditions, the nature and extent of the insult (Infection), the nature and magnitude of the host Response, and the degree of Organ dysfunction, was proposed. The use of organ failure scores was encouraged to quantitatively describe organ dysfunction developing over the course of critical illness. The previous definitions of sepsis, severe sepsis, and septic shock were maintained essentially unchanged. Although much debate has focused on the non-specific nature of the SIRS criteria, no clear improvements could be offered. In fact, a number of additional criteria indicative of physiologic derangements were added to the four traditional SIRS criteria. These further non-specific alterations consist of clinical abnormalities (altered mental status, ileus) and biochemical evidence of involvement (procalcitonin [PCT], C-reactive protein [CRP], creatinine, or cytokines). Standard definitions for organ dysfunction are still lacking and several controversies regarding the best way to quantify organ dysfunction in the ICU remain unresolved [8].

The Epidemiology of Organ Dysfunction in the ICU

Organ dysfunction is common in the ICU [1–3]. A large European, multicenter, observational study, Sepsis Occurrence in Acutely ill Patients (SOAP) [3], reported

that up to 71% of ICU patients have a considerable degree of organ dysfunction (sequential organ failure assessment [SOFA] score >2 for the corresponding organ); 81% of which was present on ICU admission. Sepsis contributed to 41% of the reported organ failures. MOF (failure of ≥ 2 organs) occurred more in patients with sepsis (75 vs. 43%) compared with other ICU patients. The incidence of 2, 3, and ≥ 4 organ failures was higher (38, 24, and 13 vs. 28, 12, and 4%, respectively), and all forms of organ failure were more common, in patients with sepsis compared with other ICU patients. In contrast, isolated single organ failure occurred more in patients with no sepsis (57 vs. 25%), with more renal (27 vs. 9%), respiratory (15 vs. 12%), and central nervous system (CNS) failure (11 vs. 2%) compared with patients with sepsis. Patients with no organ dysfunction on admission had ICU mortality rates of 6% while those with four or more organ failures had mortality rates of 65%. Patients with severe sepsis had higher mortality rates (32 vs. 21%) compared to patients with organ failure without sepsis. Occurring alone or in combination, renal, respiratory, cardiovascular, and hepatic failure were associated with higher ICU mortality rates in patients with severe sepsis (41 vs. 23%; 35 vs. 26%; 42 vs. 34%; 45 vs. 28, respectively) compared to patients with no sepsis.

The results of this multinational study underscore the common occurrence of organ dysfunction/failure in the ICU and its close relation to poor outcome in ICU patients. Sepsis appears to be an important associate of organ dysfunction in the ICU. The incidence of organ dysfunction/failure seems, unfortunately, not to be decreasing overtime, as seen when comparing the results of the SOAP study [3] with those with a similar cohort of ICU patients reported by Vincent et al [9] more than a decade ago.

Quantifying Organ Dysfunction in the ICU

Numerous physiological parameters and therapeutic interventions have been used to define multiple organ dysfunction [5]. The pulmonary, cardiovascular, renal, hepatic, hematologic, and central nervous systems were the organs most commonly considered in describing organ dysfunction/failure in the ICU. But other organs considered in this context included gastrointestinal, metabolic, endocrine, and immunologic function. As early as 1980, Fry et al. [10] proposed a system of four organ failures for surgical patients (pulmonary, hepatic, gastrointestinal, and renal failure). The sepsis severity score proposed by Stevens [11] comprised seven organ systems, each with five severity levels. Knaus et al. [12] defined organ system failure using a dichotomous score for five organ systems (cardiovascular, respiratory, renal, hematologic and neurologic failure). Goris et al. [13] proposed a score based on seven organ systems and three categories (0=normal organ function, 1=organ dysfunction, and 2=organ failure). In 1993, Fagon et al. [14] included infection in their organ dysfunction and infection model (ODIN). Other definitions were proposed by Hebert et al. [15], Pine et al. [16], Moore et al. [17] and Bernard et al. [18]

One of the widely used organ dysfunction scores, the multiple organ dysfunction score (MODS) was proposed in 1995 by Marshall and collaborators [19] as an objective scale to measure the severity of MOF in critical illness. Descriptors of organ dysfunction were identified according to a systematic review of the literature and included PaO_2/FiO_2 ratio for the respiratory system, serum creatinine concentration for the renal system, serum bilirubin concentration for the liver, platelet count for the hematologic system, and Glasgow coma scale (GCS) for the CNS. The cardiovas-

cular system was evaluated using the pressure-adjusted heart rate, which is calculated as the product of the heart rate and the ratio of central venous pressure (CVP) to mean arterial pressure (MAP). The relative complexity of the cardiovascular component may be a drawback and is commonly modified using simple parameters such as heart rate, the use of inotropes, and serum lactate levels [20]. Nevertheless, this score was shown to successfully describe organ dysfunction in the ICU, was correlated strongly with the ultimate risk of ICU and hospital mortality rates [19], and reflected organ dysfunction development when measured sequentially in the ICU [19–21].

Another commonly used score was developed by a working group of the European Society of Intensive Care Medicine [9]: the SOFA score, comprising six organ systems, graded from 0–4 according to the degree of dysfunction failure. Organ systems considered in the SOFA score are: respiratory (PaO_2/FiO_2), cardiovascular (blood pressure, vasoactive drugs), renal (creatinine and diuresis), hematological (platelet count), neurological (GCS) and liver (bilirubin). The reliability and accuracy of calculating the SOFA score among ICU physicians was shown to be good [22], probably due to its simplicity. It was further validated in various groups of critically ill patients [23–26].

Unlike the previous scores, which were established by consensus, the logistic organ dysfunction system (LOD) was developed by Le Gall et al. [27] using logistic regression techniques in a cohort of 13152 adult ICU patients. This score includes physiological variables of six organ systems – GCS, PaO_2/FiO_2 ratio, heart rate, blood pressure, serum urea, creatinine, urine output, white blood cell (WBC) count, bilirubin, platelet count, and prothrombin. The assignment of points in this score took into account both the relative severity among organ systems and the degree of severity within an organ system. Despite the complexity of this score, its prognostic value was not proven to be superior to the other scoring systems [28].

Oda et al. [29] developed the cellular injury score (CIS) as an index of cellular dysfunction in critically ill patients. CIS is derived from three parameters of intracellular metabolism: Arterial ketone body ratio, osmolality gap, and blood lactate. Each parameter is assigned 0–3 points according to arbitrarily defined cut off points. The usefulness of CIS in mortality prediction was reported in 157 patients with MOF [29] and was found to be correlated and comparable to the SOFA score in terms of mortality prediction, in another study by the same authors [30]. This has not been investigated in a large cohort of unselected ICU patients.

Similar scoring systems have been developed and validated in pediatric ICU patients including organ failure index [31] and pediatric logistic organ dysfunction (PELOD) [32] scores, comprising age specific criteria, adapted to the pediatric population.

The Utility of Organ System Failure Scores

Scoring systems for organ dysfunction/failure have been designed primarily as a descriptive tool, aimed at establishing standardized definitions to stratify and compare patients' statuses in the ICU in terms of morbidity rather than mortality. The LOD is one exception to this, as it was developed using a statistical procedure to maximize its predictive value in terms of mortality prediction. Accordingly, organ dysfunction scores can also be used statistically to adjust analyses for baseline characteristics, to control for time dependent changes in matched cohort studies [33], to

define subgroup analyses [34], and to directly compare organ dysfunction between groups as a secondary outcome in randomized trials [35, 36].

Organ dysfunction is a dynamic process; thus, sequential evaluation of organ dysfunction during the ICU stay may be helpful in tracing disease progression in the ICU and has been shown to be highly correlated to the subsequent outcome [20, 37]. As early as the 24 hours following ICU admission, changes in organ dysfunction as assessed by the SOFA score were found to predict eventual survival in severe sepsis [38]. The course of organ dysfunction/failure could also be useful in identifying patients who remain unresponsive despite appropriate treatment for several days, where intensive therapy may be considered futile [39]. In addition, as many of the components of the aforementioned scoring systems are readily affected by the various therapeutic maneuvers, they may be theoretically useful in the determination of therapeutic effects and setting therapeutic targets in the management of critically ill patients.

Indeed, the severity of organ dysfunction is strongly correlated with outcome in critically ill patients [19, 27, 40, 41]. Interventions shown to have improved outcome exhibited similar favorable effects on organ function [42]. Accordingly, organ dysfunction scores can be used to predict outcome from critical illness [43]. Because comorbidities and baseline characteristics on ICU admission usually do not contribute to these scoring systems and may play a major role in determining the subsequent outcome in the ICU, the performance of organ dysfunction scores in terms of outcome prediction may be inferior to that of severity scores such Acute Physiology and Chronic Health Evaluation (APACHE) II [44] and Simplified Acute Physiology Score (SAPS) II [45], that were designed primarily to efficiently predict outcome. It may also be worthy to note that the contribution of each organ dysfunction in association with outcome seems not to be equal. The cardiovascular system has persistently been shown to have the highest impact followed by the renal, neurological, and respiratory systems [14, 27, 46, 47].

The correlation between organ failure and outcome may justify the use of the former as a surrogate end point for clinical studies. The relatively common occurrence of organ dysfunction in the ICU may permit a decrease in the sample size needed for future interventional studies, if organ dysfunction is used as the primary endpoint. However, the Food and Drug Administration (FDA) and the Retirement Medical Benefit Accounts (RMBA) have, until now, not accepted differences in organ dysfunction as primary endpoints in sepsis studies.

Which Score is Best?

The aforementioned organ dysfunction scores vary in the strategy of development (consensus vs. statistical techniques), characteristics of the validation-set, reason for development (descriptive vs. outcome prediction), data collection (admission values vs. sequential or summary of values all over the ICU stay), and weights of each organ system according to the utilized cut-off points. Several studies have evaluated the comparative prognostic value of the commonly used organ dysfunction scoring systems (Table 1). According to the current evidence, these scores are quite similar in terms of outcome prediction.

Table 1. Studies comparing the predictive value of various organ dysfunction scores in terms of mortality prediction.

Author	n	Setting	Evaluated scores	Main findings
Oda et al [30]	47	General ICU	SOFA and CIS	SOFA and CIS similar discriminated poor outcome.
Timsit et al [28]*	1685	General ICU	LOD and SOFA	LOD and SOFA scores had good accuracy and internal consistency. No difference in discrimination between the two scores during the first week in the ICU
Tsai et al [24]	160	Critically ill patients with liver cirrhosis	OSF, SOFA, and Child-Pugh score	OSF and SOFA scores were closely correlated. Both OSF and SOFA scores displayed similarly excellent discriminative power compared to the Child-Pugh score.
Peres Bota et al [47]	949	General ICU	APACHE II, MODS, and SOFA	Outcome prediction of the APACHE II score was similar to the initial MODS and SOFA score in all patients and slightly worse in patients with shock. MODS and SOFA scores were similar in outcome prediction. Cardiovascular component of SOFA score performed better than that of MODS in outcome prediction.
Petilla et al [48]	520	General ICU	APACHE III, SOFA, MODS and LOD	Highest outcome prediction with total maximum scores. Discriminative power was good and comparable between all organ dysfunction scores and that of APACHE III.
Hantke et al [26]	874	Surgical ICU	APACHE II, MODS, and SOFA	SOFA, MODS and APACHE II scores similarly discriminated poor outcome.

* Multicenter study; ICU: Intensive care unit; SOFA: Sequential Organ Failure Assessment; CIS: Cellular Injury Score; LOD: Logistic Organ Dysfunction; MODS: Multiple Organ Dysfunction score; APACHE: Acute Physiologic and Chronic Health Evaluation; SAPS: Simplified Acute Physiology Score; PELOD: Pediatric Logistic Organ Dysfunction; OSF: Organ System Failure

Several criteria should be taken into consideration when judging the value of any scoring system in clinical practice. Reliability and validity are important issues that allow confident use of a scoring system in ICU patients with different case-mixes and baseline characteristics. Responsiveness is also an important criterion and signifies the ability of a scoring system to unmask temporal changes in organ dysfunction if measured sequentially. Dichotomous scores, such as the OSF score, may be less flexible in detecting such changes during the ICU stay. Simplicity and availability of the various components of the scoring system are also crucial. The use of complex criteria, such as with the LOD, without any proven advantage for this complexity, may limit its widespread use in everyday practice. Likewise, using criteria that may not be available in all ICU patients, as in the cardiovascular component of MODS, may also be a practical limitation of its use.

Dynamic measures of cellular response to insults such as apoptosis could be considered in the future to describe organ dysfunction at the cellular level [7].This could be particularly useful in developing therapies that target the injurious cellular processes.

Conclusion

Organ dysfunction is common in the ICU and is strongly correlated to outcome. Sepsis is associated with a significant degree of organ dysfunction and subsequently worse outcome. Despite the variability between various organ dysfunction scoring systems, their prognostic value seems to be similar in terms of mortality prediction. The presence and use of different scoring systems makes the comparison of different descriptive and mortality predictive studies very difficult. This is of particular importance when these scores are used as entry characteristics, as criteria for stratified randomization, or as a surrogate end point for interventional or therapeutic studies in the ICU. Describing organ dysfunction in light of the PIRO system is promising. Further effort is required towards setting standard definitions that could have useful clinical and therapeutic utility.

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Lipids

Sphingolipid Metabolism in Systemic Inflammation

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Introduction

The inflammatory response – induced and regulated by a variety of mediators such as cytokines, prostaglandins, and reactive oxygen species (ROS) – is the localized host's response of the tissue to injury, irritation, or infection. In a very similar and stereotyped sequence, the mediators are thought to induce an acute phase response orchestrated by an array of substances produced locally or near the source or origin of the inflammatory response. Despite its basically protective function, the response can become inappropriate in intensity or duration damaging host tissues or interfering with normal metabolism. Thus, inflammation is the cause and/or consequence of a diversity of diseases and plays a major role in the development of remote organ failure. Better knowledge of the underlying mechanisms of these processes is, therefore, a fundamental pre-requisite fostering the molecular understanding of novel therapeutic targets or diagnostic variables.

Over the past decades, immense attempts have been made to better understand the inflammatory response at the cellular and extracellular level. In the course of these studies, a multitude of lipid mediators of inflammation, such as prostaglandins, leukotrienes, and lipoxins, has been identified and characterized. Much attention has been focused on the function of another major class of lipids, the sphingolipids, which are involved in key regulation processes such as cellular stress response and apoptosis. This chapter highlights the relevance of sphingolipids, their possible role in the regulation of the inflammatory response, and suggests key questions for further research.

Sphingolipids: Structure and Function

Sphingolipids are ubiquitous, inert membrane components of all eukaryotic cells, but are also major constituents of lipoproteins. Most of their functional properties are still being discovered, but there are at least three crucial aspects: structure, recognition, and signal transduction. Originally, sphingolipids were thought to play merely structural roles with rather inert metabolism. However, there has been a fundamental shift in the understanding of their role in modulating various cellular processes such as proliferation, differentiation, induction of apoptosis, and inflammation. The metabolism of sphingolipids comprises a set of highly regulated pathways that serve to control the levels of the individual molecule, their interconversions, and their functions. Most notable of these bioactive molecules are ceramide, ceramide-1-phosphate, sphingosine, sphingosine-1-phosphate, sphingosyl phosphorylcholine, and other derivatives (Fig. 1) [1–3].

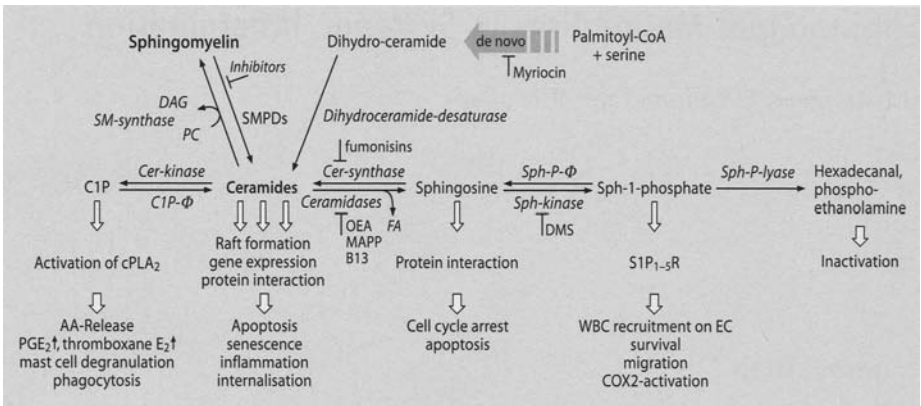

Fig. 1. Sphingolipid metabolism and interconversions. C1P: ceramide-1-phosphate; C1P-Φ: C1P-phosphatase; DAG, diacylglycerol; DMS: N,N-dimethyl-D-erythro-sphingosine; EC: endothelial cell; FA: fatty acid; MAPP: (1S,2R)-D-erythro-2-(N-myristoylamino)-1-phenyl-1-propanol; PC: phosphocholine; PDMP: D-threo-1-phenyl-2-decanoylamino-3-morpholino-1-propanol; PGE_2: prostaglandin E_2; SMPDs: sphingomyelin phosphocholine diesterases = sphingomyelinases; Sph: sphingosine; Sph-1-P: sphingosine-1-phosphate; Sph-1-P-Φ: Sph-1-P-phosphatase; WBC: white blood cell.

The term 'sphingolipid' generally refers to a number of lipids consisting of a polar head group, which is attached to the primary hydroxy-moiety of ceramides. Ceramides, the central building block of all sphingolipids, consist of a sphingoid base, which is *N*-acylated with fatty acids, differing in length (14–32 carbon atoms) and functionalization (degree of saturation, hydroxylation, etc.). The *de novo* synthesis of ceramides starts with the rate-limiting condensation of palmitoyl-CoA with serine, yielding to dihydroceramide after addition of a long chain fatty acid. The lipid mediator, resulting from desaturation, serves as a precursor for all known sphingolipids, and is further functionalized by addition of a polar head group such as carbohydrates (not referred to in this review) or phosphocholine. In eukaryotic cells, a 4,5-*trans* double bound is essential for bioactivity in lipids with sphingosine backbone. Sphingomyelin (=*N*-acyl sphingosyl phosphocholin) is the other central, but inert storage pool with crucial biophysical functions, which is localized in the outer leaflet of membranes by active flip-flop mechanisms. Removal of the head group – resulting in the generation of ceramide – is catalyzed by the function of several isoforms of sphingomyelinases, which are outlined in detail in Table 1. Accumulation of ceramides in cellular membranes results in the formation of lipid rafts and functional clustering of surface receptors. Another mode of action is performed by direct interaction with proteins in the activation of kinases (e.g., protein kinase C, isoformζ) coupling the action of ceramide to activation of transcription factor, nuclear factor-kappa B (NF-κB) and stress activated protein kinases. In addition, the activation of phosphatases or cathepsin D result in signaling, which enables stimulation of adequate and fine tuned cellular responses. For ceramide clearance, ceramidases hydrolyze the *N*-acyl fatty acid, resulting in the generation of sphingosine, which can be phosphorylated at the primary hydroxy-moiety by isoforms of sphingosine-kinase (SphK) forming sphingosine-1-phosphate (S1P). S1P is cleaved by S1P lyase in an irreversible manner, whereas ceramides or sphingosine can be reversible synthesized by the action of S1P-phosphatase or ceramide synthase, respectively. A

Table 1. Sphingomyelin hydrolyzing enzymes. In human tissues, five RefSeq validated proteins have so far been described, differing in pH-optima, cellular localization and functions. *SMPD1* (sphingomyelin phosphodiesterase 1) codes for the acidic sphingomyelinase, which is either localized in lysosomes by mannose-6-phosphate-receptor/sortilin-dependent trafficking or plasma secreted due to pro-inflammatory stimulation. *SMPD2* codes for a neutral sphingomyelinase-1 (NSM1); however SMPD3 is the *bona fide gene* for NSM2 [83]. A variety of isoform specific inhibitors is described in the right column.

HUGO name (Genbank ID)	pH	cofactor (s)	Localization	Function	Inhibitor(s)
SMPD1 (NM_000534)	~5	none	lysosomes, caveolae, platelets	Metabolic degradation of sphingomyelin; induction of apoptosis, differentiation, inflammation. Congenital deficiency: Nieman-Pick Disease	desipramine, SR33557, NB6, DTT
SMPD1 (NM_000534)	5–7	Zn^{++}	plasma secreted enzyme	Atherogenesis, hemophagocytic lymphohistiocytosis, diabetes, ARDS, sepsis	
SMPD2 (NSMI) (NP_003071)	6.5–7.5	Mg^{2+}	membrane (multi-pass membrane protein)	Differentiation, permeability barrier function	
N.N.	~7	Triton X-100, deoxycholat, phosphatidylserine, (*in-vitro*)	cytoplasma	Differentiation, 1,25-Dihydroxyvitamin D_3, IFNγ and TNF-α induced signal transduction in HL-60 cells (no RefSeq validation)	Cu^{2+}, Fe^{3+}, Zn^{2+}
SMPD3 (NSMII) (NP_061137)	~7	Mg^{2+}, Mn^{2+} (millimolar); phosphatidylserine, arachidonate	membrane	Hydrolysis of membrane bound SM; differentiation, proliferation, inflammation, apoptosis, lung injury; *bona fide gene*	scyphostatin, glutathione, manumycin, ubichinol, chloro-gentisylchinone, GW4869, C11AG
ENPP7 (NP_848638)	8.0–9.5	bile salts	intestinal lumen; bile, mucosal membrane;	degradation of dietary sphingomyelin; intestinal PAF-degradation, role in intestinal inflammation or inhibition of colonic tumorigenesis?	

ARDS: acute respiratory distress syndrome; DTT: dithiothreitol; HUGO: Human Genome Organization; IFNγ: interferon gamma; PAF: platelet activating factor; TNF-α: tumor necrosis factor alpha.

major metabolite of ceramide is ceramide-1-phosphate (C1P), which is formed by direct phosphorylation of ceramide by the action of a specific kinase. C1P plays a critical role regulation proliferation, differentiation, apoptosis and generation of eicosanoids *via* direct binding to target proteins, e.g. phospholipase A_2. There is also increasing evidence of a key role in phagocytosis [4], and most importantly, inhibition of SMPD1 by physical interaction has been observed [5].

A major principle in understanding ceramide metabolism (which applies to all other bioactive lipids) is the distinct subcellular localization and topology, which is outlined, e.g., in a review by van Meer and Lisman [6]. In recent years, considerable attention has been given to other types of sphingosine base derivatives, namely 'lyso'-types or *N*-methyl derivatives, which are highly bioactive, however little is known about their origins or function(s).

Beyond the stimulus-dependent activation of sphingolipid metabolism, considerable evidence has been gathered regarding the role of subcellular compartmentalization of the generation and accumulation of individual metabolites. Numerous studies point to a tightly controlled localization of enzyme and lipid pools within the cell, e.g., suggesting a specific, apoptogenic role for endogenously generated ceramide from mitochondrial membranes [7]. Within the different compartments, distinct isoforms of sphingomyelinases perform specific functions *via* generated ceramide, such as inhibition of protein kinase C translocation, inhibition of NF-κB, and aggregation of the Fas receptor [6, 8]. Another mode of action is the endosomal generation of ceramide by acid sphingomyelinase as a direct activator of cathepsin D [9]. Considering that *de novo* synthesis of ceramide is mainly restricted to the endoplasmatic reticulum as well as to membranes associated with mitochondria and the nucleus, the localization and the effects of synthesis inhibitors, such as fumonisins, suggest a pivotal role for ceramide subfractions in mediating apoptosis. Furthermore, recent evidence has indicated the contribution of the mitochondria and the nucleus as major sites in the initiation of apoptosis by ceramide [10].

Due to the dynamic equilibrium of lipid mediators, cells may respond to an extracellular stimulus with perturbation of the balanced 'rheostat' as a consequence of activation of sphingomyelinases followed by accumulation of ceramide and related metabolites. For a better understanding of the sphingolipid flux during the cellular stress response as well as the effects of pharmacological or molecular manipulations of critical enzymes (see below), it is imperative to use reliable measuring systems. Recent molecular advances in the identification of enzymes involved in sphingolipid metabolism and improvements in the analytical equipment, e.g., mass spectroscopy, have facilitated a better understanding of the role and function of sphingolipids [11].

Step by Step: The Hydrolysis of Sphingomyelin

Sphingolipid metabolism is a constitutive process beginning with removal of the head group, phosphocholine, yielding the intermediate product and lipid mediator, ceramide, a reaction catalyzed by a family of enzymes termed sphingomyelinases (or systematically sphingomyelin phosphocholine diesterase, SMPD). The isoforms are distinguished by different pH-optima, localization and cation dependence (Table 1) [12]. Of the five human sphingomyelinases identified so far, the neutral Mg^{2+}-dependent and the lysosomal acid isoform are the most thoroughly studied and, therefore, seem to appear most relevant for generation of ceramide during the stress response [12, 13].

The activity of a neutral pH-optimum, Mg^{2+}-dependent sphingomyelin hydrolyzing enzyme was initially described four decades ago [14]. The maintenance of this isoform's specific activity in both the $SMPD1^{-/-}$ model as well as in cells obtained from patients affected with Niemann-Pick disease Type A, verified that these isoforms are distinct products of different genes [15]. The purified enzyme, termed

neutral sphingomyelinase 1 (NSMI), exhibits an optimal pH at 7.4, an estimated molecular mass around 60 kDa, and a specific activity for hydrolysis of sphingomyelin, but not phosphatidylcholine. The presence of divalent cations such as magnesium or manganese as well as a proper lipid composition containing anionic lipids and unsaturated fatty acids, especially phosphatidylserine and arachidonic acid, are essential for its activity. Recently, it was reported that in caveolae-enriched membrane fractions derived from bovine lung microvascular endothelial cells, a caveolar isoform of neutral sphingomyelinase cross-reacts with specified antibodies against an isoform with a higher molecular weight purified from brain [16].

Two years after the cloning of NSMI, the sequencing and characterization of another mammalian, brain-specific Mg^{2+}-dependent sphingomyelinase, NSMII, was reported [17]. NSMII is also activated by phosphatidyl-serine and other anionic phospholipids, suggesting an enrichment of the protein in the inner leaflet of the plasma membrane, at the mitochondria as well as the endoplasmatic reticulum. Using deletion mutants of the p55 tumor necrosis factor (TNF) receptor, a neutral sphingomyelinase activation domain (NSD) was identified, which is juxtaposed to the death domain of the TNF receptor. A corresponding protein, termed 'factor associated with neutral sphingomyelinase activation' (FAN), was described which binds to NSD, resulting in a functional coupling of the TNF receptor with neutral sphingomyelinase activation [18]. Results demonstrating an interaction of neutral sphingomyelinase with activated C-kinase 1 as well as caveolin-1 further suggest that the formation of multiprotein complexes are involved in the signal transduction from TNF-α to neutral sphingomyelinase, thus regulating its activity [19]. Most recently, it was demonstrated that hydrogen peroxide (H_2O_2)-induced apoptosis of endothelial cells was completely blocked in NSMII-loss of function models [20], highlighting the essential role of NSMII in the induction of apoptosis.

Recent studies have shown that SMPD1 has characteristics of a lysosomal and secretory sphingomyelinase, which both derive from the same gene (*smpd1*) exhibiting differences in the oligosaccharide structure and *N*-terminal proteolytic processing with subsequent differential protein trafficking [21, 22]. As a result the proteins are present in both blood plasma and intracellular lysosomes [12]. Previous studies suggested that the lysosomal mannose-6-phosphate receptor is implicated in SMPD1 trafficking [23]. However, the type I transmembrane glycoprotein, sortilin, is also involved in targeting of the protein: Truncated sortilin partially inhibits lysosomal trafficking and enhances the secretion of SMPD1 [24]. Among the known types of eukaryotic sphingomyelinases, only the secretory variant, SMPD1, has so far been shown to be responsible for extracellular hydrolysis of membrane and lipoprotein bound sphingomyelin [22]. SMPD1 is secreted by macrophages, human skin fibroblasts, and human vascular endothelial cells; the latter are assumed to be the chief source of the enzyme [12, 25]. In endothelial cells, apical as well as basolateral secretion of SMPD1 is stimulated by a variety of pro-inflammatory mediators, including interleukin (IL)-1β, interferon (IFN)γ, IFNβ, TNF-α, platelet activating factor (PAF), and ROS as endogenous mediators, as well as endotoxin as an exogenous mediator [25]. The secreted form of SMPD1 is stimulated by zinc-ions, whereas the lysosomal form is already tightly bound to the cation. Thus, the source of the activity can be distinguished as originating, e.g., from damaged and disintegrating endothelium or from activated cells driven by a pro-inflammatory impetus. An increase to pH 7.4 which is far beyond the optimum for the lysosomal protein as found in plasma, appears to affect only the substrate affinity (i.e., the K_m), but not the activity (V_{max}) of SMPD1 [24], a fact important in estimating the extralysosomal activity of the enzyme [21, 27].

In the following section, cellular mechanisms of the action of sphingolipid mediators are briefly discussed.

Receptor-mediated Effects

The lysosphingolipid, S1P, functions as a ligand for at least five G-protein species coupled to cell surface receptors termed $S1P_{1-5}R$ regulating cell proliferation, apoptosis and motility. Activation by S1P binding results in sequestering of lymphocytes from the circulation to lymph nodes and Peyer's patches. This redistribution effectively reduces T cell numbers at the sites of inflamed tissue or graft sites. TNF-α induced sphingosine kinase 1(SphK1) activity is followed by increased S1P levels and subsequent cyclooxygenase (COX)-2 activation. The inhibition of S1P clearance, e.g. by targeting S1P lyase or S1P phosphatase, augments COX-2 activation and consequently prostaglandin (PG)E2 generation. Exogenous S1P addition dose-dependently reproduced COX-2 induction observed subsequent to TNF-α addition. In neutrophils, the hydrolysis of sphingomyelin increased SphK activity, and S1P generation appears to be a key event in neutrophilic priming by TNF-α and other stimuli [28, 29]. During activation of mast cells, S1P levels increase resulting in the release of inflammatory mediators such as leukotrienes and cytokines. In contrast, sphingosine has an opposing effect on mast cell activation [30].

Cell migration is crucial to the proper functioning of the cells involved in the course of inflammatory processes. The SphK/S1P pathway plays an important role in chemoattractant signaling in myeloid differentiated HL-60 cells [31]. In addition, S1P was found to act as a specific and effective regulator of migration of freshly isolated human neutrophils across endothelial cells [29]. This transmigration, which is essential for the recruitment of white blood cells to the site of inflammation, is enabled by the expression of adhesion molecules, such as intracellular adhesion molecule (ICAM)-1, on endothelial cells.

Interest in sphingolipids as signaling molecules in immune cells increased as it became evident that sphingosine, as well as ceramide, induces apoptosis in T cells, whereas S1P emerged as a counterregulatory principle; in Th2 cells sphingosine (but not ceramide) exerts inhibiting effects on proliferation, implying that individual immunological responses depend on the dynamic balance of sphingolipids [2].

Raft Formation: Ceramide-induced Reorganization of Membrane Receptors

There is currently no defined receptor for ceramide; however, the lipid has been shown to be involved in signal transduction by altering membrane organization and fluidity. Ceramide has the tendency to self-associate and to form ceramide-enriched microdomains that spontaneously fuse to large ceramide-enriched macrodomains, also termed ceramide-enriched platforms. These biophysical properties act to reorganize very small distinct domains in the cell membrane, termed rafts, which serve in the spatial organization of signaling molecules. Thus, ceramide enriched membrane platforms have been shown to mediate clustering (i.e., tighter packing) and recruitment of signaling molecules, while excluding others, and to reorganize the topology of proteins and aggregation of receptors inducing the transduction of signals.

These specialized domains of the cell membrane are central for the spatial organization of receptors and signaling molecules. Upon stimulation, acid sphingomyelinase is translocated to the outer leaflet of the cell membrane, apparently mediated by a fusion of sphingomyelinase containing vesicles with the cell membrane resulting in the cell surface exposure of the enzyme. Generated ceramide then contributes to the formation of both small and large rafts, ceramide-enriched platforms, which in turn may amplify signaling in response to stress, irradiation, ultraviolet light, gamma irradiation, doxorubicin, cisplatin, disruption of integrin-signaling, TNF receptor, CD40, LFA-1, DR5/TRAIL, CD20, FcγRII, CD5, LFA-1, CD28, TNF, IL-1 receptor, PAF-receptor, and CD14 [32].

CD95-dependent apoptosis requires a pre-association of CD95, the formation of the death-inducing signaling complex (DISC), and clustering of CD95 in specific membrane domains. In this context, the acid isoform, activated upon CD95 ligation and initial caspase 8 activation, is translocated and functions upstream of the DISC to mediate CD95 clustering in ceramide-enriched membrane platforms, an event required for DISC formation, yielding full caspase 8 activity and apoptosis [33].

Identical mechanisms seem to be operative in the signaling of apoptosis by other death receptors or stress suggesting a general role of ceramide-enriched platforms in apoptosis and explaining the function of SMPD1 and ceramide in multiple signaling pathways [34, 35].

The metabolic precursor, sphingomyelin, is important for Fas receptor clustering through aggregation of lipid rafts, leading to Fas-mediated apoptosis. Experiments with sphingomyelin synthase (SMS)-defective WR19L cells transfected with the human Fas gene (WR/Fas-SMS$^{-/-}$), and cells that have been functionally restored by transfection with SMS1 (WR/Fas-SMS1), show that expression of membrane sphingomyelin enhances Fas-mediated apoptosis through increasing efficient translocation of Fas into lipid rafts, Fas clustering, DISC formation, and subsequent activation of caspases [34].

Role of Sphingolipids in Regulation of the Immune Response, Susceptibility to Infection, and Triggering of Pathogen-associated Apoptosis

Some pathogens activate SMPD1, which releases ceramide in membrane rafts, structures which enable a host/pathogen interaction by formation of negative membrane curvatures. An abundance of evidence indicates that the formation of ceramide-enriched membrane raft structures facilitates the invasion of various pathogens [37–39]. Often, the final result of ceramide-mediated cellular entry is containment and/or inactivation of the pathogen. The importance of ceramide in pathogen invasion is underscored by studies investigating *Neisseria gonorrhoeae*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Sindbis virus*, which have been shown to activate SMPD1 resulting in rapid ceramide formation. The strength of these studies was the demonstration that inactivation of SMPD1 nearly completely hindered pathogen internalization. As a result, all pathogen-associated alterations of membrane scaffold have been shown to mediate internalization of bacteria, viruses, and parasites into the host cell, to initiate apoptosis of the host cell upon infection, and to regulate the release of cytokines from infected mammalian cells [37]. On the other hand, ceramide-enriched membrane platforms are also central to the host defense against potentially lethal pathogens like *P. aeruginosa* which, upon infection, trig-

gers activation of SMPD1 and the release of ceramide in sphingolipid-rich rafts within minutes [39]. Failure to generate ceramide-enriched membrane platforms in infected cells results in an unabated inflammatory response, such as massive release of IL-1β and septic death in mice.

In addition, the protozoan, *Leishmania donovani*, was shown to induce ceramide formation by both *de novo* synthesis and SMPD1 activation, resulting in elevated ceramide levels which facilitate the survival of the parasite in the intramacrophageal milieu [41]. Rafts, in addition to playing a crucial role in pathogen entry, have also been shown to serve as platforms for viral assembly or budding and in the intracellular trafficking of phagosomes [42]. It has also been shown that ceramide-enriched membrane platforms are involved in the infection of human cells with pathogenic rhinoviruses [42]. The infection of human epithelial cells with rhinovirus strains triggers a rapid activation of SMPD1, the formation of ceramide in the cell membrane and, finally, the formation of large ceramide-enriched membrane platforms. These events correlate with microtubule- and microfilament-mediated translocation of the enzyme from an intracellular compartment onto the extracellular leaflet of the cell membrane. In agreement with a key role of SMPD1 and ceramide in the infection of human cells with rhinoviruses, genetic deficiency or pharmacological inhibition of the SMPD1 prevented infection of human epithelial cells by rhinoviruses.

The susceptibility to infections of individuals with these diseases, and with elevated plasma levels of SMPD1, which has been observed during the course of sepsis and systemic inflammation (see below), might contribute to an altered immune response rendering individuals susceptible to other, secondary infections, e.g. from colonizing bacteria. All these studies support the notion that rafts and ceramide-enriched membrane platforms function as central structures involved in the infection of mammalian cells by pathogens and as targets for the development of anti-infective drugs.

Role of the Oxidative Balance

The tripeptide, glutathione (GSH), plays a major role in cellular redox homeostasis. Several lines of evidence suggest that ROS, such as superoxide radical ($O_2^{\cdot-}$), H_2O_2, and the hydroxyl radical (·OH), as well as reactive nitrogen species, such as the peroxide radical (·$ONOO^-$) and the cellular redox potential, which is mainly regulated by GSH concentration, are tightly linked to the regulation of sphingolipid hydrolysis.

NSMI activity requires the presence of reducing agents, and recent studies have shown that it is reversibly inhibited by ROS and oxidized glutathione, whereas it is irreversibly inhibited by peroxynitrite [43]. As described above, NSMII activity and trafficking are tightly regulated by the oxidative intracellular status in a complex dynamic process [20]. On the other hand, sphingolipids are also known to play an important role in maintaining cellular redox homeostasis through regulation of plasma membrane oxidants, such as NADPH oxidase, with subsequent disturbance of mitochondrial integrity and induction of apoptosis [44]. Post-translationally, both recombinant and plasma borne SMPD1 are also directly activated in the presence of oxidizing agents by modification either of a 'cysteine switch' or by a copper induced dimerization via disulfide bond formation responsible for an increase in enzymatic hydrolysis [45, 46]. It is, therefore, tempting to speculate that at least some of the above-named stress stimuli stimulate SMPD1 via redox processes. Ceramide itself is

known to trigger the release of ROS, from, for example, endothelial cells [47]. Thus, under oxidative conditions, it might be part of a self-perpetuating and positive feedback mechanism for the post-translational activation of SMPD1. Interestingly, in neutrophils, ceramide generation, CD95 clustering, and apoptosis were dependent on ROS suggesting that an altered redox status initiates ligand-independent death receptor signaling via activation of SMPD1 and clustering of preformed DISC components in lipid rafts [48].

Increased SMPD1 Activity: Cause or Consequence in Organ Failure?

A 2–3-fold increase in plasma sphingolytic activity has been observed in animal models after application of endotoxin or pro-inflammatory cytokines (TNF-α, IL-1β) [49–51]. In a human setting, there is indirect evidence for altered SMPD1 activity during systemic disease. An increased ceramide/sphingomyelin ratio has been reported in septic patients as well an association with a poor clinical outcome [52]. At a cellular level, increased ceramide concentrations have been reported in circulating mononuclear cells of septic patients that were positively correlated with plasma TNF-α levels [53]. In this study, patients with multiple organ failure (MOF) exhibited a more pronounced ceramide accumulation. Furthermore, raised SMPD1 levels have also been reported in children with hypercytokinemia due to hemophagocytic lymphohistiocytosis [54]. In order to achieve a deeper insight into the functional role of SMPD1 during sepsis, we analyzed its presence and activity in patients with various degrees of different concomitant diseases, variable sources of infection, and a broad range of disease severity as reflected by differing levels of inflammatory markers. Circulating SMPD1 was found to be markedly elevated on the first day of sepsis. During the course of the disease, an inverse trend between survivors (decrease) and non-survivors (further increase) as well as a positive association with organ failure scores was observed [45].

The first clinical implications for inhibition of sphingolipid hydrolysis came from studies dealing with the lysosomal activity of SMPD1 in leukocytes obtained from patients with major depression [55]. In addition to an increase in the constitutive activity in peripheral blood mononuclear cells dependent on illness severity, *ex vivo* treatment of the cells with tricyclic antidepressive drugs, such as amitryptiline or imipramine, resulted in a rapid reduction in SMPD1 activity. As reported above, ceramide generation changes the composition of membrane structures thus mediating the formation of platforms, which facilitate receptor clustering and signaling. Thus, alterations in SMPD1 activity may have clinical implications for the regulation of serotonin and dopamine reuptake transporter activity. The pathophysiological significance of altered SMPD1 activity in major depression remains to be further elucidated. The observed increase, however, supports the concept that SMPD1 activity and ceramide generation may function in these molecular phenomena, may contribute to subsequent interference with other enzymes such as phospholipase A_2 and isoforms of protein kinase C, as well as synaptic transmission. It is, therefore, tempting to speculate that SMPD1 activity may be a molecular target for antidepressant drug therapy.

There is also an increasing body of evidence that extracellular hydrolysis of sphingomyelin due to secreted SMPD1 activity may be involved in mediating systemic effects. Induced by the massive release of cytokines and other factors into the circulation, this phenomenon, termed generalization, leads to functional effects

occurring outside the genuine locus of actual infection or treatment, which is often observed in the pathogenesis of MOF or subsequent to radiotherapy for various malignancies. Sathishkumar et al. demonstrated, in patients who underwent high dose spatially fractionation radiation, an elevation of both the level of plasma secreted SMPD1 activity and the concentration of lipoprotein bound ceramide, with a correlation of SMPD1 activity and clinical outcome [56]. Most importantly, a role of SMPD1 and ceramide in the generation of PAF-mediated pulmonary edema was shown by Goggel and colleagues [57]. In an animal study, it was clearly demonstrated that SMPD1 plays a critical role both locally in ceramide generation in the stimulated tissue as well as in the increase of vascular permeability resulting in edema formation. Accumulation of ceramide was paralleled by synthesis and release of aspirin-inhibitable prostaglandin production. SMPD1 deficient mice exhibited 50% less pulmonary edema than wild type animals. Consistent with these findings, agents interfering with ceramide generation as well as anti-ceramide antisera reduced edema formation initially triggered by exogenously administered PAF, bacterial endotoxin, or intratracheal instillation of acid. The latter two models are relevant to the increased permeability during lung edema observed in the clinical condition of sepsis or aspiration-induced pneumonia, which are common and often lethal precipitators of acute lung injury (ALI) in humans [58]. On the other hand, systemic effects were proven by the release of the enzyme into circulation via venous efflux enabling extra-pulmonary ceramide generation at the outer leaflet of remote endothelial membranes [57]. In this context, it is noteworthy that until now plasma secreted SMPD1 is the only enzyme shown to be responsible for extracellular sphingomyelin hydrolysis; activity of NSMII or other sphingomyelinase isoforms has not been observed in plasma [49, 57]. Additionally, SMPD1 translocates to cell surfaces and has activity in the outer leaflet of the cellular membrane; the membrane-associated enzyme may behave differently to recombinant protein administered in solution [34]. However, it is not yet clear whether the enzyme translocated onto the plasma membrane upon CD95 stimulation [33] is identical to the form described by Tabas as the plasma secreted isotype [21].

Signaling by ceramide is also critically involved in molecular mechanisms triggering ischemia/reperfusion injury as well as TNF-α induced organ damage. In the liver, ceramide levels transiently increased in galactosamine/TNF-α-induced liver damage or after the reperfusion phase of ischemia due to early activation of hepatic SMPD1. Inhibition of sphingolytic activity decreased ceramide generation and subsequent increase in surrogates for tissue damage, hepatocellular apoptosis, and mitochondrial targeting of apoptosis-triggering gangliosides, resulting in cytochrome c release [59, 60]. Thus, modulation of sphingolipid signaling may be of therapeutic relevance, e.g., in hepatic tissue injury.

In addition, thrombin activated thrombocytes release SMPD1 [61] and these cells may be a candidate source for SMPD1 because they are critically involved in triggering thrombotic and inflammatory events, e.g., resulting in lung edema [58]. By triggering Weibel-Palade body exocytosis, ceramide activated endothelial cells release von Willebrand factor and P-selectin, which induce leukocyte rolling as well as platelet adhesion and aggregation [62]. This phenomenon, which was also observed after addition of sphingomyelinase, suggests a novel and intriguing mechanism by which ceramide may contribute as an intermediary to vascular inflammation raising a thrombophilic state. A hypothetical schema of the functions of plasma secreted SMPD1 and subsequent ceramide generation during systemic inflammation and infection is outlined in Fig. 2.

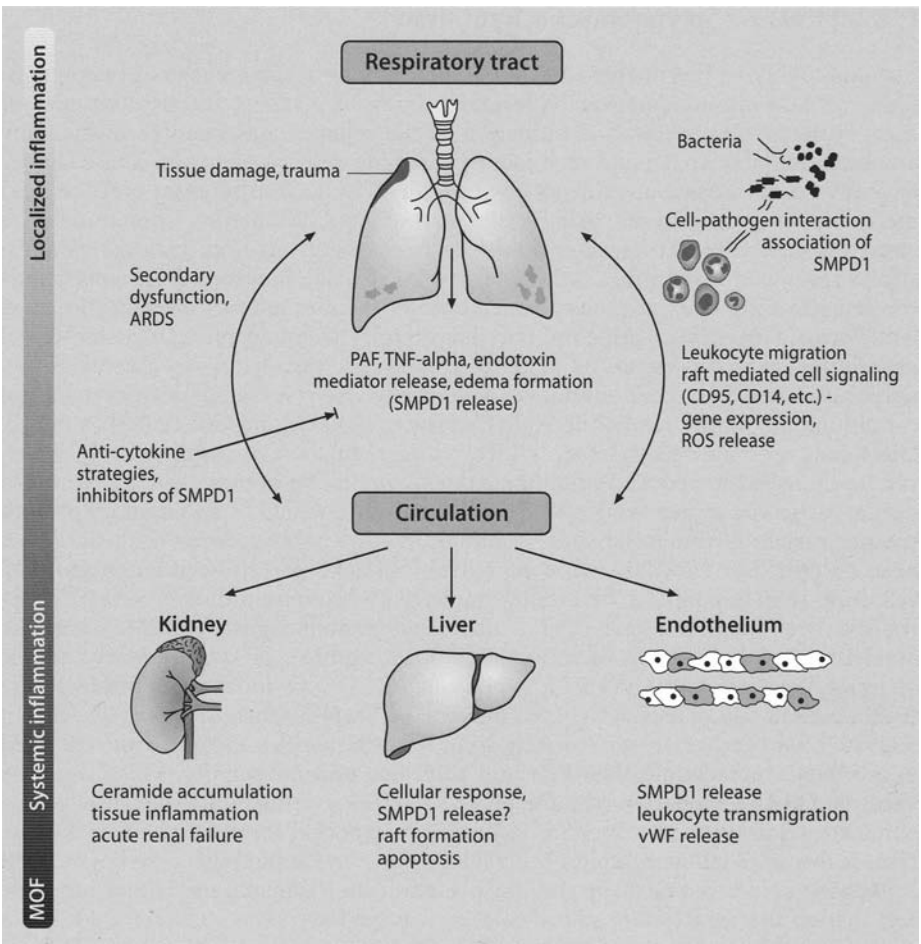

Fig 2. Plasma secreted sphingomyelinase in inflammation. Hypothesized impact of sphingomyelin phosphocholine diesterase (SMPD)1 in the development and exacerbation of systemic inflammation. ARDS: acute respiratory distress syndrome; vWF: von willebrand factor; MOF: multiple organ failure.

It is noteworthy that the observed plasma activities in patients with inflammation of different origins clearly exceed the enzymatic activity reported to induce biological effects in cell culture systems [63, 64]. SMPD1 has been shown to hydrolyze sphingomyelin in low density lipoprotein (LDL) particles [27], which is important considering that most of serum sphingomyelin and nearly half of the ceramides are known to be localized in circulating LDL [49]. Therefore, the accumulation of ceramide in both circulating cells and in lipoproteins may serve as a persistent reference pool reflecting elevated plasma secreted SMPD1 activity. Though not proven, it might, therefore, be possible to discriminate between transient/short-term effects and long lasting/even mild variations by determination of enzyme activity levels in a functional assay or resulting ceramide accumulation. According to our own observations and data from other groups, there is only marginal degradation of plasma ceramide, resulting in the mentioned accumulation of the mediator in lipophilic compartments, such as plasma lipoproteins.

Inhibitors of Sphingomyelin Hydrolysis

The availability of specific pharmacological inhibitors of some enzymes of sphingolipid metabolism and ongoing molecular cloning of some of the key enzymes of these pathways, has allowed examination of the cellular consequences of inducing accumulation of endogenous ceramides or ceramide clearing enzymes. These studies have provided substantial evidence for the role of sphingomyelinases and the lipid mediator, ceramide, in initiating cellular responses. Numerous compounds are known to inhibit NSMI. Structural analogs of the naturally occurring compounds, scyphostatin and manumycin A, have been used [65, 66]; however, these compounds contain a reactive epoxide moiety, which enables them to interact directly and covalently with a variety of proteins, thus hampering the interpretation of biological experiments. For this reason, a panel of compounds was synthesized containing a polyunsaturated fatty acid bound to a chemically less reactive, under physiological conditions, 1,2-amino alcohol derivative with a cyclohexenone moiety [65]. A redox-dependent reversible mechanism is involved in regulation of NSMI activity, which can be abolished by reduced glutathione during induction of programmed cell death due to ischemia in neuronal cells [13]. Structural analogs of sphingomyelin with reactive structural moieties, such as difluoromethylenephosphonic acid, have also been described as effective, non-competitive inhibitors of TNF-α-induced neuronal cell death [67]. In addition, various low molecular weight inhibitors of SMPD1 activity have been identified, including 5'-adenosine monophosphate (5'-AMP); tricyclic antidepressive drugs, such as imipramine, amitryptiline, or desipramine; cationic amphiphilic drugs, such as NB6, L-α-phosphatidyl-D-myo-inositol-3,5-bisphosphate (PtdIns3,5P2), and phosphatidyl-myo-inositol 3,4,5-triphosphate [PtdIns (3,4,5)P(3)]; SR33557; and derivatives of α-mangostin. Tricyclic antidepressants induced rapid intracellular degradation of SMPD1 and abolished enzyme activity, which could be abrogated by preincubation with the protease inhibitor, leupeptin. Interestingly, data obtained using plasmon resonance technology supported the concept of an interaction of the amphiphilic inhibitor with the enzyme and immobilized sphingomyelin containing lipid bilayers, displacing the protein from its membrane bound substrate and rendering it susceptible to proteolytic cleavage [68].

In an experimental model of endotoxic shock, specific inhibition of SMPD1 by the carbazol derivative, NB6, resulted in decreased hepatocellular apoptosis and improved survival rate, providing further evidence for a crucial role of SMPD1 in the pathogenesis of systemic inflammation and subsequent organ failure [45]. Remembering that SMPD1 fulfils crucial functions in host defense, a non-beneficial effect of SMPD1 inhibition during infection of mice with living bacteria in a model of polymicrobial peritoneal contamination and infection with overwhelming mortality after instillation of human feces is feasible (Bunck et al., unpublished data).

FTY720, a substrate for SphK has shown tremendous promise as a regulator of multiple levels of inflammation. FTY720 is chemically derived from myriocin, an ascomycete metabolite, and is metabolized to the phosphor-derivative, FTY-P, by sphingosine kinases to become active as an S1P agonist at four of the five known S1P receptors [69]. FTY720 has been tested as an immunomodulator in renal transplant patients and patients with emphysema. The Sph1P mimetic produces lymphopenia by reducing recirculation of lymphocytes and sequestering them into lymph nodes. FTY-P induces $S1P_1R$ internalization of lymphocytes, which abrogates the interaction with the naturally occurring ligand, S1P, regulating lymphocyte trafficking between lymphoid organs and the sites of inflammatory response. The com-

pound also induces CD31 and β-catenin expression in subcapsular sinus endothelial cells in lymph nodes. The modulation of the inflammatory response by FTY720 may be a useful intervention in a number of inflammatory conditions by targeting bioactive sphingolipid signaling function.

Cracking the Enigma of the Sphingolipids

Distinct analysis of the biological effects mediated by variations in the key sphingolipid enzyme activity or its localization, as well as the complex and structurally diverse composition of the mediators, was hampered for a long time by the lack of an accurate and reliable methodology to measure the sphingolipid factors relevant in, for example, pro-inflammatory signaling. A plethora of papers stress the importance of distinguishing between *de novo* synthesized sphingolipids and degradation products derived from turnover or metabolism from more complex sphingolipids. Over the past decades, sphingolipids, e.g. ceramide, have been discovered to have not only structural but also signaling properties, especially during the stress response. The dynamic balance between sphingolipid metabolites existing in a phosphorylated or dephosphorylated as well as in an acylated or deacylated form, has been recognized as a fundamental factor determining cell fate because of its ambiguous and often opposing properties. On one hand, consistent with their functions as bioactive lipids, ceramide and its metabolites are present in very low levels in the cell's common lipid machinery. On the other hand, the interconvertibility of these mediators and the highly variable kinetics in metabolism present a difficult technical challenge for examining the absolute concentrations at operator-defined time points. This is of particular relevance when various agents for stimulation or analysis of cells consisting of a multitude of cellular subpopulations such as circulating leukocytes are tested. Biological examinations frequently focus on pro-apoptotic or cyto-protective effects alone when mediators are studied. Thus, for a more complete picture it is imperative to analyze all or at least a majority of the lipid mediators potentially responsible for the effects of interest. For this purpose, a number of relatively specific enzymatic methods have been developed based on the use of lipid kinases. These assays are relatively insensitive, time consuming, and require huge amounts of lipid material [70–72]. Derivatization and subsequent separation by high performance liquid chromatography and fluorescence detection have improved detection sensitivity [73, 74]. The problem of co-elution of related, very similar or interfering compounds, however, has to be addressed. In addition, there is an urgent need for the preparation of internal standards for each unique mediator of interest. Methods based on metabolic labeling of the cellular sphingolipid pool with radioactive or fluorescently labeled compounds are also burdened with concerns regarding improper distribution within the cell and the cellular substructures, uneven behavior in the cellular membrane, and questionable metabolic and enzymatic properties in comparison to the naturally occurring structure of interest. Overall this may be an inefficient and inaccurate method for absolute mass level determinations [75–77].

The limitations of these 'one single lipid experiments' can now be addressed by the use of recently developed methodologies, such as liquid chromatography, coupled to subsequent tandem mass spectroscopy resulting in an approach called 'sphingolipidomics' [78, 79]. The use of mass spectroscopy is an intriguing feature for more detailed study of numerous agonists and antagonists and to better address

the effects of previously undetectable variations in concentrations of mediators with a sphingolipid backbone. In fact, the methods of sphingolipidomics also facilitate the determination of isotype specific effects, such as the induction of mitochondrial apoptosis exclusively by C16:0 ceramide [80]. Additional information is also obtained by the analysis of the biological significance of the presence or absence of a double bond in the backbone. This difference cannot be distinguished by conventional methods due to strong structural similarity and the poor discrimination when using enzymatic methods. Saturated analogs, however, are usually biologically inactive [81]. Sphingolipidomics will provide thrilling information on the source and the 'job history' of a unique metabolite by comparative analysis of the levels of its *de novo* precursors versus its degradation products. The convenience of the method can also be applied for the discrimination of cellular effects of extra- or intracellular sphingolytic activity as well as for the determination of the biological effects, e.g., of a selected sphingomyelinase isoform targeted to distinct sub-cellular compartments. Such studies are mandatory for probing the impact of different intracellular sphingomyelin pools [7, 82]. We are convinced that sphingolipidomics will provide useful information on the role, the origin, and the fate of a variety of sphingolipids orchestrating cellular signaling.

Conclusion

There is increasing evidence suggesting a pivotal role of sphingolipids as mediators regulating apoptosis, the cellular stress response, and inflammation. Key regulating enzymes in these processes are sphingomyelinases and ceramidases, generating a fine tuned 'rheostat' between lipid mediators often responsible for opposite cellular effects. The generation of knock-out models and administration of specific low molecular weight inhibitors has enabled the detailed study of the effects at a cellular level. Accumulating evidence emphasizes a critical role of ceramide in systemic inflammation mediated by a plasma secreted isoform of acid sphingomyelinase, SMPD1. An intriguing functional concept for the role of plasma secreted SMPD1 in receptor signaling activation pathways suggests that the enzyme modifies membrane fluidity by the formation of ceramide enriched rafts. Subsequent structural alterations of membrane morphology may then allow rapid and efficient signaling inside the cell, explaining the function of the enzyme in a variety of effects in cellular stress response, but also in the development of MOF during systemic inflammation. Accordingly, plasma secreted SMPD1 is hypothesized not only to function as a signaling molecule *per se*, but also to be involved in the host response and development of remote organ failure. However, for future therapeutic interventions, it is very important to specifically target the enzyme and the ceramide pool in the precise tissue or cell. A therapeutic intervention of ceramide generation might be envisioned to prevent tissue damage during development of organ failure and to prevent infection of mammalian cells with *P. aeruginosa* and other pathogens.

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Statins in Sepsis and Acute Lung Injury

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Introduction

Severe sepsis is a common cause of critical illness and death on the intensive care unit (ICU). It is estimated that severe sepsis accounts for more than 9% of all annual deaths in the United States, comparable to the figure for myocardial infarction. Interestingly, recent human studies suggested that 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors or statins, widely used in the treatment of hypercholesterolemia and atherosclerosis, have some protective effects in bacteremia, sepsis, and related problems, independent of their cholesterol-lowering effects [1–17]. The growing body of evidence gives rise to the question of whether statins have a role in established sepsis as an adjuvant therapy, in the primary prevention of sepsis or both, and what the mechanisms of action are.

In this chapter, an update is given on the possible mechanisms whereby statins might affect sepsis and related disorders, such as acute lung injury (ALI) and acute respiratory distress syndrome (ARDS). There will be some considerations regarding adverse events and withdrawal of statin therapy.

Statin Therapy Reduces the Incidence of and Mortality from Sepsis

In recent years, several observational reports have been published regarding prior statin therapy and sepsis [4, 12], bacteremia [2, 9, 11], multiple organ dysfunction syndrome (MODS) [10], pneumonia [6], and ICU-acquired infections [14]. The largest observational study was recently reported by Hackam et al. [12]. The authors included 69,168 patients in their population-based cohort analysis of whom half received a statin and half did not. They showed that the use of statins in patients with atherosclerosis was associated with a reduced risk of subsequent sepsis (hazard ratio 0.81; 95% CI 0.72–0.90 if adjusted for demographic characteristics, sepsis risk factors, comorbidities, and health-care use) even in high risk subgroups (Fig. 1). Reductions in severe sepsis were also observed. The reduction in the development of severe sepsis needing intensive care was confirmed by Almog et al. [4]. Obviously, all statin-using patients had cardiovascular disease. Results are, therefore, hard to interprete and to extrapolate to patients not suffering from cardiovascular disease. Indeed, hyperlipoproteinemic mice are more susceptible to sepsis than wild-type mice [15], so that patients with cardiovascular disease or hyperlipoproteinemia might be more susceptible to sepsis. The protective effect of statins is, therefore, likely to be under- rather than overestimated.

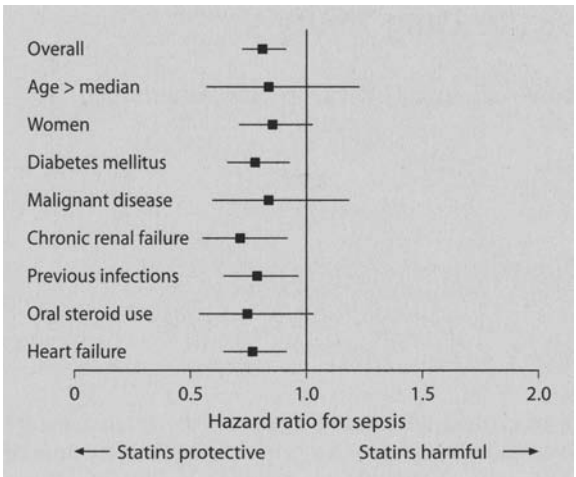

Fig. 1. Decreased risk of sepsis in high-risk subgroups. The protective association between statins and sepsis persisted in high-risk subgroups, including patients with diabetes mellitus, chronic renal failure, or a history of infections. Hazard ratios represent ratio of risk of patients treated with statins to patients not treated with statins as the reference group. Horizontal lines show 95% CI. From [12] with permission.

Furthermore, prior statin therapy decreased mortality in patients with bacteremia, relatively independent of underlying disease [2, 9, 11]. However, Thomsen et al. [11] reported that statin use did not have an effect on short-term mortality after bacteremia, but in contrast, was associated with a decreased mortality between 31 and 180 days after bacteremia, while Liappis et al. [2] and Kruger et al. [9] found that statin use was associated with a survival benefit already (at 28 days) in the hospital. In addition, statin use appeared to be associated with a decreased 28-day mortality in patients developing MODS [10] and decreased 30-day mortality in patients hospitalized for community-acquired pneumonia (CAP), seemingly independent of comorbidity [6]. In contrast to these, often retrospective, observational studies reporting beneficial effects of statins on mortality, Fernandez et al. [14] found higher hospital mortality in patients using statins (61% vs 42%) even after adjustment for predicted risk on the basis of the APACHE II score. The authors concluded that statin therapy may be a marker rather than a mediator of a worse outcome, probably because of incomplete mortality prediction by scoring systems [14].

Until now, no randomized clinical trials have been performed to support the potentially beneficial effects of statins in the prevention and adjuvant treatment of sepsis, by decreasing its prevalence, severity, and mortality. The prospective evidence available regarding the use of statins as adjuvant therapy is limited to studies on other inflammatory diseases and to animal work. Statins have been tried in the treatment of rheumatoid arthritis and multiple sclerosis. In a randomized clinical trial in patients with rheumatoid arthritis, treatment with statins decreased disease activity, C-reactive protein (CRP), and the erythrocyte sedimentation rate [16]. Six months of simvastatin treatment in multiple sclerosis decreased the number and volume of brain lesions on magnetic resonance imaging (MRI) [17]. These results underline the anti-inflammatory properties of statins.

Merx et al. [18] studied treatment with statins after the onset of sepsis in mice. They showed that treatment, starting 6 h after induction of sepsis by cecal ligation and perforation (CLP), improved survival by preservation of cardiac function and hemodynamics. This could be attributed to improved endothelial nitric oxide (NO) synthase (NOS) function and reduced endothelial adhesion of leukocytes [18]. In

contrast, Yasuda et al. [19] studied sepsis-induced acute kidney injury in mice after CLP and they did not observe improvement in kidney function after delayed simvastatin treatment, although pretreatment in this model partly prevented renal hypoperfusion, increased permeability, renal dysfunction, tubular injury and mortality.

Mechanisms of the Beneficial Effects of Statins during Sepsis and Acute Lung Injury

Increasing evidence suggests that statins have beneficial effects beyond lowering of serum cholesterol, the so-called pleiotropic effects, including immunomodulation and endothelial protection (Fig. 2). Furthermore, statins may have antibacterial, antifungal, and antiviral properties and could thereby limit an influenza pandemic, for instance [18, 20–22]. Pleiotropic effects can be explained by the isoprenoid intermediates playing a role in the cholesterol pathway. Cholesterol synthesis begins with the transportation of acetyl-CoA from the mitochondria to the cytosol, which is then converted to HMG-CoA. The conversion of HMG-CoA to mevalonate by the enzyme HMG-CoA reductase is rate limiting and can be inhibited by statins. This does not only disrupt cholesterol synthesis, but also the synthesis of the isoprenoid intermediates (Fig. 3). Isoprenoid intermediates are necessary for the addition of the farnesyl or geranylgeranyl groups (prenylation) to several proteins involved in fundamental cellular processes such as regulation of actin filament (F-actin) cytoskeleton, apoptosis, proliferation, migration, and gene expression.

Statins are able to modulate the immune response and ameliorate inflammation by a variety of mechanisms. For example, they repress major histocompatibility complex II (MHC II)-mediated T-cell activation, inhibit the interaction between leukocytes and endothelial cells by reduction of the expression of various adhesion molecules, shift the T-helper (Th)-1/2 balance towards Th2 leading to suppressed secretion of pro-inflammatory cytokines interleukin (IL)-2, -6, -12, interferon (IFN)-gamma and tumor necrosis factor (TNF)-α [5, 23, 24]. Furthermore, Niessner et al. [13] showed that statin treatment of healthy volunteers blunted monocyte, lipopolysaccharide (LPS)-induced activation of Toll-like receptors (TLR)-4 and -2. Steiner et al. [7] showed in healthy volunteers that simvastatin was able to inhibit LPS-induced elevation of serum high-sensitive CRP and monocyte chemoattractant protein-1 (MCP-1). In addition, they reported that statin pretreatment blunted the expression of monocyte tissue factor expression in response to LPS, attenuating activation of coagulation by LPS. Statins may also increase fibrinolytic activity in the endothelium. In contrast to anti-inflammatory effects, Erikstrup et al. [25] reported that simvastatin treatment had no effect on the rise in circulating cytokines and leukocyte counts in response to endotoxemia in healthy volunteers.

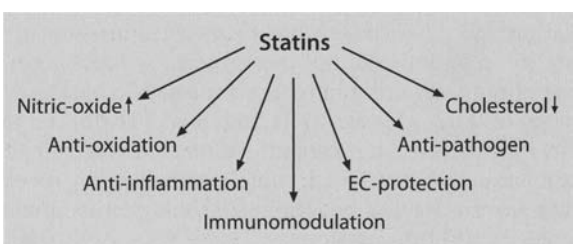

Fig. 2. Pleiotropic effects of statins. Statins are most often prescribed for their cholesterol-lowering effects, but they have also a variety of cholesterol-independent effects, including immunomodulatory, anti-inflammatory, anti-oxidative, endothelium-protective, and antimicrobial properties. EC: endothelial cell

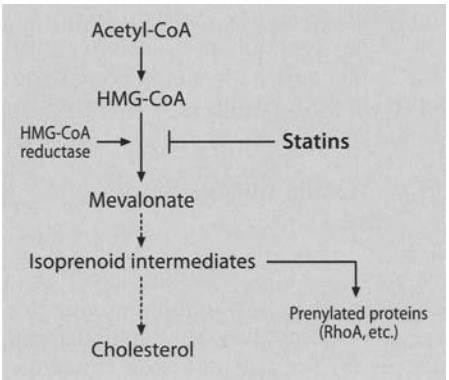

Fig. 3. Pathway of cholesterol biosynthesis. Acetyl-CoA is converted to 3-hydroxy-3-methyl-glutaryl-CoA (HMG-CoA) by HMG-CoA reductase. This enzyme can be inhibited by statins. By doing so, statins do not only inhibit the synthesis of cholesterol, but also of the isoprenoid intermediates, which are necessary for the prenylation of several vital proteins, such as RhoA.

Reactive oxygen species (ROS) are involved in the pathogenesis and manifestations of sepsis. They react with various biological substrates causing membrane dysfunction, tissue damage and ultimately, perhaps, MODS. Durant et al. [3] reported that superoxide anion production upon neutrophil stimulation was greater in critically ill, septic patients than in non-septic patients and healthy volunteers and that simvastatin reduced *in vitro* superoxide production by 40%, through inactivation of NADPH oxidase, as also found by others [5, 26, 27]. Landmesser et al. [8] reported that statin treatment of patients with chronic heart failure doubled the activity of superoxide dismutase (SOD), thereby reducing oxidative stress and improving flow-dependent dilation, as compared to low density lipoprotein (LDL) cholesterol lowering by a non-statin drug. Recently, the pro-inflammatory effects of statins, via T cells secreting IFNγ, have been described also [28].

Endothelium

The endothelium is the first organ that comes into contact with circulating bacterial toxins in sepsis and it coordinates the inflammatory response. Dysfunction of the endothelium is thought to play a major role in the pathogenesis of sepsis. Leukocyte adherence and activation, vasodilation and vasoconstriction, the balance between coagulation and fibrinolysis, and microvascular permeability changes during sepsis, are regulated by the endothelium. A major problem in sepsis is endothelial barrier dysfunction and increased vascular leakage, for instance in the lungs, thought to be associated with ALI/ARDS. Indeed, a number of sepsis-related factors such as LPS, cytokines, and thrombin can impair endothelial barrier function *in vitro* [29, 30]. One of the pathways that may contribute to barrier dysfunction by activation of the F-actin cytoskeleton is the RhoA/Rho-kinase pathway [30]. RhoA has a geranylgeranyl anchor required for translocation from the cytosol to the membrane upon activation. We have shown that simvastatin attenuated thrombin-induced hyperpermeability in endothelial cell monolayers by prevention of translocation of RhoA to the membrane, resulting in reduced formation of stress fibers and conserved focal adhesions [29]. In addition, Dell'Omo et al. [1] showed that pre-treatment with simvastatin normalized the increased permeability of capillary endothelium in hypercholesterolemic, atherosclerotic men. Furthermore, several recent experimental *in vitro* and *in vivo* studies have reported that statins attenuate increased vascular leak, by preventing RhoA activation [1, 29–32].

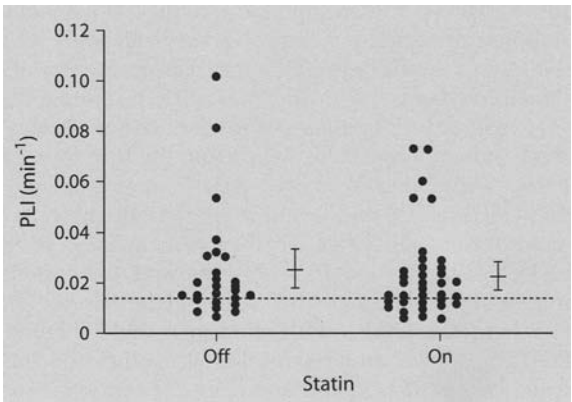

Fig. 4. Prior statin therapy does not affect vascular leakage. Prior statin therapy did not affect nor ameliorate the mildly increased pulmonary leak index (PLI) in 37 patients using statins and 27 patients not using statins, after cardiac or major vascular surgery. The PLI was supranormal (>0.014: values above this line are considered elevated) in 21 (57%) and 16 (59%) patients, respectively. Individual data are shown; horizontal bars represent mean and vertical bars the 95% confidence interval. From [32] with permission.

In murine, intratracheal LPS-induced ALI, statin pretreatment ameliorated lung vascular leak and inflammation, but impaired host defense [33, 34]. Jacobson et al. [31] treated mice with simvastatin prior to and concomitantly with intratracheally-administered LPS and found protection of barrier function of the lung vessels and prevention of changes in a variety of genes involved in the inflammatory and immune response. In ischemia/reperfusion models, both of lungs and remote organs, prior statin therapy seemed to ameliorate ALI, inflammation, and increased permeability [27]. In a clinical, prospective and observational study involving 64 patients after cardiac and major vascular surgery, known risk factors for increased permeability in the lungs and ALI/ARDS [32], prior statins had been administered in 68 and 44% of patients, respectively. Prior statin therapy did not ameliorate mildly increased pulmonary permeability after surgery, as measured using a non-invasive radionuclide method [32] (Fig. 4). Since the patients did not fulfil ALI/ARDS criteria, it cannot be excluded that statins have beneficial effects when permeability is severely increased, during sepsis for instance.

RhoA does not only play a role in endothelial dysfunction by activation of the F-actin cytoskeleton, but also by negatively affecting eNOS mRNA expression and impairing release of NO. Endothelium-derived NO mediates vasodilation and inhibits leukocyte adhesion, platelet aggregation, and smooth muscle proliferation. Statins improve endothelial function by upregulating eNOS, downregulating vasoconstriction endothelin, and increasing responses to vasodilators, also in endotoxin-challenged humans [1, 5, 27, 35]. Statins may also reduce NO scavenging by ROS [26]. Statins may ameliorate endotoxin-induced inducible NO expression mitigating vasoconstrictor responses, in animals and man [5, 36].

Cholesterol-dependent Effects

Statins might not only be beneficial in sepsis because of the pleiotropic effects, but also because of their ability to raise high-density lipoprotein (HDL) cholesterol levels while decreasing total and LDL cholesterol, and triglyceride levels. Accumulating evidence indicates the protective role of plasma lipoproteins such as HDL cholesterol in sepsis. HDL cholesterol has higher binding capacity for LPS than other lipoproteins, via LPS-binding protein [37]. Berbee et al. [38] described that the protein moieties of lipoproteins, the apolipoproteins, are responsible for modulating effects of

LPS. HDL-associated apolipoprotein CI [38] enhances the early inflammatory response to sepsis and improves survival, while apolipoprotein E [39] and apolipoprotein A-I [40] reduce the LPS-induced production of cytokines and ameliorate hemodynamic changes, ALI and mortality, in animal models. Interestingly, Chien et al. [41] reported that patients who died within 30 days had lower levels of HDL cholesterol and apolipoprotein A-I during the first four days of severe sepsis, as compared to survivors. Furthermore, HDL cholesterol correlated inversely with IL-6 and TNF-α levels. A low serum level of HDL cholesterol was an independent predictor of 30-day mortality rate. Furthermore, infusion of recombinant HDL in animal models or healthy volunteers blocks many of the pathophysiological effects of endotoxemia or sepsis, but a positive clinical trial has not yet been reported [42]. In addition to raising the level of HDL cholesterol and thereby increasing the binding capacity for LPS, Spitzer and Harris. [43] hypothesized that statins enhance LPS clearance from the circulation and attenuate the septic response by promoting the expression of LDL receptors enhancing the uptake of lipoprotein-LPS complexes. This may result in inhibition of nuclear factor-kappa B (NF-κB) nuclear translocation and thereby amelioration of the pro-inflammatory response [43]. Finally, HDL cholesterol in particular may be a precursor for adequate cortisol synthesis, necessary to cope with stress.

Adverse Events and Withdrawal of Statins

Statins have an excellent safety profile, because more than 50,000 individuals, primarily middle-aged and older persons, have been randomized to either placebo or statin in several trials and no severe morbidity or increased mortality was observed in the drug treatment group [44]. Reported adverse effects are neuromuscular, including rhabdomyolysis, axonal neuropathy, myopathy, elevations of hepatic enzymes, without clinically significant liver disease and, possibly in the long term, cancer [45]. The dose of statins should be well balanced, because for most of the pleiotropic Rho-dependent effects, high doses are necessary, while, on the other hand, neuromuscular adverse effects are more likely to occur at higher statin doses. Rhabdomyolysis is a severe, but fortunately very rare adverse event [44]. The adverse neuromuscular effects might contribute to the development of critical illness polyneuromyopathy, if statin administration is continued throughout critical illness [46]. Patients with sepsis may be more susceptible to statin-associated neuromuscular disease due to a reduced statin metabolism, the additive effect of other processes inhibiting neuromuscular function and the use of drugs associated with increased toxicity of statins. Other risk factors may also apply, such as those described before [44], including advanced age, frailty, multisystem disease, multiple medications, and peri-operative periods.

Despite the growing evidence that statin therapy lowers the incidence, severity, and mortality of sepsis [12], little is known about the effects of statin withdrawal in patients. Recent studies have suggested that acute withdrawal of statin therapy may result in deterioration of endothelial function with resultant propensity for thrombosis and impaired vasodilation [35, 47, 48]. In addition, Fonarow et al. [49] reported an increased risk of mortality in patients whose statin therapy was discontinued during the first 24 hours of hospitalization for acute myocardial infarction. This may be explained by a rebound-like increase in oxidative stress, decrease in NO bioavailability, and increase in coagulability [26, 35, 50]. The implication for the sep-

tic and postoperative, critically ill patient is that sudden discontinuation of statins may be harmful.

Conclusion

Prospective randomized clinical trials, also in normocholesterolemic patients, are necessary to study the effect of statins in the prevention and treatment of sepsis. Study variables might include fluid balance, lung permeability, occurrence and severity of ALI/ARDS, and pro-inflammatory factors, among others. Prior statin therapy should not be discontinued in the critically ill (postoperative) patient with sepsis. Continuation, however, carries the presumably small risk of aggravation of critical illness polyneuromyopathy, however.

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Potential Mechanisms by which Statins Modulate the Development of Acute Lung Injury

T. Craig, C. O'Kane, and D. McAuley

Introduction

Acute lung injury (ALI) and the acute respiratory distress syndrome (ARDS) are characterized by acute hypoxemic respiratory failure and bilateral pulmonary infiltrates that are not attributable to left atrial hypertension [1]. ALI/ARDS is a heterogeneous disease with a complex pathophysiology that may occur in response to a direct pulmonary or indirect systemic injury [1]. ALI and ARDS are different spectrums of the same condition. ALI is characterized by a PaO_2/FiO_2 ratio of less than 300 mmHg (40 kPa). ARDS, the more severe end of the spectrum on the basis of oxygenation criteria, is defined by a PaO_2/FiO_2 ratio of less than 200 mmHg (26 kPa). A recent prospective cohort study estimated the incidence of ALI to be 79/100,000 person years [2]. Mortality remains high although more recent trials have reported a lower mortality [3, 4].

Limiting tidal volume and, thereby, lung over-distension is the only maneuver that has been proven by clinical trials to improve the mortality of patients with ARDS [3]. Despite advances in mechanical ventilation strategies, it is becoming increasingly apparent that even the best ventilation strategy further damages the injured lung [5]. There is, therefore, a major need to develop a pharmacological agent to improve clinical outcome in ALI/ARDS.

Many treatment options including anti-inflammatory agents, antioxidants, pulmonary vasodilators, and surfactant replacement have been studied; however, despite extensive and ongoing research, no therapeutic option has been convincingly shown to decrease mortality in ALI/ARDS. Although, a small phase II clinical study has suggested that beta-agonists may have a potentially beneficial role [6], larger clinical trials are required to confirm this finding.

Inhibitors of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, or statins, were introduced into clinical practice in the 1980s. They were introduced as cholesterol lowering agents and considerable research has been focused on them. Secondary prevention trials have provided insights into future potential applications of statin therapy. For example, the Long-term Intervention with Pravastatin in Ischemic Disease (LIPID) [7] study suggested that the degree of cardiovascular benefit was in excess of the cholesterol lowering properties. There are now a large number of publications describing the pleiotropic effects of statins and as a result they may have other applications in clinical practice. This chapter will focus on interesting recent *in vitro* and *in vivo* animal and human studies that suggest that statins may modulate mechanisms important in ALI/ARDS.

Pathogenic Mechanisms of ALI/ARDS

The alveolar capillary unit is the site for gas exchange in the lung. This interface consists of two closely related barriers, the vascular endothelium and the alveolar epithelium. The alveolar epithelium consists of two cell types, the more common type I cell (90%) important in gas exchange and the type II cell (10%) involved in alveolar fluid clearance, surfactant production, and regeneration of type I cells following injury.

The pathogenesis of ALI/ARDS remains poorly understood. The majority of evidence suggests that neutrophil-mediated injury is central to the development of ALI/ARDS [1]. Furthermore, the persistence of neutrophils in the bronchoalveolar lavage (BAL) fluid of patients with ALI/ARDS is associated with a higher mortality at day 7 [8]. Alveolar macrophages play a pathogenic role in conjunction with the influx of neutrophils [9]. These inflammatory cells release inflammatory cytokines, proteases, and, in particular, matrix metalloproteinases (MMPs), and reactive oxygen species (ROS) [1].

Inflammatory cytokines play an important role in the pathophysiology of ALI/ARDS. Tumor necrosis factor (TNF)-α, interleukin (IL)-1β, IL-6, and IL-8 are found in BAL fluid and plasma of patients with ALI/ARDS. At the onset of ARDS, non-survivors have significantly higher BAL fluid concentrations of TNF-α, IL-1β, IL-6, and IL-8 (CXCL8); over time, pro-inflammatory cytokine levels remain persistently elevated in non-survivors [10]. Indices of endothelial permeability correlate with plasma and BAL fluid cytokines. However, no single cytokine predicts either the onset or the outcome of ARDS.

MMPs are a group of diverse zinc containing, proteases enzymes capable of degrading the extracellular matrix. They are produced by cells important in the pathogenesis of ALI/ARDS, including epithelial and endothelial cells, alveolar macrophages, neutrophils, and fibroblasts [11]. MMPs are regulated in part by tissue inhibitors of matrix metalloproteases (TIMPs). MMPs are classified according to their substrate specificity. The gelatinases (MMP-2 and MMP-9) are capable of degrading type IV collagen, the major extracellular matrix component of the basement membrane, which is characteristically disrupted in ARDS, appear to play a role in ALI/ARDS [12, 13]. More recently MMP-1, -2, and -8 have been identified in BAL fluid from patients with ALI/ARDS. MMP-1 and -3 are associated with disease severity, including mortality [14].

This uncontrolled local inflammatory response causes alveolar epithelial and capillary endothelial barrier damage [15, 16] central to the development of lung injury. The small GTPases, Rho and Rac, are involved in signal transduction linking extracellular stimuli to epithelial and endothelial barrier function [17]. Consequently, there is impaired gas exchange with resultant hypoxemia, reduced lung compliance, and the need for mechanical ventilation.

Classically, the fibro-proliferative phase follows, characterized by organization of the alveolar exudate and by fibrosis. There is type II cell proliferation. Lymphocytes and fibroblasts are the predominant cell types with evidence of matrix reorganization. However, it is now considered that these subdivisions of ALI/ARDS may not be clearly demarcated and there may be a degree of overlap in these pathological divisions.

Mechanism of Action of Statins

HMG-CoA reductase catalyzes the rate limiting step in the production of cholesterol. Although the chemical composition of the statin may vary, they all inhibit the reductase site in the same manner; they inhibit the conversion of HMG-CoA to mevalonate. As a consequence of this action, the intermediates of the mevalonate pathway are also reduced (Fig. 1).

In addition to cholesterol production, the mevalonate pathway leads to the formation of isoprenoids such as geranylgeranylpyrophosphate (GGPP). Isoprenoids regulate prenylation, the addition of hydrophobic molecules to a protein. Protein prenylation involves the transfer of either a farnesyl or a geranylgeranyl moity to C-terminal cysteine(s) of the target protein. Lipid modification of proteins is necessary for interaction with cellular membranes. The isoprenoid intermediates are important in several signaling pathways, and regulate function of G proteins and the small GTP binding proteins, Ras, Rho, and Rac. The small GTP binding proteins control multiple cellular activities, including cell proliferation, generation of ROS and acti-

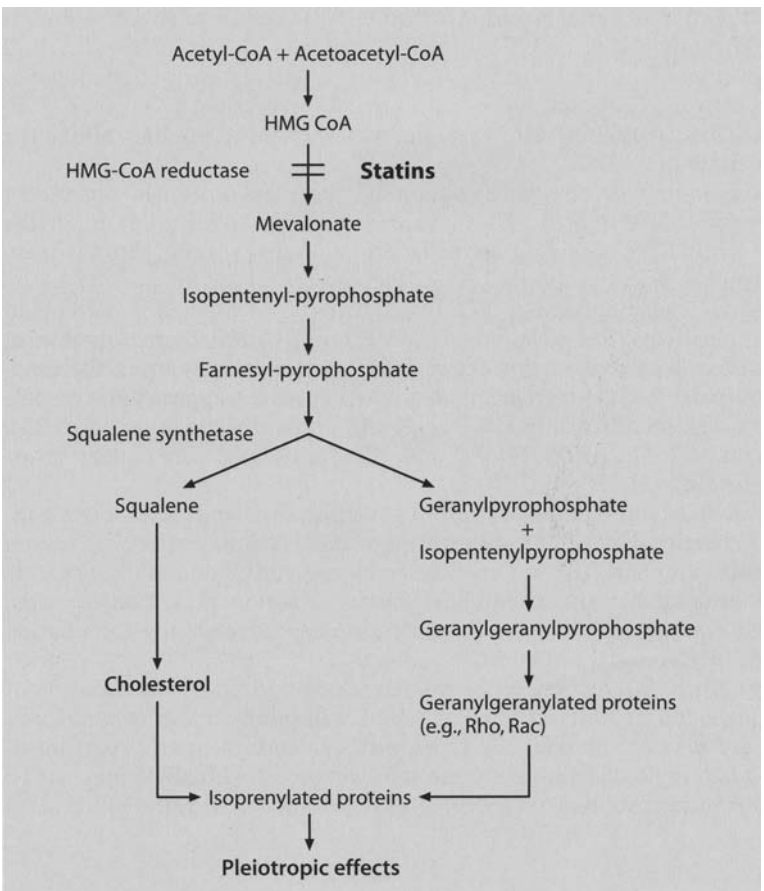

Fig. 1. The mevalonate pathway

vation of pro-inflammatory cytokines, all of which are important in the pathogenesis of ALI [1]. Inhibition of isoprenoid formation by statins, therefore, may have significant anti-inflammatory effects.

Most of the anti-inflammatory effects of statins are mediated by inhibition of the mevalonate pathway, as shown by the reversal of anti-inflammatory properties with the addition of mevalonate, GGPP, and farnesylpyrophosphate (FPP).

However, statins have additional properties independent of their HMG-CoA reductase inhibition. Lymphocyte function associated antigen-1 (LFA-1) or integrin $\alpha_1\beta_2$ (also known as CD11a or CD18) is a member of the integrin family and is found exclusively on leukocytes in an inactivated state. It plays a role in leukocyte extravasation and in T cell activation by antigen presenting cells. The main ligand of this adhesion molecule is the intracellular adhesion molecule 1 (ICAM-1). Following binding of ICAM-1, LFA-1 provides a potent co-stimulatory stimulus for T cell receptor (TCR) activated T cells. Simvastatin inhibits the binding of LFA-1 to ICAM-1 [18]; as a result there is reduced lymphocyte adhesion to ICAM-1 and reduced T cell costimulation. This action is only partially reversed by the addition of mevalonate, indicating that HMG-CoA reductase inhibition-independent mechanisms are involved. Interestingly, pravastatin did not affect the LFA-1-ICAM-1 interaction, indicating specificity among the statins rather than a class effect.

Molecular Mechanisms for the Anti-inflammatory Effects of Statins

Statins may modify the critical pro-inflammatory intracellular signaling pathways described below.

Transcription Factors: Nuclear Factor-kappa B and Activator Protein

Several pro-inflammatory stimuli converge on a few key transcriptional pathways. Nuclear factor-kappa B (NF-κB) is one of the major transcription factors. It is found in the cytoplasm bound to its inhibitor, IκB. In response to inflammatory stimuli, the inhibitor is degraded with the active portion, leaving NF-κB free to translocate to the nucleus, where it binds to the promoter sequences of pro-inflammatory genes, inducing gene expression.

Activator protein-1 (AP-1) is another key transcription factor family regulating the induction of a series of pro-inflammatory genes. Like NF-κB the family consists of a series of subunits, such as Jun and Fos. Upregulation in AP-1 occurs in response to a series of stimuli including IL-1, which is a key mediator in the pathogenesis of lung injury. In turn AP-1 regulates the induction of many cytokines, including CXCL8, and some MMPs, which are also described in the pathophysiology of ARDS.

Simvastatin, lovastatin, and atorvastatin inhibit the binding of nuclear proteins to NF-κB and AP-1. They also upregulate the cytoplasmic inhibitor of NF-κB (IκB-α) in endothelial cells, preventing nuclear translocation and, therefore, induction of NF-κB-dependent genes. Statins also reduce the expression of c-Jun, a component of AP-1 [19].

Kruppel-like Factor 2

KLF2 is a member of the Kruppel-like family of transcription factors. These factors are important in the control of endothelial gene expression and in the regulation of multiple endothelial functions. Statins induce KLF2 expression via the Rho pathway [20]. Statins can induce factors including thrombomodulin and endothelial nitric oxide synthase (NOS).

Peroxisome Proliferator Activated Receptor-alpha

The activation of peroxisme proliferator activated receptor-(PPAR)-α leads to the inhibition of inflammatory pathways. PPARs act by negatively interfering with NF-κB and AP-1 signaling pathways. Statins activate PPARs [21]. The addition of mevalonate reverses the PPAR-α activation by statins indicating that it is the downstream products of the mevalonate pathway that inhibit PPAR-α activity.

Mitogen-Activated Protein Kinases

The mitogen-activated protein kinases (MAPKs) are a group of intracellular signaling intermediates, activated by inflammatory cytokines and growth factors, which regulate gene expression, differentiation, and apoptosis. Key inflammatory cytokines, such as CXCL8, and most MMPs are regulated by MAPKs. Statins can directly inhibit the MAPK pathways, resulting in reduced cytokine/MMP gene expression. For example, simvastatin inhibits granulocyte-macrophage colony stimulating factor (GM-CSF) induced by the Ras and Rho-p38 MAP kinase signaling cascade. This in turn results in inhibition of macrophage proliferation [22].

Statins and Leukocyte Activity

Neutrophil Function

Statins reduce neutrophil numbers in animal models of ALI/ARDS. In an endotoxemic model of ALI/ARDS, mice were pre-treated with 5 mg/kg or 20 mg/kg simvastatin or placebo injected intraperitoneally. This was administered 24 hours before and again concomitantly with intratracheal lipopolysaccharide (LPS, 2 μg/g body weight). Compared to placebo, mice treated with 20 mg/kg simvastatin had lower levels of BAL neutrophils and myeloperoxidase (MPO) activity (a marker of neutrophil activity) with an associated reduction in endothelial permeability as reflected by the 50% reduction in BAL albumin. A reduction in lung injury was confirmed histologically [23].

In an ischemia-reperfusion model, rats were pretreated with simvastatin 10 mg/kg daily or distilled water via an orogastric tube for 3 days. The intestinal ischemia-reperfusion injury was performed by occlusion of the superior mesenteric artery for 60 minutes followed by 90 minutes of reperfusion. Rats pretreated with simvastatin had improved oxygenation compared to the control group. There was also evidence of reduced lung injury indicated by a reduction in neutrophils and end products of free radical mediated injury and an improvement in endothelial permeability with a lower wet to dry ratio in the statin subgroup [24].

In a lung ischemia/reperfusion model (90 minute ischemic period and 4 hours of reperfusion) rats were pretreated with simvastatin 0.5 mg/kg orally. After 5 days of

simvastatin there was an 85% reduction in vascular permeability. There was a significant reduction in MPO content and presence of leukocytes in the alveolar space in the statin treated group [25].

Statins can also inhibit leukocyte migration in response to chemotactic agents and also across the vascular endothelium. Cerivastatin has been demonstrated to reduce leukocyte chemotaxis to CXCL8. Migration was restored with the addition of mevalonic acid. Cerivastatin reduced transendothelial migration of neutrophils. With elevated concentrations of cerivastatin, the rate of apoptosis in neutrophils and monocytes was also increased [26].

Monocyte Function

Statins regulate monocyte function. Pre-treatment of monocytes with lovastatin (20–40 mg) reduced the expression of CD11b and inhibited CD11b-dependent monocyte adhesion to the endothelium. Cd11b/CD18 is a β_2 integrin important in cell adhesion and signal transduction, and is found on the surface of monocytes. The interaction of this integrin with ICAM-1 is important in the adhesion and migration of activated monocytes across the endothelium. Co-incubation with mevalonate but not low-density lipoprotein (LDL) reversed the effects of lovastatin. This suggests that early precursors and not cholesterol mediate this effect [27].

Lymphocyte Function

In the later stage of ARDS, the number of neutrophils declines and there is an increase in numbers of lymphocytes and macrophages in BAL fluid. As discussed earlier, simvastatin, but not pravastatin, inhibits lymphocyte adhesion to ICAM-1 resulting in decreased T cell activation [18]

Statins: Effects on C-reactive Protein and Inflammatory Cytokines

Concentrations of C-reactive protein (CRP), a marker of inflammation, are decreased 15–30% by pravastatin in patients with cardiovascular disease [28]. The level of CRP reduction does not correlate with the lipid lowering properties. This again supports the hypothesis that anti-inflammatory effects exist that are independent of cholesterol reduction.

In a murine model of systemic inflammation, lovastatin and simvastatin were administered 0.5 hours, 8 and 20 hours before the introduction of LPS. Both inhibited IL-6 and CCL2 secretion. The effect of blocking the activity of the enzyme that catalyzes the first step of the cholesterol pathway, after it branches to various non-sterol products, was also investigated (Fig. 1). Administration of a squalene synthetase inhibitor, squalestatin did not inhibit inflammatory cytokine secretion [29].

There is also *in vitro* and *in vivo* work indicating that statins can reduce monocyte derived IL-6, CXCL8, and CCL2 [30]. Patients with hypercholesterolemia were pre-treated with simvastatin 20–40 mg for 6 weeks. Following treatment there was a significant reduction in plasma CCL2, IL-6, and CXCL8. There was a similar reduction in the expression of IL-6, CXCL8, and CCL2 mRNA in peripheral blood mononuclear cells. Similar results were obtained *in vitro* by using cultured human umbilical vein endothelial cells and peripheral blood mononuclear cells from healthy normolipemic donors. Exposure to simvastatin, atorvastatin, or cerivastatin caused

downregulation of the expression of cytokine mRNA in a time- and dose-dependent manner.

The effect of pre-treatment with simvastatin prior to exposure to intravenous LPS was investigated in a double-blind, placebo controlled trial in healthy individuals. Compared to placebo, after 4 days treatment with 80 mg simvastatin, the inflammatory response was blunted. CRP and CCL2 were significantly suppressed and the concentration of monocyte tissue factor was also inhibited in the statin group [31]. Published data show that a single dose of 80 mg simvastatin can reduce CRP levels in patients with unstable angina within 48 hours [32].

Statins and MMP Activity

As discussed above, MMP-9 has been implicated in the development of ALI/ARDS. Macrophages are a major source of MMP-9. *In vitro* incubation of mice and human macrophages with statins is associated with reduced quantities of MMP-9 secretion [33, 34]. The effect is again reversible with the addition of mevalonate. In addition, cerivastatin inhibits macrophage MMP -1, and -3 [34] and neutrophil MMP-9 [33].

In patients with hypercholesterolemia and coronary artery disease randomized to receive 20 mg simvastatin or 200 mg fenofibrate for 8 weeks, both lipid-lowering agents resulted in a reduction in plasma TNF-α. However, only the simvastatin group had a significant reduction in MMP-1, and -9 compared to the fenofibrate treated group [35]. Data suggest that statins may more effectively modulate the key inflammatory pathways driving ALI than fibrates, and further support the hypothesis that this effect is independent of lipid lowering.

Statins and Oxidative Stress

Increased oxidative stress is important in the development of ALI. ROS are detectable in BAL fluid of patients with ALI. Through the inhibition of Rac isoprenylation, statins lead to a reduction in NADPH oxidase with a resultant reduction in ROS which may be beneficial in ALI [36].

Statins and the Endothelium

Endothelial dysfunction is central to the development of ALI [1]. The vascular endothelium has several important roles including regulation of vascular tone, permeability, blood flow, coagulation and inflammation. The effect of statins on the endothelium has been studied extensively. Statins improve endothelial function by increasing the bioavailability of NO with resultant increased vasodilatation [37]. This effect is mediated by various mechanisms including upregulation of eNOS expression.

Actin-myosin cytoskeletal organization determines endothelial permeability with Rho mediating increased permeability and Rac decreasing permeability in response to injury with thrombin [38]. Simvastatin attenuates the endothelial barrier dysfunction induced by thrombin. As expected by its mode of action (Fig. 1), simvastatin inhibited Rho but paradoxically Rac was activated. One potential explanation for this paradoxical observation is that the inhibition of prenylation preferentially

inhibits Rho and, via a normally tonic inhibitory effect on Rac, effectively increases Rac activation. Alternatively, specific inhibitors of Rac may be induced by simvastatin independent of the prenylation pathway. In addition, in a murine inflammatory model of ALI, simvastatin reduced BAL albumin by 50% indicating an improvement in alveolar epithelial-capillary endothelial barrier permeability [23].

Von Willebrand factor (vWF), a marker of endothelial injury/activation is elevated in pulmonary edema fluid and plasma samples in individuals with ALI/ARDS. Statins reduce plasma vWF levels over a sustained time period. In hyperlipidemic patients treatment with simvastatin resulted in a significant reduction in vWF after three months, which was sustained during a two-year follow up period [39].

Statins also affect the endothelium by their action on coagulation. Abnormalities of the clotting system occur in ALI [1]. Higher baseline levels of plasminogen activator inhibitor 1 (PAI-1) in ALI/ARDS are associated with a longer duration of ventilation and higher mortality [40]. Statins inhibit the expression of PAI-1 from human smooth muscle cells and endothelial cells *in vivo*. This effect is reversed by the addition of mevalonate and GGPP but not FPP, suggesting that GGPP is required in the expression of PAI-1 [41].

Statins and the Alveolar Epithelium

In ALI/ARDS, an intact functioning alveolar epithelium is associated with improved outcomes, with regards to survival and duration on a ventilator [42]. Although there are few published data on the effect of statins on alveolar epithelial cell function, preliminary work published in abstract form has shown that statin treatment inhibits alveolar epithelial cell CXCL8 production [43]. Intact epithelial function requires tight junctions, and an intact basement membrane. Both tight junctions and basement membrane are degraded by MMPs (in particular MMP-2/-3/-7/-9) and given that statins reduce expression of these MMPs *in vivo* and *in vitro*, it is possible that statin treatment will improve epithelial barrier function.

Statins in Sepsis: Implications for ALI/ARDS

ALI is the most lethal complication of sepsis and has the highest mortality. There is now emerging evidence that statins may have a beneficial effect on clinical outcomes in sepsis.

In a murine model of sepsis, pre-treatment with simvastatin markedly increased survival time. Mice treated with simvastatin at 0.2 μg/g body weight had a median survival of 108 hours as opposed to 28 hours in the placebo group following cecal ligation and perforation. This appeared to be the result of preservation of cardiac function [44].

In addition, there have been several human observational studies, which have suggested a benefit with statins in septic patients [45–48]. The largest trial analyzing data from over 69,000 patients found a 19% risk reduction of developing sepsis if pre-treated with a statin. This also applied to high risk groups defined by the presence of diabetes, renal impairment, and a history of recurrent infections. Interestingly, no benefit was noted with non-statin lipid-lowering agents [48].

Furthermore, statins decreased the risk of progression to severe sepsis [45] and reduced mortality attributable to sepsis [46]. Importantly, in one study, the reduc-

tion in both all-cause hospital mortality and death attributable to bacteremia was more marked in the patients who continued to receive statin therapy after the diagnosis of bacteremia [47].

These studies support the concept that statins may have a potential role in the treatment for ALI/ARDS.

Statin Safety Profile

Most statins are metabolized by hepatic cytochromes. Pravastatin is metabolized by sulfation and not via the cytochrome pathway. Drugs that interfere with hepatic cytochromes, therefore, need to be used with caution in conjunction with statins. Statins should be used with caution in those with liver disease. Treatment should be withheld or discontinued if serum transaminase concentrations persist three times above the upper level of normal.

Myopathy is the other important adverse effect associated with statin use. Treatment with statins should be withheld or discontinued if the creatine kinase (CK) is elevated five times above the upper level of normal [49].

The large quantities of data on the use of statins in cardiovascular disease can provide further insights into statin safety. One study randomized 2265 patients following a coronary event to receive simvastatin 80 mg or placebo. Even with high dose (80 mg) simvastatin, myopathy (CK >10 times the upper limit of normal associated with muscle symptoms) occurred in only 0.4% and rhabdomyolysis (CK >10,000 units/l with or without muscle symptoms) in 0.13% of patients [50]. Of note, treatment in this study lasted up to 24 months with follow-up only at months 1, 4, and 8, and every 4 months, thereafter, until trial completion.

There are concerns that critically ill patient may be at higher risk of adverse effects related to statins. However, the finding of greater reduction in all-cause hospital mortality in patients with sepsis who continued to receive statin therapy [47], and the fact that the duration of treatment in the critically ill patient with ARDS will be much shorter and that patients will be intensively monitored provides some reassurance that adverse effects from statins may not be a greater problem in an intensive care patient population. However, ongoing pharmacovigilance is clearly required.

Which Statin?

Pleiotropic actions have been demonstrated for most statins but to date there have been no studies directly comparing these effects. Although the mode of action among the statins is similar, there do appear to be differences among their non-lipid lowering effects. For example, pravastatin does not inhibit LFA binding [18].

In the four observational sepsis studies [45–48], which statin was used was not consistently reported. However, in the reports which presented this information, although a range of statins was used, simvastatin was the most common.

Furthermore, in a double-blind, placebo-controlled study, simvastatin was effective in inhibiting the systemic inflammatory and procoagulant responses important in the development of lung injury [31]. No other clinical studies have been published.

Therefore, although there is limited evidence as to the most appropriate statin for use in ALI, the currently available data support the use of simvastatin.

Statin Dosage

Although there are large amounts of data suggesting statins may be beneficial in models of ALI, only a single animal study has compared two doses of simvastatin (5 or 20 mg/kg). Only the higher dose was effective in attenuating lung injury [23]. On a dose per unit body mass basis these doses are significantly higher than those used in humans.

The data from Steiner et al. involving healthy human volunteers exposed to LPS suggested an improvement in inflammatory indices after four days treatment with simvastatin 80 mg [31]. No other clinical studies demonstrating that a lower dose is effective have been published. The dosages of statins used in the observational sepsis studies were variable and although lower doses were more common, higher doses were also used [45–48].

Therefore, although clearly further work is necessary to determine the appropriate therapeutic dose which may be beneficial in the setting of ALI, on the basis of current available data, higher doses seem appropriate.

Duration of Treatment

Further research is required to determine the appropriate timing and duration of statin therapy, which may be effective in ALI/ARDS. Assuming that statins modulate mechanisms that are more important in the development and early phase of ALI/ARDS these agents are more likely to be effective if they are commenced early after the onset of ALI/ARDS or as a prophylactic therapy in those at risk. Pleiotropic effects are seen early in the course of treatment with statins. In fact, in one study a reduction in CRP was seen by 48 hours after a single dose of simvastatin 80 mg [32]. The median duration of ventilation in ALI/ARDS is 6 (2–12) days [2] which has implications for the proposed duration of therapy. It is likely that a treatment duration of up to 14 days will be required.

Potential Limitations to Therapy

There are significant changes in lipid metabolism in patients with sepsis. It appears that while triglyceride levels are elevated, there is a reduction in total cholesterol. The mechanism for this remains unclear but it may have implications for statin use in sepsis-induced ALI/ARDS. It is possible that HMG-CoA reductase is already maximally downregulated and that any benefit of additional inhibition by statins will be limited. However, the beneficial effects in sepsis [45–48], in addition to the actions independent of HMGCoA reductase inhibition [18], would suggest that they will provide additional potential benefit.

Conclusion

There is considerable evidence from *in vitro, in vivo* animal, and observational human studies to suggest that statins may have a role in the management of ALI/ARDS. However, at present there are insufficient clinical data to recommend their use in ALI/ARDS.

A phase II clinical trial is currently underway examining the effect of treatment with simvastatin 80 mg in patients with ALI/ARDS (the Hydroxy-methyl glutaryl CoA reductase inhibition in ALI to Reduce Pulmonary edema (HARP) study-ISRCTN 70127774). A further study investigating whether simvastatin can prevent the development of ALI/ARDS is planned in at risk patients. Results from these clinical trials will help define the role of statins in the prevention and treatment of ALI/ARDS.

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Acute Lung Injury

Matrix Metalloproteinases in Acute Lung Injury

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Introduction

Matrix metalloproteinases (MMPs) are a family of zinc-dependent endopeptidases belonging to the metzincin superfamily of metalloproteinases. MMPs can degrade most of the components of the extracellular matrix and basement membrane. In addition, these enzymes can cleave some inflammatory mediators. This variety of substrates gives MMPs a wide number of functions during physiologic and pathologic processes. In this sense, many of the MMPs are not expressed in normal tissues, but expression and activity increases dramatically during matrix turnover, inflammation and repair.

Acute lung injury (ALI) is a devastating condition that leads to an acute inflammation of the lungs. In some cases, mechanical ventilation is needed, thus causing an additional stress to the lung with potential for the so-called ventilator-induced lung injury (VILI). The repair process after this injury can lead to near-normal resolution or, in other cases, an increase in the collagen content of the lung interstitium and, thus, pulmonary fibrosis. MMPs may be involved in this sequence of events, from the initial events until the resolution of the disease. In this chapter, we will review the functional role of MMPs in ALI and discuss the derived therapeutic implications.

Matrix Metalloproteinases

There are 24 human genes encoding 23 different MMPs. The typical structure of a MMP consists of a propeptide region (about 80 amino acids), a catalytic domain (170 amino acids, with a zinc ion bounded to histidine residues), and a 'hinge region' that links a hemopexin domain (200 amino acids). MMP-7, -23 and -26 are exceptions to this model, lacking the linker and hemopexin domains. Addition of other domains leads to a diversity of proteins with different substrates and activities. Based on their structure and preferential substrates, MMPs can be divided into different groups (Table 1).

Most of the MMPs are not expressed in quiescent cells, but MMP transcription occurs in tissues in response to different stimuli. No single factor responsible for the expression of MMPs has been identified. Instead, a variety of cytokines, growth factors, and oncogene products induce MMP synthesis and release (e.g., tumor necrosis factor [TNF]-α, interleukin [IL]-1). The pathways responsible for this expression are also diverse (see [1] for a review). Cell deformation or mechanical stress can lead to MMP expression through a pathway dependent on nuclear factor-κB (NF-κB), one of

Table 1. Matrix metalloproteinases.

	Extracellular matrix substrates	Other substrates
Collagenases		
MMP-1 (interstitial collagenase)	Native collagen	MCP-1, IL-1, pro-TNF-α
MMP-8 (neutrophil collagenase)		
MMP-13 (collagenase-3)		
Gelatinases		
MMP-2 (gelatinase A)	Gelatin (denatured collagen)	MCP-3, pro-IL-8, pro-TNF-α, pro-TGFβ, pro-IL-1, plasminogen
MMP-9 (gelatinase B)		
Stromelysins		
MMP-3	Laminin, fibronectin, gelatin	MCP (1–4), pro-TNF-α
MMP-10		Pro-MMP-1, pro-MMP-8, pro-MMP-10
MMP-11		E-cadherin, cell surface bound Fas-L
Membrane-type MMPs		
MMP-14 (MT1-MMP)	Native collagen, gelatin	MCP-3, CD44
MMP-15 (MT2-MMP)	Proteoglycan	
MMP-16 (MT3-MMP)	Type III collagen, fibronectin	
MMP-17 (MT4-MMP)	Gelatin, fibrin	
MMP-24 (MT5-MMP)	Fibronectin, proteoglycans	
MMP-25 (MT6-MMP)	Type IV collagen, gelatin	
Matrilysins		
MMP-7	Fibronectin, laminin, type IV collagen, gelatin	Pro-TNF-α, E-cadherin, cell surface bound Fas-L
MMP-26		
Other		
MMP-12 (macrophage elastase)	Elastin, fibronectin, laminin	Plasminogen, pro-TNF-α
MMP-19	Type IV collagen, gelatin, laminin	
MMP-20	Amelogenin	
MMP-23	Gelatin	
MMP-27		
MMP-28		

TNF: tumor necrosis factor; IL: interleukin; TGF: transforming growth factor; MCP: monocyte chemoattractant protein

the transcription factors related to the inflammatory response [2]. Other molecules, like transforming growth factor (TGF)β, interferon (IFN)γ and glucocorticoids can block MMP expression. MMPs are usually secreted as a proenzyme, thus requiring activation to be fully functional. This process can be mediated by reactive oxygen species (ROS), endogeneous proteases (including MMPs) or even bacterial proteases [3].

Classically, the main function of MMPs was thought to be the degradation and turnover of the extracellular matrix. However, this paradigm has changed, in view of the variety of substrates and the effects caused by their enzymatic activity [3]. Currently, MMPs are considered to regulate the cell-cell and cell-matrix interactions, through the cleavage of inflammatory mediators (TNF-α, IL-8, insulin-like growth factor [IGF]-1, among others) [4], or through breakdown of components of the extracellular matrix and the release of growth factors bound to the matrix. Cleavage

of collagen and elastin is required for cell migration, and it has been demonstrated that expression of epitopes of the extracellular matrix after proteolysis can induce the chemotaxis of inflammatory cells. Supporting this view, MMPs have been shown to be involved in cell migration, apoptosis, morphogenesis, inflammation, neovascularization, etc.

The main MMP plasma inhibitor is α2-macroglobulin. However, there is a family of more selective inhibitors, the tissue inhibitors of metalloproteinases (TIMPs). There are four TIMPs; although all of them inhibit virtually all MMPs, there are differences in their activity. For example, mice lacking TIMP-3 develop emphysema-like lung injury, while mice lacking TIMP-1 or -2 have no severe abnormalities. These findings emphasize the relevance of TIMP-3 *in vivo*.

Matrix Metalloproteinases in Acute Lung Injury

ALI and its more severe form, the acute respiratory distress syndrome (ARDS), can be viewed as being the result of an inflammatory process within the lungs, caused by pulmonary or extrapulmonary diseases. The lung epithelium and endothelium respond to this aggression with an increase in permeability, thus affecting the interstitium and the alveolar spaces. Inflammatory cells release cytokines and chemokines, responsible for cell chemotaxis and the propagation of the response. As discussed in the previous section, some of the inflammatory mediators involved in the acute phase response, as well as bacterial products or ROS, can precipitate the expression of MMPs. A variety of cells in the respiratory system can synthesize MMPs (Table 2) [5].

After the acute phase, repair can lead to chronic inflammation and deposition of collagen in the interstitium, thus causing pulmonary fibrosis. All these processes can be mediated by MMPs (Fig. 1). In some patients, mechanical ventilation is needed. If this is the case, high positive pressures in the lung can cause further damage, the so-called VILI. The implications of MMPs in this process will be discussed separately.

The role of MMPs in ALI has been studied in different animal models and patients. An upregulation of MMPs in bronchoalveolar lavage (BAL) fluid has been documented in different models of ALI. Following exposure of rats to 100% oxygen, there is an increase in BAL fluid levels of MMP-9, and MMP-2, -7, -8 and -9 in lung tissue [6]. Similar results were found in a model using intratracheal endotoxin [7].

The expression of TIMPs also increases after a challenge, in order to maintain the normal equilibrium between the enzymes and their inhibitors [8]. The critical importance of this relationship is demonstrated in models of endotoxin-induced lung injury in mice deficient in TIMP-3 [9]. In this case, the increased activity of MMPs has no natural inhibitor. These animals develop severe airway enlargement

Table 2. Sources of matrix metalloproteinases (MMP) and tissue inhibitors of metalloproteinases (TIMPs) in the respiratory system

Cells	MMPs released
Fibroblasts	MMP-2, MMP-1, TIMP-1
Bronchial epithelial cells	MMP-2, MMP-9, TIMP-1
Alveolar epithelial cells	MMP-1, TIMP-2
Alveolar macrophages	MMP-1, MMP-9, MMP-12, TIMP-1
Neutrophils	MMP-8, MMP-9, TIMP-1

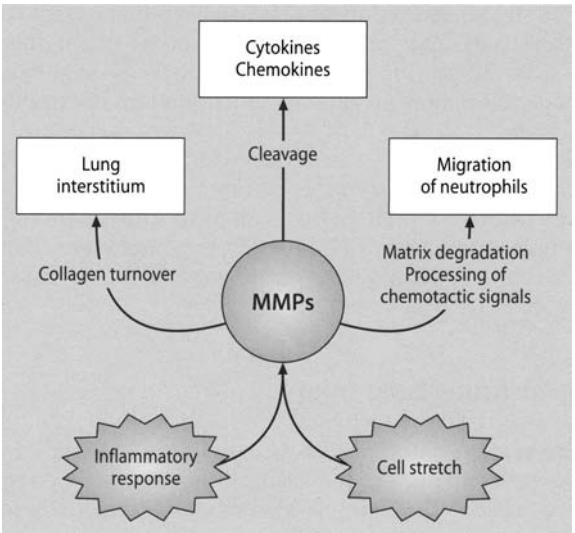

Fig. 1. Effects of matrix metalloproteinases (MMPs) in acute lung injury.

and a decrease in the collagen content of the lungs. Similarly, blocking TIMP-2 in a model of immunocomplex-mediated alveolitis worsens lung injury [8].

MMP-9 may also be involved in lung injury from an extrapulmonary origin. In a pancreatitis model [10], high levels of MMP-9 were found in lung parenchyma, originating from primed neutrophils. Treatment with an MMP inhibitor decreased neutrophil concentration and capillary leakage in the lung. In a similar manner, gelatinase and elastase activities increase in lung injury after abdominal sepsis [11] or cardiopulmonary bypass [12]. Blockade of gelatinases using COL-3 (a chemically modified tetracycline that inhibits gelatinases) decreased the degree of lung injury. These results suggest a possible role of MMPs (and specifically MMP-9) in the loss of compartimentalization of the inflammatory response seen in different pathologies.

Neutrophils and macrophages can be a source for these enzymes. Neutrophils are rapidly recruited within the lungs during ALI. These cells contain MMP-8 and MMP-9 in their secondary granules, and both enzymes are expressed on the surface (mainly at the active pole of the cell) and secreted [13]. In models of ALI, there is a correlation between the number of neutrophils and MMP levels in the BAL fluid [14].

Alveolar macrophages are also important contributors to the development of the lung inflammatory response. In a macrophage-dependent lung injury model (IgA immune complex-mediated alveolitis), there is an increase in BAL fluid gelatinases, parallel to neutrophil recruitment. Moreover, lung injury can be attenuated by treatment with TIMP-2 [15]. These results suggest that alveolar macrophages release gelatinases that can favor the migration of neutrophils.

In contrast, mice lacking MMP-8 have more neutrophils in BAL fluid and an increase in lung permeability after endotoxin challenge, suggesting an anti-inflammatory role of this enzyme [16]. MMP-8 deficiency has been related to a slightly delayed, but persistent, inflammatory response in a model of carcinogenesis [17]. This could explain the protective effects of MMP-8 deficiency in hyperacute inflammation, but also its harmful effects in the long-term. These important properties of MMP-8 will be discussed later.

The implication of MMPs in ALI in patients has been addressed by different studies. The levels and activity of MMP-8 and -9 are elevated in BAL fluid from children with ALI, compared to healthy controls, with no differences in MMP-2 levels [18]. MMP-9 levels in BAL fluid were also elevated in a sample of adult patients with ARDS at different stages (early versus late), and in patients with a risk factor for developing lung injury (but normal lung function) [19]. TIMP levels were also increased in this cohort. Interestingly, the ratio MMP-9/TIMP was above 1 in patients with risk factors and in those at a late stage of the syndrome. However, patients with early ARDS had a ratio lower than 1 [20]. It has been suggested that ratios higher than 1 could facilitate lung repair [19]. A recent study by Fligiel et al. [21] confirmed an increase in MMP-2, -8 and -9 in patients with ALI. In addition, these authors showed that a subgroup of these patients with increased levels of MMP-1 and MMP-3 have an increased morbidity and mortality.

The elevation of MMP-2 in BAL fluid from patients with ARDS receiving mechanical ventilation has been correlated to the levels of type III procollagen and to an impairment on the mechanical properties of the respiratory system (i.e., the compliance measured on an inspiratory pressure-volume curve) [22]. This relationship follows a logarithmic pattern, with an abrupt increase in MMP-2 and procollagen in patients with a compliance less than a critical value (28 ml/cmH_2O). This suggests that collagen turnover, matrix remodeling, and, therefore, MMP activity, play a critical role in the maintenance of the mechanical properties of the respiratory system during lung injury.

Matrix Metalloproteinases in Ventilator-induced Lung Injury

Application of relatively high pressures and volumes to the lungs during mechanical ventilation can cause or augment lung damage. Although the initial pathogenetic mechanism may be mechanical (including cell overstretching and changes in the cytoskeleton), it is clear that there can be a release of mediators that not only perpetuates, but also disseminates, the inflammatory response. The lung matrix supports part of this initial mechanical stress and can contribute to this form of injury. As in other lung diseases, MMPs are thought to play an important role.

Mechanical stress and cell deformation can release MMPs from various types of cells. Using endothelial cells exposed to stretch, Haseneen et al. [23] showed an increase in MMP-1, -2 and membrane type-1 MMP (which could be related to cell migration). Disruption of the cytoskeleton of fibroblasts increased MMP-1 expression [2]. The pathway responsible for this process is NF-κB dependent. It is noteworthy that this and other pathways related to MMP expression (for example, those related to p38 mitogen activated protein kinase [MAPK]) have also been implicated in the pathogenesis of VILI [24].

Different models of high pressure ventilation in animals have shown an increase in the expression of gelatinases in lung tissue and BAL fluid (MMP-9 and MMP-2, respectively) (Fig. 2). Moreover, this increase and the degree of lung injury can be attenuated by treatment with MMP inhibitors (either Prinomastat, a broad spectrum MMP inhibitor, or COL-3) [25, 26]. This protective effect was related to a decrease in neutrophil recruitment within the lungs, supporting the role of MMPs in cell migration.

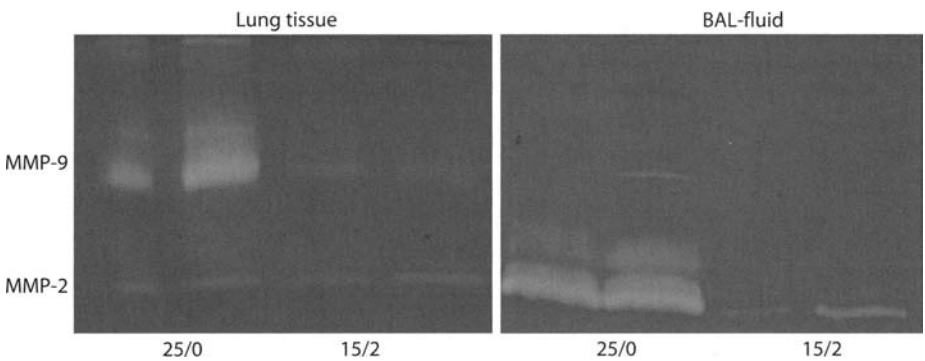

Fig. 2. Gelatin zymographies of lung tissue (left) and bronchoalveolar lavage (BAL) fluid (right) from mice ventilated using either high inspiratory pressures with zero PEEP (25/0 cmH_2O) or low inspiratory pressures and PEEP (15/2 cmH_2O). White bands, which represent gelatinolytic activity correspond to MMP-9 and MMP-2. Note the significant increase of MMP-9 in lung tissue and MMP-2 in BAL fluid after injurious ventilation.

Therapeutic Possibilities

Based on their effects in ALI, MMPs could be an interesting therapeutic target. There are different MMP inhibitors and some of them have been tested in clinical trials in cancer patients, but none has achieved a significant benefit. Although one can argue that cancer and ALI are different diseases, there could be some similarities in the reasons for this lack of benefit.

There are an increasing number of papers documenting a benefit of MMP inhibition (either using genetically modified animals or pharmacologic inhibitors) in ALI and VILI. Mice lacking MMP-9 have less lung injury in different models, ranging from endotoxin [27] to immune complexes [28], and a higher survival after high doses of endotoxin [29]. In a similar way, inhibition of gelatinases with drugs decreased lung injury after sepsis [11], pancreatitis [10], cardiopulmonary bypass [12], and high tidal volume ventilation [25, 26]. These results would support a trial using a MMP inhibitor (more specifically, against MMP-9) in patients with ALI.

However, MMP inhibitors are poorly selective. Results using MMP-8 deficient mice show that this MMP may have a dual effect. MMP-8 may inhibit hyperacute inflammation, as MMP-8 knockout mice have less neutrophil infiltration and enhanced survival after acute liver injury [30], and a delay in skin inflammation after injection of a mutagen [17]. However, these animals have a sustained inflammatory response over time, with an increase in BAL fluid neutrophils 24 hours after endotoxin administration [16] and, in the case of carcinogenesis, persistent skin inflammation and development of tumors [17]. It should be noted that MMP-8 and -9, which seem to have opposite effects (anti-inflammatory and pro-inflammatory, respectively), are both released from activated neutrophils.

These data show that we need to take into account the targets (specific enzymes) and the time frame when planning a therapeutic strategy with MMP inhibitors. It is possible that early inhibition of one MMP results in one effect, while late inhibition results in the opposite. Likewise, it seems unlikely that non-selective inhibition of different enzymes with antagonistic functions would result in a net beneficial effect. Before planning this kind of study, we need to acquire a deeper knowledge of the role and implications of MMPs in ALI.

Conclusion

MMPs are a family of enzymes involved in many of the pathophysiological responses after ALI and VILI, including modulation of the inflammatory response, cell chemotaxis, and turnover of the extracellular matrix. MMPs are also a therapeutic target in these diseases. Although non-selective inhibition of these enzymes has yielded interesting results in animal models, the diversity of enzymes, and the contrasting roles of some of them make this strategy difficult in the clinical arena. A deeper knowledge of their functions is needed, in order to define the precise therapeutic targets.

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The Role of Vascular Endothelial Growth Factor in Lung Injury and Repair

J. Varet and A.B. Millar

Introduction

Acute lung injury (ALI), along with its most severe form acute respiratory distress syndrome (ARDS), is one of the most challenging conditions in critical care medicine. ARDS continues to have a mortality of more than 35% despite improvements in ventilator strategies and management of sepsis [1]. Inflammation and increased vascular permeability are characteristics of ARDS. Vascular endothelial growth factor-A (VEGF-A) is a multi-functional cytokine known to play a pivotal role in angiogenesis and vascular permeability leading to interest in its potential role in ARDS. There is a body of work suggesting that VEGF plays a major role in lung development; however, it is expressed more highly in the healthy adult lung than any other organ suggesting a physiological role [2]. This apparent contradiction leads to controversy about the role of VEGF in ARDS.

Acute Lung Injury and Acute Respiratory Distress Syndrome

The characteristics of ARDS as first described by Ashbaugh et al. are well known [3]. A lack of stringency in the definition of this condition made it difficult to undertake comparative studies. In 1994, the American–European Consensus Conference Committee proposed the currently used definition of ARDS, which is not limited to adults. They described ARDS as a 'syndrome of inflammation and increased permeability' and suggested the term ALI to describe the continuum of pathological responses to pulmonary parenchymal injury. They defined ARDS as a severe form of ALI and a syndrome of acute pulmonary inflammation and resultant increased capillary endothelial permeability [4].

ARDS is characterized by inflammation and pulmonary edema, resulting from increased permeability of the alveolar-capillary membrane. The precise sequence of events occurring during ARDS is still unclear, however, several phases can be distinguished in the time course of the ARDS process. The earliest morphological abnormalities are injury to the lung microvascular endothelial cells and diffuse aggregation of polymorphonuclear leukocytes [5]. Activation of the leukocytes into the pulmonary interstitium causes lung injury resulting in plasma proteins leaking into the interstitium and the alveolar spaces. This acute or exudative early phase lasts only for a few hours. As early as 24 h afterwards, a proliferative phase starts, in which fibroblasts infiltrate and remodel the site of inflammation. After the acute phase, some patients will show a rapid resolution, however, other patients will develop a fibrotic response that results in consolidation and fibrosis of the pulmonary paren-

chyma in the late phase of ARDS, around day 5–7. Importantly, several studies have emphasized the critical importance of the degree of alveolar epithelial injury and its potential for repair in the pathogenesis and recovery from lung injury [5].

VEGF Biology

In mammals, the superfamily of VEGF proteins consists of five members: Placental growth factor (PlGF), VEGF-A, VEGF-B, VEGF-C, and VEGF-D, that are structurally homologous. However, there are molecular and functional diversities among these subtypes [6]. This chapter is confined to the importance of VEGF-A, termed VEGF throughout the text. VEGF is a dimeric 34–46 kDa glycoprotein, produced in various cell types: Cancer cells, inflammatory cells, fibroblasts, smooth muscle cells and epithelial cells. VEGF stimulates endothelial cell proliferation and is also a well-known pro survival factor for endothelial cells, inducing the expression of anti-apoptotic factors such as Bcl2 [7]. Importantly, VEGF mediates the secretion and activation of enzymes involved in matrix degradation. It also stimulates endothelial cell migration and their organization in capillary tubes *in vitro* and *in vivo*. VEGF critically regulates vasculogenesis such that embryos lacking a single VEGF allele have a lethal phenotype due to abnormal vascular development, including that of the lung [8]. In addition, VEGF increases microvascular permeability, up to 20,000 times more potently than histamine. This VEGF-increased vascular permeability also accounts for its active role in inflammation. It also stimulates arteriole vasodilatation via enhanced production of nitric oxide (NO). Although initially described as a specific growth factor for endothelial cells, targets for VEGF bioactivity outside the vascular endothelium have been discovered [6].

VEGF Isoforms

Alternate splicing of the VEGF transcript leads to the generation of several isoforms of differing sizes, the subscript relating to the number of amino acids present ($VEGF_{121}$, $VEGF_{145}$, $VEGF_{148}$, $VEGF_{165}$, $VEGF_{183}$, $VEGF_{189}$ and $VEGF_{206}$). $VEGF_{165}$ is physiologically the most abundant splice variant. $VEGF_{121}$, lacking exons 6 and 7, does not bind heparin sulfate and is freely diffusible. In contrast, the longer isoforms ($VEGF_{189}$ and $VEGF_{206}$) have heparin binding sites and are cell surface and extracellular matrix associated. $VEGF_{165}$ has intermediate properties [7]. Recently, a new family of VEGF isoforms has been identified, $VEGF_{xxx}b$, with differing amino acids in the exon 8 position, and some inhibitory properties [9].

VEGF Receptors and Co-receptors

VEGF isoforms bind to the tyrosine kinase receptors, VEGF receptor 1 (VEGF-R1 or flt1) and VEGF receptor 2 (VEGF-R2 or KDR or flk-1) [7]. Although VEGF affinity for VEGF-R1 is ten-fold higher than that for VEGF-R2, VEGF-R1 is a weak kinase compared to VEGF-R2 that exhibits a strong autophosphorylation in response to VEGF binding. This has lead to the hypothesis that VEGF-R1 may act as a decoy receptor, by preventing binding of VEGF to VEGF-R2. This hypothesis has been reinforced by the fact that PlGF, which only binds VEGF-R1, potentiates the effect of

VEGF by displacement of VEGF from VEGF-R1 binding. Further evidence of differing functions comes from knock out murine studies [6]. Several groups have reported crosstalk between VEGF-R1 and 2 [6]. Currently VEGF-R2 is regarded as the main signaling receptor for VEGF bioactivity, most evidence coming from endothelial cell studies.

The function and activity of the VEGF-R can be modulated by co-receptors. The binding of $VEGF_{165}$ to VEGF-R2 is enhanced by heparin and heparin amplifies signaling by $VEGF_{165}$ but not $VEGF_{121}$ [6]. In addition, VEGF can bind cell surface glycoproteins called neuropilin (NRP): NRP-1 and NRP-2. In contrast to the VEGF-R, the neuropilins bind VEGF in an isoform specific manner [6]. They are expressed by endothelial cells in many adult tissues but lack the intracellular component containing tyrosine kinase activity. NRP-1 is expressed mainly in arteries whereas NRP-2 is expressed on venous and lymphatic vessels. However, several studies have reported their presence on numerous other cell types [6]. NRP-1 binds $VEGF_{165}$ through its exon 7 and enhances the effect of $VEGF_{165}$ by increasing its binding to VEGF-R2 [10]. In addition, it has been shown recently that NP-1 is an essential mediator for $VEGF_{165}$-mediated endothelial cell permeability through VEGF-R2 in the lung vasculature [11]. These results may account for the permeability properties and greater mitogenic potency of $VEGF_{165}$ compared with the $VEGF_{121}$ isoform, unable to bind NP-1. NRP-2 can bind $VEGF_{165}$ and $VEGF_{145}$ but not $VEGF_{121}$. It has also been suggested that NRP-2 could interact with VEGF-R1.

Regulation of VEGF Bioactivity

Ubiquitous cell types produce VEGF, and hypoxia is currently regarded as the major factor inducing VEGF expression and is certainly the most widely studied. HIF-1 and HIF-2, the hypoxia inducible factors, are transcription factors regulating gene expression according to oxygen tension. HIF is composed of two subunits: HIF-β, a nuclear protein constitutively expressed and HIF-α, whose activity is oxygen dependent. There are mainly two possible subunits for HIF-α: 1α, which is ubiquitously expressed and 2α, notably expressed in endothelial cells and type II pneumocytes. Under hypoxic conditions, HIF-1α stability and bioactivity are increased and after heterodimerization with HIF-α it stimulates VEGF gene transcription by binding to the hypoxia-responsive element located in its promoter [12]. However, hypoxia can also stimulate VEGF expression by another mechanism, transcription independent. This has been described in retinal epithelial cells, where hypoxia increased VEGF mRNA stability [13]. Several growth factors have been involved in stimulation of VEGF production, such as platelet-derived growth factor, transforming growth factor (TGF) -α and -β, insulin like growth factor and keratinocyte growth factor. In addition, VEGF can be induced by reactive oxygen species (ROS), glucose deprivation, inflammatory cytokines, such as tumor necrosis factor-α (TNF-α), interleukin (IL)-6 and interferon (IFN) gamma and mechanical forces *per se* [14].

Another very important mechanism regulating VEGF activity is the splicing of VEGF RNA, leading to this diversity of isoforms [15]. However, the mechanisms by which splicing occurs and is regulated remain to be elucidated.

Post-translational control of VEGF activity has also been described. Proteolytic processing also regulates the bioactivity of VEGF. It has been demonstrated that native $VEGF_{189}$ requires maturation by urokinase to bind to VEGF-R2 and stimulate endothelial cell proliferation. In contrast, plasmin digestion of $VEGF_{165}$ yields an

amino-terminal homodimer (VEGF1-110) containing binding sites for VEGF-R1 and VEGF-R2, and a carboxyl-terminal fragment having a binding site for NRP. This VEGF1-110 exhibits a reduced mitogenic activity [16].

Regulation of VEGF Receptor and Co-receptor Activity

Hypoxia induces VEGF-R1 expression, whereas VEGF-R2 does not have hypoxia responsive elements in its promoter [17]. However, hypoxia could indirectly up regulate VEGF-R2 expression. Endothelial cell VEGF-R2 expression is increased by ischemia. TGF-β1 has been shown to decrease endothelial VEGF-R2 expression [18]. Conflicting results about TNF-α-regulated VEGF-R2 and NRP-1 expression in endothelial cells have been reported [19]. Like VEGF expression, VEGF-R expression could be modulated directly or indirectly by mechanical forces [14]. In macrophages, lipopolysaccharide (LPS) upregulates the expression of VEGF-R1 mRNA and increases specific binding for VEGF [20]. However, the mechanisms regulating VEGF-R and NRP expression remain largely to explore. Alternate splicing or proteolytic processing of VEGF-R gives rise respectively to soluble variants of VEGF-R1 (sflt1) or VEGF-R2. sflt1 lacks the cytoplasmic and membrane part of VEGF-R1, but it still has the same binding capacity to VEGF as VEGF-R1 and acts as an inhibitor of VEGF activity [7].

It is readily apparent from this brief review that VEGF bioactivity is complex as befits such a potent molecule, particularly in an organ such as the lung.

VEGF in the Lung

The main function of the lung is gas exchange. This function critically depends on the 'fine-tuning' between ventilation and perfusion. The integrity and functionality of the alveolar capillary barrier is, therefore, crucial. The normal alveolar barrier is composed of three different structures: The capillary endothelium, the interstitial space (basement membrane and the extracellular matrix), and the alveolar epithelium. The alveolar epithelium is constituted of alveolar epithelial type I and type II cells (respectively pneumocytes type 1 and 2 or AT1 and AT11). The AT1 are flat cells and cover more than 90% of the alveolar surface area. Their thin cytoplasm is optimized for respiratory gas exchange. The AT11 are cuboidal cells, located in the corners of the alveolar space, and they constitute about 60% of alveolar epithelial cells while they cover only about 5% of the alveolar surface in adult mammals. These AT11 have several functions: They secrete surfactant, are the progenitor cells of the alveolus, and possess the engineering required for active alveolar liquid clearance [21]. However, the lung is the organ where the highest concentrations of VEGF are found. Why is VEGF present in an organ where angiogenesis and vascular permeability are unusual? In healthy human subjects, VEGF protein is highly compartmentalized within the lung. The alveolar levels of VEGF are even 500 times higher than in plasma. It has been hypothesized that this could function as a physiological reservoir. VEGF would be slowly released across the alveolar epithelium to stimulate the lung microvascular endothelial cells, maintaining the integrity of the capillary structure. However, in case of alveolar injury, this spatial compartmentalization would lead to a strong induction of VEGF-stimulated endothelial cell permeability resulting in pulmonary edema [22].

The Epithelial Surface

In vitro studies have confirmed the abundant secretion of VEGF by human AT II [23]. In A549 cells (a tumor derived lung epithelial cell line), VEGF secretion is increased in response to LPS, neutrophil elastase, keratinocyte growth factor and TGF-β, and hypoxia [24, 25]. Moreover, hypoxia stimulates apical VEGF secretion by primary rat alveolar AT II *in vitro* and VEGF in bronchoalveolar lavage (BAL) *in vivo* [23].

In another study, rats exposed to hyperoxia showed a significant decrease in VEGF expression [26]. Distal airway epithelial cells from human fetal lung express VEGF-R2 and NRP-1. Interestingly, both cytoplasmic and nuclear staining of VEGF-R2 were detected in many of the distal airway epithelial cells [27]. Immunohistochemical studies in normal mice lungs have shown that AT I can weakly express VEGF-R1 but not VEGF-R2. In contrast, AT II display a strong expression of VEGF-R1 and a weak expression for VEGF-R2. Alveolar macrophages express VEGF-R1 and could occasionally express VEGF-R2 [28]. These results are in accordance with the work of Fehrenbach et al., done in rat lungs [29]. In addition, it has been shown by immunohistochemistry that, in the adult lungs, alveolar cells express NRP-1 [30].

The Endothelial Surface

Lung microvascular endothelial cells express VEGF-R1 and 2 and at least NRP-1. VEGF is well known for its ability to stimulate endothelial cell survival, proliferation and chemotaxis [6]. In addition, VEGF increases lung endothelial permeability, via a mechanism that strictly depends on the presence of NRP-1. Interestingly, NRP-1 enhances, but is not essential to, VEGF-induced cell proliferation and chemotaxis through VEGF-R2 [11].

Role of VEGF in the Alveolar Space

Pneumotrophic Effect of VEGF

It has been shown that VEGF is mitogen for human retinal pigment epithelial cells and is a survival factor for podocytes, cells involved in the glomerular capillary barrier [31, 32]. Trophic paracrine activity of VEGF has also been described in the liver, where VEGF stimulates the release of hepatocyte growth factor (HGF) by endothelial cells, promoting the growth of hepatocytes [33]. Therefore, the potential of VEGF as a pneumotrophic factor has been considered.

The tumor derived epithelial cell line, A549, expresses both VEGF-R1 and VEGF-R2. In an acid exposure model of injury *in vitro* leading to suppression of A549 proliferation and VEGF secretion, exogenous $VEGF_{165}$ is capable of restoring cellular proliferation. VEGF-R neutralizing antibodies of either VEGF-R1 and 2 suppressed proliferation of acid exposed A549 without altering control cell proliferation. These results suggest that healthy A549 cells are less dependent on VEGF for their proliferation than acid injured cells [34]. It has recently been shown that downregulation of either VEGF or VEGF-R1 by small interfering RNA (siRNA) in A549 cells reduced their proliferation and induced morphological changes [28]. These results suggest that VEGF may be a survival factor for the A549. However, it is still not clear if these effects occur via a direct autocrine pathway or indirectly via the stimulation of secretion of other growth factors by these same cells. Similar mechanisms have been observed in several other malignant cell types.

In non-cancerous models, it has been shown that exogenous VEGF acts as a growth factor on human fetal lung explants *in vitro*. This study suggests a possible role of VEGF as an epithelial cell growth factor [27]. In contrast, no such effect was detected in isolated fetal rat type 2 pneumocytes, one explanation being a paracrine rather than an autocrine role of VEGF [35].

In summary, there is conflicting and limited evidence regarding the pneumotrophic role of VEGF in the human lung.

Regulation of Surfactant Production

This also remains a controversial topic. Compernolle et al., showed that HIF-2a -/- mice developed neonatal fatal respiratory distress syndrome due to insufficient surfactant production by type 2 pneumocytes [36]. These mice had lower VEGF levels in alveolar cells than controls suggesting that HIF-2a primarily regulates VEGF expression in fetal type 2 pneumocytes and, therefore, VEGF might regulate surfactant production. Subsequently, VEGF directly increased the transcription of surfactant protein B and C, in cultured rat type 2 pneumocytes [36]. In contrast, Raoul et al., showed that VEGF could directly stimulate only surfactant B protein transcription, in fetal rat AE2 *in vitro* [35]. Brown et al., had reported enhanced surfactant protein A and C expression in fetal lung explants, but unchanged surfactant protein B expression [27]. Recently, a study on cultured ovine type II pneumocytes *in vitro* has reported no direct effect of VEGF on surfactant protein transcription [37]. Many of these contradictions may be related to differing species and experimental design.

Effect of VEGF on Alveolar Structure

Compernolle et al., have described HIF-2a -/- mice suffering from respiratory distress syndrome and demonstrated abnormal alveolar epithelium, attributable to impaired cellular differentiation [36]. In a model of prematurity, the same group showed that intra-uterine delivery of VEGF prevented development of respiratory distress syndrome.

Furthermore, intra-amniotically injected VEGF-R2 neutralizing antibody, remaining in the alveolar space, led to the development of respiratory distress. The beneficial effect of VEGF was associated with more normal alveolar septa through differentiation of alveolar cells, crucial for gas exchange, and inhibited by the addition of VEGF-R2. Similarly, mice with a deficiency of $VEGF_{164}$ or $VEGF_{188}$ isoform or of the HIF-binding site in the VEGF promoter died from respiratory distress syndrome. These data strongly suggest an essential role of VEGF ($_{164}$ or $_{188}$) in lung maturation [36]. However, Zeng et al., have shown that over-expression of VEGF targeted to the developing pulmonary epithelium in transgenic mice resulted in disruption of the lung branching morphogenesis and a lack of type I cell differentiation [37, 38]. These apparent discrepancies suggest that a tight regulation of the VEGF system in the alveolus is crucial to lung maturation and, therefore, possibly to lung repair.

VEGF in Lung Injury and Repair (Figure 1)

Lung Injury and the VEGF System

The main features of lung injury are inflammation and vascular leakage. Since VEGF strongly stimulates microvascular endothelial cell permeability and is an inflamma-

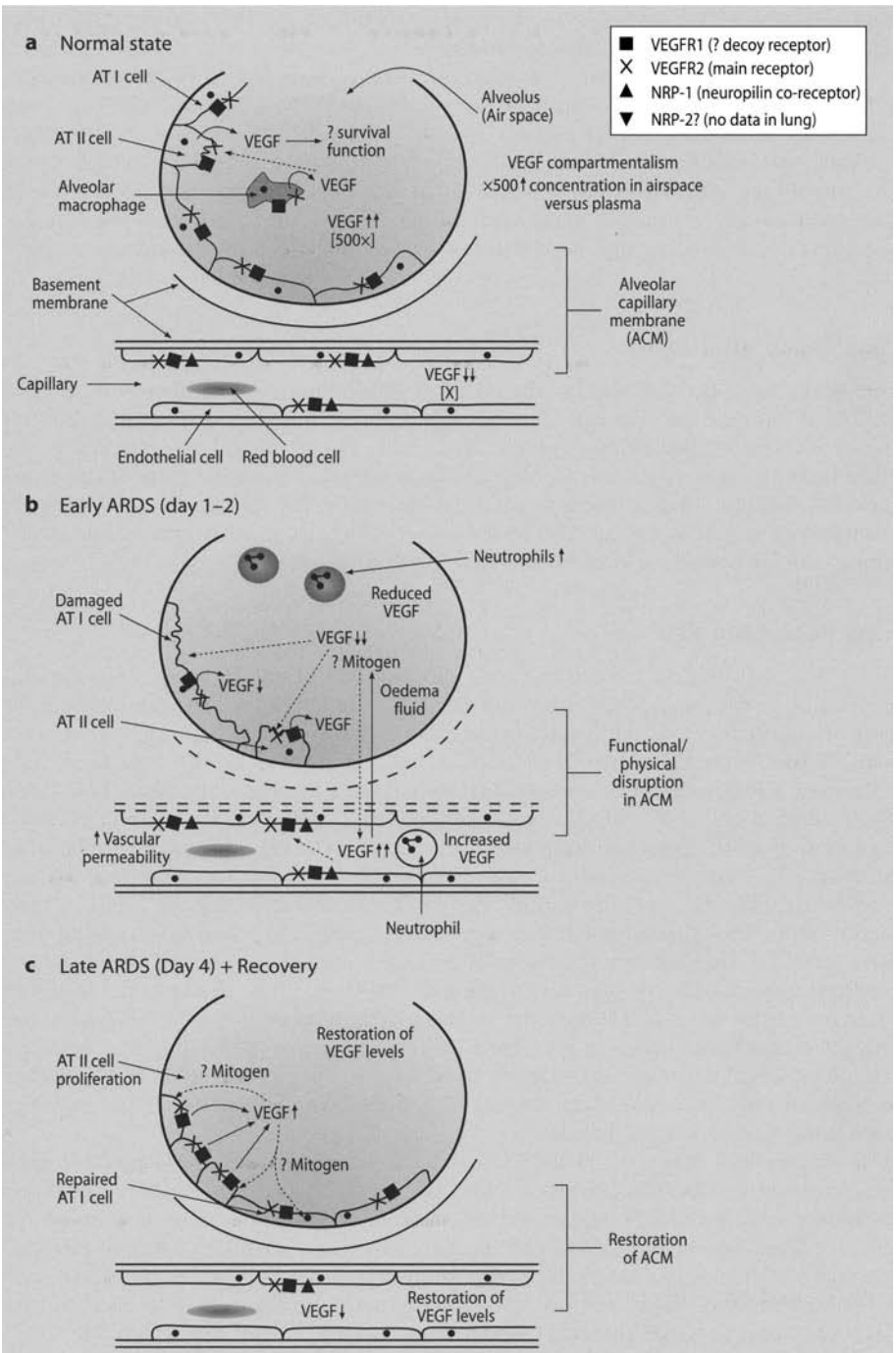

Fig. 1. Diagrammatic representation of the human alveolar capillary unit in (**a**) normal health subjects, (**b**) subjects with lung injury, and (**c**), during the recovery phase. From [48].

tory mediator (via its monocyte chemoatactic effects), its role during lung injury has been the focus of much investigation.

Adenoviral $VEGF_{165}$ delivery to murine neonatal lung led to pulmonary edema and increased pulmonary capillary permeability [39]. Similarly, $VEGF_{164}$ over-expressing mice, targeted to respiratory epithelial cells, demonstrated pulmonary hemorrhage, endothelial destruction, and alveolar remodelling in an emphysema-like phenotype [40]. However, a recent study has demonstrated that NRP-1 inhibition reduced VEGF-induced permeability [11]. This result emphasizes the fact that the effects of VEGF are tightly modulated by the specific combination of its receptors.

Lung Repair after Injury

One of the main determinants of the outcome of lung injury is the degree of alveolar epithelial injury. After damage of the alveolar barrier, the proliferative phase of lung injury is characterized by hyperplastic AT II cells. The AT II cells migrate and proliferate trying to restore epithelial integrity. This is crucial since integrity of the alveolar epithelium is essential for alveolar fluid clearance, the AT II cells possessing the engineering required for active ion transport. Integrity of this epithelium is also important for surfactant metabolism and for immune functions [4].

Lung Repair and VEGF

VEGF has been widely studied in repair mechanisms of organs other than the lung in relation to its angiogenic effects. In the lung the necessity for close approximation of endothelial and epithelial surfaces can be considered in an angiogenic context. It has been described that after hyperoxia injury, in the rat, there is a decreased VEGF expression in the lung associated with apoptotic endothelial cells and epithelial cells [26]. Inhibition of angiogenesis and specific blocking of VEGF signaling leads to abnormal lung structure in rats [41–43]. In contrast, hyperoxia-induced lung damage in newborn rats is rescued by intratracheal adenovirus-mediated VEGF [43]. In a similar model, the intramuscular injection of $VEGF_{165}$ transiently worsened the lung edema, but subsequently improved recovery of lung structure [44]. In adult rats treated with a VEGF-R blocker, increased apoptosis and emphysematous like changes were found in the lung [45]. Finally, in transgenic mice over-expressing IL-13 exposure to hyperoxia reduces lung injury. This protective effect has been linked to increased VEGF production in the lung and administration of VEGF neutralizing antibody decreases this protective effect of IL-13 over-expression [46]. All these data suggest a complex crosstalk between the endothelium and the alveolar epithelium.

If we consider human data then it has been shown that in ARDS patients there are increased numbers of apoptotic endothelial cells and a reduced endothelial area compared to controls [47]. Moreover, in several models of lung injury decreased pulmonary levels of VEGF are observed compared to control, with recovery of intrapulmonary VEGF levels to pre-injury levels following recovery [48]. Several other studies of lung injury in humans also describe a reduction in free VEGF levels in epithelial lining fluid in ARDS patients compared to controls, in addition to similar reductions in intrapulmonary VEGF levels in other forms of lung injury [49, 50]. All these data suggest that impaired VEGF expression or bioactivity could lead to endothelial cell loss and, therefore, might compromise alveolar repair. Since the major source of

VEGF in the lung is the alveolar epithelium, this adds to the concept that the degree of epithelial injury is determinant for the outcome of the disorder. However, a tight regulation of the VEGF system is certainly involved in this positive feedback between epithelial and endothelial cells.

Conclusion

VEGF is a multi-functional growth factor. Several isoforms and receptors of VEGF have been described with potential to regulate its bioactivity. The normal lung expresses high concentrations of VEGF and has got a strong compartmentalization of the growth factor in the alveolar space. VEGF is well recognized to be angiogenic and increase permeability of capillary endothelial cells leading to interest in its role in ALI/ARDS, characterized by a disruption of the alveolar capillary membrane and an inflammatory reaction. Furthermore, the degree of epithelial injury appears to be crucial to the outcome of the disorder. However, the precise role of VEGF in normal lung function and during lung injury and repair remain unclear. Increased understanding of both the complex biology of the VEGF family and crosstalk between the cellular constituents within the lung are needed to establish the role of VEGF.

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Cell Regeneration in Lung Injury

Z. Bromberg, C.S. Deutschman, and Y.G. Weiss

The acute respiratory distress syndrome (ARDS) is a lethal inflammatory disorder of the lung. Its incidence is estimated at 75 cases per 100,000 population and appears to be increasing [1]. Even with optimal treatment, mortality is about 30% [1–3]. As such, ARDS represents a major public health problem. The effects of two recent crises created by unusual viral infections of the respiratory tract – the severe acute respiratory syndrome (SARS) epidemic caused by the novel SARS coronavirus [4, 5] and the bird flu [6] highlight the importance of research into ARDS. Both viruses cause an ARDS-like picture. Because lung repair and regeneration contribute substantially to the pathophysiology of ARDS, understanding these processes is essential [7]. This chapter focuses on specific cell populations and markers involved in cell division and regeneration. In addition, a brief review of two pathways intimately associated with cell division is provided because of their potential for pharmacologic manipulation.

The Alveolar Epithelium in Acute Lung Injury

ARDS is primarily a disease of disordered inflammation. Early ARDS is characterized by increased inflammation where alveolar epithelial cells are damaged and ultimately may be destroyed [1–3]. While some mechanisms contributing to the pathophysiology of ARDS have been identified, most are poorly understood. As a result, treatment is largely supportive. A better understanding of the fundamental biological changes leading to ARDS would be of scientific and therapeutic value. The magnitude of injury to the alveolar epithelial barrier is one of the most important determinants of the severity of lung injury [8]. Similarly, early repair of epithelial injury may be a major determinant of recovery. Most recent therapeutic approaches were developed to attenuate pulmonary inflammation and thus minimize the initial injury [8]. Unfortunately, specific interventions to accelerate alveolar epithelial repair do not exist. This reflects our limited understanding of the cellular mechanisms that modulate alveolar epithelial repair in ARDS.

Histological sections from patients dying of ARDS and from animal models of the disease demonstrate that the first abnormality is interstitial edema. This is followed by severe damage that is characterized primarily by extensive necrosis of alveolar type I (ATI) cells [9]. Pathological examination of lung tissue from patients with SARS was similar to changes seen in established ARDS. This included diffuse alveolar damage, desquamated epithelial cells, ATII hyperplasia, fibrin and collagen deposition in the alveolar spaces, and a loss of the normal barrier crucial for gas exchange [5, 10–13]

Cell regeneration is a fundamental biological response to cell damage. Through adult life, multicellular organisms must generate new cells to maintain the structure and function of their tissues [14]. This is especially important in the lung. The adult lung is a vital and complex organ that normally turns over slowly. Nevertheless, it is able to respond to specific injuries that mimic damage caused by environmental or infectious agents [15]. In most cases, pulmonary injury predominantly affects ATI cells. These highly differentiated and flat cells facilitate gas exchange. In contrast, the cuboidal, metabolically-active ATII cells that produce surfactant and other products essential to pulmonary function may be relatively spared. Following injury, regeneration of alveolar epithelial cells proceeds via an organized paradigm where ATII cells and other specific stem cells appear to function as progenitor cells for ATI cells [16–17] (Fig. 1). Most research in ARDS has focused on the finding that ATII cells

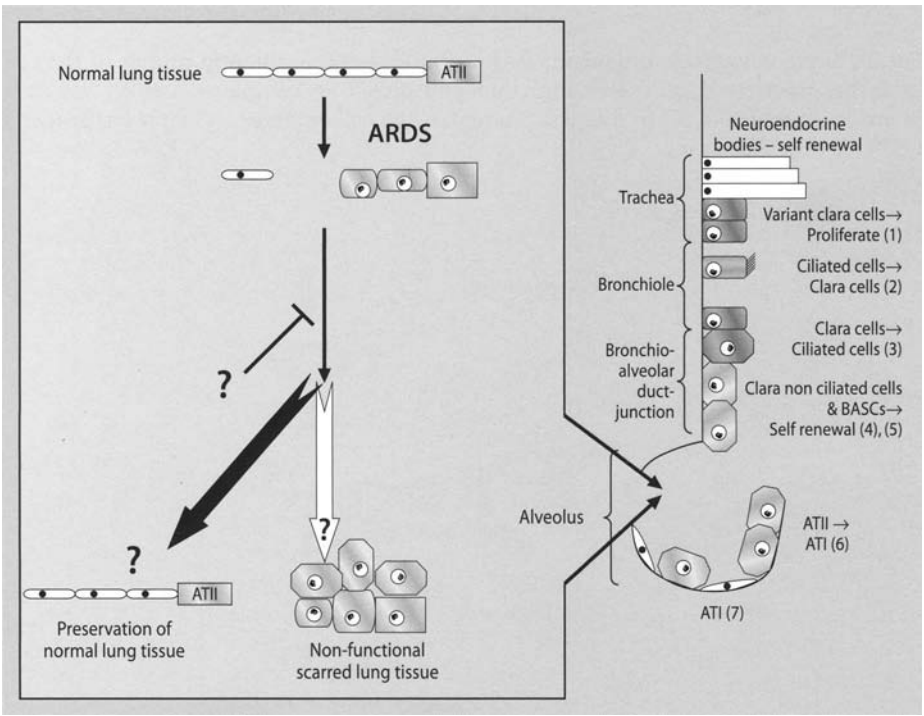

Fig. 1. Right panel: Cell populations participating in lung regeneration. In the trachea, variant Clara cells (1) can be found adjacent to neuroendocrine bodies. Within proximal bronchioles, two types of cells can transdifferentiate. Ciliated cells may proliferate and transdifferentiate into Clara cells after injury (2) while Clara cells may proliferate after injury and give rise to ciliated cells (3). At the bronchioalveolar duct junction (BADJ) between the conducting and respiratory epithelium, columnar Clara cells (4) can serve as progenitors. A sub-population of Clara cells termed bronchioalveolar stem cells (BASCs) (5), retains features of stem cells and may also participate in lung repair. In the alveolus, ATII cells (6) give rise to ATI cells (7) after injury. Left Panel: Following lung injury ATII cells may reenter the cell cycle, differentiate into ATI cells and spread along alveolar septa. This results in coverage of denuded basement membrane and re-establishment of epithelial continuity. In severe inflammation or pulmonary fibrosis, however, proliferation of ATII cells may become excessive. This can prevent appropriate replacement of ATI cells and lead to fibrosis and scarring. The precise control mechanisms and pathways involved in these processes are unknown. Modified from [14] with permission

reenter the cell cycle, differentiate into ATI cells and spread along alveolar septa. This results in coverage of denuded basement membrane and re-establishment of epithelial continuity [16, 18, 19]. In severe inflammation or pulmonary fibrosis, however, proliferation of ATII cells may become excessive (Fig 1). This can prevent appropriate replacement of ATI cells and lead to fibrosis and scarring [19, 20]. In such a situation, the fibrinous alveolar exudate characteristic of acute lung injury (ALI) will be covered by the migrating ATII cells. This transforms the intra-alveolar debris into interstitial tissue and stimulates fibrosis [21–23]. The significant morbidity and mortality associated with these pathological changes accentuates the importance of deciphering the mechanisms involved in cell division, repair and differentiation.

The Cell Cycle

For all living eukaryotic organisms it is essential that the different phases of the cell cycle be precisely coordinated and that one phase be completed before the next phase is entered (Fig 2). In the first phase, G1, the cell enlarges. When it has reached

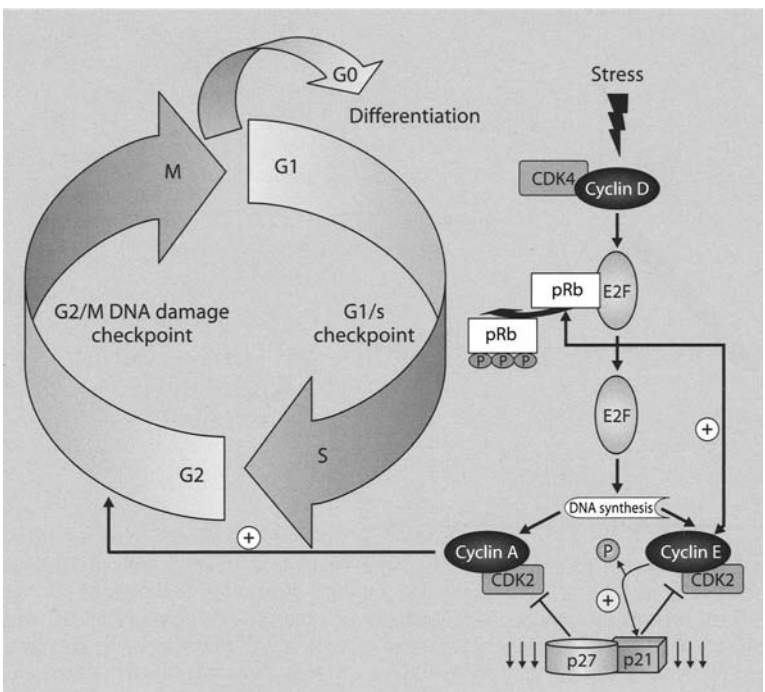

Fig. 2. The E2F-retinoblastoma (Rb) cell signaling pathway controlling the G1/S restriction point of proliferating cells. Passage through the restriction point and transition to S phase is triggered by the activation of the cyclin D1/cdk4 complex. This phosphorylates Rb. Phosphorylated Rb dissociates from E2F and is degraded further by the proteasome. E2F binds to the chromosome and initiates DNA replication. Cyclin E/cdk2 accumulates during late G phase and triggers passage into S phase. The entire genome is replicated during the S phase. Cyclin A/cdk2 accumulates during S phase and activates transition to the G2 phase. This results in inhibition of DNA replication, cell growth and new protein synthesis

a certain size, it enters S phase, in which DNA is replicated. This is followed by the G2 phase, where there is an internal check to assure that DNA-replication is completed and that the cell is prepared to divide. Finally, in the mitosis or M phase, chromosomes separate and cell division occurs. After M phase, most cells exit the cell cycle and enter a resting stage [G0]. However, some re-enter the cycle and remain in the G1 phase for a prolonged period, awaiting a signal to proceed on to the S phase. This resting point in the G1 phase is often referred to as the 'G1 restriction point'. Cell division is initiated when the integration of diverse metabolic, stress and environmental signals stimulate a transition past the G1 restriction point and facilitate entry into S phase [24].

Several pathways control pulmonary cell replication at the G1 restriction point. We will briefly describe two major pathways: E2F-retinoblastoma (Rb) and Wnt/β-catenin. These pathways may prove to be important sites for future pharmacological interventions.

The E2F-Rb pathway

The E2F-Rb pathway is critical in controlling progression beyond the G1 restriction point (Fig. 2) [25, 26]. Passage through the restriction point and transition to S phase is triggered by the activation of the cyclin D1/cdk4 complex that phosphorylates Rb. Phosphorylated Rb dissociates from E2F. E2F binds to the chromosome and initiates DNA replication. Cyclin E/cdk2 accumulates during late G phase and triggers the passage into S phase. The entire genome is replicated during S phase. Cyclin A/cdk2 accumulates during S phase and its activation triggers the transition to G2, a phase characterized by the accumulation of cyclin B/cdc2, which results in the inhibition of DNA replication, cell growth and new protein synthesis [26, 27]

The Wnt/βcatenin Cell Signaling Pathway

The Wnt/β catenin cell signaling pathway has been shown to be fundamental for cell division, regeneration, and differentiation processes [28]. Within this pathway, β-catenin is a key effector of the Wnt signaling pathway (Fig. 3), and persists as an important regulator of homeostasis in adult self-renewing tissues. β-catenin has been shown to participate in signal transduction in epithelial cells. Specifically, activation of β-catenin results in a loss of differentiation and trans-differentiation of mammary epithelial cells into epidermis-like structure [29]. Others have shown that the Wnt/β-catenin cell signaling pathway is activated in idiopathic pulmonary fibrosis [30]. Further, β-catenin has been shown to regulate differentiation of respiratory epithelial cells *in vivo*. An activated form of β-catenin was expressed in respiratory epithelial cells of the developing lung. Activation of β-catenin caused ectopic differentiation of ATII-like cells in conducting airways, goblet cell hyperplasia, and airspace enlargement, demonstrating a critical role for the Wnt/β-catenin signal transduction pathway in the differentiation of the respiratory epithelium in the postnatal lung [31].

Cell Populations Participating in Lung Regeneration

Stem cells are cells capable of limited self-renewal. They can develop into more differentiated cell types [32]. Stem cell turnover is relatively slow, allowing them to act

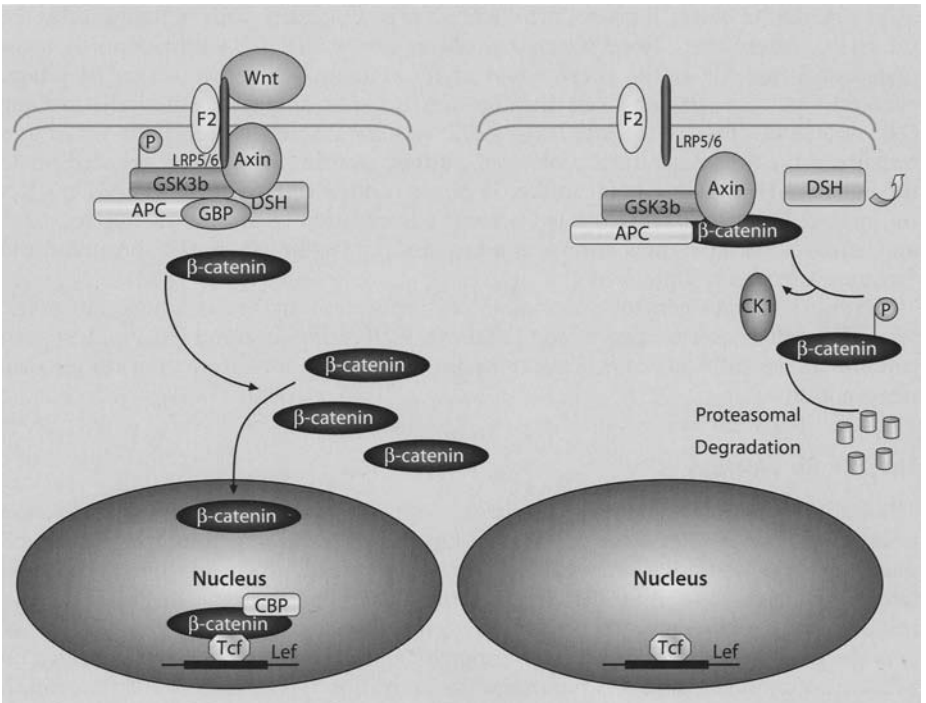

Fig. 3. Cell replication at the G1 restriction point-Wnt/β-Catenin cell signaling pathway. Left panel: Wnt-stimulation leads to reduction of GSK-3β kinase activity via phosphorylation. As a result, β-catenin is retained in the cytoplasm. Once β-catenin accumulates it can further translocate to the nucleus. In the nucleus, β-catenin binds to Tcf/Lef and acts as a co-activator to stimulate transcription of target genes such as *c-myc* and Cyclin D1. This facilitates cell proliferation. Right panel: in the absence of Wnt activation, *Dsh*, through its receptor *frizzled*, causes GSK-3β dissociation from Axin. Axin and adenomatous polyposis coli (APC) gene products serve as a scaffolding for phosphorylation of β-catenin by the enzyme GSK-3β. The phosphorylated form of β-catenin is targeted for ubiquitination and proteasomal degradation. This prevents transcription of β-catenin target genes.

as a source for differentiated cells throughout the lifespan of the organism [33]. Embryonic stem cells are divided from the inner cell mass of the blastocyte and are considered 'totipotent' in that they can regenerate all three germ layers of an organism. In contrast, adult stem cells are considered multi-or 'unipotent', able to give rise to one or several mature cell types [33, 34]. Two major categories of tightly regulated adult stem cells have been described: The 'dedicated' stem cells capable of long term self renewal and the transient amplifying (TA) daughter cells characterized by a high rate of proliferation. TA cells can self-renew over a short period [14, 35]. In addition, adult stem cells, called 'progenitor cells', are found in a number of adult tissues, including the lungs [33, 34, 36, 37], where constant exposure to potential toxic agents and pathogens in the environment may require that cells regenerate rapidly and effectively. These progenitor cells are patterned very early in embryogenesis [33, 34]. There is evidence that some differentiated epithelial cell types can act as progenitor cells and proliferate and 'transdifferentiate' in response to specific conditions [14].

The pulmonary tree contains cells with potential stem cell properties in distinct anatomical regions of the respiratory tree and lung [38, 39]. These include the submucosal gland ducts and intercartilagenous region of the tracheobronchial tree, neuroepithelial bodies in the bronchioloes, and the bronchoalveolar duct junctions [38, 39]. In the trachea and bronchioles, secretory progenitor cells can be found. Immunostaining for the nuclear proliferative marker, Ki67, expressed in proliferating cells, has been shown in human proximal airways to correlate with the most highly proliferative cells [40]. Within the proximal area, ciliated Clara cells are present in small numbers adjacent to neuroendocrine bodies. Non-ciliated, columnar Clara cells located at the junction between the conducting and respiratory epithelium (bronchioalveolar duct junction [BADJ]) label with bromodeoxyuridine (BrdU, a thymidine analog incorporated into DNA during the S phase). Such label retaining cells could repair the tracheal airway epithelium after polidocanol detergent or inhaled SO_2 injury [36]. Another mouse model of lung injury using naphthalene inhalation resulted in loss of most of Clara cells of the BADJ area. However, these cells can be divided into two distinct populations, based on their susceptibility to naphthalene injury [39]. One sub-population of Clara cells retains features of stem cells. This regional pulmonary stem cell population was termed bronchioalveolar stem cells (BASCs) [39]. These cells, identified by a dual expression of the Clara cell secretory protein (CCSP) and surfactant protein-C (SP-C) [39], are resistant to bronchiolar and alveolar damage and proliferate during epithelial cell renewal.

Circulating progenitor cells also may have a role in lung repair. Recently, one distinct population of blood-borne, mesanchymal stem cells was found to be associated with engraftment of donor derived ATII cells [41]. Thus use of exogenous cells to supplement the regenerative process in the lung may be feasible.

Type II pneumocytes also may function as stem cells. ATII cells have been shown to self renew and to give rise to ATI cells after lung injury [32, 42]. This ATII progenitor function may depend on the nature of the airway injury and the microenvironment [32, 42]. Specifically, there appear to be two subpopulations of ATII cells. These are distinguished by expression of a specific marker. Hyperoxic injury in rats induces expression of E-cadherin in some ATII cells [43]. This E-cadherin positive subpopulation has minimal levels of telomerase activity, indicating a low proliferative index. In contrast, the E-cadherin negative subpopulation expresses high levels of telomerase activity and proliferates well in culture. Several well differentiated cell lines in the lung can undergo 'transdifferentiation'; these include the Clara cells that differentiate into ciliated cells and the ATII cells that differentiate into ATI cells.

Cellular Markers and Factors Regulating Lung Epithelial Repair

Accumulated evidence suggests that epidermal growth factor (EGF), transforming growth factor-β (TGF-β) and the related receptor, epidermal growth factor receptor (EGFR), may regulate epithelial repair *in vivo* and *in vitro*. TGF-β is elevated in pulmonary edema fluid from patients with ARDS and has been shown to induce alveolar epithelial repair *in vitro* [8]. Other studies have reported an increased concentration of cytokines (tumor necrosis factor [TNF]-α, interleukin [IL]-6, IL-8, and IL-10) in the broncholaveolar lavage (BAL) fluid of patients with acute phase ARDS [44]. Among the cytokines implicated in lung fibrosis, TNF-β, a multifactorial peptide capable of enhancing mesenchymal cell proliferation and extracellular matrix synthesis [45], plays a fundamental role. The presence of receptors for this protein

after lung injury may contribute to the upregulation of TGF-β expression [46]. Additionally, TGF-β has been associated with the pathogenesis of pulmonary fibrosis [45].

A variety of pro-inflammatory cytokines have been shown to upregulate keratinocyte growth factor (KGF) and hepatocyte growth factor (HGF). The roles of these proteins have been investigated widely and it appears that they play an important role in both normal lung development and in injured lung repair. Indeed, KGF and HGF may have therapeutic potential in lung disease. Endogenous KGF plays an important role in epithelial repair. Studies in animal models of hyperoxia, demonstrated a 12-fold increase in KGF mRNA [47]. This increase was followed by increased ATII cell proliferation, suggesting that KGF stimulates ATII hyperplasia [47]. Endogenous HGF from both bronchial epithelial cells and alveolar macrophage participates in the reparative response to lung injury [48]. It is of interest that the lung may be a source of HGF after injury to other organs. Six hours after partial hepatectomy, HGF levels within the lung were increased [49]. Similar elevations in lung, liver, and kidney were noted in acute pancreatitis [49]. These findings suggest that the lungs serve as an endocrine organ, contributing to organ repair and regeneration by excreting HGF [50]. A feedback mechanism may be operative as ATII cells express the c-met receptor for HGF [50].

IL-6 plays a key role in liver regeneration [51]. For example, this cytokine appears to initiate HGF synthesis [52]. Absence of IL-6 has been associated with failed regeneration in septic liver injury [53]. IL-6 is elevated in lung injury and also may impact on repair mechanisms in chronic pulmonary inflammatory disorders. Previously published studies have examined the role of IL-6 on proliferation and cell-cycle kinetics in primary human lung fibroblasts obtained from patients with idiopathic pulmonary fibrosis. IL-6 was mitogenic for idiopathic pulmonary fibrosis fibroblasts. This effect appears to involve a sustained activation of mitogen-activated protein kinase (MAPK) that, in turn, inhibited the production of $p27^{Kip1}$. This allowed activation of cyclin D_1 and hyperphosphorylation of Rb protein [54] (Fig 2). In an ozone/cigarette smoke model of lung injury, BrdU labeling within terminal bronchiolar epithelium and proximal alveolar regions was significantly reduced in IL-6 knowck-out mice compared to IL-6 sufficient mice. Further, CCSP abundance was markedly reduced in the terminal bronchiolar epithelium of these IL-6 knock-out mice [55].

Pulmonary surfactant forms the surface-active film that is crucial for normal lung function. This substance consist of complexes of phospholipids and four protein components known as surfactant-associated proteins [1, 56]. Among them, SP-A has important autocrine effects on cells of the lung epithelium. ATII cells produce and secrete pulmonary surfactant proteins. SP-A signals through an ATII cell surface receptor and regulates anti-apoptotic gene expression. Hence, surfactant proteins may represent a local regulatory system for cell regeneration.

Conclusion

In ARDS, cell proliferation may be either beneficial or detrimental (Fig. 1). Early in the disease process, when loss of pulmonary epithelial cells may contribute to pathology, enhancing cell division may be of value. However, cell division also may increase vulnerability to oxidative stress-induced DNA damage. In contrast, in the fibroproliferative phase of the disease, cell overgrowth contributes to pathological

scarring and fibrosis. Hence, increased knowledge on the mechanisms and pathways of cell division and regeneration may stimulate the development of novel pharmacological interventions. Due to the complex nature of the mechanisms and time course involved in the pathophysiology of ARDS, understanding of the role of ARDS-associated cellular proliferation is essential.

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The Extracellular Matrix of the Lung: The Forgotten Friend!

P. Pelosi, P. Severgnini, and P.R. Rocco

Introduction

The extracellular matrix represents the three-dimensional scaffold of the alveolar wall, which is composed of a layer of epithelial and endothelial cells, their basement membrane, and a thin layer of interstitial space lying between the capillary endothelium and the alveolar epithelium [1]. In the segment where the epithelial and endothelial basement membranes are not fused, the interstitium is composed of cells, a macromolecular fibrous component, and the fluid phase of the extracellular matrix, functioning as a three dimensional mechanical scaffold characterized by a fibrous mesh consisting mainly of collagen types I and III, which provides tensile strength, and elastin conveying an elastic recoil [2, 3]. The three-dimensional fiber mesh is filled with other macromolecules, mainly glycosaminoglycans (GAGs), which are the major components of the non-fibrillar compartment of the interstitium [4]. In the lung, the extracellular matrix plays several roles, providing: a) mechanical tensile and compressive strength and elasticity; b) a low mechanical tissue compliance, thus contributing to the maintenance of normal interstitial fluid dynamics [5]; c) low resistive pathway for effective gas exchange [2]; d) control of cell behavior by binding of growth factors, chemokines, cytokines, and interaction with cell-surface receptors [6].

Study of the extracellular matrix is important to improve: 1) pathophysiological knowledge about the development of edema and specific interstitial lung diseases; 2) early diagnosis of extracellular matrix alterations and lung remodeling processes; and 3) ventilatory and pharmaceutical therapeutic strategies.

Organization of the Extracellular Matrix

The extracellular matrix is not only a scaffold, having a mechanical role in supporting and maintaining tissue structure, but also a complex and dynamic meshwork influencing many biological cell functions such as development, migration, and proliferation. Molecules of the extracellular matrix include fibrous proteins (collagen and elastin) and structural or adhesive proteins (fibronectin and laminin) embedded in a hydrated polysaccharide gel containing several GAGs (Fig. 1).

Collagen

Collagen fibers constitute the main component of the extracellular matrix. Collagen is a fibrous protein that consists of three α-chains, which form a rope-like triple

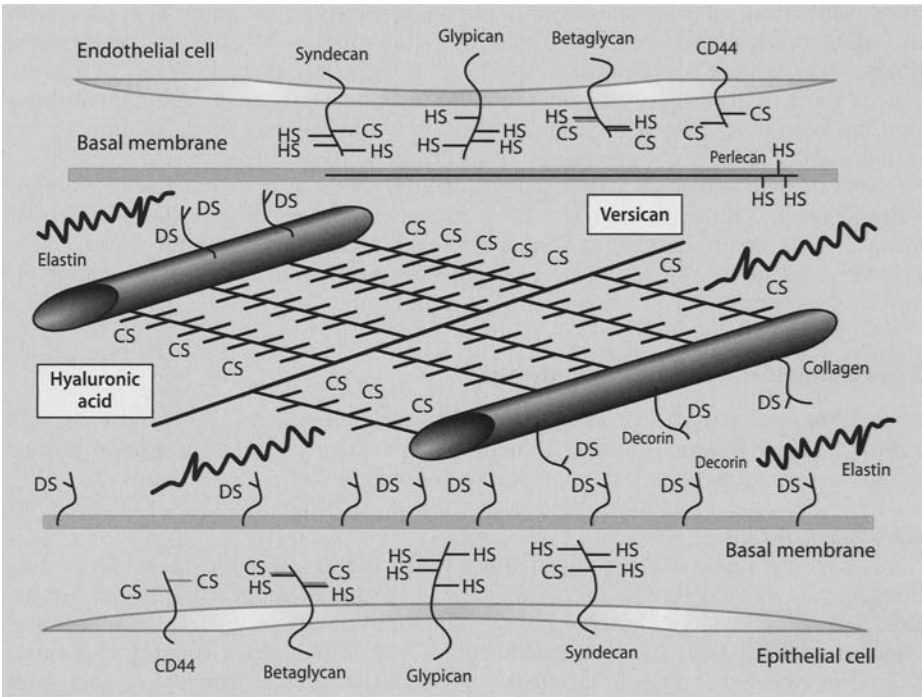

Fig. 1. Extracellular matrix components in the lung parenchyma. CS: Chondroitin sulphate, HS: Heparan sulphate; DS: Dermatan sulphate

helix, providing tensile strength to the extracellular matrix. α chains contain glycine, praline, and hydroxyproline. The synthesis of collagen molecules begins on the rough endoplasmatic reticulum. The pro-α-chains are made on the rough endoplasmatic reticulum, and are hydroxylated and glycosylated in the Golgi. Procollagen forms three α-chains, and possesses terminal 'propeptides'. This procollagen is then secreted from vesicles, and undergoes proteolysis at its ends in the extracellular space, to form mature 100 nm long collagen molecules. Collagen molecules are then crosslinked into fibrils, which link molecules together. Despite their broad diversity in the connective tissue, types I, II, III (fibrillar), and IV, V, VI (non-fibrillar or amorphous) represent the main collagen molecules. The turnover of the collagen fibers is a dynamic process, necessary to the maintenance of normal lung architecture [7]. The final collagen accumulation does not depend only on its synthesis, but also on its degradation [3]. Consequently, the extracellular matrix is a dynamic structure, and equilibrium between synthesis and degradation of extracellular matrix components is required for the maintenance of its homeostasis [8].

Elastin

Elastic fibers represent another component of the extracellular matrix. Elastic fibers comprise three components defined according to the amount of elastin and fibril orientation: 1) oxytalan fiber composed of a bundle of microfibrils; 2) elaunin fibres made up of microfibrils and a small amount of elastin; and 3) fully developed elastic

fibers consisting of microfibrils and abundant elastin [9]. Many types of cells, including condroblasts, myofibroblasts, and smooth muscle cells synthesize these fibers. Due to their mechanical properties, elastic fibers provide recoil tension to restore the parenchyma to its previous configuration after the stimulus for inspiration has ceased. In normal alveolar septa, a subepithelial layer of elastic fibers composed mainly of fully mature elastic fibers, confers a great elasticity to the alveolar tissue in normal situations [10]. Elastosis could be a result of repair and remodeling following septal inflammation and fiber fragmentation yielding to derangement in alveolar wall architecture [11]. Thus, the elastic component of the extracellular matrix could be one of the structures potentially involved in alveolar remodeling and in the biomechanical behavior of the lung tissue.

Glycosaminoglycans and Proteoglycans

In the connective tissue, proteoglycans form a gelatinous and hydrated substance embedding the fibrous proteins. Proteoglycans are comprised of a central protein bound to one or more polysaccharides, denominated GAGs.

Glycosaminoglycans

GAGs are long, linear and heterogeneous polysaccharides, which consist of repeating disaccharide units with sequences that vary in the basic composition of the saccharide, linkage, acetylation, and N- and O-sulphation: galactose, galactosamine, *N*-acetylgalactosamine-4-sulfate, and galacturonic acid. Their chain lengths can range from 1 to 25,000 disaccharide units, the molecular weights of which vary over three orders of magnitude, implying that the polymer chains can contain as many as 10^4 units with a huge variability in size and structure. There are two main types of GAG: 1) Non-sulphated GAG (hyaluronic acid), and 2) sulphated GAGs (heparan sulphate and heparin, chondroitin sulphate, dermatan sulphate, and keratan sulphate). With the exception of hyaluronic acid, GAGs are usually covalently attached to a protein core, forming an overall structure referred to as proteoglycans.

Hyaluronic acid: Hyaluronic acid is the most abundant non-sulphated GAG in the lung extracellular matrix. Hyaluronic acid differs from the other GAGs because: 1) it is spun out from the cell membrane, rather than being secreted through the Golgi, and 2) it is enormous (10^7 Da – much larger than other GAGs). Hyaluronic acid is a naturally occurring, linear polysaccharide composed of up to 10,000 disaccharides constituted by a uronic acid residue covalently linked to an N-acetyl-glucosamine, with a flexible and coiled configuration. Hyaluronic acid is a ubiquitous molecule of the connective tissue that is primarily synthesized by mesenchymal cells. It is a necessary molecule for the assembly of connective tissue matrix and an important stabilizing constituent of loose connective tissue. A unique characteristic of hyaluronic acid, which relates to its variable functions, is its high anion charge that attracts a very large solvation volume, which makes hyaluronic acid an important determinant of tissue hydration. Excessive accumulation of hyaluronic acid in the interstitial tissue might, therefore, immobilize water and behaves as a regulator of the amount of water in the interstitium [12]. Hyaluronic acid is present in the extracellular matrix, on the cell surface, and inside the cell and its functions are related to its localization [13]. Hyaluronic acid is also involved in several other functions, such as tissue repair [14] and protection against infections and proteolytic granulocyte enzymes [15].

Sulphated glycosaminoglycans: These other GAGs are synthesized intracellularly, sulphated, secreted, and usually covalently bound into proteoglycans. They are sulphated polysaccharides made of repeating disaccharides, which consist of uronic acid (or galactose) and hexosamines. The proteoglycan core proteins may also link carbohydrate units including O-linked and N-linked oligosaccharides, as found in other glycosylated proteins. The polyanionic nature of GAGs is the main determinant of the physical properties of proteoglycan molecules, allowing them to resist compressive forces and to simultaneously maintain tissue hydration. They are much smaller than hyaluronic acid, usually only 20 to 200 sugar residues long [16].

Within the lung parenchyma, the most abundant sulphated GAG is heparan sulphate, a polysaccharide that is expressed on virtually every cell in the body and comprises 50% to 90% of the total endothelial proteoglycan [17]. Heparan sulphate has the highest variable structure, largely because of variations in the sulphation patterns of its chains. In addition to sequence diversity, its size ranges from 5–70 kDa. Although it is initially produced in a cell-surface-bound form, it can also be shed as a soluble GAG. The mechanism of action of heparan sulphate includes specific, non-covalent interactions with various proteins, a process that affects the topographical destination, half-life, and bioactivity of the protein. Furthermore, heparan sulphate acts on morphogenesis, development, and organogenesis [18]. Heparan sulphate is also involved in a variety of biological processes including cell-matrix interactions and activation of chemokines, enzymes, and growth factors [17].

Heparin is the most highly modified form of heparan sulphate. This GAG, which can be considered an over-sulphated intracellular variant of heparan sulphate, is commonly used in patients as an anticoagulant drug [18]. Heparin and heparan sulphate are very closely related and share many structural and functional activities. The lung is a rich native source of heparin. Heparin's abundance may be explained by the fact that the lung is rich in mast cells, which may be heparin's sole cell of origin [19]. Mast cell heparin resides in secretory granules, where most of the GAG chains are linked to a core protein (serglycin), forming macromolecular proteoglycans much larger than commercial heparin. Very little heparin is incorporated into the cell surface proteoglycan of epithelial and endothelial cells, which are more likely to contain heparan sulphate, which is under-sulphated compared with heparin. Some heparan sulphate chains of vascular endothelium contain short heparin-like sequences [17]. However, most native lung heparin is locked up in mast cells as large proteoglycans. This does not necessarily mean that heparin's physiological action is exclusively within cells, because stimulated mast cells secrete heparin outside the cell along with granule-associated mediators, such as histamine, chymase, and tryptase [20].

Proteoglycans

In the lung, three main proteoglycan families may be distinguished based on GAG composition, molecular weight, and function: 1) chondroitin suphate-containing proteoglycan (CS-PG, versican), heparan sulphate-containing proteoglycans (HS-PGs, perlecan and glypican), chondroitin and heparan sulphate-containing proteoglycans (CS-HS-PGs, syndecan), and dermatan-sulphate-containing proteoglycans (DS-PG, decorin). The proteoglycans are localized in different areas of the extracellular matrix: Versican in the pulmonary interstitium, perlecan in the vascular basement membrane, decorin in the interstitium and in the epithelial basement membrane linked with collagen fibrils, syndecan and glypican in the cell surface.

Versican is a large molecule (>1000 kDa) found around lung fibroblasts and blood vessels in regions not occupied by the major fibrous protein, collagen, and

elastin. Thus, it is localized mainly in the interstitium creating aggregates with hyaluronic acid [21]. The precise function of versican is unclear but it is thought to be involved in tissue hydration in mature tissues. It may form aggregates with hyaluronic acid, fibronectin, and various collagens, playing an important role in cell to matrix interaction. It has been shown to link with smooth muscle cells in the walls of airways and pulmonary vessels, to inhibit cell-matrix adhesion [22], to regulate differentiation of mesenchymal cells, and to play a specific role in matrix synthesis, favoring wound healing.

Perlecan is the largest proteoglycan in the lung, with its core possessing about 4400 amino acids. Perlecan is a typical component of vascular basement membrane [23], although it has been also identified within the extracellular matrix of some tissues, remote from the basement membrane. Certainly its complex core protein provides the potential to interact with numerous proteins. In the basement membranes it provides a filtration barrier interacting with collagen IV, limiting flow of macromolecules or cells between two tissue compartments. It also regulates the interaction of the basic fibroblast growth factor with its receptor and modulates tissue metabolism.

Syndecan and glypican are more densely arranged in the cell surface [24]. The function of syndecan is commonly associated with its heparan sulphate chains and its interaction with heparin binding growth factors or extracellular proteins like fibronectin and laminin, and plays a relevant role in wound healing [25].

Decorin is the smallest dermatan-sulphate-containing proteoglycan. The presence of decorin alters the kinetics of fibril formation and the diameter of the resulting fibril [21], modulating tissue remodeling. Indeed its name was derived from its surface decoration of collagen fibrils when viewed with the electron microscope.

These data indicate that the function of proteoglycans and GAGs in the lung is not limited to the maintenance of the mechanical and fluid dynamic properties of the organ. These molecules also play a relevant role in tissue development and recovery after injury, interacting with inflammatory cells, proteases, and growth factors. Thus, the extracellular matrix transmits essential information to pulmonary cells, regulating their proliferation, differentiation, and organization. The structural integrity of the pulmonary interstitium largely depends on the balance between the regulation of synthesis and degradation of extracellular matrix components.

Extracellular Matrix Metalloproteinases

Although many proteases can cleave extracellular matrix molecules, the family of Zn^{++} matrix metalloproteinases (MMPs) and their inhibitors are likely to be the physiologically relevant mediators of extracellular matrix degradation [26]. They can degrade many proteins including collagens, fibronectin, laminin, proteoglycans, entactins, and elastin. In particular, they play a major role in: a) the breakdown of extracellular matrix and basement membrane; b) in tissue remodeling and angiogenesis; and c) the restoration of functional connective tissue in the wound-healing process. Several subclasses of MMPs (23 enzymes) have been identified, including interstitial collagenases, gelatinases, stromelysins, and membrane-type MMPs. MMPs are secreted in a latent form, as inactive proenzymes, and are activated by the loss of the propeptide under physiologic conditions. At least two MMPs play a relevant role in the extracellular matrix remodeling: MMP-2 ubiquitously distributed in the lung parenchyma, and MMP-9 produced by free alveolar macrophages and epithelial cells. The proteolytic activity of MMPs is precisely controlled by endogenous physiologic inhibitors, which include the broad-spectrum serum inhibitor $alpha_2$-

macroglobulin and a special class of tissue inhibitors of metalloproteinases (TIMPs). Loss of coordination in the expression of proteinases and inhibitors is believed to generate tissue degradation in inflammatory diseases [27].

Pulmonary Interstitial Fluid Dynamics

The maintenance of the steady state interstitial fluid turnover results from the balances between several factors, such as: 1) the transendothelial fluid and solute filtration; 2) the convective outflows into the lymphatic system; and 3) the mechanical and hydrophilic properties of the solid elements of the extracellular matrix.

The fluid bulk flow (J_v) between the pulmonary capillaries (*c*) and the surrounding interstitium (*i*) is described by the well known Starling law:

$$J_v = L_p \cdot S \cdot [(P_c - P_i) - \sigma(\pi_c - \pi_i)] \qquad \text{(Eqn 1)}$$

where P and π are the hydraulic and colloid osmotic pressures in the two compartments, L_p is the hydraulic filtration coefficient of pulmonary endothelium, S its surface area, and σ the reflection coefficient of the endothelium for total proteins, a correction factor accounting for the protein to endothelial pore radii ratio. The factor in the square parentheses gives the net pressure gradient across the membrane, ΔP_{net}.

In Table 1, an example of hydraulic (P) and colloid osmotic (π) pressures from the microvasculature (*c*) and surrounding interstitial space (*i*) under normal conditions or during attainment of mild or severe interstitial pulmonary idraulic or lesional edema are shown.

The hydraulic pressure of the free liquid phase of the pulmonary interstitium (P_i) reflects the dynamic situation resulting from the complex interaction between such factors and represents, therefore, a key variable in understanding the mechanisms controlling lung fluid balance. P_i has been measured in anesthetized supine rabbits with lungs physiologically expanded in the intact pleural space at zero airways pressure [28]. The end-expiratory P_i is significantly lower than pleural (P_{liq}) and extrapleural (P_{epl}) liquid pressure, indicating that the lung parenchyma is relatively 'dehydrated' compared to the other two tissue compartments. The P_i distribution is not uniform within the lung parenchyma, decreasing by ~0.7 cmH_2O/cm of lung height. The gravity-dependent P_i distribution reflects: a) the uneven mechanical stress developing in lung tissue at various lung heights; b) the inhomogeneous perfusion of the lung parenchyma; and, in analogy with what is found in the pleural space, c) a greater drainage of interstitial fluid into the lymphatic system in the lowermost regions. On inspiration, sustained by lowered pleural surface pressure (Ppl), both P_{liq} and P_i become more subatmospheric, but P_{liq} and P_i drops are greater then expected on the basis of the change in Ppl. From the mechanical standpoint this

Table 1. Example of hydraulic (P) and colloid-osmotic (π) pressures (cmH_2O) from the microvasculature (*c*) and surrounding interstitial space (*i*) under normal conditions or during attainment of mild or severe interstitial pulmonary hydraulic or lesional edema.

	P_c	P_i	σ	π_c	πi	ΔP_{net}
Control	10	−11	0.8	30	12.5	+10.5
Interstitial edema	10	+4	0.6	20	12	0
Severe edema	10	0	0.4	20	10	+2

indicates that, with increasing lung volume, complex mechanical deformations arise both between the sliding pleurae and within the tight fibrous matrix [29–34].

The Extracellular Matrix and Lung Edema

The early phase of interstitial edema implies an increase in interstitial fluid pressure with no significant change in interstitial fluid volume due to the low tissue compliance. A low compliance provided by the structure of the matrix represents an important 'tissue safety factor' to counteract further progression of pulmonary edema. As the severity of edema progresses, P_{ip} drops back to zero and subsequently remains unchanged despite a marked increase in the wet weight to dry weight ratio of the lung. As edema develops toward a more severe condition, fluid filtration occurs down a transendothelial Starling pressure gradient that is smaller compared with the control condition, due to the progression increase of the interstitial fluid pressure. Hence, at least two factors interact to determine the development of pulmonary edema: the loss of the tissue safety factor and the increase in microvascular permeability [35, 36].

As shown in Figure 2, different types of injury leading to lung edema (hydraulic, lesional, or ventilator-induced) affect the extracellular matrix in different ways. In hydraulic edema, the biochemical analysis of tissue structure reveals an initial fragmentation of chondroitin sulphate proteoglycan due to mechanical stress and/or proteolysis. In lesional edema, the partial fragmentation of heparan sulphate proteoglycan is mainly due to enzymatic activity. Progression toward severe edema is similar for both kinds of edema because the activation of tissue metalloproteinases leads to extended fragmentation of chondroitin sulphate proteoglycan, causing a marked increase in tissue compliance and, therefore, a loss in tissue safety factor, and of heparan sulphate proteoglycan, leading to an increase in microvascular permeability [37]. In hypoxic edema, the development of interstitial edema is associated with a prevalent degradation of the heparan-sulphate proteoglycans paralleled by an increase in the interstitial pressure [38].

Recent data also suggest that the integrity of the heparan-sulphate proteoglycan components of the pulmonary extracellular matrix is required to maintain the three-dimensional architecture of the matrix itself, and in turn to guarantee its mechanical response to increased fluid filtration [39].

Effects of Mechanical Ventilation on the Extracellular Matrix

Mechanical ventilation is essential to sustain respiratory function both during general anesthesia and in patients with respiratory failure. Compared to spontaneous breathing, during mechanical ventilation a reversed distribution of forces around the alveolar-capillary barrier occurs with a simultaneous increase in airway and thoracic pressures. Hence, the external mechanical load to which the pulmonary extracellular matrix is exposed during mechanical ventilation at different tidal volumes may represent '*per se*' an important factor promoting extracellular matrix macromolecular rupture and progressive lung injury.

The majority of studies investigating this issue have focused on the effects of mechanical ventilation on the alveolar-capillary layer, while studies investigating the role of macromolecular components of the extracellular matrix are, at present, scanty.

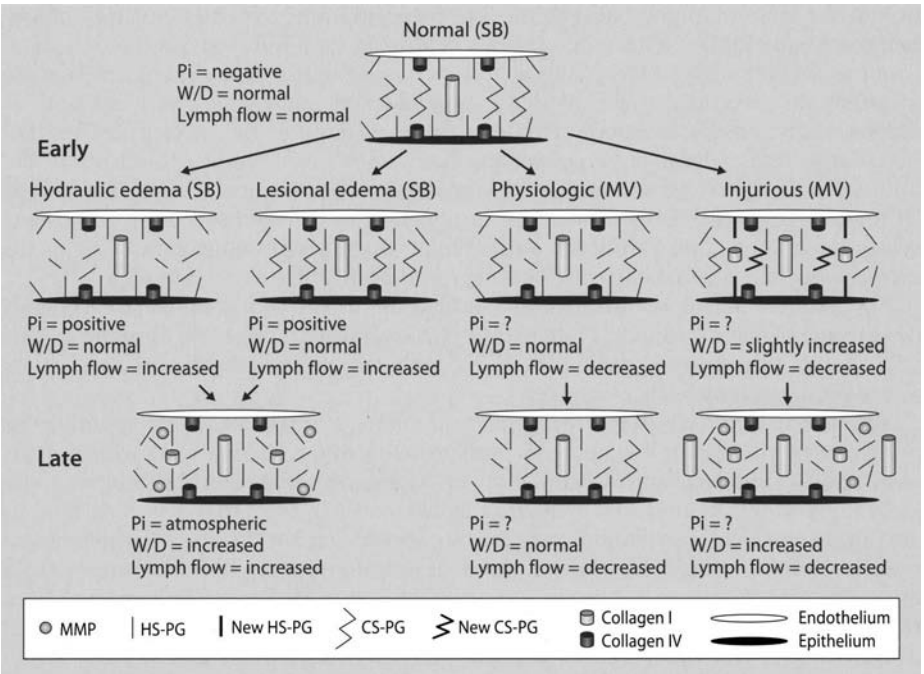

Fig. 2. Changes in extracellular matrix during hydraulic and lesional edema in spontaneous breathing (SB) and physiologic and injurious mechanical ventilation (MV) early and late in the course of lung injury. Bold lines represent the new synthesis of heparan sulphate-proteoglycan (HS-PG) or chondroitin sulphate-proteoglycan (CS-PG). During hydraulic edema and in the early phase, the prevalent lesion is the fragmentation of chondroitin sulphate, whilst in lesional edema, heparan sulphate is damaged. In physiologic mechanical ventilation, mainly chondroitin-sulphate proteoglycan was fragmented, but the ongoing mechanical ventilation yields the fragmentation of both GAGs. During injurious mechanical ventilation, although HS-PG and CS-PG are injured, collagen fiber content increases early and late in the course of lung injury. Thus, we hypothesize that collagen fiber synthesis may be beneficial to avoid the rupture of GAGs minimizing interstitial edema formation. Pi: interstitial pressure, W/D: wet-to-dry weight ratio, HS: heparan sulphate, PG: proteoglycan, CS: chondroitin sulphate. MMP: matrix metalloproteases.

Effects of Mechanical Ventilation with 'Physiologic' Tidal Volumes on the Extracellular Matrix

Serious damage to different lung structures has been reported as a consequence of mechanical ventilation at 'physiological' (6–8 ml/kg) tidal volumes and in the absence of positive end-expiratory pressure (PEEP) in otherwise previously healthy lungs:

a) injury to the epithelial cells with leukocyte infiltration in the alveolar septa and increase in the percentage of abnormal alveolar-bronchiolar attachments [40];
b) damage to the endothelial cells promoting right ventricular dysfunction with increased microvascular leakage [41];
c) peripheral airway injury [42], not mediated by pro-inflammatory process [43].

Other studies have specifically investigated the effects of mechanical ventilation on the extracellular matrix. Negrini and colleagues [44] observed a marked fragmenta-

tion of the interstitial and basal membrane proteoglycans, with no activation of systemic or tissue MMPs in anesthetized rats ventilated for 4 hours at 'physiologic' tidal volumes. Interestingly, these changes were not associated with a significant increase in pulmonary wet-to-dry weight ratio. Al Jamal and Ludwig [45] did not find an increase in proteoglycan synthesis after 1 hour of 'physiologic' mechanical ventilation. Farias and colleagues [46] observed that 1 hour's ventilation without PEEP did not increase type III procollagen mRNA expression. The N-terminal peptide of type III procollagen has been used as a biologic marker of collagen synthesis [47]. Many cell types in lung, e.g., fibroblasts and alveolar macrophages, may contribute to the increase in lung parenchyma mRNA for type III procollagen.

The lesional effect of mechanical ventilation on the extracellular matrix may depend upon several factors: 1) increased transpulmonary pressure; 2) reversed distribution of intrathoracic pressures, 3) inhomogeneous distribution of ventilation; 4) reduction in lymphatic drainage.

The transpulmonary pressure to inflate the lung is attained during spontaneous breathing by decreasing intrapleural pressure with minor changes in alveolar pressure, and during mechanical ventilation by a positive increase in both airway and pleural pressures. In addition, formation of atelectasis in the dependent lung regions during mechanical ventilation may cause overdistension of the non-dependent regions and inhomogeneous distribution of ventilation [48, 49]. Therefore, since increased transpulmonary pressure and regional alveolar overdistension has been reported to be one of the major factors influencing the stress and strain of alveolar structures [50] it is likely that both phenomena may expose the extracellular structures to excessive mechanical stress leading to local macromolecular fragmentation. Finally, a possible reduction in the lymphatic drainage during mechanical ventilation [51] could enhance the development of interstitial edema and further GAG fragmentation in the extracellular matrix. In summary, mechanical ventilation with 'physiologic' tidal volumes in healthy lungs leads to mechanical damage of proteoglycans in the extracellular matrix, not associated with the activation of either the inflammatory or the fibrogenic processes (Fig. 2).

Effects of Mechanical Ventilation with 'Injurious' Tidal Volumes on the Extracellular Matrix

Ventilator-induced lung injury (VILI) is a well recognized complication of mechanical ventilation. The mechanisms of VILI are not completely elucidated, but can be attributed in part to the effects of excessive airway pressure and alveolar distension. In fact, VILI may be considered an *in vivo* cause of excessive mechanical stress and strain on extracellular matrix components. Large tidal volumes and high inspiratory airway pressures with increased transpulmonary pressures are associated with increased mechanical stress that may damage the endothelial [52] and epithelial cells [53], due to the development of inflammatory response [54, 55] and/or to the inactivation of surfactant [56].

Berg and colleagues [57] observed higher levels of mRNA for α1 (III)- and α2 (IV)-procollagen, fibronectin, basic fibroblast growth factor, and transforming growth factor (TGF)-β1 in lungs ventilated with high-PEEP levels (compared with low-PEEP and untreated groups). In contrast, α2 (I)-procollagen and vascular endothelial growth factor (VEGF) mRNA levels remained unaltered. These authors concluded that high lung inflation for 4 hours increased mRNA levels for extracellular matrix components and growth factors in lung parenchyma. Parker and colleagues

[58] found that ventilation with high peak airway pressures and low perfusion pressures led to increased type III procollagen mRNA expression in comparison with unperfused lungs. Garcia and colleagues [59] demonstrated that the increase in the tissue stress induced by oscillation force, but not amplitude, increased procollagen type III mRNA expression in rat lung parenchymal strips.

Al Jamal and colleagues in an '*in vivo*' healthy lung rat model [45] found a significant increase in the protein component of versican (CS-PG), basement membrane HS-PGs and byglycan during mechanical ventilation only at extremely high tidal volume (30 ml/kg) and respiratory rate (90 breaths/min). However, in addition to the proposed increase in proteoglycan synthesis, the observed augmented proteoglycan extraction might also be explained by matrix fragmentation inducing an easier extraction of proteoglycans. Furthermore, the possible effects of hyperoxia, instead of the high tidal volume, leading to these extracellular matrix changes could not be ruled out [60].

In another study in anesthetized rats with previously healthy lungs, Negrini and colleagues [44] reported a degradation of proteoglycans in the basal membrane and interstitium after 4 hours of injurious mechanical ventilation, associated with an increase in wet-to-dry weight ratio.

In short, injurious mechanical ventilation with high stress or strain in previously healthy lungs induces proteoglycan synthesis and increased mRNA procollagen in the early phases. Then, with the course of mechanical ventilation a major degradation of proteoglycans occurs associated with a further increase in fibrogenesis (Fig. 2).

The Extracellular Matrix and the Inflammatory Process

The cells in the lung react to increased stress and strain activating their mechanosensors, i.e., the integrins, the cytoskeleton, and the ion channels, transducing the mechanical signal in biochemical events, via a complicated network of signaling molecules. This phenomenon is defined as 'mechanotransduction' [61, 62].

The mechanical stress of the extracellular matrix is transferred to biochemical cell activation, by the link between the basal lamina and the extracellular matrix. The basal lamina of pulmonary cells is extremely complex and it is composed of different molecules like laminin, nidogen, and perlecan (Fig. 3).

However, it is not clear if the damage induced by mechanical ventilation to the extracellular matrix is mainly 'mechanical' or 'inflammatory'. In predominantly 'mechanical' damage, the increased amount of GAG fragments in the interstitium is not caused by an increased activation of MMPs and inflammatory mediators. However, it can subsequently further promote the activation of MMPs through a positive feed-back mechanism. In case of predominant 'inflammatory' damage, it has been proposed that high local tissue stress may trigger the production of MMPs favoring the degradation of GAGs [34]. Hence, the progressive CS-GAG and HS-GAG cleavage observed with injurious ventilation might be associated with a progressive activation of MMPs due to increased tissue stress.

The data about the inflammatory response induced by injurious mechanical ventilation are conflicting [63]. *In vitro* studies showed that excessive stretch of the epithelial cells and macrophages activates the release of inflammatory mediators through neutrophil recruitment [64, 65]. In isolated unperfused previously healthy rat lungs during injurious mechanical ventilation, Tremblay et al. [66], but not Ricard and colleagues [67], found an increase in lung inflammatory mediators. On the other hand, *in vivo* experiments in previously healthy lungs [68, 69] did not

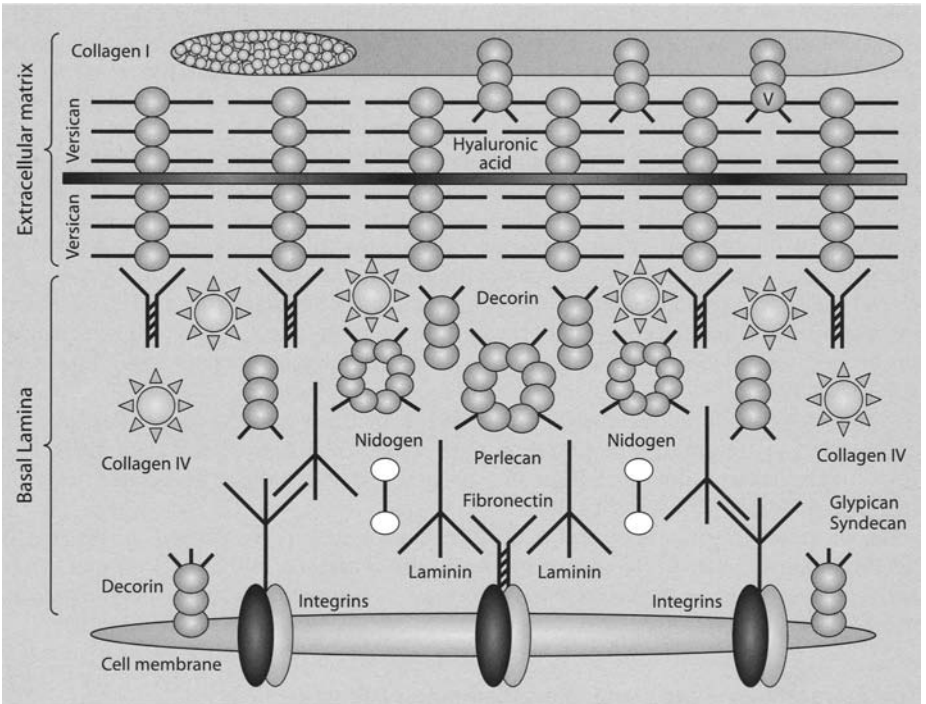

Fig. 3. Links between the cell membrane, the basal lamina, and the extracellular matrix. The mechanical stimuli induced by stress and/or strain are transferred into biochemical and biomolecular alterations by means of the basal lamina structure.

reveal local or systemic cytokine release when ventilating with an injurious tidal volume. A significant increase in IL-6 expression across the pulmonary epithelium [70] and in the serum [71] together with epithelial apoptosis [72] was found in an acid aspiration lung injury model in rats ventilated at high tidal volume. However, other authors did not find a significant increase in inflammatory mediators in the lung during injurious mechanical ventilation in rat lavaged lungs [73].

Conclusion

The extracellular matrix of the lung plays an important role: 1) in regulating hydration and water homeostasis; 2) in maintaining structure and function; 3) in modulating the inflammatory response, and 4) in influencing tissue repair and remodeling.

The fibrous extracellular matrix components, in particular chondroitin sulphate (versican) and heparin sulphate (perlecan) proteoglycans, play a major role in the maintenance of tissue fluid homeostasis, providing: a) a perivascular and interstitial highly restrictive sieve with respect to plasma proteins, thus modulating both interstitial protein concentration and transendothelial fluid filtration; b) mechanical support to lymphatic vessels sustaining and modulating their draining function; and c) a rigid three dimensional, low compliance scaffold opposing fluid accumulation into

the interstitial space. Fragmentation of proteoglycans induced by different stimuli, like fluid overload, exposure to proteolytic or inflammatory agents, hypoxic or hyperoxic gas mixtures, or increased tissue strain/stress, invariably results in the progressive loosening of proteoglycan intermolecular bonds with other extracellular matrix components. The loss of the proteoglycan regulatory functions compromises the protective role of the extracellular matrix, progressively leading to interstitial and, eventually, severe lung edema.

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Mechanical Ventilation

Advances in Translaryngeal Tube Technology

P.J. Young and M.C. Blunt

Introduction

Intubation of the trachea with a cuffed tube can be performed by the translaryngeal route (endotracheal tube) or through a tracheal stoma (tracheostomy tube). Tracheal intubation by one of these routes is the only way to simultaneously provide a secure airway, ventilatory support, and convenient access to the trachea. Unfortunately, the presence of an artificial airway bypasses many of the patient's natural defenses and so increases the chances of upper and lower airway colonization, aspiration, and infection [1]. Sedatives, analgesics or muscle relaxants may be required to improve tolerance of the airway; this risks cardiovascular, respiratory and neuromuscular complications. It is, therefore, desirable to avoid the use of artificial airways, for example, by using facemask oxygen or an external airway interface to achieve non-invasive ventilation (NIV). Indeed it has become clear that NIV as opposed to tracheal intubation can reduce morbidity and mortality in the critically ill. When an artificial airway is required it is the responsibility of both the medical devices industry and the clinician to minimize the complications consequent to its use.

The major complications directly related to the artificial airway in the critically ill are:

1. Airway colonization – risk of ventilator-associated pneumonia (VAP)
2. Airway injury – risk of laryngeal and tracheal pathology.
3. Tube occlusion – risk of hypoxemia, hypercapnea, and sudden death.

The design and quality of care of the artificial airway is critical in preventing these complications.

Endotracheal Tubes

Physiological Impact of the Endotracheal Tube

The curvature of stiff polyvinyl chloride (PVC) endotracheal tubes causes pressure injuries at the contact points of the palate, posterior larynx, the cricoid cartilage, and the trachea at the level of the cuff and the tube tip [2]. Regurgitation of bile and gastric secretions may exacerbate these injuries and delay healing [3]. This mucosal injury predisposes to bacterial adhesion [1]. As well as providing a physical barrier to mucus clearance, endotracheal intubation can reduce tracheal mucus velocity [4] and prevents effective cough by preventing cord opposition and by increasing the need for sedation or muscle relaxation. The universal problem of aspiration past (high-volume low-pressure) cuffs [5–7] leads to rapid tracheal colonization [8].

Pneumonia results if the nature of aspirated material and the pathogenicity of bacteria within it are sufficient to overcome lung defenses [1]. Feldman and co-workers investigated the sequence of colonization [9] beginning with the oropharynx after 1–2 days, followed by the stomach, then the lower respiratory tract (2–4 days), and thereafter the endotracheal tube. Bacteria within secretions attached to the tube lumen are protected from systemic antibiotics and are propelled into the lung by the shear forces of gas flow and by the passing of tracheal suction catheters [10]. Tube blockage with secretions is common [11] and may be delayed in larger bore tubes compared with smaller tubes. Larger internal diameter tubes produce a lower airway resistance but at the expense of more erosions of the laryngeal inlet and increased patient discomfort.

Securing the Tube

A multicenter Spanish Study showed an 8% incidence of unplanned extubation, and accidental extubation carried a relative risk of 5.3 for the development of VAP [12]. Although endotracheal tubes are commonly secured using adhesive tape or cloth ties, commercial devices are available and provide more effective tube fixation [13, 14].

Cuff Types

The large diameter high-volume low-pressure cuff has been the standard in intensive care for nearly 40 years. There is no tension within the wall of an inflated high-volume low-pressure cuff and so all the intra-cuff pressure is transmitted to the tracheal wall, enabling easy monitoring of the tracheal wall pressure by direct measurement. High-volume low-pressure cuffs reduce the incidence of associated tracheal injury compared with low-volume high-pressure cuffs [15]. Unfortunately there is an inherent design fault with these high-volume low-pressure cuffs in that they allow pulmonary aspiration to occur even when correctly inflated (Fig. 1) [5, 6, 16]. The rate of aspiration, however, is reduced with thinner cuff wall material and if unintentional falls in cuff pressure are prevented [6, 17].

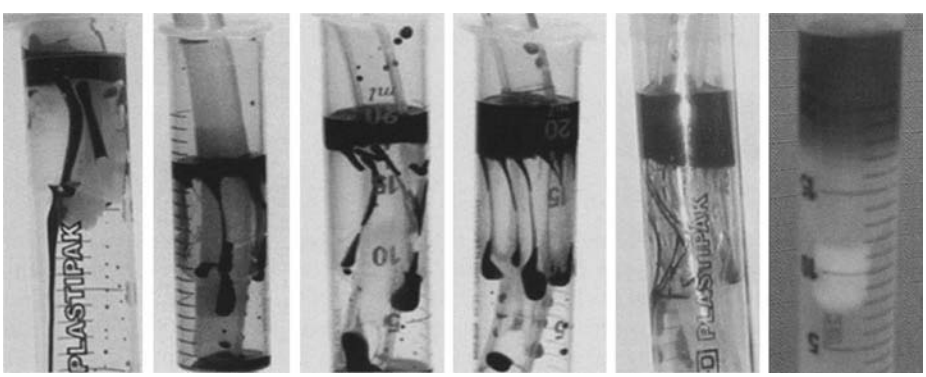

Fig. 1. Left: Five high-volume low-pressure cuffs manufactured of material of decreasing thickness from left to right all inflated to 30 cmH_2O. Note channels in folds within cuff walls increase in size with cuff material thickness. All high-volume low-pressure cuffs permit leakage. Far right: silicone LoTrach cuff prevents leakage because there are no folds.

Preventing Iatrogenic Injury From Endotracheal Tubes

Preventing Aspiration

Aspiration past the inflated tracheal tube cuff is commonplace in the ICU by the mechanism described above. Most intubated critically ill tube-fed patients aspirate gastric contents; using tracheal pepsin as a marker, aspiration was identified in 89% of patients and those who aspirate frequently are 4 times more likely to have pneumonia develop than those who aspirate infrequently [18]. Preventing gastric overdistension, attention to patient position, avoiding patient transport, oral hygiene, and maintaining adequate cuff pressures have all been used in an attempt to limit regurgitation and aspiration [8]. Tube [19] and cuff [7, 20, 21] technologies have been developed to reduce aspiration.

Preventing Mechanical Injury

Measures include control of tracheal wall pressure, using smaller external diameter tubes, appropriately supporting circuit connecting equipment, and maintaining good oral hygiene to reduce chemically damaging material [3]. Tracheal wall pressure should be routinely monitored and controlled at 20–30 cmH_2O in all intubated patients in the intensive care unit (ICU). This reduces the late complication of tracheal stenosis related to high intracuff pressures and also reduces the quantity of tracheobronchial soiling related to low intracuff pressures. Tracheal tube design should ensure that the tube is flexible enough to fit the patient's anatomy with minimal pressure points at the level of the palate, arytenoids, cords, or trachea at the level of the tube tip.

Cuff Pressure Control

Surveys show that cuff pressure is not routinely monitored in many ICUs [22–24], leading to inappropriately high cuff pressures [25] and causing tracheal injury [26]. Too low a cuff pressure is also an important problem due to an increased rate of aspiration [6, 27] and an intracuff pressure persistently less than 20 cmH_2O has been shown to be associated with the development of pneumonia within the first 8 days of mechanical ventilation (relative risk = 4) [17].

Manual Inflators

The most common technique used for cuff pressure regulation is intermittent measurement and adjustment with a handheld cuff inflator/manometer. Temporary loss of cuff pressure can occur when measurement technique is poor. Manual cuff pressure inflators differ in bias and precision [28]. The compressible volume within the device will cause a fall in cuff pressure as soon as the pilot valve is connected. Manufacturers should keep the compressible volume to a minimum. Accidental depression of the deflation button on the inflator can also occur when inexpertly used.

Lanz Balloon

The Lanz inflation balloon is an integral component of a brand of tracheal tube and has been available for nearly 30 years. This is an ingenious constant pressure balloon

combined with a pressure-regulating valve and protected in a PVC sleeve. The device maintains the cuff pressure in the desirable range preventing over- and under-inflation. It is surprising that this is not more widely used by clinicians as concerns regarding over- and under-inflation are eliminated.

Foam Cuffs

A foam-filled high-volume low-pressure cuff [29] is also available. Air is aspirated prior to use, and after intubation of the trachea the pilot channel is opened allowing the cuff to inflate under the force of the expanding foam maintaining continuous inflation. Aspiration can still occur by the same mechanism as with conventional cuffs [6].

Electronic Cuff Pressure Controllers

These are available to maintain cuff pressure in the desirable range but they are costly. The Tracoe cuff pressure controller (www.tracoe.com) is a portable electrical device with a battery back-up designed to attach to the pilot valve of a tracheal tube and maintain the cuff pressure at a value set by the operator. An audible alarm detects unintentional disconnections and battery power failure. There are two potential disadvantages of this device. First, if the tube is unintentionally withdrawn into the larynx the device will further inflate the cuff preventing re-intubation (until the situation is recognized, the cuff deflated, and the tube re-positioned). Second, the inflator has a standard luer-locking connector. If this is accidentally misconnected to an intravenous line then a fatal air embolus will occur. A simple modification of the luer valve prevents attachment to an intravenous connector but allows connection to the tracheal tube connector.

Subglottic Secretion Drainage Tubes

The Mallinckrodt HiLo Evac endotracheal tube has a dorsal port above the cuff designed to allow suctioning of secretions from the subglottic space to reduce the volume available for pulmonary aspiration. Studies show a reduction in pneumonia rates (particularly early onset VAP). A meta-analysis of five studies with a total of 896 patients showed subglottic secretion drainage reduced the incidence of VAP by a half [19]. There was a shortened duration of mechanical ventilation (2 days), length of ICU stay (3 days), and a delay in the onset of pneumonia (7 days). There are, however, some major disadvantages with this tube. The port of the HiLo EVAC tube is prone to blockage. This is most commonly due to the application of suctioning to a closed space allowing the tracheal mucosa to be drawn into the suction channel opening. Unfortunately, animal studies have shown that continuous suctioning causes tracheal injury even at lower negative pressures [30]. Therefore, only intermittent and not continuous suctioning can be recommended. This is likely to reduce pulmonary aspiration but will not eliminate it as aspiration past the cuff will be possible between episodes of subglottic secretion drainage. The incorporation of the subglottic port into the wall of the tube increases the rigidity of the tube. The death of a patient due to erosion of the stiff tip into the tracheal wall causing perforation and innominate artery fistula development has been reported [31] and tube displacement during ventilation has also been attributed to this stiffness [32].

New Technologies and Future Advances

The key initial steps in the pathogenesis of VAP that can be influenced by the design of the artificial airway are:

1. Colonization proximal to the cuff (the stomach, sinuses, dentition, oropharynx, larynx, and subglottis)
2. Aspiration of this microbial laden fluid past the tracheal tube cuff
3. Biofilm accumulation
4. Security of airway (avoidance of unintentional extubation)

Simple pragmatic measures such as hand washing, upper airway decontamination, and the reduction of aspiration by means of semi-recumbency and control of gastric volumes are currently the mainstay of defense against VAP. Recently researchers have turned their attention on improving the function of the simple tracheal tube by improvements in design.

Antimicrobial Coating of Tracheal Tubes

Both antiseptic [33] and silver [34] coated endotracheal tubes have recently been evaluated in animal studies with regard to biofilm and pneumonia prevention. Berra and colleagues [33] investigated bacterial colonization of the ventilator circuit, the tube, and the lungs with a silver-sulfadiazine and chlorhexidine coated endotracheal tube in a sheep model. Coated endotracheal tubes had less biofilm and the ventilator circuits were protected from colonization. There was no difference in tracheal colonization. This is not surprising as coating of the tube lumen may reduce biofilm progression and re-inoculation of the tracheobronchial tree but will not prevent upper airway colonization and aspiration. Similarly Olson and co-workers reported the effect of using silver hydrogel coated endotracheal tubes on the lung bacterial burden of mechanically ventilated dogs challenged with buccal administration of *Pseudomonas aeruginosa* [34]. The silver coating delayed the appearance of bacteria on the inner surface of the endotracheal tube and the bacterial burden and inflammation in the lung was reduced. A recent prospective, randomized, single-blind, multiple-center study using a sliver-coated endotracheal tube in 149 patients showed a reduced colonization rate and decreased bacterial burden but failed to demonstrate a reduction in the incidence of VAP [35]. These tubes can only impact on the incidence of VAP by reduction in bacterial burden but will not prevent aspiration of upper airway material past the cuff.

Modification to the Hi-Lo Evac Tube

This tube has recently been modified to bring the dorsal opening closer to the cuff and to increase the luminal cross-section [36]. This is an attempt to address the common problem of the subglottic port becoming blocked by in-drawing of the tracheal mucosa.

Tracheostomy Tubes with Subglottic Ports

Tracheostomy tubes are now also available with subglottic ports allowing intermittent suctioning. This is likely to offer advantages related to a reduction in aspiration. Logically one should perform subglottic aspiration prior to times when aspiration

past the cuff is likely, such as before tracheal suctioning, circuit disconnections, deflations of the cuff, or the loss of positive end-expiratory pressure (PEEP) [6].

Mucus Shaver

This is an experimental inflatable silicone rubber 'razor' designed to pass down an endotracheal tube and shave the endotracheal tube lumen free of mucus [37]. Regular use was associated with reduced accumulation of mucus/secretion and bacterial growth within the endotracheal tube during mechanical ventilation in a sheep model [38] (Fig. 2).

Mucus Slurper

To reduce the build up of mucus within the endotracheal tube, Kolobow and co-workers have developed a prototype tracheal tube with an integral mucus aspirator ring at the distal tip [39], connected to a suction source. Preliminary studies in six mechanically ventilated sheep have shown that the mucus slurper reduced secretion accumulation within the endotracheal tube compared with a conventional tube and open suctioning. Human studies are awaited (Fig. 3).

Thin-walled Cuffs

Cuffs made of thicker material have larger channels within the folds in the cuff wall, and, therefore, a more rapid rate of aspiration [6] (Fig.1). Innovative manufacturers are utilizing this effect in an attempt to reduce aspiration. (e.g., Seal Guard and Microcuff). These cuffs are made of very thin, yet robust material. Dullenkopf and co-workers have recently introduced the Microcuff endotracheal tube (Microcuff

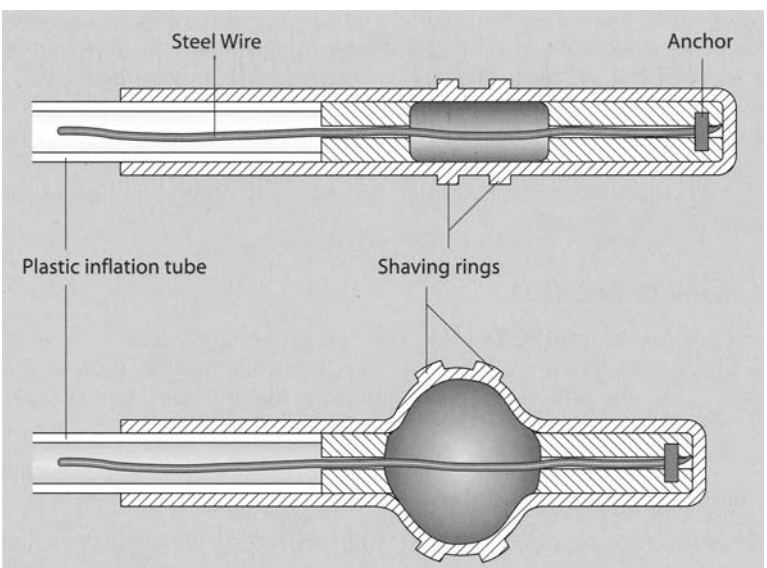

Fig. 2. Mucus shaver. Above deflated, below inflated (courtesy of Dr T. Kolobow).

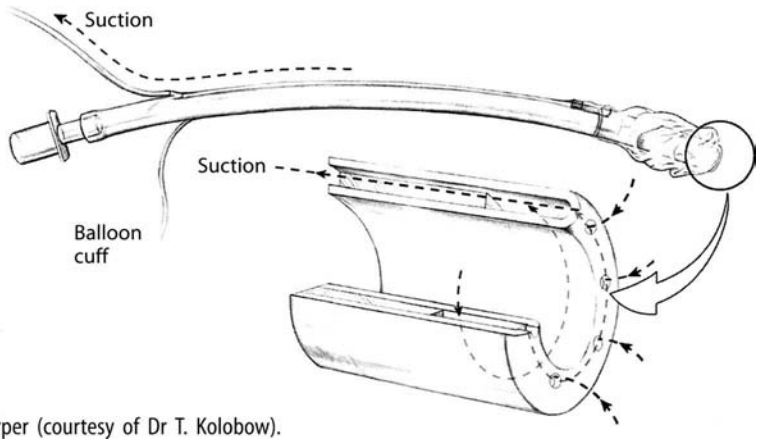

Fig. 3. Mucus slurper (courtesy of Dr T. Kolobow).

PET, Weinheim, Germany) [40], a thin polyurethane cuff that is likely to reduce the rate of subglottic to tracheal leakage. These appear particularly useful in pediatric practice where very low intracuff pressures are possible [40]. There are currently no clinical trials with outcomes of aspiration, lung injury, or infection.

Kolobow Tube

Theodor Kolobow and colleagues have designed an ultrathin-walled, non-kinking, crush proof, wire reinforced tube with an oropharyngeal-section diameter larger than the diameter of the tracheal section, to reduce airway resistance. The tube has no cuff, but instead airway seal is achieved at the level of the glottis through a no-pressure seal made of 'gills'. The gills are made of numerous soft, pliable, rings of polyurethane to occlude voids for potential air leaks from within the larynx. This tube has been extensively tested in animal models and in this setting has been shown to provide an effective airway seal and to prevent aspiration of oropharyngeal indicator dye. These tubes are not currently commercially available (Fig. 4).

LoTrach Tube

The LoTrach endotracheal and tracheostomy tube [7] (www.LoTrach.com) has been designed to reduce the risk factors associated with upper airway colonization, aspiration, and tracheal wall pressure control (Fig. 5).

Low-volume low-pressure cuff: The cuff is calibrated during the manufacturing process such that at a single working intracuff pressure the tracheal wall pressure is

Fig. 4. Kolobow's ultrathin walled 'gilled' tube (courtesy of Dr T. Kolobow).

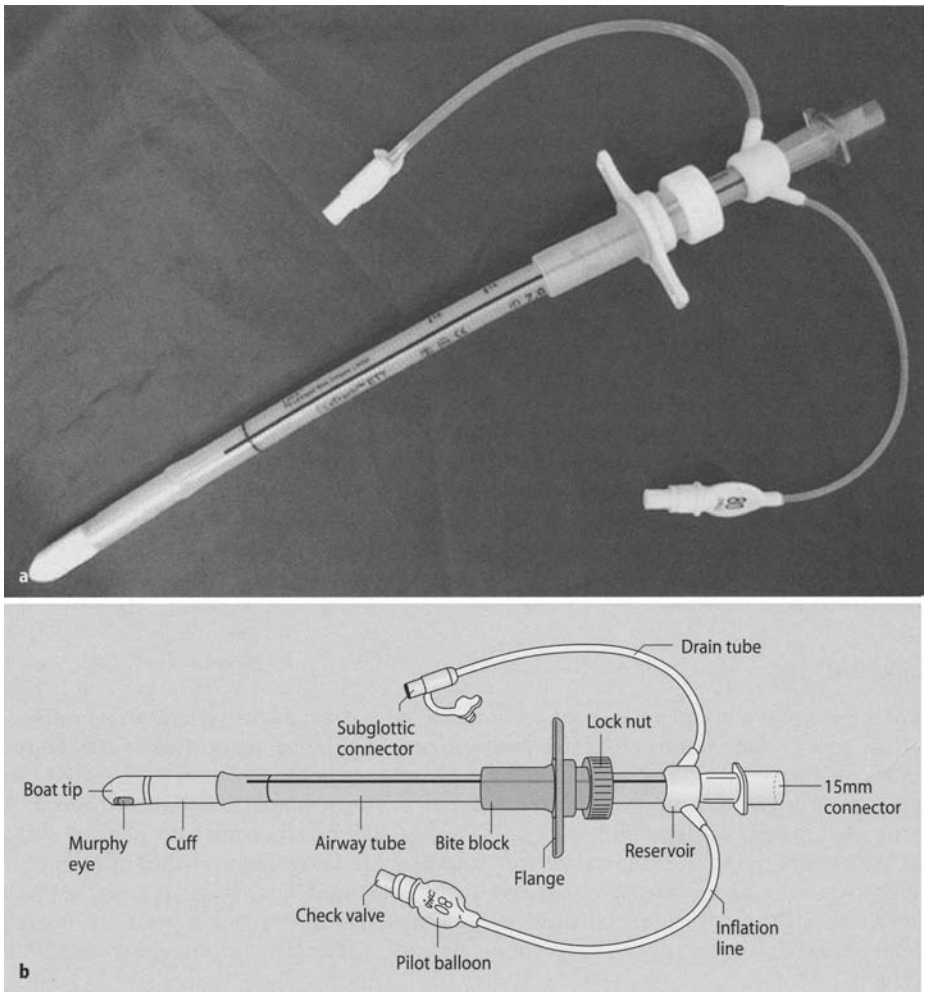

Fig. 5. (a) LoTrach tube; (b) Diagram of LoTrach Tube

kept at a desirable level of 20–30 cmH_2O [7]. There are no folds within the cuff wall to allow fluid to pass and leakage is prevented in a model trachea, in anesthetized patients, and in the critically ill mechanically ventilated patient [7]. To maintain inflation, the LoTrach can be used with either a constant pressure inflation device or with regular careful monitoring and correction of pressure using a manual manometer/inflator device.

Subglottic ports: The LoTrach tube has three integral fine bore subglottic ports which open distally in a position on three quadrants of the circumference of the tube immediately above the cuff. This provides maximum clearance of subglottic secretions independent of the geometry of the tube. The three ports join to one to allow intermittent suctioning with a standard syringe.

Upper airway cleansing: Despite best nursing care, it is impossible to provide adequate oral, pharyngeal, and laryngeal hygiene due to difficulty with access. This can lead to colonization. Because the low-volume low-pressure cuff completely prevents leakage, the subglottic ports can be used to irrigate the upper airway. Normal saline can be injected into the subglottis (taking care not to increase the subglottic pressure to over 30cmH_2O). The fluid refluxes through the laryngeal inlet and into the oral and/or nasal cavity carrying secretions with it. A suction catheter at the anterior oral cavity and/or nares is used to remove the effluent. Between 50 ml and 500 ml is typically required to remove all the offensive material and irrigations are normally performed 1–3 times daily.

Conclusion

Aspiration is the pivotal step in the development of VAP [8, 41], the most common cause of nosocomial mortality in the ICU [42]. Conventional, low cost, high-volume low-pressure cuffed tubes do not stop the ubiquitous problem of aspiration in the critically ill [18]. New technologies should address aspiration and also the multiple other factors implicated in the pathogenesis of VAP. Critical care specialists and hospital administrators need to understand the impact of VAP in terms of mortality (doubled), length of stay (increased by 6 days), and cost [43]. A conventional tracheal tube costs less than a dollar to produce, whereas newer technologies cost considerably more. Price is, therefore, a barrier to the introduction of new devices into routine practice and this inhibits the development of this technology by the industry. However, in their recent editorial in *Critical Care Medicine*, Drs Shorr and Wunderink state "even marginally beneficial preventative interventions are likely to yield significant net savings" [44]. The justification for investment in prevention is easy, each episode of VAP costs $10,000 [43], and so, if a preventative measure were estimated to reduce the VAP rate by just 2% (e.g., a fall in incidence from 20% to 18%), then it would be logical to invest $200 per patient to gain clinical benefits whilst remaining cost neutral.

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Is One Fixed Level of Assist Sufficient to Mechanically Ventilate Spontaneously Breathing Patients?

C. Sinderby, L. Brander, and J. Beck

Synchronous versus Asynchronous Assist

Mechanical ventilation delivers pressure, flow, and/or volume to the patient with the aim of improving ventilation and reducing inspiratory work. Depending on various circumstances, such as the level of sedation, paralysis, or if the ventilator support is patient triggered or not, the goals of mechanical ventilation (improved ventilation and reduced work of breathing) may be achieved in different ways.

In its simplest form, the mechanical ventilator delivers assist according to a preset level of assist, ventilator frequency, and duty cycle (controlled mode). If the patient is not breathing (i.e., no neural inspiratory effort) the ventilator must assume all the work of breathing to overcome inspiratory loads, and the tidal volume and the frequency of breath delivery is adjusted to maintain adequate blood gases. When no neural inspiratory effort is present, unloading is achieved by sedation and mandatory ventilation keeping CO_2 levels lower than what is necessary to breathe spontaneously. Figure 1 (left panel) illustrates a patient ventilated in the volume control mode who is not triggering any inspirations or showing any electrical activity of the diaphragm (EAdi), i.e., the patient is not performing neural inspiratory efforts. The right panel of Figure 1 shows the same patient on volume control 40 minutes later, and at this time the diaphragm is very active and the patient tries to inspire, however, since the volume delivered to the patient is fixed, and not adaptable, all the patient's inspiratory efforts are performed in vain.

It is reasonable to assume that patients who are not paralyzed, although sedated, are likely to have changes in respiratory drive and that modes that deliver fixed frequencies and volumes are not ideal in this situation. Modes of mechanical ventilation where inspiratory efforts trigger and cycle the ventilator have, therefore, been introduced to better meet patient requirements by attempting to deliver assist in synchrony to patient effort. Patient-ventilator interaction has two dimensions: The first dimension relates to ventilator timing in relation to a patient's neural timing and the second relates to how the magnitude of the assist level adapts with neural inspiratory effort [1].

Timing of Assist Delivery

When mechanical ventilation is applied in a patient who is breathing, the ventilator assist could either be delivered at the same time as the patient's inspiratory effort (so called patient-ventilator synchrony) or the assist could be delivered asynchronously with the patient's inspiratory effort (i.e., not delivered at the same time as the

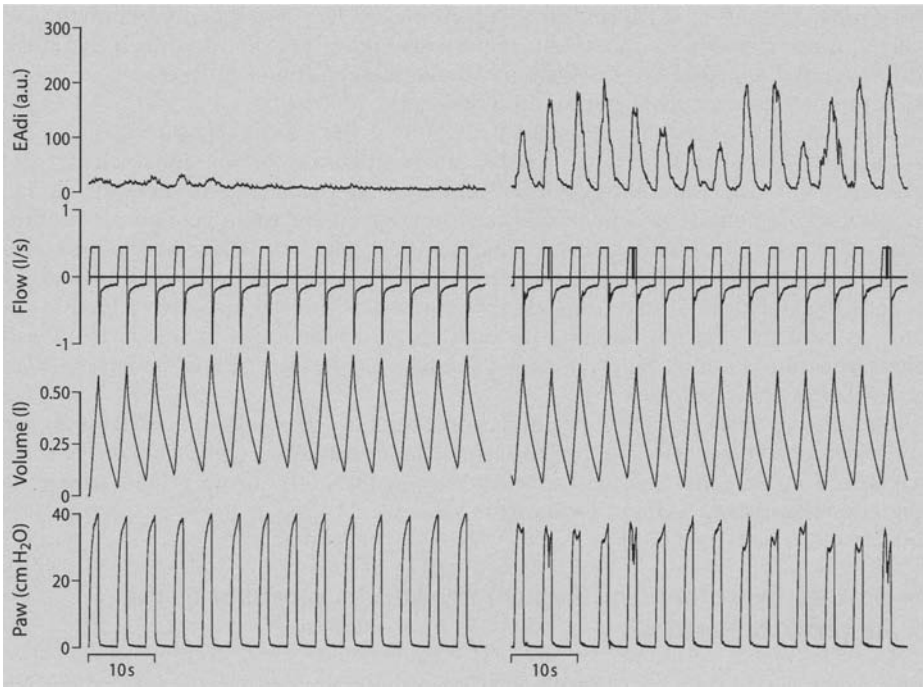

Fig. 1. From top to bottom: Diaphragm electrical activity (EAdi), flow, volume and airway pressure (Paw) in a patient with respiratory failure ventilated on volume control. The left and right panels illustrate measurements in the same patient 40 minutes apart. As indicated in the left panel, the diaphragm is neurally apneic. Forty minutes later the diaphragm is very active, indicating increased inspiratory efforts; however the ventilator mode does not allow the patient to change his tidal volume regardless of whether he makes inspiratory efforts or not.

patient is inspiring). Examples of mild forms of asynchrony in terms of timing include late triggering of assist as well as early or delayed off-cycling of the assist. Severe forms of asynchrony can involve inspiratory efforts that do not trigger the ventilator at all, so called wasted inspiratory efforts [2] or the inability to cycle-off the assist, so called hang-ups [3].

Magnitude of Assist

The second dimension of patient-ventilator interaction relates to the adjustment of the assist level in relation to the magnitude of the patient's neural inspiratory effort. Today, mechanical ventilation is typically associated with delivery of one fixed level of assist (i.e., targeted pressure or volume) to the patient, where the frequency of adjustment is arbitrarily determined and the level of assist is adjusted according to changes in the patient's clinical status.

Regardless of whether the patient is breathing or not, there are a number of factors that may vary with time and which can influence the need for an adjustment in the assist. For example, respiratory mechanics, i.e., elastance and resistance of the respiratory system, can alter. Insufficient exhalation time in relation to the expira-

tory time constant of the respiratory system may induce dynamic hyperinflation so called intrinsic positive end-expiratory pressure (iPEEP), a threshold load that must be overcome to initiate the inspiration. Altered metabolism, e.g., fever, may increase CO_2 production and, thus, the need for increased ventilation.

If a patient is breathing spontaneously, and if the mechanisms controlling the respiratory drive are functional, he/she can compensate for the above changes in respiratory status. If the settings of the ventilator are inadequate to meet the altered respiratory demand, the patient will need to compensate when changes in respiratory status occur, something that a patient with respiratory failure may not be prepared for if the demand is high (e.g., respiratory muscles are weak and inspiratory load is high). Recent studies indicate that patients with high inspiratory load [4] as well as patients who fail weaning [5] have marked reductions in the variability of their breathing pattern, whereas patients who wean successfully demonstrate more variable breathing patterns [5].

In order to avoid respiratory failure, it is, therefore, likely that the assist level has to be set to a level where the patient can sustain ventilation when demand is at its highest. Consequently, this level of assist may be too high during periods when the patient's respiratory demand for assist is lower.

How do Synchrony and Asynchrony Affect Unloading of the Respiratory Muscles?

One example of severe asynchrony in terms of both timing and magnitude is when a fixed level of ventilatory assist is delivered during the neural exhalation period. This means that the ventilator would provide a more or less fixed tidal volume during the period when the patient is not making an inspiratory effort. Thus, the ventilator cannot aid the patient's inspiratory effort in terms of sharing the work of inspiration and, with most modes currently used, the patient's inspiratory efforts would be performed against closed inspiratory valves. Despite such wasted inspiratory efforts, the ventilator will deliver tidal volume at a breathing frequency that could be sufficient or even excessive with regards to satisfying the patient's ventilatory requirements. If the asynchronous ventilation provokes the patient to recruit expiratory muscles to interrupt the assist delivered by the ventilator, which is not uncommon [6], work of breathing may actually increase [7], it becomes difficult to ensure that appropriate assist is delivered, and sedation may be required.

If the ventilator's assist period coincides with the inspiratory effort of the patient, this will act to unload the patient's inspiratory muscles since the ventilator will assume a part of the work to reduce the respiratory system load. The combination of the patient's inspiratory effort, which increases the distending pressure around the lungs (pleural pressure, Ppl), and the pressure delivered by the ventilator, which increases the airway pressure (Paw), will increase the transpulmonary pressure (Ptrans = Paw-Ppl), and hence increase the tidal volume.

If the ventilator's assist is delivered at the same time as the patient is inspiring, i.e., the ventilator's trigger and cycling off is synchronized to neural inspiratory effort, the ventilator and the patient will share the work of breathing to overcome inspiratory loads. However, truly synchronized assist occurs when the sharing of load between the ventilator and patient adapts in relation to patient effort, i.e., both the timing and magnitude of the assist delivered by the ventilator are synchronized to the patient's inspiratory effort.

Typical features of asynchronous assist could thus be identified as maintenance of ventilation without aiding the patient's inspiratory efforts while maintaining patient comfort through sedation. In other words, asynchronous ventilation of a patient involves suppression of respiratory drive through ventilation and sedation. On the other hand, synchronous assist delivery will aid the patient to overcome inspiratory loads, i.e., the ventilator is 'pushing' when the patient is 'pulling'. If optimal patient-ventilator synchrony is present, the ventilator should provide assist and share the patient's inspiratory effort, i.e., the ventilator should act as an inspiratory muscle prosthesis which can deliver support in proportion to inspiratory demand, allowing the patient to control both timing and depth of inspiration.

Sedation is another factor that helps unload respiratory muscles (by inhibiting respiratory drive) but also affects control of breathing. Breathing is controlled by multiple feedback loops including neural networks and chemical reflexes as well as by direct voluntary control. The central chemoreflex (i.e., the ventilatory response to carbon dioxide mediated by the central chemoreceptors), and the peripheral chemoreflex (i.e., the ventilatory response to carbon dioxide and hypoxia mediated by the peripheral chemoreceptors) are the key components of models describing the control of breathing [8]. Sedation not only suppresses voluntary control of breathing but may also alter the sensitivity of chemoreceptors for their specific stimuli (e.g., hypercapnia or hypoxia).

Ideally, a comfortable level of analgesia and sedation should be achieved that allows the patient to control respiratory drive while the ventilator unloads the respiratory muscles by overcoming elastance and resistance of the respiratory system. However, in clinical practice, a variety of reasons necessitate that the level of sedation is increased, including difficulties to achieve synchrony between the patient and the ventilator, especially when the respiratory drive is high [9]. Most of the commonly used sedative drugs and opioids depress the response of the respiratory centers to breathing stimuli in a dose-dependent manner, although differences between substance groups may exist [10–12].

Increasing the level of sedation reduces the load on the respiratory muscles and reduces the production of CO_2, but also deprives the patient of the control over the respiratory drive. For example, Grasso and colleagues [13] increased the sedation level stepwise to achieve a Richmond agitation-sedation scale (RASS) of -1 (drowsiness), to -2 (light sedation), and to -3 (moderate sedation). These investigators found that an increase in the sedation level resulted in a monotonous breathing pattern (i.e., a progressive loss in variability of tidal volume, respiratory rate, and inspiratory time) [13].

According to the above discussion, factors that influence unloading can be summarized to:

a) unloading by delivering a fixed level of assist asynchronous to the patient's demand, which is in essence maintaining ventilation but not assisting patient efforts. Unloading thus takes place by only ventilating the patient, reducing CO_2 levels, and reducing the central respiratory drive
b) increasing sedation, which reduces the respiratory drive and the sensitivity to respiratory stimuli and hence reduces the load on respiratory muscles, but also deprives the patient of the control over the respiratory drive
c) Unloading by delivering assist in synchrony and in proportion to the patient's demand overcoming inspiratory loads while allowing the patient to maintain control over his/her breathing pattern.

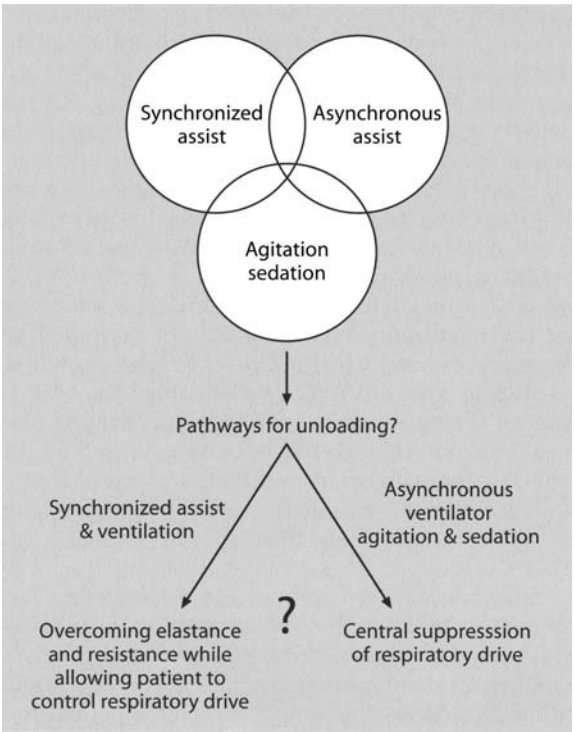

Fig. 2. Different ways of unloading respiratory muscles in breathing patients are indicated by the three circles. Unsynchronized mechanical ventilation suggests that the patient is being ventilated, which satisfies the ventilatory demand without assisting during the patients inspiratory efforts. Agitation often increases respiratory muscle efforts whereas sedation can reduce the respiratory drive (i.e. decreases respiratory muscle effort) and thus unloads respiratory muscles. Combination of asynchronous mechanical ventilation and sedation likely acts to unload the respiratory muscles through suppression of respiratory drive not necessarily assisting to overcome inspiratory loads during the patient's inspiratory effort; moreover, it may hinder the patient in maintaining control of ventilaton. Synchronized mechanical assist + ventilation suggests that assist is delivered to overcome inspiratory loads, aiding inspiratory muscles during inspiration while allowing ventilation to be controlled by the patient.

Today's clinical practice of mechanical ventilation in spontaneously breathing patients likely involves a combination of all these factors as indicated by Figure 2. Introducing changes in respiratory demand would certainly add complexity to this model increasing the likelihood that patients will alter between the different types of unloading described in Figure 2.

Patient-ventilator Interaction and Adaptation to Changes in Respiratory Demand with Frequently used Modes of Ventilation

In order to adapt the assist to changes in respiratory demand, modes of mechanical ventilation must adapt to both changes in respiratory timing and magnitude of inspiratory effort. Few studies comment on how current modes perform with respect to timing of assist in relation to patient effort. For example, synchronized intermittent mandatory ventilation (SIMV) is actually poorly synchronized to a patient's neural inspiratory effort [14]. Increasing pressure support causes off-cycling to be prolonged into the neural exhalation period [2, 15]. Asynchronous off-cycling interferes with breathing pattern [14–16]. Furthermore, it appears that delayed off-cycling also has a negative impact on pneumatic triggering of ventilatory assist [6]. In fact, the Cochrane review on synchronized mechanical ventilation in neonates remarked on limited evidence that modes that are considered to deliver assist synchronously to patient effort actually are doing what they are intended to do [17].

A recent modification of mechanical ventilation to overcome wasted inspiratory efforts during asynchronous ventilation is by using two levels of continuous pressure, allowing the patient to breathe freely regardless of the pressure level. Though this eliminates the negative impact of occluded inspiratory/expiratory efforts, the assist delivery could still be asynchronous in terms of timing and is asynchronous in terms of magnitude of assist for the same reasons as during pressure support. Moreover, the assist is not adjusted in response to changes in patient inspiratory effort.

Today, there are few modes that deliver patient-triggered assist that adapts the level of assist in relation to the patient's inspiratory effort. The first mode to be introduced was proportional assist ventilation (PAV) [1]. During PAV, the ventilator delivers positive pressure throughout inspiration in proportion to the air flow and volume generated by the patient where the magnitude of unloading is based upon measurements of elastance and resistance [1]. Unlike other modes assisting spontaneous breathing, air flow, tidal volume, and airway pressure are not preset. PAV has been shown to be effective in unloading the respiratory muscles [18].

Neurally adjusted ventilatory assist (NAVA) [19] is directly driven by the neural output to the diaphragm. During NAVA, positive pressure is instantaneously applied

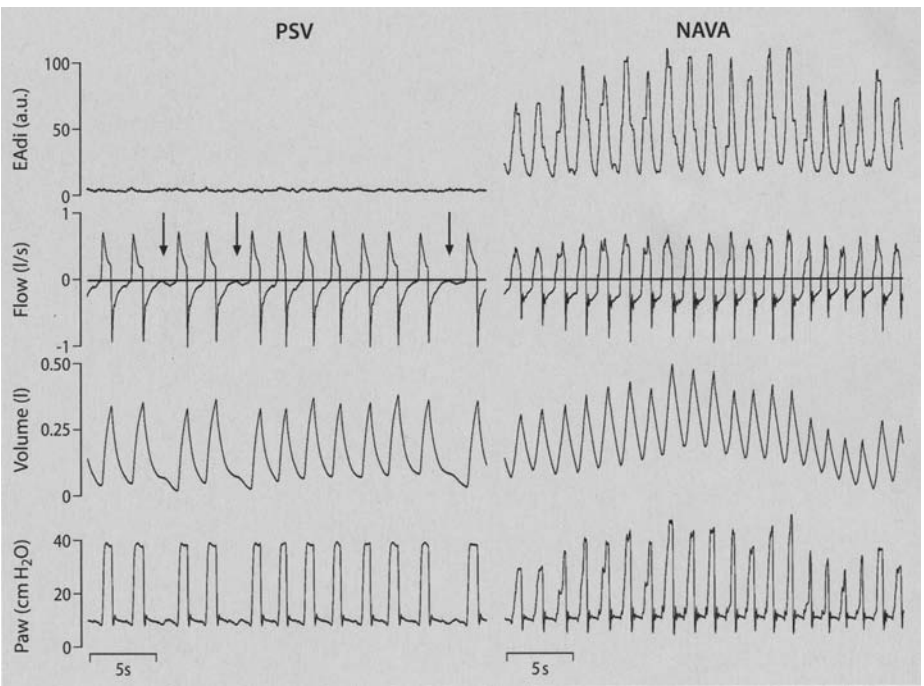

Fig. 3. From top to bottom: Diaphragm electrical activity (EAdi), flow, volume, and airway pressure (Paw) in a patient with respiratory failure on pressure support ventilation (PSV, left panel) and neurally adjusted ventilatory assist (NAVA, right panel). All delivered breaths were triggered during PSV period and, as indicated by the arrows, there were also indications of wasted inspiratory efforts in the flow and pressure tracings. The EAdi, however, indicated that the diaphragm was not active at all during PSV. After switching the patient to NAVA, diaphragm activity was restored (right panel), suggesting that the patient ventilator asynchrony was because of overassist during PSV and that waveform analysis of flow and pressure without EAdi may be misleading.

to the airway opening in proportion to the measured amplitude of the EAdi. The EAdi constitutes the temporo-spatial summation of the phrenic nerve activity from the brain's respiratory centers to the diaphragm motor units [20] and is influenced by both facilitatory and inhibitory feedback loops controlling respiratory drive. EAdi provides a reliable estimate of inspiratory timing [21] and drive [20, 22]. Hence, during NAVA, the ventilator support is synchronous with and proportional to the respiratory drive and, therefore, acts as an external 'respiratory muscle pump' controlled by mechano- and chemo-receptors as well as by voluntary and behavioral inputs [23, 24].

To emphasize the importance of a patient driven assist, the left panels of Figure 3 shows EAdi and airway pressure, flow, and volume during pressure support ventilation (PSV). The patient is receiving inspiratory assist at a frequency of 24 breaths per minute and each breath is triggered by the patient. The flow tracing in the left panel of Figure 3 also indicates that the patient suffers from wasted inspiratory efforts (indicated by arrows). However, the EAdi tracing does not reveal that the diaphragm is active, i.e., with regards to EAdi the patient is not using his diaphragm to breathe. In the right panel of Figure 3, the patient has been switched to NAVA, i.e., no assist is delivered unless the diaphragm is electrically active. With NAVA the patient is making noticeable inspiratory effort as indicated by the EAdi, confirming the possibility that the patient was over-assisted during PSV.

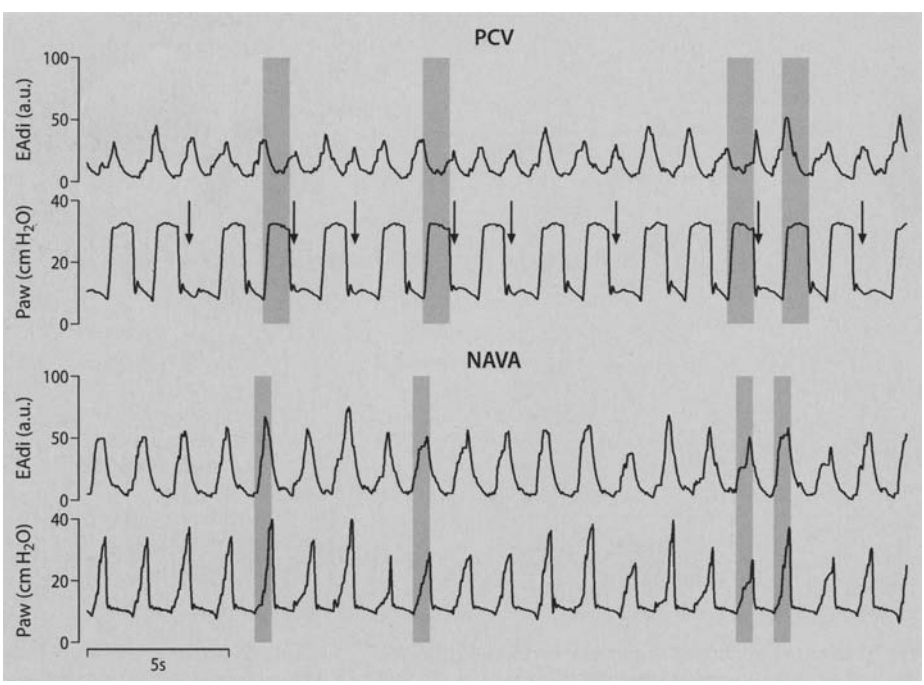

Fig. 4. Diaphragm electrical activity (EAdi) and airway pressure (Paw) in a patient with respiratory failure on triggered pressure control ventilation (PCV, top panel) and neurally adjusted ventilatory assist (NAVA, lower panel). Despite a strong EAdi signal, the delivered ventilator breaths are poorly synchronized to the EAdi as indicated by the shaded areas in the upper panels. Moreover, arrows indicate a high frequency of wasted inspiratory efforts. When switching to NAVA (lower panels), all EAdi efforts are synchronized with the ventilatory assist.

In this case it appeared that the flow trigger was so sensitive that the patient was able to trigger the ventilator with other muscles than the diaphragm and then received a full breath without using the diaphragm. In contrast to PSV, NAVA would not deliver assist if the patient is not making inspiratory efforts with the diaphragm. Since the diaphragm normally is recruited in every breath in healthy subjects, one would assume that a patient with respiratory failure should recruit the diaphragm to breathe. Absence of EAdi during PSV hence suggests that the patient was either over-assisted or too sedated and that NAVA restored the normal activation of the diaphragm.

From one extreme to another, Figure 4 (upper panel) shows poor patient ventilator synchrony with numerous wasted inspiratory efforts during pressure control ventilation despite significant levels of diaphragm activity in every breath. When switching to NAVA, the ventilatory assist becomes synchronized to the patient's demand and every inspiratory effort of the patient is shared by the ventilator's pressure delivery.

Finally, as an example about adaptation of assist level over time, Figure 5 top panel illustrates how highly variable neural inspiratory efforts over time are not met by alteration in assist during pressure support whereas during NAVA (lower panel) each inspiratory effort is shared by the ventilator both in time and magnitude.

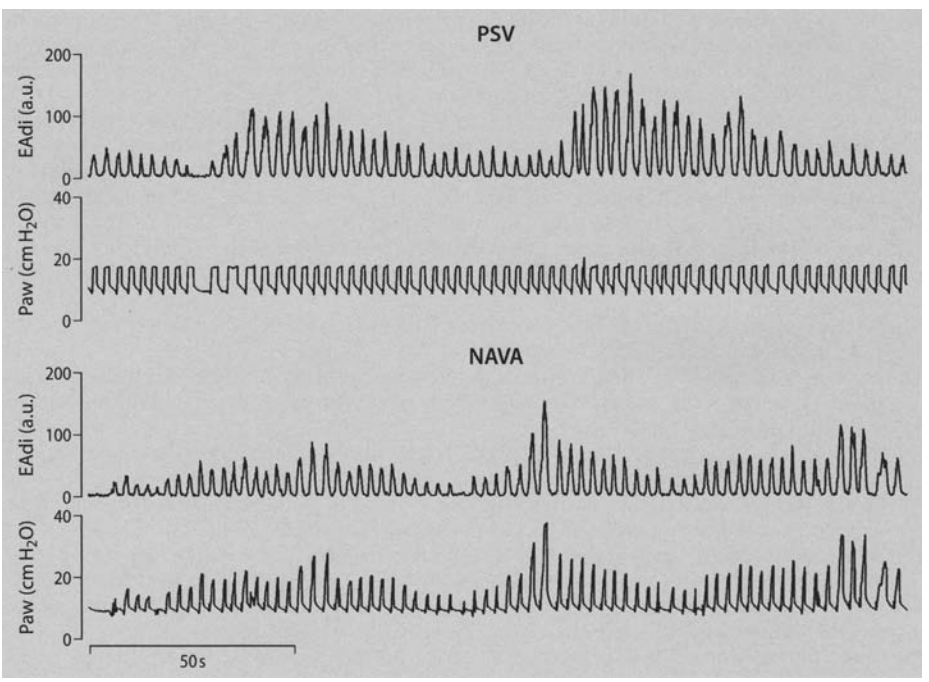

Fig. 5. Diaphragm electrical activity (EAdi) and airway pressure (Paw) in a patient with respiratory failure on pressure support ventilation (PSV, top panel) and NAVA (lower panel). During PSV assist is constant at one level despite large variability in the EAdi. During NAVA, the pressure delivery is in proportion to EAdi such that low pressure is delivered during low neural inspiratory efforts (low EAdi) and high pressures are delivered during high inspiratory efforts (high EAdi). In other terms, PSV delivers monotonous assist regardless of patient needs, whereas during NAVA the mechanical ventilator is sharing the work with the patient.

Conclusion

One fixed level of assist may be insufficient to satisfy a patient's ventilatory demand and there is a need for ventilator modes that deliver assist in time and in proportion to patient inspiratory effort. However, modes of mechanical ventilation used today are poorly evaluated with respect to timing of assist and the majority do not adapt to changes in patient respiratory demand. Moreover, there is a lack of monitoring devices to evaluate respiratory drive and determine if the assist is delivered in synchrony with patient effort.

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Patient-ventilator Interaction During Non-invasive Ventilation with the Helmet Interface

O. Moerer, C. Sinderby, and F. Brunet

Introduction

Non-invasive ventilation (NIV) for the treatment of acute and chronic respiratory failure has achieved an increasingly important role over the last decade. Until the mid-eighties, mechanical ventilation in intensive care unit (ICU) patients with acute respiratory failure was generally delivered invasively via an endotracheal or tracheostomy tube. With growing knowledge of pathophysiology, it became apparent that there are also risks and complications, not only related to mechanical ventilation itself (volu- and barotrauma), but especially if mechanical ventilation is delivered invasively, such as the increased rate of nosocomial pneumonias [1]. Hoarseness, sore throat or vocal cord dysfunction becoming apparent after extubation may also result in long term complications [2]. Therefore, the application of NIV techniques seems logical.

Since the first clinical application of NIV in ICU patients [3, 4] the spectrum of indications has broadened and the percentage of mechanical ventilation applied non-invasively has continuously increased. Today NIV is used in the treatment of acute and chronic respiratory failure and during weaning from invasive ventilation [5–8]. It has been applied in patients with acute hypoxemic respiratory failure, severe cardiogenic pulmonary edema [9], and acute exacerbation of chronic obstructive pulmonary disease (COPD) in order to decrease the need for, and complications of, endotracheal intubation. A large body of clinical studies has proven the value of NIV although it became evident that careful patient selection and monitoring is crucial, with patients with acute respiratory distress syndrome (ARDS), for example, being less likely to benefit from NIV [10, 11].

Some problems related to higher gas leakage and dead space are observed with NIV. Specific modes of ventilation have been developed in recent intensive care ventilators to overcome these issues, as will be discussed later. However, the role of the interface used to deliver NIV should not be underestimated! In a study by Navalesi and coworkers [12], the choice of interface improved the performance of NIV more than the mode of ventilation. Today a large number of different masks are available. Problems with the widely used face masks result partially from air leakage [12, 13], discomfort for the patient [14], and pressure-related ulceration of the nose [15, 16]. These problems may limit the duration of use and account for a large proportion of NIV failures.

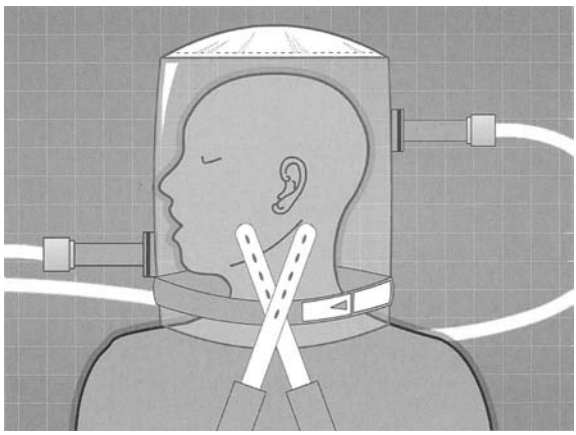

Fig. 1. The Helmet interface for non-invasive ventilation.

The Helmet Interface

Recently, a helmet interface was developed in order to improve NIV tolerance and patient comfort (Fig. 1). Compared to conventional interfaces, it offers increased patient comfort. The patient is able to communicate with his/her environment, see or even read while being ventilated. There is also a lower risk of skin lesions due to the special fixation system, with no direct pressure on facial structures and it can be used regardless of the anatomical structure of the face [17]. To our knowledge there are currently two manufacturers distributing a helmet for NIV or continuous positive airway pressure (CPAP) (Castar R, StarMed, Mirandola, Italy, and 4Vent Rüsch, Rüsch GmbH, Kernen, Germany), and another distributor (Series 500 Sea-Long Medical Systems, Inc., Louisville, Kentucky), offering a helmet device for hyperbaric medicine and oxygen therapy. The two models allowing administration of NIV or CPAP are transparent, latex free hoods, joined by a soft polyvinyl chloride collar in the Castar R and polyurethane collar in the 4Vent helmet. The collar of the Castar R has inflatable cuffs, whereas the 4Vent has no cuffs. Inspiratory and expiratory tube connectors are connected at the upper part of the Castar R, and at lower part of the 4Vent helmet. Two underarm laces attach to a ring at the lower end of the helmet and prevent the helmet from lifting when it is inflated. A plastic collar fitting around the neck prevents leakage during ventilation. Inspiratory and expiratory tube connectors are connected in the upper part of the helmet. The Castar R Helmet (size M) has an internal volume of 7.5 l with inflated cuffs. The 4Vent Rüsch Helmet has an internal volume of about 8.0 l. Depending on the size of the patient's head, this volume is reduced by about 50% when put on. Both helmets contain special ports for a nasogastric tube or to enable patients to drink through a straw. Some Starmed models are also equipped with a safety valve with automatic opening which allows immediate access to the patient without taking off the helmet.

Recent Studies using the Helmet Interface

The helmet has been successfully used in different clinical situations (Table 1).

Table 1. Recent clinical studies and published reports using the helmet interface for continuous positive airway pressure (CPAP) or non-invasive ventilation (NIV). Clinical improvements and improvements in PaO_2, $PaCO_2$ are presented qualitatively as: ++ = good improvement; + = moderate improvement; – = insufficient improvement; -- = deterioration; 0 = no improvement or stable; x = no data presented. ACPE: acute pulmonary edema; ARF: acute respiratory failure; IS: immunosuppressed; ER: emergency room; ICU: intensive care unit; NPSV: non-invasive pressure support ventilation; NPPV: non-invasive positive pressure ventilation. Results about comparison with face mask (if performed) are presented within the text.

	Patients	Location	Study design	NIV_{Helmet} Number of patients [n]	Vent. mode	Max. (mean) NIV duration [hours]	Early/late mean improvement PaO_2	$PaCO_2$	Clinical qualitative	Intubation rate Total	Helm intolerance % (n)	ICU Mortality % (n)	Complications related to NIV_{Helmet} % (n)
Foti (18)	ACPE	Out Hosp.	Prospect. study	27	CPAP	x (0.37+0.12)	++/ x	x	++/ x	0	0	x	0
Antonelli (23)	Hypox. ARF	ICU	Prospect. pilot study/ Retrosp. matched control	33	NPSV	x (36+29)	++/+	+/0	+/+	24 (8)	0	9 (3)	0
Tonnelier (19)	ACPE	ICU	Prospective pilot Retrosp. control	11	CPAP	23 (13 ±4)	++/+	0/+	++	0	0	9.1	0
Pelosi (29)	ACPE	ER	Prospect. Study	23	NPSV	x (6±1)	x /++	x /+	x /++	4 (1)	0	x	0
Rabitsch (20)	ARF IS	ICU	Prospect. Study	10	CPAP	x	++/++	+/+	++/++	20 (2)	0	60 (2)	0
Antonelli (26)	Acute exacerb. COPD	ICU	Prospect. study Historical control	33	NPSV	23 (39 ±42)	++/++	+/-	++/+	30 (10)	0	9 (3)	3 (1)
Piastra (27)	Pediatric	ICU	Pilot study	4	NPSV	x	++/++	x	++/+	50(2)	0	50 (2)	0
Tüller (30)	ACPE	Angioplasty room	Case report	1	NPSV	x	++/x	x	++/x	0	0	0	0
Rocco (24)	ARF IS	ICU	Case control study Matched control	19	NPPV	x (13 ±6)	++/++	0/0	+/+	37 (7)	14 (1)	31 (6)	10 (2)
Klein (25)	ARF weaning	ICU	Case report	1	NPPV/ CPAP	24	++	++	++	0	0	0	0
Squadrone (22)	Hypoxemia	ICU	Randomized clinical study	105	CPAP	x (19±22)	++/++	0/0	++/++	1 (1)	0	0	0
Piastra (21)	Pediatric	ICU	Case report	1	NPSV	x	++/++	++/++	++/++	0	0	0	0
Codazzi (28)	ARF Pediatric	ICU	Prospect. study	15	CPAP	x (40 ±30)	++/+	0/0	++/+	13 (2)	0	0	0

CPAP with the Helmet Interface

Initially, the helmet was introduced as a CPAP device [18]. In addition to the good improvement in oxygenation, shown in eleven patients admitted with acute respiratory failure due to acute cardiogenic pulmonary edema, and a good tolerance to the interface, the improvement in $PaCO_2$ was delayed when compared to a control group ventilated with a conventional face mask [19]. These findings were attributed to the huge dead space of the device (9–15 l). A study by Rabitsch et al. also showed good results regarding PaO_2 improvement and tolerance in immunosuppressed patients with acute respiratory failure [20]. This approach was also successfully applied in a pediatric patient with an acute crisis of myasthenia gravis [21]. Recently, the helmet has been studied in patients who postoperatively developed hypoxemia after major elective abdominal surgery [22]. In this study, 209 patients with a $PaO_2/FiO_2 < 300$ were randomized to receive either oxygen through a Venturi mask or CPAP of 7.5 cmH_2O delivered by the helmet. This study, was stopped early after an interim analysis showed that the application of CPAP resulted in a significantly reduced intubation rate (1 vs. 10%, $p < 0.005$) and lowered the incidence of infectious complications.

Non-Invasive Pressure Support Ventilation with the Helmet Interface

Using the helmet for non-invasive pressure support ventilation (PSV), Antonelli and co-workers studied the helmet interface in patients with hypoxemic acute respiratory failure in comparison to matched control patients treated with the face mask [23]. In both groups, oxygenation improved during non-invasive PSV. $PaCO_2$, pH, and respiratory rate were equal in the two groups over time. There was a tendency towards an increased need for intubation in the group ventilated via a face mask (32 vs. 24%), which did not reach significance. Interestingly, the duration of continuous application was longer in the helmet group (36 ± 29 vs. 26 ± 13 hours). The reason for intubation was related to NIV intolerance in 38% of the patients treated with the face mask while none of the helmet patients were intubated for this reason. The percentage of complications (skin necrosis, gastric distension, eye irritation) was significantly increased in the patients who were treated with a face mask (21% vs. 0%). Comparable results were reported by Rocco et al. [24], who noticed a sustained improvement of PaO_2/FiO_2 ratio in 74% of the patients treated with a helmet. Intubation rate was related to helmet intolerance in one case while it was attributable to the face mask in four patients. In three helmet patients, hypoxia could not be corrected sufficiently over time and thus required invasive ventilation. Recently non-invasive PSV with the helmet was effectively used for weaning in patients with acute respiratory failure after extubation [25].

The helmet has also been used in patients with acute exacerbations of COPD treated with non-invasive PSV [26]. Again this study showed a better tolerance of the helmet interface compared to face-mask, with a lower number of complications (3 vs. 36%) and comparable results regarding improvement of oxygenation. However, there was a lesser decrease in $PaCO_2$ in the helmet group and significantly higher $PaCO_2$ at discontinuation of non-invasive respiratory support. Especially in those patients treated unsuccessfully, $PaCO_2$ levels remained high during non-invasive PSV with a helmet.

The Helmet Interface for Pediatric Patients

In pediatric patients, the helmet has been used in children in a pilot study including four patients with acute leukemia who suffered from acute respiratory failure [27]. In this study non-invasive PSV was applied with a helmet with a positive end-expiratory pressure (PEEP) between 7 and 10 cmH_2O and a pressure support of 12 and 23 cmH_2O, adjusted to achieve a reduction in oxygen need, decrease in respiratory rate, and disappearance of accessory muscle activity. In all patients, an improvement in oxygenation was observed immediately. No complications attributable to the use of the helmet were reported. Despite these promising results, it is too early to recommend the helmet for a broader use in pediatric ICU patients. CPAP was also effectively applied in 15 children (nine$\leq$9 yr old) predominately treated for post extubation hypoxemic acute respiratory failure [28]. After a CPAP trial (at 5 and 10 cmH_2O) the patients were ventilated at the lowest level that ensured improvements in gas exchange. Helmet-CPAP was well tolerated and improved oxygenation in all but two patients who had to be intubated due to respiratory muscle fatigue.

Helmet Use Outside the ICU

There are several reports where the helmet has been successfully used outside the ICU. Foti and coworkers successfully used the interface in 26 patients for emergency treatment outside the hospital during transport to the emergency ward [18]. It has also been applied in patients with acute cardiopulmonary edema directly after admission to the emergency department [29]. In 23 patients, non-invasive PSV with a helmet was able to improve PaO_2/FiO_2 (from 92+4 to 265 mmHg), $PaCO_2$ (from 49+5 to 41 ± 6 mmHg), with mean pressure support levels of 15 ± 2 cmH_2O (PEEP 10 ± 2 cmH_20) over 6+1 hours. Recently the helmet has been used during angiography and stenting of the coronary arteries in a patient admitted to the hospital with acute myocardial ischemia and cardiopulmonary edema [30]. Although the findings of these reports need to be confirmed by controlled clinical trials, they underline the applicability of the helmet and provide a perspective for its early use outside the ICU setting.

Problems and Drawbacks Regarding the Helmet Interface

The use of a helmet as an interface to deliver CPAP or NIV is effective and seems to be superior to the face mask as reported above. However, there are some disadvantages of the helmet interface which may limit its use in some ICU patients.

Noise Exposure and Middle Ear Function

One of the concerns with the use of the helmet is the noise exposure and a potential impact on middle ear function. Cavaliere and coworkers investigated whether the pressure which is applied to the whole head in the NIV-helmet might lead to an inward displacement of the tympanic membrane, causing middle ear pressure to oscillate from peak pressure to PEEP and, therefore, affect middle ear function [31]. In this study, NIV with a helmet caused a limited but reversible increase in acoustic compliance and no significant depression of the acoustic reflex. Unfortunately, due to the short exposure time of one hour and a pressure support of 10 cmH_2O at a

PEEP of 5 cmH_2O, no conclusion could be drawn regarding the effect of longer periods of NIV or higher pressure settings. In a second study, the same group studied the noise exposure which was significantly increased to >100 dB compared to <70 dB if a mask was used [32]. Thus, the authors suggested the use of ear plugs in patients at risk or during longer periods of NIV with a helmet.

Carbon Dioxide Rebreathing

Clinical studies using the helmet for CPAP showed that the helmet is less effective in reducing elevated $PaCO_2$ levels. These results were confirmed by two studies on human volunteers [33, 34]. Patroniti and coworkers [33], who studied the performance of the helmet at different gas flow rates (20-30-40 l/min) and CPAP levels (0-5-10-15 cmH_2O) in healthy volunteers, showed significantly higher inspiratory CO_2 levels at every setting compared to the face mask. With a flow of 60 l/min, inspiratory CO_2 was significantly reduced (2.5 ± 0.6 mmHg) but still more than doubled compared to any setting when using the face mask (maximum 1.0 ± 0.9mmHg at a flow of 20 l/min). They concluded that higher gas flow rates should be considered to overcome the problem of CO_2 accumulation. Taccone and coworkers [34] confirmed these results in a recent lung model and volunteer study. Independent of the internal volume, the helmet predisposes to CO_2 rebreathing, which can be minimized by using a continuous high flow CPAP system. Thus during CPAP with a helmet, minute ventilation and $PaCO_2$ should be monitored closely.

Patient-ventilator Synchrony and Work of Breathing

The helmet for NIV may affect the trigger and cycling-off sensitivity due to its large compressible volume. Furthermore, in addition to the specific settings of the ventilator, individual patient characteristics such as compliance and resistance of the respiratory system and respiratory rate as well as the amount of gas leakage, may affect the performance of the system and could potentially result in desynchronization between the ventilator and the patient. Consequently, during non-invasive PSV the patient may inspire with delayed or even without any support from the ventilator (wasted efforts) and thus might be forced to increase his/her inspiratory effort. Chiumello and coworkers did not find missed respiratory efforts using either helmet or face mask in a study with human healthy volunteers. However, the respiratory rate did not exceed 14.9 ± 4.1 in this study [35]. In a recent *in vitro* study using a lung model capable of spontaneous breathing, it was shown that inspiratory trigger delays were more than twice as long with the helmet compared to face mask non-invasive PSV [36]. However, during the initial phase of inspiration, the inspiratory effort was actually lower during helmet ventilation compared to face mask ventilation while it was similar for the whole inspiratory phase. These findings are explained by the large reservoir of the helmet on one hand and the less effective pressurization on the other. By only studying the assisted phase of the breath, the face mask was more effective during non-invasive PSV than the helmet. Unassisted inspiratory efforts occurred at respiratory rates ≥ 20 and were influenced by the level of pressure support, the sensitivity of the flow trigger, and the respiratory compliance of the patient. These results are partially reflected by Racca and coworkers who showed that helmet non-invasive PSV was less effective in unloading the inspiratory muscles with the helmet when volunteers were breathing with an increased resistive load [37]. These investigators found significant asynchrony between the beginning

and the end of the inspiratory effort, a relevant number of auto-cycled breaths, and increased CO_2 concentrations with the helmet. Thus, the authors concluded that partial rebreathing and impaired patient-ventilator interaction are the underlying mechanisms responsible for the less efficient unloading of the respiratory muscles during non-invasive PSV with helmet and proposed that other ventilatory assist modes should be tested with the helmet interface.

Neural Control of Mechanical Ventilation

One possible way to overcome issues related to pneumatic triggering systems is by neurally controlling mechanical ventilation, which has recently been proposed to overcome problems of failure or delayed pneumatic detection of inspiratory and expiratory efforts [38]. Neurally adjusted ventilatory assist (NAVA) is a mode of partial ventilatory assist, where the ventilator is neurally controlled by the electrical activity of the diaphragm (EAdi). With NAVA, the pressure during inspiration is delivered in proportion to the EAdi, i.e., if the patient increases respiratory drive, the assist will increase. Since NAVA is controlled by EAdi, its ability to control assist delivery is not disadvantaged by leaks in the respiratory circuit or complex pneumatic interfaces as the helmet.

Neurally Triggered Non-invasive PSV with the Helmet Interface

Neural triggering and cycling-off of pressure support was compared to conventional triggering and cycling-off of pressure support, at different levels of assist (5-10-20 cmH_2O) and increasing respiratory rates (10, 20, and, 30 bpm) in healthy subjects [39] breathing with the helmet interface. The onset of the ventilatory support as well as the offset was delayed if triggered conventionally. Interestingly, wasted inspiratory efforts only occurred at high levels of assist and respiratory rates. The inspiratory effort of the volunteers during the unassisted phase was significantly increased and even exceeded the effort of the whole inspiratory phase at higher assist levels and respiratory rates. Thus, during pneumatic triggering, healthy subjects tend to increase their inspiratory effort in order to 'force' the ventilator into synchrony. In a second study, in order to simulate a clinical situation where a patient with acute respiratory failure might not be able to increase their inspiratory effort without further decompensation, subjects were coached to keep there breathing effort below 10% of maximum EAdi [40]. This led to a worsening of subject-ventilator synchrony especially with regard to off-cycling and the number of unassisted inspiratory efforts during non-invasive PSV with helmet (Fig. 2). In comparison, synchrony was assured with neural triggering. The comfort of breathing was significantly improved during neurally-triggered NIV with a helmet.

Conclusion

The recently introduced helmet interface has several advantages compared to other interfaces. It allows relatively free movement of the head while maintaining a good seal without compression to the face or head. Consistent through all clinical studies is a high patient tolerance and almost no failures or complications directly contributed to the interface itself. The helmet was shown to be as efficient as the facemask

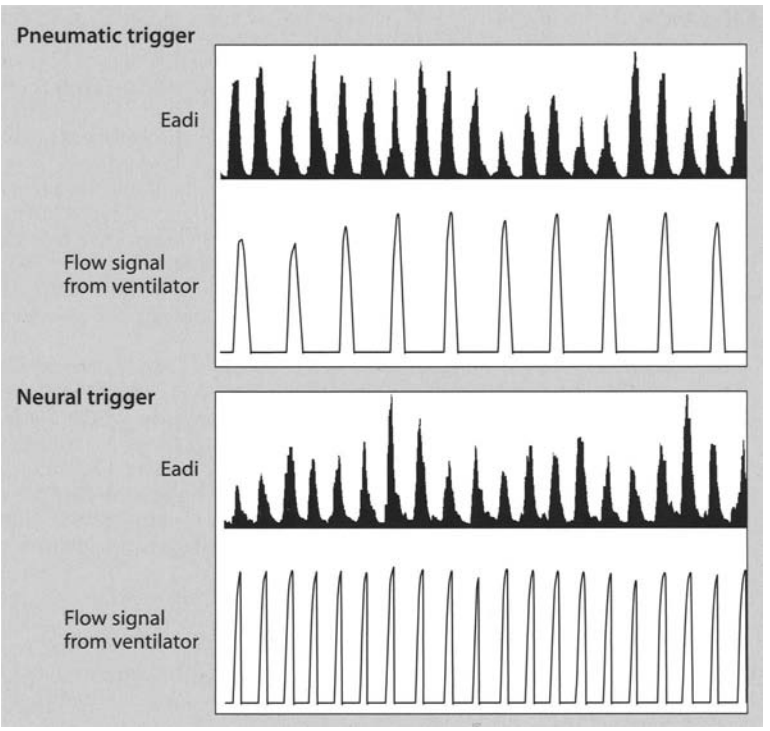

Fig. 2. Example of subject-ventilator synchrony during non-invasive pressure support ventilation with a helmet interface during neural vs. pneumatic triggered ventilation (pressure support 20 cmH_2O, respiratory rate 30 bpm). Tracing of the subject's diaphragmatic activity (EAdi) and the on and offset of the ventilator assist during a period of 40 seconds. Note that during pneumatic-triggered non-invasive pressure support ventilation with helmet, 50% of the inspiratory efforts were unassisted in this volunteer.

in increasing oxygenation. However, it is unclear if CO_2 accumulation within the helmet contributes to the insufficient decrease in $PaCO_2$ levels in some patients. In summary, clinical results are promising but a randomized clinical trial addressing both primarily hypercapnic and hypoxemic patients performed in adults as well as in pediatric patients would be desirable. As reported in healthy volunteers and *in vitro* studies, patient ventilator interactions can be impaired during non-invasive helmet ventilation, probably resulting in increased inspiratory efforts of the patient. Neural control of the ventilator might overcome some of the problems related to NIV in general, but especially with the helmet interface. At low and controlled inspiratory and expiratory efforts, neural triggering and cycling-off significantly reduced ventilator delays. Neural triggering and cycling-off improved subject-ventilator synchrony and comfort during PSV with a helmet interface compared to conventional pressure trigger and flow cycling-off. Future studies are required to demonstrate that these results are applicable to the clinical setting.

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Protective Ventilation in Respiratory Failure

Dynamic Lung Imaging Techniques in Mechanically Ventilated Patients

I. Cinel, S. Jean, and R.P. Dellinger

Introduction

Endotracheal intubation and mechanical ventilation are required for the majority of critically ill patients in tertiary care intensive care units (ICUs) [1]. During mechanical ventilation, patients often have imbalances in regional lung ventilation due to heterogeneity of lung mechanics. The current methods generally available for assessing lung function in mechanically ventilated patients include arterial blood gas analysis and graphic waveforms from ventilators (flow, pressure and volume over time as well as pressure-volume, pressure-flow and flow-volume loops). At best, these methods reflect only overall lung function, while failing to give information on disparate regional functionality. Unlike data collected from the ventilator or the blood, lung imaging allows for regional assessment of anatomy or function. Methods which provide the capability of quantifying these regional differences in mechanically ventilated patients are of great interest.

A lung imaging technique such as dynamic computed tomography (dCT) provides valuable anatomic information about lung heterogeneity and is well validated, but lacks the bedside monitoring capabilities ideal for the ICU. Electrical impedance tomography (EIT) and vibration response imaging (VRI) have emerged as new non-invasive and radiation-free imaging tools providing real time functional lung assessment. The dynamic nature of these techniques provides information on lung function and has advantages over static techniques and may better illustrate patient-ventilator interactions. Although more realistic assessment of ventilatory processes is obtained compared to static measurements, these methodologies are not currently available for routine use in clinical practice. Bedside tools for the adjustment of mechanical ventilation with the capability of measuring regional ventilation, if validated, offer significant potential utility in the ICU. For example these technologies can be used to guide and manage, and to implement lung-protective ventilation strategies.

Dynamic Computerized Tomography

CT has opened a new era in our understanding of the pathophysiological and clinical aspects of lung injury in mechanically ventilated patients. Although traditional chest radiograph shows diffuse involvement of lung parenchyma in acute respiratory distress syndrome (ARDS), our understanding of this pathology was changed after CT studies showed heterogeneity of lung involvement in ARDS [2]. CT scan provides axial images of the thorax, allowing visualization of lung parenchyma. Based on

acquiring CT images at a subsecond speed (50 to 500 msec) in a quasi-continuous fashion, CT scanning is used in dynamic conditions, including analysis of mechanical ventilation and lung perfusion [3–6]. dCT imaging is a highly sensitive approach to image-based analysis of the processes of spontaneous respiration and mechanical ventilation. This approach allows the variations of functional anatomy and their correlation with gas exchange during tidal breathing to be investigated without interrupting mechanical ventilation [7, 8].

Dynamic scanning can be performed during continuous respiration and raw CT data are then reconstructed with a predefined temporal increment (i.e., 100 ms). As the scanner table is immobile during the dynamic multiscan acquisition, chosen slices move slightly over time with the cranio-caudal respiratory motion of the lung thus affecting image quality. In the near future, multislice CT scanners will allow simultaneous acquisition of several slices.

In contrast to static breath-hold imaging, dCT acquisitions allow the assessment of several complete respiratory cycles. In light of this new technique, it becomes clear that the analysis of static CT images does not accurately reflect physiological reality during cyclic ventilation. Breath-holding is required to avoid image artifacts during conventional static CT imaging whereas fast dynamic acquisition allows a cine-type visualization of the lung inflation and deflation processes during continued respiration. dCT can also provide valuable information about regional differences in dynamic distribution during mechanical ventilation [8].

The indications for using dCT scan in clinical practice are not yet completely clear. Concerns exist about the risks of moving patients out of the ICU. The most important feature of safe transport is to ensure that adequate equipment and personnel are immediately available to cope with a catastrophic emergency such as accidental extubation, interruption of critical intravenous infusions, or extraction of venous, arterial, or enteral catheters. Patients requiring vasopressors or high inspired oxygen concentrations or positive end-expiratory pressure (PEEP) are at particular risk. Although portable CT could offer the advantages of bed-side monitoring, its availability is currently very limited. Costs of dCT imaging is another issue as well, as are the costs of transport. Using the same scanner and similar acquisition parameters, preliminary data on humans showed approximately the same radiation doses for dCT measurement and spiral CT of the thorax, which are considerable [9]. Further investigations will be necessary to optimize imaging procedures, and to reduce radiation exposure during dynamic acquisition.

Electrical Impedance Tomography

EIT has emerged as a non-invasive and radiation-free imaging technique for potential bedside use in the ICU [10, 11]. EIT uses the variability in electrical impedance between tissue, air, and fluid to provide a map of impedance. The EIT hardware injects small amounts of electrical current sequentially, using electrodes applied circumferentially to the patient's chest. A standard set up of 16 electrodes receiving small currents in a rotating fashion is currently used and can generate up to 44 cross-sectional images per second with a typical resolution of more than 10 Hz [12]. The receiving electrode calculates the voltage differential and determines the impedance between the transmitting and receiving electrodes [13]. This creates a tomogram depicting the distribution of tissue electrical properties in a cross-sectional image. Air is a poor conductor of electric current and causes high impedance,

whereas water or blood are good conductors. The difference makes it possible to detect changes in air and tissue content, enabling the assessment of ventilation distribution. The shape of the lungs is indirectly accessible by the use of the functional images, showing regions with very low changes outside and high changes within the lung. In general, an increase in aerated lung volume results in a positive impedance change and a decrease in aerated lung volume produces a negative impedance change. The cardiac-related impedance signal can be significant and may, therefore, interfere with the accuracy of the ventilation-induced impedance signal. During mechanical ventilation, the displacement of the blood away from the thorax will increase measured impedance [14]. In a recent study to limit the effect of cardiac events on measured impedance changes, a low-pass filter of 2 Hz (120 cycles per minute) was applied and the center of the EIT image containing the heart was excluded in the analysis [15].

In pigs, EIT-derived impedance changes correlated very closely with whole lung pressure-volume relationships quantified by strain gauge plethysmography [16]. The ratio of anteroposterior lung impedance changes during recruitment and derecruitment was also described in the same animal model [17]. In an animal model of acute lung injury (ALI), comparison of atelectasis using EIT and CT (as a reference method) revealed the correlation of impedance changes and volume changes [11]. A dynamic approach to assess regional recruitment was also described in patients with ALI. Victorino et al have presented results in 10 severely ill patients with marked inhomogeneity of ventilation distribution [18]. There was excellent reproducibility of the measurement of ventilation distribution when partitioning the lungs into four zones, with a variation of only 4 to 7%. Bias was minor and the difference between the EIT and CT was less than 10% for detecting imbalances between the right and left lung. The only disadvantage of this investigation was that EIT and CT images were not obtained simultaneously due to electromagnetic interference of the EIT equipment in the CT scanner. However, this study confirmed that regional impedance changes are closely correlated with regional volume changes identified by CT.

The spatial resolution of EIT is generally low and cannot be easily increased by an increase in the number of electrodes because the physical limitation of the current flow through the tissue is not changed by the number of electrodes. It is best in the area near the electrodes and worsens in the targeted regions deeper in the thorax which contain a large part of the lung volume. The transverse area of the thorax, was trapezoid in the studied patients, but the algorithms are based on a circular structure and, therefore, require modification [19]. Reliable recordings of absolute air content would be valuable. Interference by other electrical devices commonly found in the intensive care environment has not been systematically investigated. Some investigators have recently applied a software modification that shifts the EIT current out of the range of the electrocardiograph (EKG) electrodes, and the signal showed less interference [20]. The difficulty in fixing electrodes equidistant from one another and maintaining the connections throughout the recording is also a concern.

Compared with dCT, EIT is cheaper, smaller, and requires no ionizing radiation. EIT can in principle produce thousands of images per second. Its major limitations are its low spatial resolution, and large variability of images among subjects. To obtain reasonable images, at least one hundred, and preferably several thousand measurements must be made.

Vibration Response Imaging

The use of acoustic signals from the thorax to evaluate the functioning of the lungs is not a new concept. In the early 1800s, Laennec invented the stethoscope and described lung sounds and this practice continues today but is considered more an art than science due to its subjective nature [21]. Attempts have been made to move this clinical tool more towards the realm of science by some investigators whose research revealed that lung sounds are associated with inspiratory flow rate [22], patient position [23], and position of sensors [24]. In this way, distribution of regional ventilation was able to be measured using breath sounds [25]. Although interesting, the computational capabilities are not yet available to develop this concept into a viable imaging modality.

VRI is a novel dynamic imaging technique that measures vibration energy of lung sounds generated during respiration and mechanical ventilation [26, 27] (Dellinger et al., unpublished data). As air enters and leaves the lungs, the vibrations propagate through the lung tissue and are recorded by surface sensors. The current device uses 36 surface skin sensors (6 rows) which are spatially distributed and attached to the patient's back. A protoptype device with 7 rows is shown in Figure 1. The vibration energy signal is transmitted to the VRI device where it is processed and a dynamic digital image is created. Each frame of the dynamic image represents 0.17 seconds of the respiratory cycle. In the dynamic image, left and right lungs are depicted side by side and the image simulates size and structure of the lungs and spine. In addition, a graph is produced that represents the average vibration energy as a function of time and is displayed under the image. Numerical raw values for vibration energy are also available and can be used to analyze and compare any regions of interest. Areas with greatest vibration energy are depicted as dark colors (black) and low energy areas are shown in light colors (light gray); minimum energy areas are defined as "white". The maximal energy frame (MEF) of the inspiratory phase typically shows the maximum area of the vibration distribution in the VRI image. The MEF image of a normal healthy non-smoker is shown in Figure 2. The VRI technology is totally non-invasive and requires no radiation.

The dynamic image produced from the VRI recording provides a sense of the air movement in the lungs. The VRI was designed to diagnose lung pathologies that

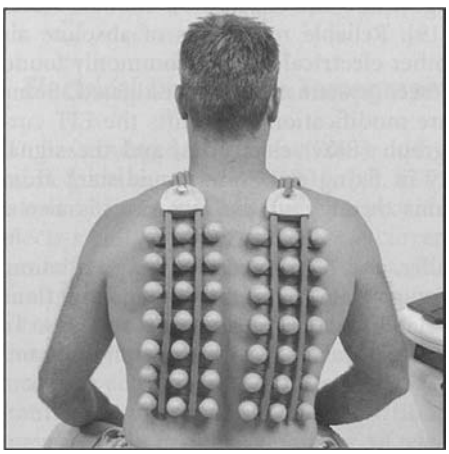

Fig. 1. Attachment of VRI sensors.

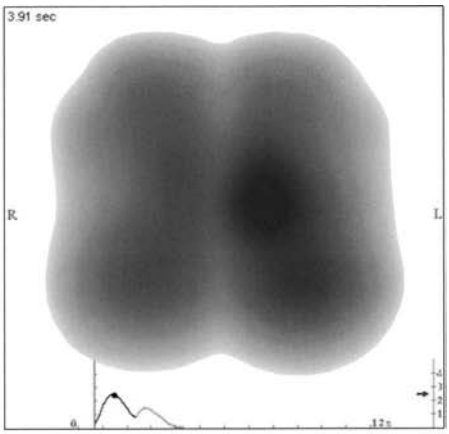

Fig. 2. Vibration response image recorded on healthy non-smoker 30 year old male during one respiratory cycle. Both image and total vibration energy graph are displayed over time. R = right lung; L = left lung

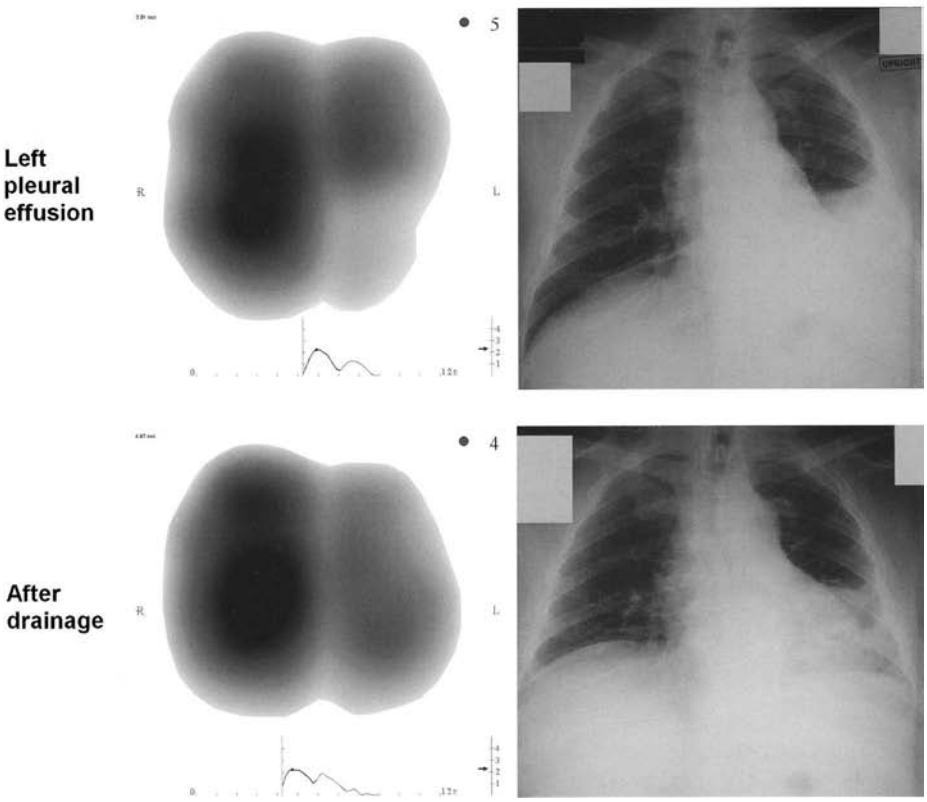

Fig. 3. VRI images and chest radiographs before and after drainage of pleural effusion.

influence lung vibration energy, such as consolidation, atelectasis, asthma, crackles, and wheezes. It allows demonstration of the effect on lung vibration when a large pleural effusion is drained (Fig. 3). It also characterizes different characteristics of vibration in chronic obstructive pulmonary disease (COPD) patients. In mechani-

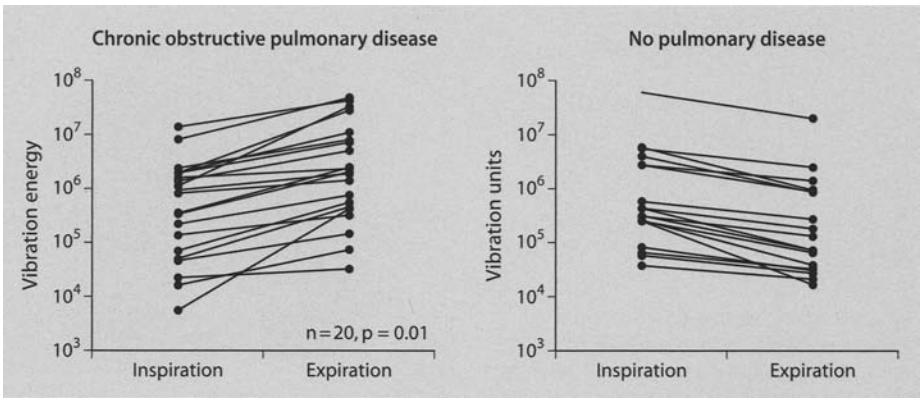

Fig. 4. Maximal vibration energy during inspiration versus expiration in patients with chronic obstructive pulmonary disease compared to patients with no pulmonary disease.

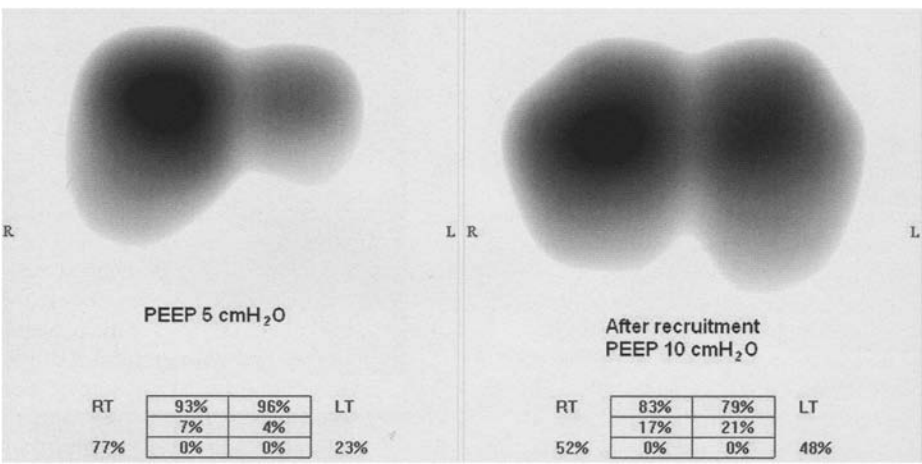

Fig. 5. VRI images of an ARDS patient before and after recruitment maneuver and increased PEEP setting. Note the ability to quantitate regional vibration energy. In table, upper boxes represent top 2 rows of sensors, the middle boxes the middle 2 rows of sensors, and the lower boxes the bottom 2 rows of sensors.

cally ventilated patients with COPD, the VRI image typically shows greater intensity of vibration during expiration, the reverse of non-COPD patients where intensity of vibration is typically greatest during inspiration (Fig. 4) [28].

In mechanically ventilated patients in the ICU, VRI has other potential uses. Our group has demonstrated changes in geographical distribution of lung vibration in different modes of mechanical ventilation [26] (Dellinger et al., unpublished data). Research is ongoing to correlate these results with effectiveness of ventilation and oxygenation and potentially clinical outcome. VRI offers potential utility in assessing the effectiveness of recruitment and PEEP settings in ARDS patients [29]. Figure 5 shows changes in vibration distribution when an ARDS patient at PEEP 5 cmH_2O underwent a recruitment maneuver and PEEP was increased to 10 cmH_2O. The ability to visualize regional distribution of vibration during recruitment may assist in judging the level of dependent lung opening. VRI offers the potential for use in the

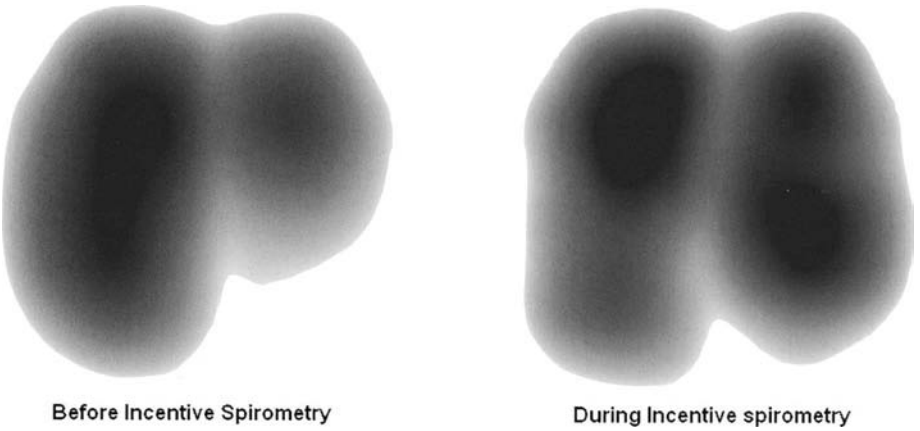

Fig. 6. Recently extubated patient with left atelectasis before and during incentive spirometry.

ICU in non-mechanically ventilated patients as well. The immediate images provided with the VRI might serve as feedback and performance incentive for patients using incentive spirometry. Figure 6 shows the extension of vibration in a patient with left lower lobe atelectasis following incentive spirometer breath [30].

The main limitation of this technology for ICU use is that, for technical reasons, the current VRI recordings are done with patients supported in the near sitting position and not the supine or intermediate (30°–45°) position where they are maintained for care. Lung sounds and vibrations would be expected to change with position due to shift in fluid and gravity effect in blood flow. However, vibration energy distribution in the near seated position is nevertheless of interest as it relates to position-independent effects of mechanical ventilation. New sensors have recently been developed that will allow VRI recording in the supine to 30° position. The patient would be able to comfortably lie on a mat of sensors at the position of mechanical ventilation. This would allow VRI to be potentially used as a continuous monitoring tool as well as to integrate it into the ICU suite. New automatic analysis tools such as regional assessment, breath to breath variability and harmony of ventilated patient with mechanical ventilator will help uncover potential clinical information captured in these recordings and may have significant impact for patient care.

Comparison of Techniques

The imaging techniques discussed have different strengths and weaknesses and all have potential application to the mechanically ventilated patient in the ICU (See Table 1 for comparison of the techniques). All three techniques provide a glimpse into the lungs during the entire respiratory cycle as the patient is being ventilated. As air moves in and out of the lungs, size, density, airflow, and conductivity change. The dynamic images produced by these techniques, unlike their static counterparts, illustrate the movement of air and can, therefore, provide information on lung function and on ventilation in the various lung regions, not just anatomy. This represents a new era in lung imaging where ventilation can be visualized directly and not assessed through remotely measured parameters in the blood or at the ventilator.

Table 1. Comparison of dynamic lung imaging techniques.

	dCT	EIT	VRI
Bedside	No	Yes	Yes
Radiation free	No	Yes	Yes
Real-time results	No	No	Yes
View	Axial	Axial	Frontal
Spatial resolution	High	Low	Low
Portion examined	One slice	One slice	Whole
Training requirement for technician	Weeks to months	Weeks	Days
Cost	High	High	Low

dCT: Dynamic computed tomography
EIT: Electrical impedance tomography
VRI: Vibration response imaging

Dynamic CT and EIT use radiation and electrical impedance signals, respectively, to reconstruct an anatomical image of an axial slice of the thorax. These techniques force some assumptions to be made concerning lung that is not visualized. Sequential images are then taken to produce a dynamic image. VRI, on the other hand, is measuring something that is inherently dynamic, vibration due to lung airflow. Since the sensors are placed over the whole lung, the resulting VRI image is a frontal view similar to a chest radiograph allowing examination of the entire lung, not just a single slice. As such, the VRI provides display of information on the function of the entire lung, not just a single slice.

While dCT provides the best resolution of lung anatomy, the greatest impediment to its use in this patient population is the current need to transport these critically ill patients to another part of the hospital to perform the test. The radiation used in dCT is also of some concern in this or any other patient group. EIT and VRI are radiation free and have no known side effects but do not produce precise anatomical images. EIT and VRI can be performed at the bedside, providing quick results while the patient remains connected to all their monitoring devices in the ICU. EIT or VRI offer the maximal potential for bedside titration of treatment or as a lung monitoring tool.

The lungs of ICU patients are not homogeneous. The new dynamic imaging techniques discussed make it possible to examine the functioning of different regions independently and begin to elucidate clinical relevance of these findings and how they might impact treatment possibilities.

Conclusion

An important need with today's sophisticated ventilatory management strategies and equipment is to determine regional ventilation for optimizing lung function, for example with recruitment, maintaining an open lung, and limiting over-distension. The novel imaging techniques discussed offer the possibility of evaluating regional lung function. Although dCT, EIT, and VRI offer significant potential utility in the ICU, all of them have limitations. There is currently no direct lung monitoring technique at the bedside but the methods examined here represent the first generation of dynamic lung imaging techniques with the potential for widespread use in the ICU. Further studies will help determine how these techniques might be integrated into ICU care.

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Can We Protect the Lung from Acute Injury?

B. Beck-Schimmer, D.R. Spahn, and T.A. Neff

Introduction

Acute lung injury (ALI) and the acute respiratory distress syndrome (ARDS) are clinical entities with a broad spectrum of increasing severity of lung injury consisting of widespread damage to cells and structures of the alveolar capillary membrane that occurs within hours to days following a predisposing insult [1]. ALI/ARDS is a major cause of acute respiratory failure with high morbidity and mortality in critically ill patients [2]. There is reason to believe that the incidence of ARDS may even increase significantly in the future because of the rising frequency of predisposing conditions such as sepsis [3]. Although mortality in patients with ALI/ARDS may have declined over the last 15 years, it remains high (30–40%) [4, 5]. Endotoxin-induced injury is a very useful experimental *in vitro* and *in vivo* model closely resembling ALI and ARDS in humans. Upon stimulation with lipopolysaccharide (LPS), enhanced expression of adhesion molecules, cytokines, and chemokines seems to play a crucial role in the inflammatory orchestration [6–9].

The lung consists of two major anatomical compartments: The vascular and the airway compartment. Endothelial cells in arteries, veins, and capillaries line the vascular system and are the cells most actively involved in the inflammatory response. Epithelial cells, on the other hand, may be regarded as the corresponding cells in the respiratory compartment. Distal airway epithelial cells, i.e., alveolar epithelial cells, are vital for maintenance of the pulmonary air-blood barrier. Type I alveolar epithelial cells, large thin cells which cover 95% of the alveolar surface, are essentially involved in gaseous diffusion. Type II cells, however, are cuboidal cells producing pulmonary surfactant. They are also progenitor cells capable of proliferating and differentiating into type I cells. Recent evidence suggests that airway epithelial cells might also act as immune effector cells in response to noxious exogenous stimuli and that the respiratory compartment plays an essential role in the pathogenesis of ALI/ARDS. Several studies have shown that airway epithelial cells express and secrete various immune molecules such as adhesion molecules, cytokines, and chemokines [10–12]. These inflammatory mediators are major players in the recruitment of effector cells (neutrophils and alveolar macrophages), which then interact with target cells (e.g., alveolar epithelial cells), thereby inducing cell death [13]. Injury to target cells can be ascribed to a complex array of mediators generated and released from activated phagocytes. Some of the primary sources of tissue injury are phagocyte-derived substances: reactive oxygen metabolites, nitric oxide (NO), and proteases [14]. Phagocytic cells can be stimulated with chemoattractants, cytokines, and bacterial LPS. Interaction of any of these stimuli with specific receptors on the cell surface activates effector cells which in turn generate cytokines (tumor necrosis

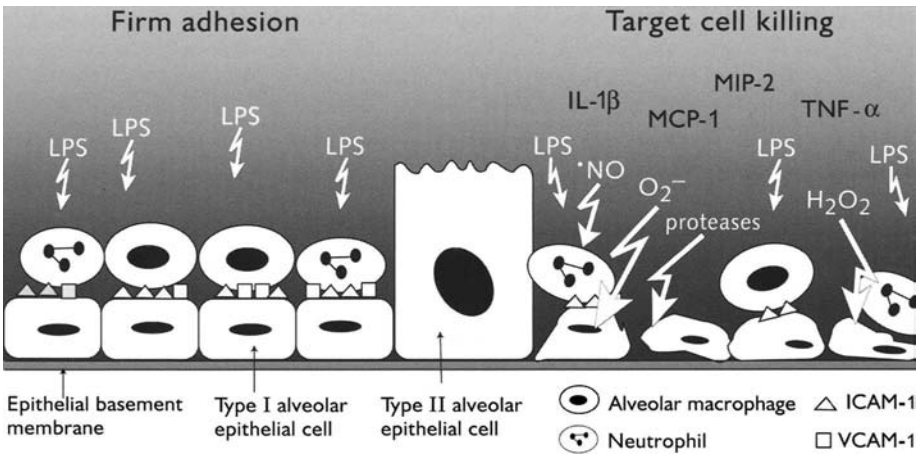

Fig. 1. Schematic diagram of effector cell (neutrophils, alveolar macrophages) and target cell (alveolar epithelial cells) interaction in the respiratory compartment of the lung after intratracheal accumulation of lipopolysaccharide (LPS). Intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) promote tight adhesion of neutrophils and alveolar macrophages to alveolar epithelial cells. This interaction triggers effector cell-induced cytotoxicity through the release of toxic products such as reactive oxygen species and proteases, which leads to alveolar epithelial cell killing. At the same time inflammatory mediators are produced by effector and target cells, recruiting thereby more effector cells.

factor [TNF]-α, interleukins [IL]) and chemokines (monocyte chemoattractant protein [MCP]-1, macrophage inflammatory protein [MIP]-2, cytokine-induced neutrophil chemoattractant [CINC]-1), phagocyte particles, secrete cytoplasmic granules, and produce oxygen metabolites [15] (Fig. 1).

Several experiments, basically performed in cardiac research, have shown that volatile anesthetics exert significant protection against myocardial injury. These are known to represent a powerful cell-protective mechanism conferring relative resistance against myocardial cell death resulting from any injury [16–21]. Organ preconditioning is a process whereby a brief antecedent event, be it transient ischemia, oxidative stress, temperature change, or drug administration, bestows on an organ a temporary tolerance to further insults by the same or a similar stressor. Although preconditioning has been clinically successful in attenuating myocardial injury, its use as a clinical protective strategy to attenuate the deleterious inflammatory cascade is limited by the inability to predict the onset of ischemia. Therefore, a novel organ protective strategy has been developed in the past, called 'post-conditioning' [22, 23]. However, unlike pre-conditioning, post-conditioning focuses on a specific phase of injury.

The model of endotoxin-induced injury with early or late application of volatile anesthetics does not exactly reflect a pre- or post-conditioning situation as in cardiac interventions. Nevertheless, the protective cellular mechanisms elicited by the application of volatile anesthetics are possibly the same in cardiac as in pulmonary tissue and have, therefore, being the focus of many studies in recent years.

Experimental Findings

In vitro Evidence

Lung epithelial cells such as alveolar epithelial cells from the lower respiratory compartment and tracheobronchial epithelial cells from the upper respiratory compartment are a potent source of inflammatory mediators within the lung on endotoxin stimulation [24]. Since alveolar epithelial cells are directly exposed to volatile agents, several studies have investigatd the potential anti-inflammatory effects of volatile anesthetics in *in vitro* models of ALI. Such studies demonstrated that halothane decreased Na,K-ATPase- and sodium channel activities and altered surfactant phospholipids and apoprotein biosynthesis in rat type II alveolar epithelial cells [25, 26]. Another study in these cells showed that on stimulation with IL-1β, exposure to vol-

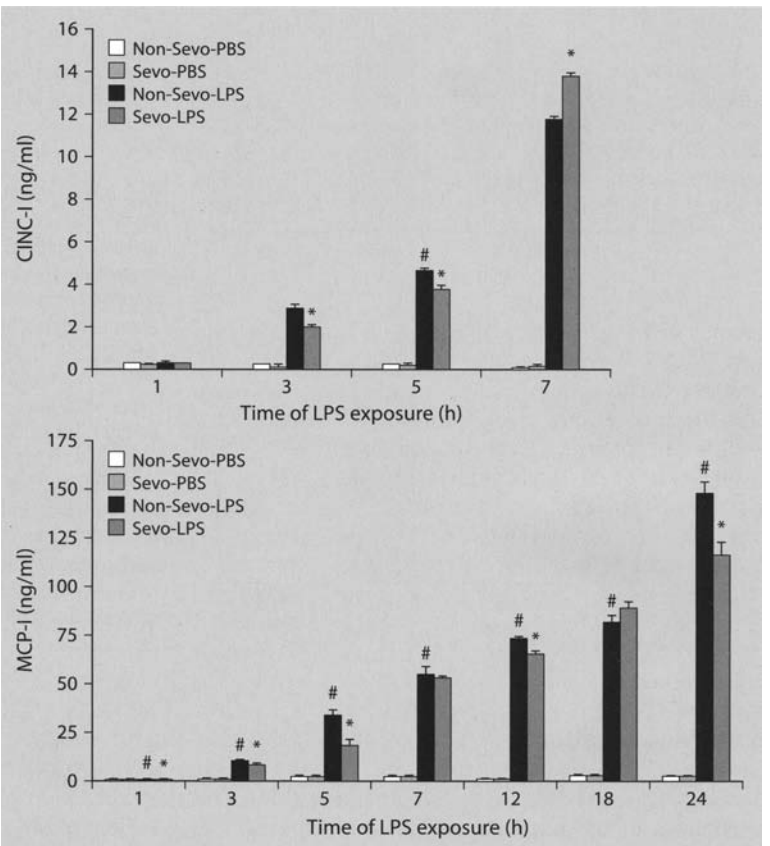

Fig. 2. Evaluation of effects of alveolar epithelial cell pre-conditioning with sevoflurane on protein expression of cytokine-induced neutrophil chemoattractant-1 (CINC-1) and monocyte chemoattractant protein-1 (MCP-1). Confluent layers of alveolar epithelial cells were pre-treated with 1.1 Vol.% sevoflurane for 0.5 h (or with a control gas), followed by stimulation with lipopolysaccharide (LPS, 20 µg/ml) or phosphate-buffered saline (PBS) as a control for 1, 3, 5, 7, 12, 18, and 24 h. CINC-1 ELISA was performed with supernatants. Values are mean ± SEM from 5 experiments. # $p<0.05$ between non-Sevo-PBS and non-Sevo-LPS, * $p<0.05$ between non-Sevo-LPS and Sevo-LPS. From [29]

atile anesthetics interfered with the secretion of inflammatory mediators [27]: Halothane, isoflurane and enflurane decreased the production of IL-6, MIP-2, and MCP-1 protein concentrations in a dose- and time-dependent manner. Additionally, halothane induced apoptosis-like changes in a lung-derived carcinoma cell line [28]. Our own data stress the downregulation of inflammatory mediators in a setting of sevoflurane preconditioning [29]. As presented in Figure 2, pre-treatment of alveolar epithelial cells with sevoflurane followed by LPS stimulation decreased expression of CINC-1 and MCP-1 proteins in a dose and time dependent way. In a different experimental approach, an attenuating effect could also be achieved by 'post-treating' alveolar epithelial cells with sevoflurane (unpublished data, Fig. 3).

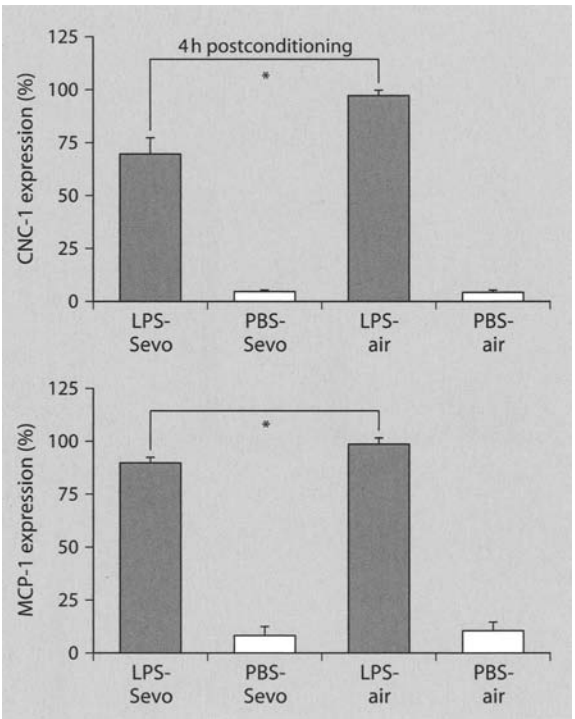

Fig. 3. Evaluation of effects of sevoflurane post-conditioning of alveolar epithelial cells on protein expression of cytokine-induced neutrophil chemoattractant-1 (CINC-1) and monocyte chemoattractant protein-1 (MCP-1). Confluent layers of alveolar epithelial cells were exposed to lipopolysaccharide (LPS) or phosphate-buffered saline (PBS, as a control) for 2 h, followed by a co-exposure to 2.2 Vol.% sevoflurane (or to a control gas = air) for 4 h. CINC-1- and MCP-1 ELISA was performed with supernatants. Values are mean ± SEM from 5 experiments. * $p < 0.05$ between LPS-air and LPS-Sevo.

In vivo evidence

In vivo results of the effect of volatile anesthetics on the expression of inflammatory mediators in the lung are varied. A comparison of gene expression of pulmonary cytokines in pigs demonstrated lower concentrations of TNF-α and Il-1β in lung tissue after sevoflurane anesthesia than with thiopental [30]. In a model of mechanically ventilated rats, halothane decreased neutrophil accumulation after LPS stimulation [31]. Giraud et al. showed that halothane-anesthetized rats had a reduced inflammatory response in LPS-induced lung injury compared to animals with thiopental anesthesia. A similar study focused on isoflurane pre-treatment in rat lungs [32]. Pre-conditioning with isoflurane markedly inhibited the LPS-induced decrease

in mean arterial pressure and damage to the vascular endothelium. Similar data were achieved in a study where rats were briefly exposed to isoflurane before sepsis was induced with LPS [33]. Endotoxemic rats with isoflurane pre-exposure had significantly lower alveolar macrophage nitrite production compared to control groups. Recent studies have confirmed the observations of anesthetic-induced inhibition of septic shock after sevoflurane pre-treatment, as well as the protective effect of isoflurane in endotoxin-induced lung injury [34, 35].

In contrast to these findings, several studies have revealed that the use of volatile anesthetics aggravates inflammation. Nader-Djalal et al. examined the effect of different anesthetics on the severity of acid-induced lung injury [36]. Application of volatile anesthetics resulted in an increase in the acute inflammatory response and leukocyte infiltration. In a model of mechanical ventilation, gene expression of pro-inflammatory cytokines was investigated by Kotani and colleagues. They found that mRNA expression of various pro-inflammatory cytokines by rat alveolar macrophages previously exposed to volatile anesthetics, was increased [37]. Sevoflurane also increased pulmonary NO_3- and NO_2 production in anesthetized pigs [38].

Findings in Patients

Only very limited data in humans exist so far. One study investigated alveolar macrophages of patients undergoing non-abdominal or non-thoracic surgery, excluding patients with a pulmonary history. Results implied that volatile anesthetics provoke a more extended and time-dependent increase in macrophage aggregation and neutrophil influx into the lung than propofol [39]. A similar study with anesthetized patients during orthopedic surgery demonstrated an attenuated pulmonary defense with the application of isoflurane as compared to propofol anesthesia [40]. However, neither study evaluated the concentrations of inflammatory mediators in bronchoalveolar lavage, but rather focused on the lavaged cell and subsequent analysis *in vitro*. Whether these observations reflect an appropriate defense against pulmonary insult or a harmful inflammatory response remains to be determined.

Conclusion and Perspective

In conclusion, substantial progress has recently been made in the understanding of ALI and ARDS. Basic investigations have presented new insights into potential attenuating effects of volatile anesthetics on the inflammatory response of pulmonary effector and target cells in general and during ALI/ARDS. However, progress in specific treatment regimens targeting patients with ALI/ARDS has lagged behind basic research. A breakthrough in new treatment modalities that bears the potential of reducing mortality in ALI/ARDS is still missing. Further strategies need to be designed to guide clinical studies with volatile anesthetics in acute pulmonary injury.

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Rationale for High-Frequency Oscillation as a Primary Lung-Protective Mode in Patients with ALI/ARDS

H. Quiroz-Martinez and N.D. Ferguson

Introduction

Clinicians and researchers are becoming increasingly conscious of the potentially harmful effects of mechanical ventilation, and more attention is being focused on methods of ventilation that may reduce these complications. Indeed the paradigm for mechanical ventilation in patients with acute lung injury (ALI) and acute respiratory distress syndrome (ARDS) has evolved in the last 10 years from a goal of normalizing blood gases to one of avoiding ventilator-induced lung injury (VILI) while maintaining adequate gas exchange. Lung protection during mechanical ventilation begins with limitation of tidal volume on conventional ventilation, but the optimal method remains to be determined [1]. One potential modality that may be useful in the avoidance of VILI is high-frequency oscillation (HFO). In this chapter, we will introduce HFO, provide a brief discussion of ARDS and VILI, and focus on the preclinical and clinical data available to date supporting the use of HFO as a primary modality to avoid VILI in adults.

Basics of HFO

HFO Mechanics and Physiology

During the second half of the 20th century, different researchers documented that ventilation (i.e., adequate CO_2 clearance) was possible for variable periods of time employing tidal volumes that were under dead space volume at high respiratory rates; observations that were theorized as long ago as 1915 [2, 3]. HFO did not appear as an alternative mode of mechanical ventilation until the early 1980s after Bohn et al. published their findings showing they could effectively ventilate dogs using a piston-driven oscillator at low mean airway pressure and tidal volumes less than anatomic dead space using variable frequencies [4, 5].

Conceptually, this mode of mechanical ventilation uses high respiratory cycle frequencies (3–15 Hz) with very low tidal volumes, maintaining a relatively constant mean airway pressure (Paw). This is achieved with rapid oscillations of a reciprocating diaphragm driven by a piston, which creates pressure waves in the ventilator circuit that ultimately determine the tidal volume. The oscillator-patient system has no intrinsic source of fresh gas; to provide adequate gas exchange a bias flow of fresh, heated and humidified gas (20–60 l/min) is incorporated as part of the ventilator circuit, and passes between the oscillating membrane and the patient (Fig. 1). This provides the desired FiO_2 and clears CO_2 from the system. During HFO the oscillating diaphragm actively pulls outward during expiration; a process that may promote

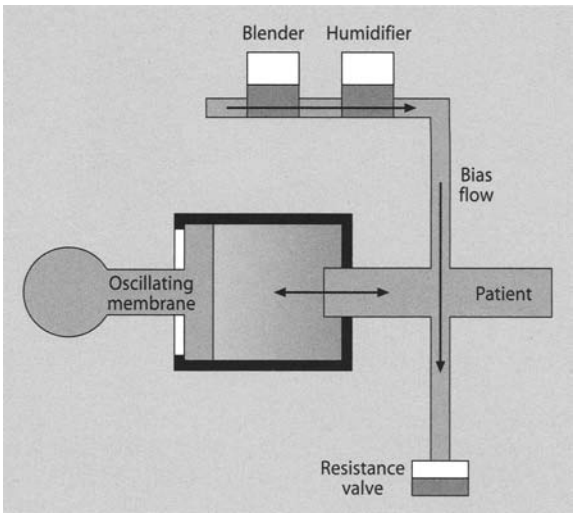

Fig. 1. Schematic overview of the high frequency oscillation (HFO) circuit. From [50] with permission.

CO_2 clearance, and help prevent gas trapping and alveolar overdistention. The oscillatory pressure amplitude (ΔP; the peak-to-peak pressure gradient generated by the membrane) is measured in the ventilator circuit; it does not reflect the pressure oscillations in the distal airways. These pressures are greatly attenuated by the endotracheal tube and high-caliber airways so the actual pressure swings in the alveoli are much lower.

During HFO, oxygenation depends on the mean airway pressure, the resultant lung volume and the FiO_2 [6, 7]. In contrast, ventilation is determined by the power set on the ventilator (and the resultant ΔP), the frequency, and the bias flow rate [8, 9]. Because frequency and tidal volume are not independent in HFO (higher frequencies lead to smaller tidal volumes because of reduced inspiratory times), and because alveolar ventilation depends more on tidal volume and less on the frequency ($V_A \approx V_T^2 \cdot f$) [9], during HFO we have a phenomenon known as 'negative frequency dependence'. This means that during HFO a decrease in the respiratory frequency leads to an increase in the tidal volume with a consequent increase in CO_2 clearance.

Gas Exchange during HFO

During spontaneous ventilation and conventional ventilation, convection and molecular diffusion are the principal mechanisms of gas movement to and in the alveoli. During HFO, the tidal volume is lower than the dead space volume so the mechanisms of gas transport in the airways are different. A number of different mechanisms have been proposed to account for the observation of adequate ventilation despite such small tidal volumes (Fig. 2) [10]. First, the most proximal alveoli may receive ventilation as usual through direct bulk convection. Second, in the conducting airways (bronchi, bronchioles, and terminal bronchioles), gas mixing and movement takes place because of differences in flow velocity profiles that occur during inspiration and expiration because of the shape of the airways, so-called asymmetric velocity profiles, with resultant net movement of fresh gas into the lung and

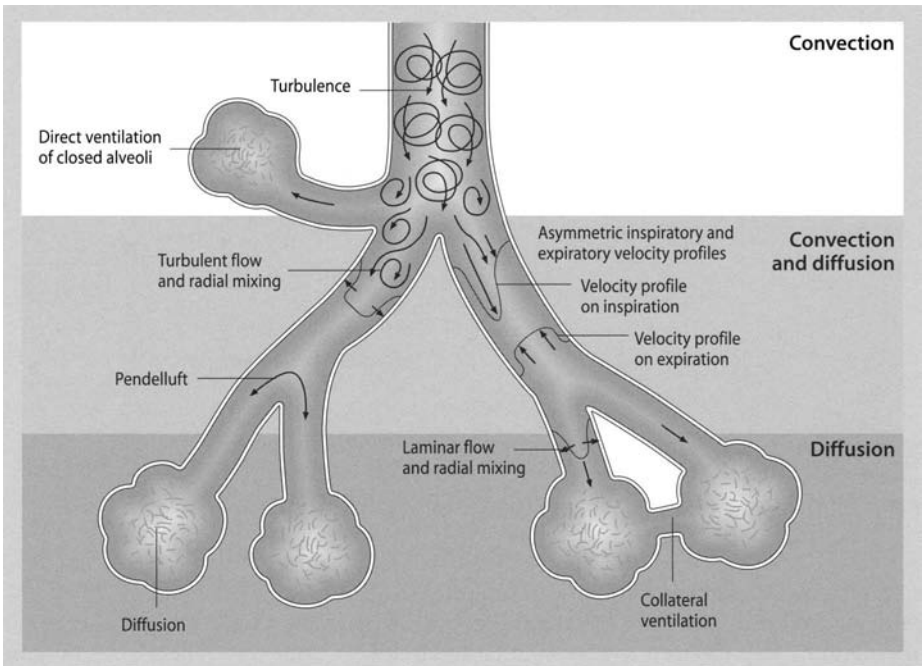

Fig. 2. Alternative mechanisms of gas exchange with high frequency oscillation (HFO). From [10] with permission.

exhaled gas moving out. Third, different alveolar units have different resistance and compliance values, and thus different time constants and rates of filling and emptying at a given pressure gradient. These differences result in asynchronous filling of contiguous alveoli, so gas can swing from rapid filling alveoli to slow filling alveoli improving the gas mixture. This mechanism of gas exchange is called Pendelluft. As usual, the principal mechanism of gas transport in the alveoli is molecular diffusion where the intermixing of molecules is due to Brownian motion. In HFO the high flow rates, turbulence and combination of convection and molecular diffusion results in enhanced molecular dispersion. Finally, transmitted cardiac oscillations also play a role in HFO gas exchange, having been shown to improve gas mixing five-fold by enhancing the molecular diffusion in the alveoli [11].

Rationale for HFO in Adults

Acute Respiratory Failure and ARDS

Acute respiratory failure is one of the leading causes of admission to the intensive care unit (ICU), and the most frequent organ dysfunction found in the critically ill patient [12]. ARDS is the most severe and life-threatening form of acute respiratory failure. ARDS is an important clinical problem for intensivists both because of its relatively common incidence (with recent reports of 65 cases per 100,000 population per year [13]), and because of its high associated mortality rate (ranging from 30–65% depending on the specific population included). Despite our improved

understanding of the pathophysiologic changes in ARDS and the advances in life support technology, the mortality rates have generally remained high in unselected populations [14]. Notwithstanding numerous laboratory and clinical investigations, no pharmacological therapy has yet been demonstrated to have an impact on mortality.

Ventilator-Induced Lung Injury

Positive-pressure ventilation is the cornerstone for support of the patient with ALI/ARDS, but it has well documented potential deleterious effects on respiratory mechanics, hemodynamics, and immune response. Indeed, since the demonstration that the manner in which mechanical ventilation is delivered directly affects mortality in patients with ALI/ARDS [1,15], our goals for ventilation have clearly changed. Today the aim is to oxygenate and ventilate without causing further VILI. VILI is thought to occur primarily through two major mechanisms: Cyclic alveolar overdistention, and repetitive alveolar collapse and reopening [16].

Gross barotrauma is defined as the presence of extra-alveolar air due to alveolar rupture; this correlates with high levels of peak airway pressure and positive end-expiratory pressure (PEEP), high tidal volume and gas trapping. More subtly, *volutrauma* results from the cyclic overdistention of lung units that leads to mechanical disruption of the alveolar-capillary barrier and inflammation, ultimately creating a histological picture that is indistinguishable from primary ARDS. Dreyfuss et al. found that high tidal volume ventilation induced pulmonary edema by causing increases in both epithelial and endothelial permeability, and that transpulmonary pressure (Pplateau – Ppleural) was the major determinant [17]. Similarly, Hernandez et al. demonstrated in immature rabbits that volume distension of the lung, rather than high peak inspiratory pressure (PIP) caused microvascular damage [18].

Meanwhile, ventilation at low lung volumes can also cause parenchymal damage due to alveolar collapse, termed *atelectrauma* [19]. The repetitive opening and closing of these atelectatic alveoli, provoked by conventional mechanical ventilation, can cause excessive alveolar wall strain that triggers the inflammatory cascade.

All forms of mechanical trauma to the alveoli may lead to surfactant dysfunction, epithelial and endothelial cell injury (necrosis/apoptosis) with increased alveolar-capillary permeability, inflammatory mediator release, neutrophil infiltration, lung macrophage activation, and bacterial translocation. Each of these mechanisms can in turn exacerbate the local injury and initiate or potentiate a systemic inflammatory response, a process known as *biotrauma* [20, 21].

Patients with ALI/ARDS are at particularly high risk of VILI since their lungs are already inflamed and heterogeneously damaged; regions of injured lung can be adjacent to relatively normal parenchyma. The injured alveolar regions may be filled with fluid and collapsed during the entire respiratory cycle, collapsed at the end of expiration but re-expanded during inspiration, or aerated throughout the respiratory cycle and susceptible to overdistention. The relatively healthy zones of the lung that have higher compliance tend to receive the bulk of delivered tidal volumes and are, therefore, submitted to more stress and strain than the consolidated alveoli. In this way, contiguous acini might be in danger of different types of ventilator-induced injury, making it more difficult to ventilate without causing further damage. In addition, using conventional ventilation, strategies to minimize volutrauma and atelectrauma can directly compete. Increasing end-*ex*piratory volumes (with high PEEP) can predispose to high end-*in*spiratory volumes and volutrauma. Meanwhile small

tidal volumes to limit volutrauma can predispose to alveolar collapse and atelectrauma. For these reasons, patients with ARDS are more susceptible to develop VILI at traditional positive pressure ventilation settings.

Laboratory Data

With an understanding of the mechanisms of VILI, we can appreciate that in order to minimize VILI, we should limit tidal overdistention and resultant volutrauma, while simultaneously keeping the lung open and avoiding cyclic collapse and atelectrauma. HFO is theoretically ideally suited to these goals. The key is the very small tidal volumes that are delivered during HFO. These should allow for the setting of a high mean airway pressure aimed at keeping the lung open, while still being able to avoid the tidal overdistention that would be inevitable even using small conventional tidal volumes. Tidal volumes are not measured routinely during HFO, and some concerning measurements in sheep raised the issue of whether the use of lower frequencies in adults (3–6 Hz compared with 10–15 Hz used in neonates) would still result in very small tidal volumes [22]. Very recently, however, Hager et al. have measured tidal volumes in adults receiving HFO at their usual settings, documenting that delivered tidal volumes are indeed low, in the range of 1–2 ml/kg predicted body weight [23].

During the 1980s and '90s several animal studies compared the effects of HFO vs. traditional (high volume/pressure) conventional ventilation in saline-lavage lung injury models. Many of these studies supported HFO as an attractive alternative to preserve adequate oxygenation, maintain ventilation, optimize lung mechanics, and minimize VILI. Almost universally, compared with what are now known to be injurious settings of conventional ventilation, HFO resulted in improved gas exchange, decreased levels of inflammatory markers, and improved pulmonary pathology scores [24, 25].

More relevant today, however, are more recent studies comparing HFO with lung-protective conventional ventilation using lower tidal volumes (6 ml/kg) with or without increased PEEP, again in saline lavage models. As expected, these investigations do not show such a dramatic benefit for HFO. Of six studies published from 1999 to 2004, two showed similar oxygenation, inflammation, and pathology between the two groups, while four favored HFO in these categories; none suggested a benefit for conventional ventilation [26–31]. Taken collectively, these animal studies still provide a strong physiological rationale for a potential benefit from HFO in terms of VILI reduction compared with optimal conventional ventilation.

Clinical Data: Neonates

With the promising results gathered from animal models, HFO was implemented in the management of hyaline membrane disease in human neonates as a ventilatory modality that could reduce VILI and promote adequate gas exchange. In 1987, Froese et al. proved sufficient efficacy and safety with HFO to warrant further investigations in this population [32]. Initial enthusiasm quickly waned when the HiFi study showed no benefits in outcomes and an increase in intracranial complications [33]; it was subsequently realized that this was likely due to a strategy targeting lower airway pressures and higher fraction of inspired oxygen (FiO_2), along with a disproportionate number of complications at less experienced centers [34]. None of the more than a dozen neonatal randomized controlled trials (RCTs) that have followed has

shown similar concerns with harm. In general, those that employed a lung recruitment strategy in the HFO arm showed better oxygenation and lung mechanics in the HFO group, with some showing significant improvement in outcomes such as chronic lung disease [35]. Despite a lack of a definitive effect on outcome, HFO remains widely used in neonatal ICUs, but is most often employed in the early management of patients with moderate to severe preterm respiratory distress syndrome who require alveolar recruitment with lung-protective ventilation at high mean airway pressures.

Clinical Data: Adults

In adults there are comparatively few data about HFO in ARDS. This is largely because until the mid-1990s commercially available oscillators were not capable of ventilating patients over 35 kg in weight, and the adult version of the neonatal/pediatric machine did not receive regulatory approval in the United States until 2001. The bulk of the published experience with adult HFO comes from observational studies where HFO was used as rescue therapy in patients who failed to improve with conventional ventilation (Table 1) [36–44]. The baseline characteristics of these study groups vary, but most of them were receiving conventional ventilation for relatively long periods of time (1.7 to 10 days) before being switched to HFO. The decision to switch a patient from conventional ventilation to HFO was generally made based on oxygenation failure ($PaO_2 < 65$ despite $FiO_2 > 60\%$) and the requirement of high airway pressures to recruit and maintain the adequate lung volumes (peak inspiratory pressures and/or high PEEP). After the switch to HFO, oxygenation, ventilation, and lung mechanics improved over all, and there were few severe or lethal complications reported. The most frequent complications were pneumothorax and hypotension. The mortality rates in these reports ranged from 31 to 81%, with most of the deaths due to multiple organ failure (MOF). As a relevant finding, the delay in the initiation of HFOV was an independent predictor of mortality. Based on these results, HFO in adults appears to be an effective and safe rescue modality for patients with ARDS who fail to improve with conventional mechanical ventilation (Table 1) [36–44].

To our knowledge only two RCTs of HFO in adults with ARDS have been carried out [45, 46]. Both of these were conceived and started prior to the landmark results of the first ARDSNet trial. As such, they compared HFO with conventional ventilation, which, by today's standards, would not be considered optimally lung-protective. The control groups in both of these studies received tidal volumes of 6–10 ml/kg of actual body weight. The primary objective of both studies was to demonstrate safety; they were both underpowered to detect mortality differences. That said, the first and largest of these studies enrolled 148 patients and showed an encouraging trend towards an HFO mortality reduction with 30-day mortalities of 37% vs. 52% ($p = 0.10$) [45]. The second smaller study was stopped early because of slow enrolment ($N = 61$) and overall showed no difference between groups (RR [95%CI]: 1.29 [0.66–2.55]) [46]; in a post-hoc analysis, patients with the most severe baseline lung disease (highest oxygenation index) may have received more benefit from HFO. Neither trial suggested any concerns with harm during HFO; both concluded that HFO was a safe and effective mode of ventilation for adults with severe ARDS. The RCT data available to date are clearly not definitive when it comes to assessing the utility of HFO as a primary lung-protective mode. Not only are the patient numbers too small to create precise estimates, but they are confounded by the use of now out-

Table 1. Summary of clinical experience with high frequency oscillation (HFO) in adults

Reference	Design	Patients	APACHE II (SD or IQR)	CV prior to HFO	Mortality
Fort 1997 [36]	Prospective	17	23.3 (7.5)	5.12 ± 4.3 days	30 day: 53%
Claridge 1999 [44]	Prospective	5	29		20%
Mehta 2001 [38]	Prospective	24	21.5 (6.9)	5.7 ± 5.6 days	30 day: 66%
Andersen 2002 [37]	Retrospective	16	27	7.2 days	3 months: 31%
David 2003 [43]	Prospective	42	28 (IQR 24–37)	3.0 (0.7–9.1) days	30 day: 43%
Mehta 2004 [39]	Retrospective	156	24	5.6 days	30 day: 61.7%
Cartotto 2004 [40]	Retrospective	6	16	4.8 days	Hospital: 83%
David 2005 [42]	Retrospective	5 trauma patients with brain injury	25	10 days	20%
Finkielman 2006 [41]	Retrospective	14	36	1.7 days	30 day: 57%
Ferguson 2005 [48]	Prospective	25	24 (19–32)	13 (6–51) hours	ICU mortality 44%
Derdak 2002 [45]	RCT	148 75 HFO 73 CV	22 (6) HFO 22 (9) CV	2.7 (3) days HFO 4.4 (8) days CV	37% HFO vs. 52% CV 30-day mortality (p=0.102)
Bollen 1997–2001 [46]	RCT	61 37 HFO 24 CV	21 (8) HFO 20 (9) CV	2.1 (3) days HFO 1.5 (2) days CV	43% HFO vs. 33% CV 30-day mortality (p=0.59)

RCT: randomized controlled trial; CV: conventional mechanical ventilation

dated conventional strategies, and by the inclusion of some patients who had already been exposed to a significant duration of conventional ventilation.

As our understanding and experience with adult HFO expands, our concept of the optimal application of HFO in the population continues to evolve [47]. Extrapolating from both the neonatal literature and adult rescue series, most experts agree that for optimal lung protection, HFO should be applied early and in combination with a strategy to recruit the lung. Our group conducted a multinational pilot study in which HFO was used in conjunction with recruitment maneuvers as a lung-protective strategy for adult patients with early ARDS. The goals were to assess safety, feasibility, and physiologic response to this HFO strategy. The main baseline differences from previous studies was the short duration of conventional ventilation prior to HFO (13 [6–51] hours), and the use of standardized ventilatory settings to judge

severe hypoxemia [48]. HFO combined with recruitment maneuvers provided rapid and significant improvements in oxygenation and respiratory mechanics. After twelve hours of HFO, the mean FiO_2 was significantly reduced compared with pre-study levels (0.5 ± 0.2 vs. 0.9 ± 0.1, $p < 0.001$). The pressure cost of oxygenation, determined by the oxygenation index ($FiO_2 \times$ mean Paw $\times$ $100/PaO_2$) was also significantly decreased over the same interval. Only 3% of the maneuvers were aborted (due primarily to transient hypotension) and good protocol adherence was demonstrated [48]. These results are promising and suggest that it is indeed feasible to enrol patients into an early trial of protocolized HFO. The other factor that we now believe is important to consider when implementing adult HFO is an effort to deliver the lowest tidal volume possible, taking advantage of the alternative mechanisms of gas exchange. To achieve this we now routinely use high power settings and titrate frequency to the maximal level that will allow a reasonable pH (e.g., above 7.25) [23, 47]. Emerging data suggest that when applied systematically it is often possible to oscillate adults at significantly higher frequencies (and therefore with lower tidal volumes) than previously believed [49].

Conclusion

In this chapter we have attempted to outline the basic physiology of high-frequency oscillation, and explain why, given our understanding of VILI, HFO is theoretically ideally suited as a lung-protective mode. Collectively, we are still on the learning curve with HFO in adults. While promising, the use of HFO as a primary mode for lung-protection in ARDS patients needs further investigation before it is widely adopted as routine clinical practice. At the current time the usual indication for HFO in adults should be as rescue therapy for patients with severe hypoxemic respiratory failure who are not responding to conventional ventilation.

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The Role of Protective Ventilation in Cardiac Surgery Patients

D. Gommers and D. dos Reis Miranda

Introduction

Cardiac surgery is associated with a pulmonary and systemic inflammatory response. The pulmonary effects of this inflammatory reaction are often modest: decreased lung compliance, pulmonary edema, increased intrapulmonary shunt fraction and decreased functional residual capacity (FRC) [1]. Less than 2% of patients undergoing cardiac surgery develop full blown respiratory failure, the acute respiratory distress syndrome (ARDS) [1]. For example, after cardiac surgery, FRC is reduced up to 40–50% during the first 24 hours after extubation [2]. However, after general anesthesia, FRC is only decreased by 20–30% [3]. This exaggerated disturbance of pulmonary function is not yet fully understood. It has been suggested that this impaired pulmonary function is the result of pulmonary inflammation, triggered by cardiopulmonary bypass (CPB), ischemia-reperfusion injury, the surgical procedure itself, or by mechanical ventilation.

The ARDS network trial has shown that mechanical ventilation with smaller tidal volumes leads to a reduction in mortality in patients with ARDS [4]. This result was somewhat surprising because the most common cause of death in ARDS is not pulmonary failure but rather multiple organ failure (MOF). There is increasing evidence that conventional mechanical ventilation itself can cause damage to the lung in critically ill patients, also known as ventilator-induced lung injury (VILI) [5]. Recent studies suggest that this could also be possible in cardiac surgery patients in whom CPB provides sufficient inflammation to sensitize the lungs to the harmful effects of conventional mechanical ventilation [6–8]. This may indicate that the exaggerated pulmonary dysfunction, as seen after cardiac surgery, is the result of two noxious hits on the lung: 1) The cardiac surgical procedure, with or without the use of CPB, and 2) mechanical ventilation of the lungs in an inflammatory environment. In this chapter, we will first discuss the two-hit model in cardiac surgery patients. Secondly, the beneficial effects of a lung protective ventilation strategy (i.e., the open lung concept) in cardiac surgery patients are discussed.

Two-hit Model

First Hit: Cardiac Surgery

The activation of the inflammatory response during cardiac surgery is an extremely complex process and has various triggers such as CPB, ischemia, and surgical trauma [9]. Although CPB does not seem to have a significant effect on pulmonary dysfunction, it triggers an important degree of cytokine and mediator release [10,

11]. Furthermore, ischemia-reperfusion injury contributes to the inflammation mainly from the myocardium, and less from the lung, as the bronchial circulation seems to meet pulmonary oxygen demands [12]. Finally, the surgical procedure itself causes a significant inflammatory response. A median sternotomy elicited greater complement release and interleukin (IL) release compared to an anterolateral thoracotomy in patients undergoing coronary artery bypass grafting (CABG) without the use of CPB [13].

Second Hit: Mechanical Ventilation

Pulmonary inflammation induced by mechanical ventilation is the result of mechanical trauma and biotrauma [5]. Mechanical trauma reflects lung injury because of atelectasis, volume or pressure; biotrauma reflects pulmonary and systemic inflammation caused by mediators airborne from the ventilated lung.

Atelectasis causes repetitive opening and closure of alveoli, and is therefore a major source of pulmonary inflammation [14, 15]. Roughly three zones can be identified (Fig. 1): A) alveoli that do not open even during inspiration; B) alveoli which remain open; C) alveoli that open during inspiration and collapse during expiration. Alveoli in zone C (Fig. 1) will be subjected to repetitive opening and closure, which is known to be a major cause of pulmonary inflammation [16]. As alveoli in zone A (Fig. 1) do not participate in tidal volume ventilation, tidal volume is distributed over alveoli in the other two zones. This may increase the risk of regional overdistention. Finally, co-existence of atelectatic and open alveoli may result in shear forces that exceed transpulmonary pressures, as predicted by Mead and colleagues [17]. Shear forces act on the fragile alveolar membrane in alveoli undergoing cyclic opening and closure. In a mathematical model, transpulmonary pressures of 30 cmH_2O will result in shear forces between atelectatic and aerated lung areas of 140 cmH_2O [17]. These shear forces, rather than end-inspiratory overstretching, may be of more importance for epithelial disruption and the loss of barrier function of the alveolar epithelium.

To further explore the role of tidal volume and pressure on mechanical trauma in the lung, Dreyfuss and colleagues [18] applied high inspiratory pressures in combination with high volumes in an experimental model. These authors concluded that: 1) High pressures together with high tidal volume resulted in increased alveolar per-

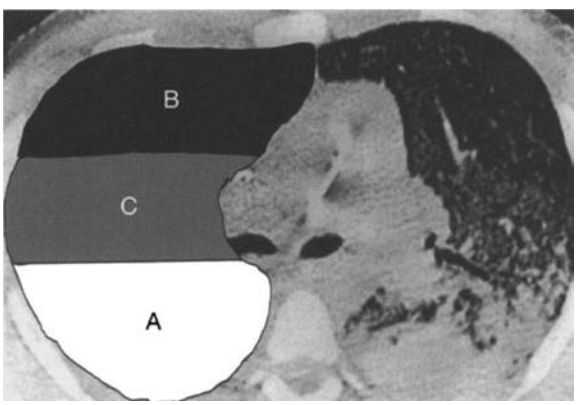

Fig. 1. CT slice of the lung with atelectasis on the dorsal side. Roughly three zones can be identified: A) alveoli that do not open even during inspiration, B) alveoli which remain open, C) alveoli that open during inspiration and collapse during expiration.

meability; 2) combining low pressure with high volume (iron lung ventilation) resulted again in increased alveolar permeability; 3) if high pressure was associated with low tidal volume (chest wall strapping) the alveolar permeability of the study group did not differ from the control group. These investigators concluded that (high) tidal volume ventilation, not pressure, is the main determinant of lung injury.

Mechanical forces such as shear forces between open and closed alveoli or alveolar overdistention cause an inflammatory response, called biotrauma. Although it is not clear how mechanical forces are converted to biochemical signals, several pathways have been suggested, such as stretch-sensitive channels, mechanoreceptors, stress-activated signaling cascade of mitogen-activated protein kinase (MAPK) [14, 19], and activation of the transcription of nuclear factor-kappa B (NF-κB) [20]. In ARDS patients, Ranieri and colleagues [21] have shown that cytokine levels (tumor necrosis factor [TNF]-α, IL-6 and IL-8) in bronchoalveolar lavage fluid (BAL) were attenuated by a protective ventilation strategy. In the protective ventilation strategy, a tidal volume of 7 ml/kg was applied with 10 cm H_2O of positive end-expiratory pressure (PEEP). In the control group, a tidal volume of 11 ml/kg was applied with 6 cmH_2O of PEEP. These authors concluded that mechanical ventilation induces a cytokine response, which can be reduced by minimizing overdistention and repetitive alveolar collapse. In a large multicenter study in 861 ARDS patients (ARDS Network trial), low tidal volume ventilation (6 ml/kg) led to lower plasma IL-6 concentrations and a significant decrease in 28-day mortality in ARDS patients [4]. Stüber et al. [22] have shown that switching from a lung protective ventilation strategy of low tidal volume and high PEEP to a conventional strategy with high tidal volume and low PEEP in patients with ALI, led to an increase in plasma cytokines within one hour, which decreased to baseline after switching back to lung-protective ventilation. In addition, Imai et al. [23] showed that injurious ventilation induced apoptosis in distal organs (kidney and small intestine) in a rabbit model of ARDS. In contrast, protective ventilation in this study was associated with much lower levels of plasma cytokines, very little apoptosis, and only minimal changes in biochemical markers. These authors concluded that protective ventilation could in fact protect distal organs from ventilator-induced end organ dysfunction. They also suggested that this mechanism might explain the decrease in mortality observed in the ARDS Network trial of low tidal volume ventilation [4]. From the results of the plasma measurements of cytokines of patients enrolled in this latter trial [4], it has been shown that the highest cytokine levels are measured in patients with ARDS due to sepsis and pneumonia and that the beneficial effect of protective ventilation was better in these patients. This provides further evidence that the pre-existing inflammatory process present at diagnosis of ARDS can be modulated by the early application of low tidal volume ventilation.

Protective Ventilation in Non-ARDS

From ARDS studies it has become clear that high tidal volume ventilation can induce a systemic inflammatory response and protective ventilation attenuates this response. Therefore, several investigators have studied the effect of protective ventilation on the cytokine network in patients without ALI/ARDS. Wrigge et al. [24, 25] performed two studies in patients with normal pulmonary function undergoing elective surgery and found no difference between injurious and non-injurious ventilation. These authors concluded that the protective effect of non-injurious ventila-

tion on the release of cytokines does not occur in healthy lungs during major non-cardiac surgery where the surgery-induced systemic inflammatory response is relatively small.

This suggests that protective ventilation modulates the cytokine network only in the presence of a more significant primary inflammatory stimulus, such as CPB. This was shown by the group of Ranieri [6] who observed lower IL-6 and IL-8 concentrations in BAL fluid (6 hrs after CPB) in a lung protective group (tidal volume 7 ml/kg, PEEP 9 cmH_2O), compared with the control group (tidal volume 11 ml/kg, PEEP 3 cmH_2O). However, Koner et al. [7] and Wrigge et al. [8] found no, or only a minor, effect of protective ventilation on systemic and pulmonary inflammatory responses in patients with healthy lungs after uncomplicated CPB surgery.

The open lung concept is also a protective ventilation strategy that combines low tidal volume ventilation with high levels of PEEP [26]. To open up collapsed alveoli, a recruitment maneuver is performed and a sufficient level of PEEP is used to keep the lung open. The smallest possible pressure amplitude is used in order to prevent lung overdistention and this results in low tidal volume (4–6 ml/kg) ventilation.

We applied this open lung concept in cardiac surgery patients and found that open lung concept ventilation (tidal volume 6 ml/kg, PEEP 14 cmH_2O), applied immediately after intubation, significantly decreased plasma IL-8 and IL-10 compared to conventional ventilation (tidal volume 8 ml/kg, PEEP 5 cmH_2O) [27]. The application of an open lung concept was accompanied by a significantly higher PaO_2/FiO_2 ratio during mechanical ventilation, suggesting a significant reduction of atelectasis [28]. We could also demonstrate that ventilation according to the open lung concept led to a significantly better preservation of FRC and better oxygenation several days after extubation when compared to conventional ventilation [29]. A decreased FRC is associated with post-operative pulmonary dysfunction. After cardiac surgery, respiratory dysfunction accounts for 40% of the readmissions on the ICU [30, 31]. Chung et al. [32] have shown that each percent increase in FiO_2 on discharge from the ICU, significantly increases the risk of readmission. Several other attempts to preserve FRC after extubation in cardiac patients have been without success.

When considering the two-hit model, one should start the open lung concept immediately after CPB and continue this ventilation strategy until extubation. When the open lung concept is initiated immediately after CPB, this approach seems to have great beneficial effects, such as decreased interleukin release [27], increased PaO_2/FiO_2 ratio during mechanical ventilation [28], an attenuated FRC decrease after extubation, and fewer episodes of hypoxemia [29]. Three days after extubation, patients were not in need of additional oxygen when ventilated according to the open lung concept peri-operatively [29]. This may indicate earlier hospital discharge.

Effect of PEEP Ventilation on Cardiac Performance

It has been shown that PEEP affects right ventricular (RV) afterload. Biondi et al. [33] have shown that the use of PEEP levels above 15 cmH_2O increased RV volume and decreased elastance, indicating an increase in RV afterload and a decline in RV contractility. Spackman and colleagues [34] have shown that during high frequency ventilation, mean airway pressure above 12 cmH_2O resulted in a decrease in the RV ejection fraction (RVEF) and was associated with an increase in the RV end-systolic

volume. The authors attributed these findings to an increase in the RV afterload due to increased mean airway pressure. Dambrosio et al. [35] found that the RVEF and the RV stroke work/RV end-diastolic volume ratio started to decrease at PEEP levels higher than 10 cmH_2O in patients with acute respiratory failure. Schmitt et al. [36] used echo Doppler data obtained by transesophageal echocardiography (TEE) to assess the effect of PEEP on RV outflow impedance. In their study, use of PEEP levels (13 ± 4 cmH_2O) caused an increased RV afterload. These studies show clearly that RV afterload is elevated during mechanical ventilation with moderate to high levels of PEEP.

Use of large tidal volumes increased RV outflow impedance as assessed by echo-Doppler of the pulmonary artery [37]. Moreover, large tidal volume ventilation is more likely to occur in the presence of atelectasis because of the so-called baby-lung effect: If one imagines a lung with 50% atelectasis, then a pre-set tidal volume of 10 ml/kg would result in a tidal volume of 20 ml/kg in aerated lung areas. Therefore, atelectasis may cause an increase in the RV afterload due to: a) an increase in the tidal volume in aerated lung areas (baby-lung effect); b) hypoxic pulmonary vasoconstriction in non-aerated lung areas. This atelectasis cannot be reversed with the use of high PEEP ventilation; only by the application of recruitment maneuvers.

In volume loaded patients after cardiac surgery, Dyhr et al. [38] found no decrease in cardiac output when applying recruitment maneuvers followed by a mean of 15 cmH_2O PEEP. This was confirmed by our results in which RV afterload, as assessed by echo-Doppler of the pulmonary artery (Fig. 2), was comparable between open lung concept ventilation with a PEEP of 15 cmH_2O compared to con-

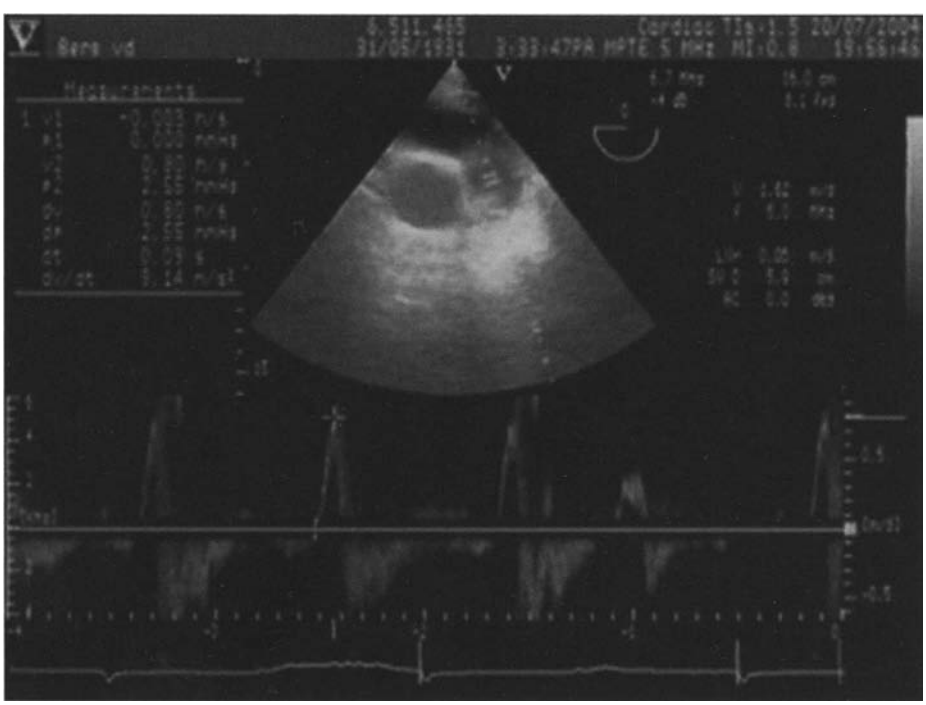

Fig. 2. Echo-Doppler of the pulmonary artery. Bottom line represents airway pressure. Dotted line in the second beat indicates the acceleration of the pulmonary flow during inspiration.

ventional ventilation using 5 cmH_2O of PEEP in cardiac surgery patients [39]. This suggests that when atelectasis is avoided, RV afterload is not increased by open lung concept ventilation. This could explain the results of Huemer et al. [40], who found no increased RV afterload using 12 cmH_2O of continuous positive airway pressure (CPAP) in healthy volunteers (without atelectasis), assessed by echo-Doppler. The separate effects of PEEP and tidal volume on RV impedance during open lung concept ventilation remain, however, unknown.

RV afterload is not only increased by high PEEP levels; also during inspiration RV afterload increment is observed. Poelaert et al. [41] showed that inspiration rather than expiration with high levels of PEEP caused RV afterload increment in cardiac surgery patients. Vieillard-Baron et al. [37] also showed that RV afterload is mainly increased during inspiration in patients with ARDS. These authors separated the effects of peak inspiratory pressure (PIP) and tidal volume by chest trapping and application of PEEP. They found that tidal volume, and not PIP or PEEP increased RV afterload. Although these results were very clear, theoretically this is hard to explain. Only intrathoracic pressure, but not volume, generates a force that could compress pulmonary capillaries, increasing RV afterload. In addition, of course, volume changes require pressure changes. The physiological explanation for the finding that tidal volume, not PIP, increases RV afterload is not known yet. However, this is more than a semantic discussion: If PIP and not tidal volume is to increase RV afterload, then elevated PEEP levels should increase RV afterload because of the increased PIP.

This phenomenon did not occur during open lung concept ventilation as assessed by echo-Doppler of the pulmonary artery. In cardiac surgery patients, mean acceleration time decreased during inspiration during conventional ventilation but not during open lung concept ventilation [39]. Ventilation according to the open lung concept was accompanied by a lower tidal volume (4 ml/kg vs. 8 ml/kg) but a higher inspiratory pressure (25 vs. 17 cmH_2O) compared to conventional ventilation [39]. The low tidal volume used during open lung concept ventilation may explain the lack of increase in RV afterload during inspiration. Also alveolar overdistention during inspiration could be reduced by application of open lung concept ventilation, despite the use of high PEEP levels. Namely, De Matos et al. [42] demonstrated, using a computed tomography (CT) scan, that tidal recruitment and degree of overdistention during inspiration in ARDS patients decreased when a recruitment maneuver was performed compared with pre-recruitment with 25 cmH_2O PEEP. This implies that during open lung concept ventilation RV afterload is not increased during inspiration due to: 1) the reduction in tidal volume ventilation in aerated lung areas due to homogenization of pulmonary gas distribution; and 2) the use of lower tidal volumes, set on the ventilator. Furthermore, these two effects of open lung concept ventilation act in synergy: Homogenization of pulmonary gas distribution reduces tidal volume ventilation of aerated lung areas which is reduced even further by the lower tidal volume ventilation set on the ventilator. In addition, in patients who have undergone cardiac surgery, the pericardium has been opened. Therefore, the effect of open lung concept ventilation on RV outflow afterload with an intact pericardium (such as in ARDS patients) still remains unknown. However, Schmitt et al. [36] have shown that the mean acceleration time did decrease significantly during inspiration using a protective ventilation strategy in patients with ARDS. This strategy used a PEEP level above the lower inflection point and a low tidal volume in order to prevent overdistention but this strategy is without a recruitment maneuver and, thus, in the presence of atelectasis.

Conclusion

Pulmonary dysfunction after cardiac surgery is probably a two-hit process: The first hit is due to the surgical procedure, the second hit due to mechanical ventilation of the lung in an inflammatory environment. Pulmonary inflammation is aggravated by non-optimal mechanical ventilation of the lung. The open lung concept is a lung protective ventilation strategy, reducing pulmonary dysfunction after cardiac surgery. The beneficial effect of this ventilation strategy is best when applied immediately after intubation. Furthermore, this ventilation strategy, using low tidal volume ventilation together with avoiding atelectasis, might attenuate the effect of airway pressure on RV afterload.

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Alveolar Pressure/volume Curves Reflect Regional Lung Mechanics

O. Stenqvist and H. Odenstedt

Introduction

The static pressure volume (P/V) curve has been regarded as the gold standard tool for assessment of the mechanical properties of the lung. On this curve, a lower inflection point (LIP) can be detected in some patients and in most patients an upper inflection point (UIP) can be seen. The most common interpretation of the LIP and the UIP is that LIP represents the point where alveoli collapse at the end of expiration and reopen at the start of inspiration and that the UIP represents the pressure above which alveoli become overdistended. It has been proposed that in order to avoid cyclic closing and opening and overdistension of alveoli, ventilation should be performed with pressures between the LIP and UIP, where the compliance of the lungs is highest.

The Lower Inflection Point

The background of LIP is complex and various hypotheses have been proposed. Gattinoni and co-workers have proposed that the weight of the edematous acute lung injury/acute respiratory distress syndrome (ALI/ARDS) lung results in a superimposed pressure, increasing vertically, causing a collapse of the most dorsal lung parts [1, 2]. The LIP should, according to this proposal, be the pressure that is high enough to counteract the threshold opening pressure and the superimposed pressure. This hypothesis has been further analyzed in a mathematical model by Hickling, who describes a continuous recruitment process during inflation [3]. Hubmayr has argued against this interpretation and favors a hypothesis where the LIP is caused by a gas/fluid interface in flooded lung parts [4].

The Volume below the Lower Inflection Point

In the literature on lung mechanics, the focus has been on the pressure level of the LIP, but very little is mentioned about the volume where the LIP is positioned. Clinical data are scarce, but from published P/V curves the volume can be estimated to lie between 50 and 150 ml [5–7]. Compliance below the LIP can be estimated to be 5–20 ml/cmH_2O based on these curves. It is important in this context to realize that compliance is closely related to the size of the lung. Thus, if you apply pressure control ventilation to a mouse or an elephant with a pressure that in a human results in normocapnia, you will have normocapnia in both these animals, as the compliance

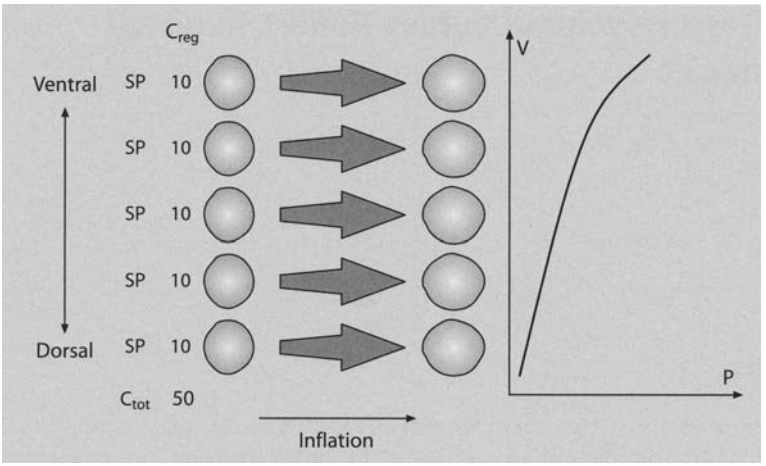

Fig. 1. Regional compliance (Creg), superimposed pressure (SP), and pressure/volume (P/V) curve in a healthy lung. No lower inflection point (LIP) is detected and a decrease in compliance is noted as inflation reaches total lung capacity.

of the elephant lung is enormous and that of the mouse is very low. Gattinoni and Pesenti have has promoted the baby lung concept, where the ALI/ARDS patient is supposed to have a part of the lung collapsed and the rest – a small but supposedly healthy lung – a 'baby lung' that is quite normal [8]. This dichotomic view of the ALI/ARDS lung may be questioned as, most likely, the open parts of the lung are also to some extent affected by a lowered compliance as a result. When a P/V curve of such a lung is obtained, the compliance below the LIP will be low, representing compliance of the baby lung. The compliance of this baby lung will be dependent on its size, the smaller the baby lung, the lower the compliance. As the inflation continues and more alveoli are recruited, compliance will increase until alveoli at the very bottom of the lung with very low compliance are recruited. The reason for elaborating on this point is that the relationship between the size of the lung and compliance is fundamental for understanding the P/V curve. If a healthy lung (Fig. 1) is divided into, for example, five horizontal planes with alveoli of the same end expiratory size and compliance is measured for each of these parts of the lung, it would be a fifth of the total compliance. If an ALI/ARDS lung (Fig. 2) with a lowered total compliance of 30 ml/cmH_2O is divided in the same way, the most ventral part would have the highest compliance and the most dependent part the lowest compliance because of the superimposed pressure from the edematous tissue. The compliance of these five parts would, when added, result in a total compliance of 30 ml/cmH_2O and could, if the superimposed pressure is increasing linearly, be 10, 8, 6, 4 and 2 ml/cmH_2O from top to bottom of the lung. If the three most dependent compartments of the lung are collapsed (Fig. 3), the total compliance would only be $10+8 = 18$ ml/cmH_2O.

Overdistension Versus Gas Compression

In ALI/ARDS, increased resistance is not a major factor, so instead of gas moving along the path of least resistance, we see the gas moving along the path of highest

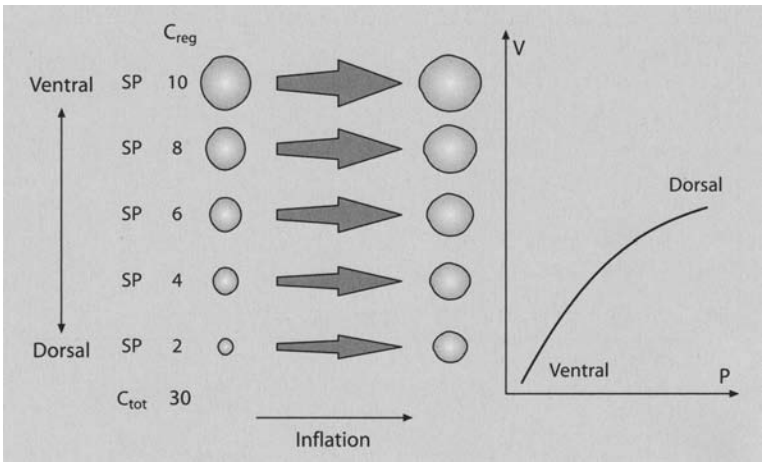

Fig. 2. Regional compliance (Creg), superimposed pressure (SP), and pressure/volume (P/V) curve in a lung with acute respiratory failure without collapsed alveoli. SP increases along the vertical axis. Functional residual capacity (FRC) is decreased and so is total lung capacity. The P/V curve shows no lower inflection point (LIP), but compliance is decreased and an upper inflection zone present, indicating that ventral lung regions are fully stretched (regional compliance ~zero) and recruitment of low compliant, dorsal alveoli at end of inspiration still on-going.

compliance. Following the path of highest compliance in an ALI lung without collapse, as in Figure 2, when inflation starts, the initial gas will naturally flow towards the non-dependent lung with highest compliance. Continuously during the inflation, when pressure increases, the alveoli of the most non-dependent lung will be expanded until not yielding any more (regional compliance = zero). Already before that, as pressure rises, gas will flow to more dorsal parts of the lung where compliance is low but, at this time point, higher than in the most ventral parts of the lung. The P/V curve of this lung will show a continuously decreasing compliance as inflation proceeds. When the pressure is high enough to inflate the most dorsal parts of the lung, the alveoli of the most ventral parts are already stretched to their structural limits and will not expand any further, i.e., compliance is decreasing towards zero. This will result in the final part of the P/V curve deflecting as a sign of low compliance in the dependent part of the lung rather than a sign of overdistension of the ventral part of the lung. The term overdistension is thus misleading as the deflection of the P/V curve indicates that pressure rises more than volume, i.e., gas is compressed.

Let us consider the behavior of another example of a five-compartment lung, where the three most dependent compartments are collapsed during the start of an inflation (Fig. 3). In this case the initial compliance will be 18 ml/cmH_2O and when the airway pressure is high enough to overcome the superimposed pressure and the threshold opening pressure of the mid compartment alveoli (the most non-dependent compartment of the three collapsed compartments), compliance will increase by 8 ml/cmH_2O, which is the compliance of that very compartment. In contrast to the situation where all compartments are open already from the start of inspiration, this sudden increase in compliance will result in a LIP of the P/V curve. As the airway pressure increases enough to open or recruit the two most dependent compartments, compliance will further increase by 6 and 4 ml/cmH_2O. When inflation pro-

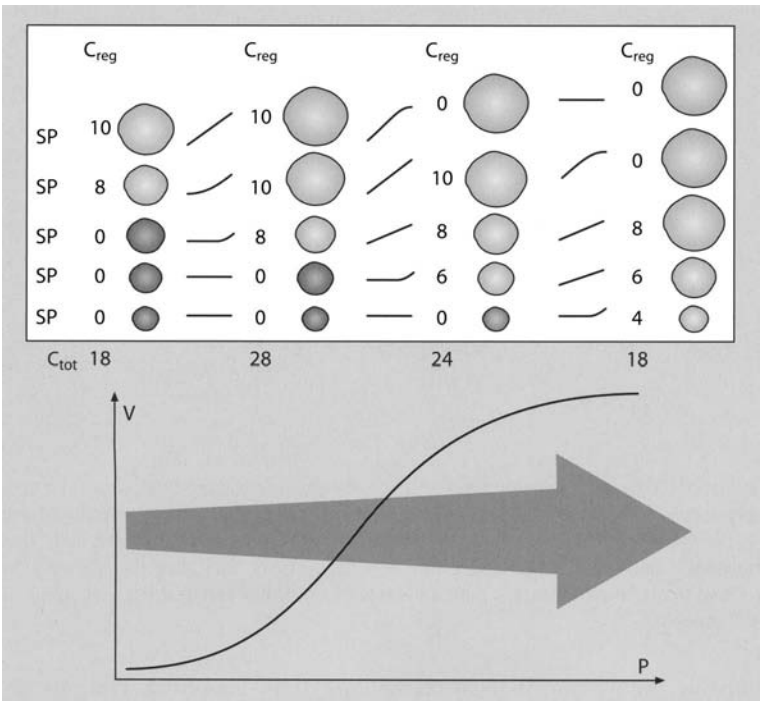

Fig. 3. Regional compliance (Creg), superimposed pressure (SP), and regional and global pressure/volume (P/V) curves in a lung with ALI/ARDS with collapsed alveoli. SP increases along the vertical axis. Functional residual capacity (FRC) is decreased and so is total lung capacity. The P/V curve shows a lower inflection point (LIP) as ventral, open parts represent a small lung volume and thus have low compliance, which increases when more dorsal parts of the lung are recruited. An upper inflection point (UIP) is present, indicating recruitment of low compliant, dorsal alveoli at end-inspiration when ventral lung regions are already stretched to their structural limits (compliance ~zero).

ceeds, the P/V curve will show a continuous decrease in compliance, as compliance in the most ventral parts of the lung decreases towards zero (as in the previous example, where all five compartments were open from the start of inflation). Compliance of the last part of the P/V curve will reflect the compliance of the dependent parts of the lung.

Volume Dependent Compliance

The changing of compliance along the P/V curve, i.e., volume-dependent compliance, reflects that different parts of the lung have different properties. Normally the regional differences are arranged along a vertical axis, so that the highest compliance of the P/V curve represents the most ventral parts of the lung and the lowest compliance the most dependent, dorsal part of the lung (Figs. 4, 5) [9]. However, in some cases the regional differences in mechanical properties of the lung occur more randomly. In any case, whether regional differences in compliance are arranged vertically or randomly, the volume-dependent compliance of the P/V curve is a measurement of these differences.

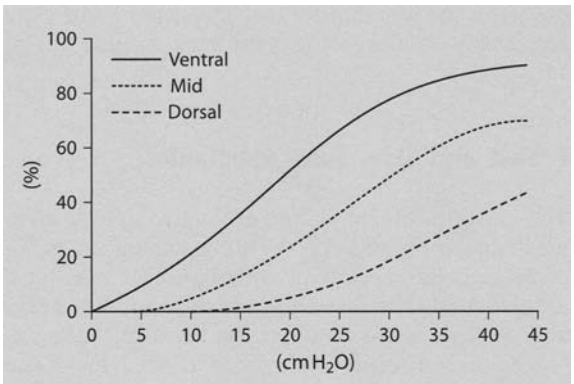

Fig. 4. Electric impedance tomography tracings from left lung of a patient with pneumonia. Relative regional tidal volume on the Y-axis and airway pressure on the X-axis. The pressure/volume (P/V) curves are obtained during a low flow inflation, which gives time for partial equilibration of visco-elastic forces. Note that the lower inflection point (LIP) is positioned at a much higher pressure in the dorsal parts of the lung. No gas enters the mid region until a pressure of ~6 cmH_2O is reached and aeration/ventilation of the most dependent region requires a pressure of about 15 cmH_2O. When compliance of the ventral lung regions are close to zero (P/V curve parallel to x-axis) compliance in the most dorsal lung regions still increases. Modified from [9] with permission

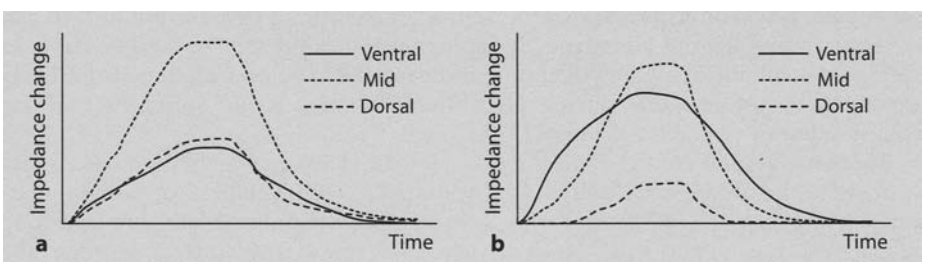

Fig. 5. Impedance changes (corresponding to lung volume changes) in the ventral, mid and dorsal region of the lung, in a pig during one tidal breath, before (**a**) and after saline lavage (**b**). In the healthy lung (**a**), gas enters all three regions from the start of inspiration. Compliance is equal in the ventral and dorsal regions, and higher in the mid region. Saline lavage (**b**) changes regional compliance and, thereby, the relative tidal volume distribution. At the start of the breath, gas will enter the ventral region with the highest compliance, now representing a proportionally larger part of the open lung. The mid region initially fills slowly due to lower compliance and the start of the inflation of the dorsal region is markedly delayed.

So far, all references to P/V curves are static P/V curves that are rarely used in clinical practice. The most common lung mechanics monitoring modality is the pressure volume loop based on pressure and flow measured in the ventilator or at the Y-piece. These dynamic loops are to a high degree influenced by the endotracheal tube resistance, which distorts the loop, resulting in a right shift of the inspiratory limb and a left shift of the expiratory limb of the loop. A loop that represents the lung mechanics more closely can be obtained by plotting the tracheal pressure versus volume instead. The tracheal pressure can either be calculated from the y-piece airway pressure and an algorithm for the endotracheal tube resistance [10] or measured directly by insertion of a narrow pressure line through the tube [11]. From the tracheal pressure loop an alveolar P/V curve can be obtained by multiple linear regression analysis of the loop, which is divided into six slices, where compliance is assumed to be constant within each slice, for volume dependent compliance calculations [12]. We have used another approach for obtaining an alveolar P/V curve from direct tracheal pressure measurements: The Dynostatic algorithm. This algorithm is

based on the assumption that inspiratory and expiratory resistance of the airway is reasonably similar at the same lung volume during inspiration and expiration [13, 14].

Fast and Slow Lung Mechanics

The most prominent feature of the dynamic alveolar P/V curve, which represents the mechanic properties of the lung during on-going therapeutic ventilation, is that compliance is lower than compliance of the static P/V curve of the same patient. Also, if any LIP is present in the dynamic P/V curve it is usually not very prominent and the UIP is not an inflection point but rather a zone of decreasing compliance.

The reason for this difference in behavior of the lung during static and dynamic conditions can probably be explained by the time factor playing a more important role than expected. As seen in Figure 6, during a low flow inflation in an ALI patient a prominent LIP at 8 cmH_2O is seen followed by a high and constant compliance throughout the inflation up to 1100 ml. In contrast, during tidal breathing in the same patient where inspiration starts from a pressure level of 4 cmH_2O no LIP can be detected and there is a tendency for an upper inflection zone in the P/V curve in spite of the volume being only half of that during the low flow inflation [15]. This is explained by the fact that during tidal breathing there is not sufficient time for equilibration of visco-elastic forces of the lung.

There are several studies indicating that low tidal volume ventilation causes less damage to the alveoli than a high tidal volume [16, 17]. The level of positive end-expiratory pressure (PEEP) seems to have less impact on ventilator-induced lung injury (VILI) as long as it is not set at very low levels or at 0 cmH_2O. However, the UIP of the static or the dynamic P/V curve does not represent overdistension, but rather recruitment of the most dorsal lung compartments with the lowest compli-

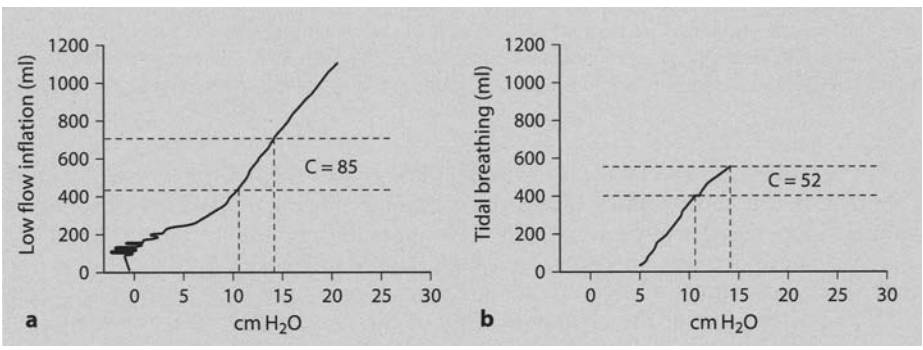

Fig. 6. Total respiratory system alveolar pressure/volume (P/V) curves obtained by the Dynostatic algorithm, in a patient with acute lung injury (ALI). During a low flow inflation (left panel) a prominent lower inflection point (LIP) is seen at ~8 cmH_2O followed by a high constant compliance without an apparent upper inflection point (UIP) even though the total volume inflated reaches 1100 ml. During tidal breathing (right panel) with inspiration starting from a pressure level of 4 cmH_2O, no LIP is seen but there is a tendency to an upper inflection zone in spite of the inflated volume being only half of the low flow inflation volume. The calculated compliance of the tidal breath at the end of inspiration, between 11 and 14 cmH_2O is 52 ml/cmH_2O but at the same pressure range during low flow inflation, it is as high as 85 ml/cm H_2O. Modified from [15] with permission

ance, during the last part of the inspiration. These alveoli are the same alveoli that collapse early at the beginning of the expiration. During static measurements this occurs at a higher lung volume than during dynamic conditions. This indicates that in patients it is important to monitor the lung mechanics during prevailing conditions to be able to set the ventilator optimally.

Conclusion

The compliance below the LIP, when present, is usually very low indicating that only a small lung volume is open when inspiration starts. The LIP of a dynamic P/V curve is usually not very prominent when compared to the LIP of a static P/V curve. There is probably no LIP without partial lung collapse. The UIP is not a sign of overdistension but rather a sign of low compliant, dorsal lung parts being recruited at the end of the inspiration, when the most compliant, ventral parts of the lung do not expand any further.

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Cardiovascular Topics

Cardiovascular Surgery in the Aging World

S. Wan and M.J. Underwood

Introduction

The world is aging fast: in 2000 there were 600 million people aged 60 and over; there will be 1.2 billion by 2025 and 2 billion by 2050 [1]. Health care for this aging population has become a vital challenge not only in industrialized societies but also in many developing countries. Today, about two thirds of all older people are living in the developing world; by 2025, it will be 75%. For instance, by the year 2036, the number of elderly Chinese people (aged 65 and above) is anticipated to surge to over 300 million and represent up to 20% of the nation's total population [1, 2]. An identical aging trend is also apparent in the developed world, where the very old (age 80+) are the fastest growing population group (Fig. 1a). Interestingly, women outlive men in virtually all societies; consequently in very old age the ratio of women to men is 2:1 [1]. By the year 2050, 1 in 12 Americans will be older than 80 years [2], which will indeed impose a major burden on health care resources (Fig. 1b) [3].

For the elderly, cardiovascular disease has been consistently ranked to be top of the killer list. Such a fact is clearly reflected by the worldwide annual increase in cases undergoing either percutaneous coronary intervention (PCI) or coronary artery bypass grafting (CABG). As far as clinical outcome is concerned, surgery may still offer the best chance to these patients. Such an important conclusion has been repeatedly confirmed, first and foremost by cardiologists rather than by cardiac surgeons. Evidence from the randomized Bypass versus Angioplasty Revascularization investigation (BARI) trial clearly showed the survival benefit of CABG over PCI in diabetic patients during a 7-year follow-up period [4]. In particular, CABG should be the preferred strategy for revascularization in the elderly diabetic patient aged 65 years and over [4, 5]. The Cleveland Clinic investigators demonstrated that in 6,033 consecutive patients with multi-vessel coronary artery disease and many high-risk characteristics, CABG was associated with better survival than PCI after adjustment for risk profile [6]. Similar observations have also been reported by a European group [7] as well as by New York State Cardiac Registries' researchers [8]. The latter study involved 59,314 patients over a 4-year period [8].

Although prospective research in the elderly population undergoing cardiac operations is less well documented, few would argue that the aging of the society and improvements in outcomes after cardiovascular procedures have resulted in a growing demand for complex surgical intervention in this group. This is reflected in the increasing number of older patients being referred for both CABG and valvular surgery (Figs. 2 and 3) in the UK [9]. Since numerous lessons have been learned over the past decade, we briefly review the English-language literature

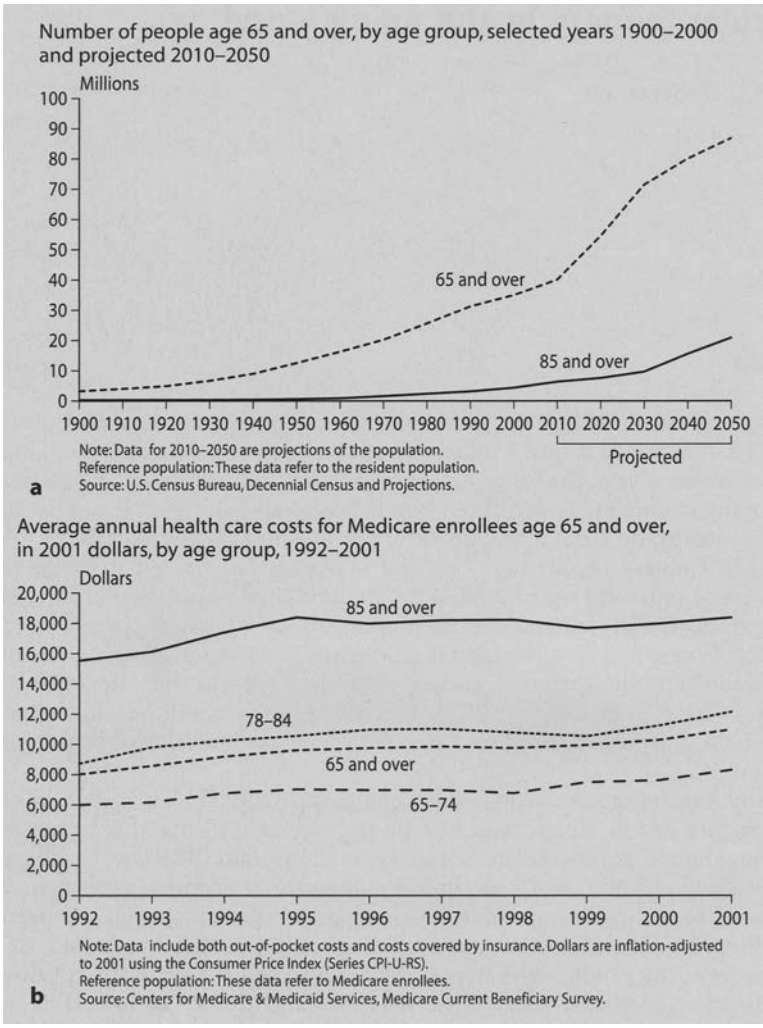

Fig. 1. [A] The growing elderly population in the USA. [B] Health care costs for elderly people in the USA. Reproduced from [3]

with particular focus on cardiovascular surgery in hexagenarians (aged 60 to 69 years), septuagenarians (aged 70 to 79 years), and octogenarians (80 years of age and older). Accumulating evidence indicates that operative mortality and morbidity following primary or re-operative CABG and valvular interventions can be limited in this high-risk patient subset. More importantly, elderly patients may benefit from improved functional status and quality of life after cardiovascular surgical therapy.

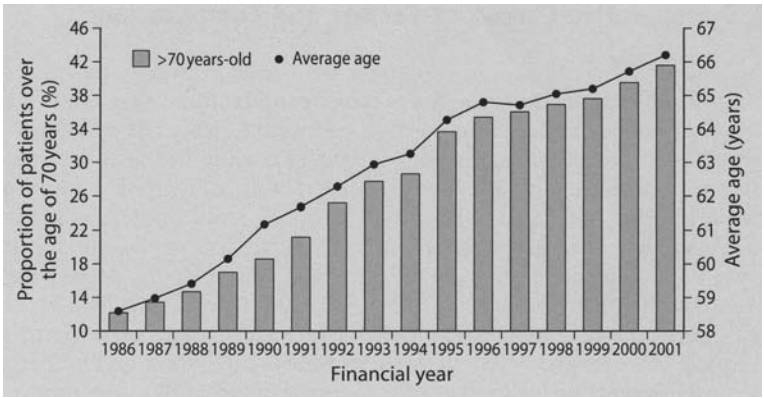

Fig. 2. Trends in age from the United Kingdom Heart Valve Registry. From [9] with permission

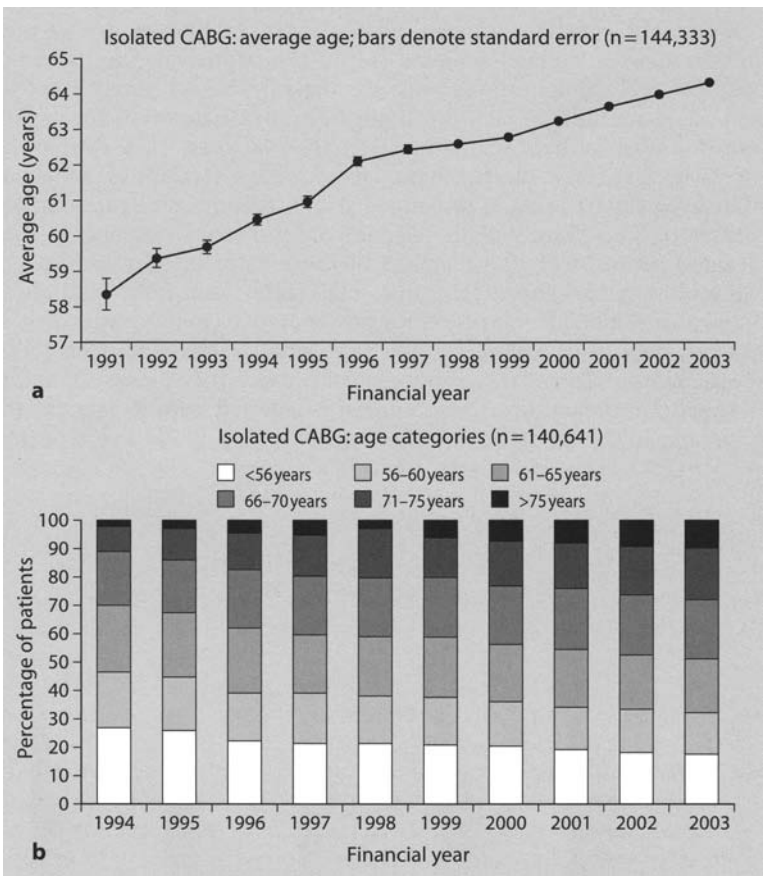

Fig. 3. [A] Average age increase for isolated CABG in the United Kingdom; [B] Age profile for isolated CABG in the UK. From [9] with permission

Perioperative Care: Risk Factors and Complications

Advanced age alone should no longer be a reason to deny patients of a potentially curative operation. However, it has long been identified that age is a crucial risk factor for patients undergoing cardiac operations, which is obviously related to the physiologic process of aging with a natural decline in the functional reserve of the organs. Advanced age is often associated with other risk factors or extracardiac comorbidities such as diabetes, hypertension, renal impairment, history of stroke, and even previous cardiac interventions. Hence, it is explicable that surgical mortality as well as postoperative complications are significantly greater for octogenarians than for their younger counterparts [10–13]. In order to best serve this group of frail patients, we must become better educated in basic geriatric principles of care. Surgical techniques must be refined, as well as case selection and perioperative care.

In the largest series to date of octogenarians (n=601) undergoing cardiac operations, Emory University group [12] reported an overall operative mortality rate of 9.1% compared with 6.7% in septuagenarians (n=5,698). Barnett and colleagues [13] also observed that octogenarians (n=444) had nearly double the mortality rate compared with younger patients (n=7,917). Moreover, the incidence of non-fatal postoperative complications was significantly higher among octogenarians than non-octogenarians. In fact, the single best univariate predictor of at least one complication after surgery was age greater than 80 years [13]. Despite recent improvements in 'fast-track' management, advanced age remains an independent predictor of delayed extubation and prolonged stay in the intensive care unit (ICU) or in hospital [14]. Therefore, patient selection for surgery in octogenarians is not always straightforward, with the majority of these patients operated upon for symptoms rather than for prognosis [15]. In a small series from Italy, Gatti and associates [16] found that the major predictors for postoperative complications in high-risk octogenarians were heart failure status (New York Heart Association [NYHA] class IV), severe angina (Canadian Cardiovascular Society [CCS] class 4), and prolonged aortic crossclamping time. These authors suggested earlier surgical therapy may be preferable in this particular group of patients [16]. It was also reported that preoper-

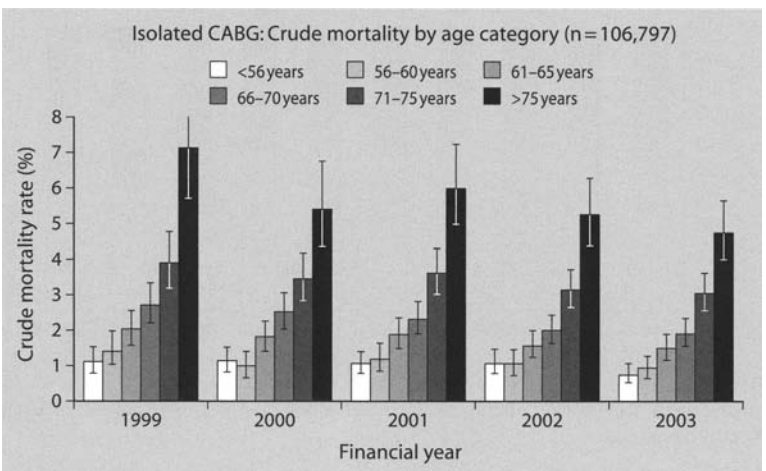

Fig. 4. Non-adjusted mortality by age for isolated CABG in the United Kingdom. From [9] with permission

ative renal failure and urgent or emergent operation significantly increased the risk of early death, while cerebral vascular disease and prolonged postoperative ventilation were the additional risk factors for late death [17]. Owing to the significantly increased lengths of stay in ICU and in hospital for octogenarians than for the younger patient subgroups, total direct costs were 26.8% higher in octogenarians (about an additional US$ 4,818 per patient) in a recent study from the USA [18]. Despite these challenges, data from the UK demonstrate that with an increasing proportion of elderly patients being referred for coronary revascularization, there has been a steady reduction in mortality in this group over time [9] (Fig. 4).

Long-term Outcome: Survival and Quality of Life

As suggested by the Emory University group [12], although there was a more rapid survival decline beyond 5 years in octogenarians, the median postoperative 5-year survival in this group of patients was 55%, compared to 69% in septuagenarians and 81% in hexagenarians. At the age of 80, for instance, the remaining life expectancy in Sweden is 8.8 years for females and 6.9 years for males [19]. Collins and colleagues [19] observed that the 36-month survival rate after open-heart surgery in 183 Swedish patients aged between 80 and 84 years was 85.6%, which did not differ from normal population matched for age and gender. More importantly, postoperative quality of life among these patients was comparable or even better than in the general population [19]. Similar findings were reported in an earlier Canadian study involving 127 patients older than 80 years at cardiac operation, with an actual 2-year survival of 80% [17]. As elderly patients benefit from improved functional status and quality of life, three fourths of these survivors rated their health as good or excellent and a majority (82.5%) of them would undergo cardiac operation again in retrospect [17].

CABG: State-of-the-art

The principles and technique of choice for surgical myocardial revascularization in elderly patients remain less absolute or even controversial, when compared with the young. Nevertheless, a growing body of evidence has impacted significantly on our decision-making process and daily practice. For example, strategies such as aggressive or more liberal preoperative use of intra-aortic balloon pumping have been shown to be beneficial in patients aged 70 years or older [20]. Arterial grafting, using the internal mammary artery or even the radial artery, has also been suggested to reduce operative mortality and improve survival as well as long-term quality of life in septuagenarians and octogenarians [21–23]. A retrospective multivariate analysis in 987 octogenarians identified that the exclusive use of saphenous vein graft was an independent predictor of operative mortality and late death [22]. Interestingly, the completeness of revascularization had no positive influence on postoperative survival, recurrent angina, or functional status among 358 octogenarians [21]. In another study, however, incomplete revascularization was identified by logistic multivariable regression as an independent risk factor for early death (within 180 days) in 859 patients aged 75 or older [24].

Over the past decade, off-pump CABG has gained enormous popularity worldwide. Numerous clinical series have proposed that the avoidance of cardiopulmo-

nary bypass (CPB) could result in a lesser degree of inflammatory injury and, in turn, minimize the incidence of postoperative myocardial, renal, or neurological injury [25]. It is believed that the off-pump approach may be particularly beneficial in high-risk patients with multiple co-morbidities, especially for the elderly. Off-pump CABG was shown to reduce postoperative ICU stay, morbidity and mortality in patients aged 75 years or older [26, 27]. A meta-analysis of eight observational studies between 1999 and 2003 in patients aged 70 years or older undergoing off-pump (n=764) or on-pump CABG (n=2253) showed that the former approach was associated with a much lower incidence of postoperative atrial fibrillation [28]. Another more recent meta-analysis of 37 randomized trials (n=3369) of off-pump versus conventional CABG also confirmed this finding [29]. In patients undergoing off-pump CABG, however, the incidence of atrial fibrillation appeared to be greater in octogenarians than in the younger age groups [30, 31].

Among many unanswered questions is whether the off-pump technique could significantly reduce postoperative brain injury [32]. Neurological deterioration following CABG could manifest in different severities, from a serious stroke to subtle cognitive impairment. The underlying etiology is obviously multifactorial, while embolization and perioperative cerebral hypoperfusion are among the commonest causes. It is evident that the incidence of aortic atheroma increases with age. Goto and coworkers [33] noted that multiple small brain infarctions could be detected before CABG in 83 out of 421 patients aged 60 years or older by magnetic resonance imaging (although 59% of them were asymptomatic), which predisposed to postoperative neurological dysfunction. Hence, off-pump CABG using a no-touch technique and total arterial grafting could theoretically be advantageous in elderly patients, particularly those with severe arteriosclerosis. Indeed, Ricci and colleagues [34] showed that octogenarians undergoing off-pump CABG (n=97) experienced remarkably fewer perioperative strokes than those receiving conventional CABG (n=172). The Montreal Heart Institute investigators [35] also demonstrated that among 125 octogenarians undergoing CABG the type of surgery (on- or off-pump) was an independent predictor of operative mortality and stroke, which occurred four times more often in the CPB group. In a recent meta-analysis including nine observational studies between 1999 and 2002 in patients aged 70 years and older who underwent CABG with (n=3,222) or without (n=1,253) CPB, the incidence of stroke appeared significantly lower in the off-pump group [36]. Nonetheless, this important observation requires validation in future prospective, randomized trials involving much larger patient populations.

Taken together, this represents both bad and good news. On the one hand, the patients now referred for CABG are generally 'older and sicker' than the same group a decade ago, as shown by the Society of Thoracic Surgeons (STS) Database – the largest voluntary database in medicine to date – which recorded that 1,154,486 patients underwent isolated CABG between 1990 and 1999 at 522 North American centers [37]. The mean age of these patients increased from 63.7 in 1990 to 65.1 in 1999, corresponding to the predicted operative risk (2.6% in 1990 and 3.4% in 1999) and the ratio of female gender (25.7% in 1990 and 28.7% in 1999) [37]. According to the STS database, surgical mortality was even higher among elderly women than men [38]. On the other hand, however, the observed operative mortality decreased from 3.9% in 1990 to 3.0% in 1999 and this trend was also true in patients 65 years and older (5.4% in 1990 to 4.1% in 1999) [37]. It is believed that significant technical advantages in surgery, cardiology, perioperative care, and better quality measures in a more dedicated and specialized team, have all contributed to the improve-

ment in outcome over the past decade [37]. In particular, evidence in favor of off-pump CABG as the approach of choice for elderly patients has been widely available [25–28, 30, 31, 39, 40].

Valvular Surgery: Type and Prosthesis of Choice

Valve replacement operations can be performed in the elderly with acceptable mortality and morbidity [41–43], although combined (often with CABG) or urgent/emergent procedures and preoperative heart failure may significantly increase surgical mortality from 8–9% to 15–34% in this patient population [41, 42]. In general, the hospital mortality rate is also higher in women than in men [43].

There is a wealth of knowledge and long-term follow-up data supporting the use of the bioprosthesis as the valve of choice for the elderly. In 2,075 patients aged 65 and older who underwent valve replacement using a porcine bioprosthesis, actuarial freedom from valve failure at 9 years was 94.4% and at 18 years was 83.7% (in total 74 valves failed from all causes) [42]. Chiappini and associates [43] noticed that the type of prosthesis was in fact a significant predictor of late mortality in 115 octogenarians undergoing aortic valve replacement. Mechanical prosthesis was commonly chosen in their study due to a narrowed aortic annulus or a concomitant atrial fibrillation, which led to an actuarial 5-year survival rate of 56.7% compared to 81.7% in the bioprosthesis group [43]. The authors suggested that thromboembolic or hemorrhagic events related to the anticoagulant therapy increased the risk of late death in octogenarians after receiving mechanical prosthesis [43]. On the contrary, elderly patients had a more favorable quality of life following bioprosthesis replacement operations compared with normal subjects matched for age and sex [42]. A significantly improved survival following stentless rather than stented bioprosthesis replacement has also been proposed in elderly patients with aortic stenosis [44], especially in those with a small aortic root [45]. Interestingly, recent evidence indicated that the use of smaller aortic valve prostheses in the elderly patient may not adversely affect the incidence of early or late mortality [46–48], which is somewhat against the conventional thinking [45]. By combining a pool of nine multi-institutional databases involving 13,258 aortic valve replacements, Blackstone and colleagues [48] concluded that using durable non-thrombogenic prosthesis is more important than concentrating on better hemodynamic performance to improve long-term survival. After aortic valve replacement with a bioprosthesis, however, anticoagulation may still be indicated in elderly patients with larger valve size ($\geq$27 mm) or preoperative endocarditis [49].

Meanwhile, the ideal treatment for isolated non-rheumatic, non-ischemic mitral regurgitation in the elderly remains largely unclear. Increased tissue fragility may make mitral repair less predictable and more complex in older patients, particularly when calcification is present. The recently published 10-year experience at the Mayo Clinic highlighted their surgical outcomes in octogenarians after mitral repair or replacement of predominantly degenerative mitral regurgitation [50]. In this study, (1) the overall 5-year survival was 61%, which was comparable with that in the general population matched for age and gender; (2) mitral repair was proven to be a reliable approach as none of the patients needed re-operation [50]. Similar to other series [41–43], preoperative left ventricular functional status had significant impact on late cardiac complications, suggesting that early surgery is preferable in octogenarians [50].

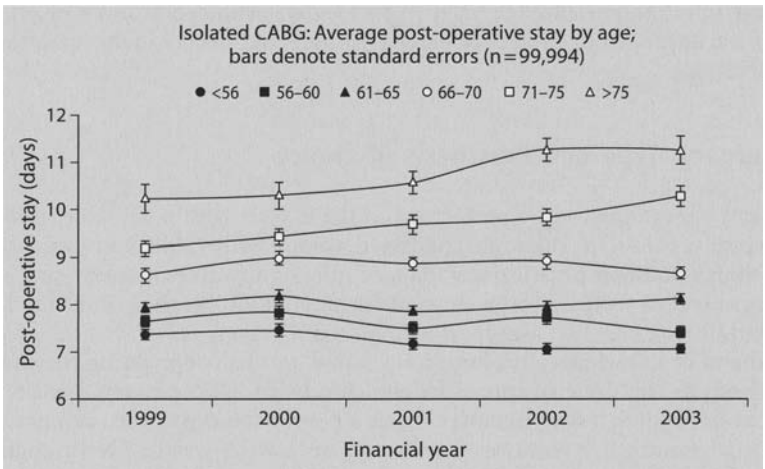

Fig. 5. Post-operative stay in days and age for isolated CABG in the United Kingdom. From [9] with permission

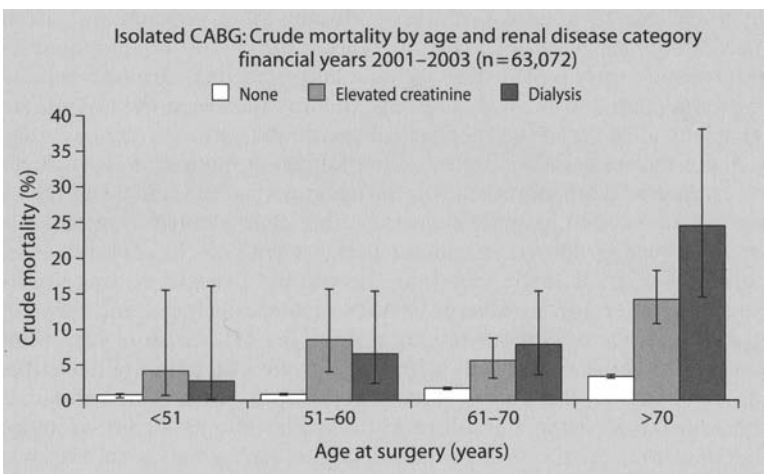

Fig. 6. Crude mortality for isolated CABG according to age and renal function. From [9] with permission

A retrospective review of the STS database, involving 31,688 patients who underwent mitral valve replacement from 1997 to 2000, revealed that surgical mortality increased from 4.1% in patients aged <50 years up to 17.0% in those aged 80 years or more [51]. Four important risk factors, including hemodynamic instability, NYHA class IV, renal failure, and concomitant CABG were identified to significantly raise operative mortality to more than four-fold among the elderly patients [51]. Indeed the combination of age and other important pre-morbidity may have a significant impact on outcome (Fig. 5), as illustrated by the combination of age and renal impairment in the UK population undergoing CABG [9] (Fig. 6). Therefore, careful pre-operative selection is mandatory. Another smaller series also suggested that chronic obstructive lung disease, preoperative use of intraaortic balloon, and postoperative stroke may be additional predictors of hospital death in octogenarians

[52]. It is noteworthy that in patients with cardiomyopathy and severe mitral regurgitation, mitral repair utilizing an undersizing overcorrecting annuloplasty ring has been advocated recently by Romano and Bolling [53] although the place of this intervention in the elderly has yet to be precisely defined.

Re-operations: An Expanding Pool

As life expectancy continues to grow, so too will the proportion of elderly patients presenting for cardiovascular re-operations which, naturally, represent a serious challenge to cardiac surgeons. Although the safety and efficacy of first-time open-heart surgical interventions in elderly patients have been well established, little is known about re-operations in this cohort. In general, the 30-day mortality is considerably higher for re-operations in the elderly [54, 55], particular in those undergoing combined CABG and valve procedures [54]. One of the largest series to date, involving 739 patients aged 70 years or older who underwent redo CABG between 1983 and 1993 at the Cleveland Clinic, revealed that preoperative renal impairment, female gender, emergency nature, poor left ventricular function, and left main disease were the major risk factors associated with increased in-hospital mortality [55]. It is believed that myocardial protection with the combination of antegrade and retrograde cardioplegia is of utmost importance in re-operative CABG, mainly because myocardial distribution of antegrade cardioplegia could be unpredictable during this particular setting and diseased but patent vein grafts may predispose to intra-operative coronary atheroembolization [56]. Recently, off-pump CABG was suggested to be a safe and potentially better approach for re-operative revascularization [57]. However, off-pump CABG may be associated with a higher incidence of incomplete revascularization even in experienced hands [58]. In fact, the 5-year clinical outcome was found to be significantly worse in the redo CABG group than in the matched first-time CABG group, likely related to the higher incidence of incomplete revascularization and greater perioperative myocardial injury in the former group [58].

In 400 patients undergoing re-operative valve replacement at Massachusetts General Hospital for failed bioprostheses, Akins and associates [59] demonstrated that age greater than 65 years, male gender, renal insufficiency, and non-elective operation were predictors of in-hospital death. Recently, the same group of surgeons also summarized their experience of first-time aortic valve replacement in 132 patients who had previous cardiac operations on CPB [60]. With an overall mortality rate of 6.7%, they suggested that routine replacement of a minimally diseased aortic valve during CABG is not necessary [60]. Excellent early results for repeat aortic [61] or mitral [62] valve replacement have also been reported by the Mayo Clinic group, supporting the selection of bioprostheses over mechanical prostheses. Similarly, in view of acceptable mortality and morbidity associated with redo mitral valve surgery, primary mitral commissurotomy or repair should be recommended even in developing countries [63].

Surgery of the Thoracic Aorta: Evolving Evidence

By summarizing data from 18 large referral centers in six countries since 1996, reports from the International Registry of Acute Aortic Dissection have provided some new insights into this old disease [64–66]. Acute type A aortic dissection is

one of the most serious medical emergencies and carries significant mortality in absence of surgical intervention. Despite improved surgical techniques and perioperative management, operative mortality remains high, particularly for elderly patients [64]. In fact, age ≥ 70 years has been identified to be an independent predictor of death for acute type A aortic dissection [65]. In patients aged 70 or older (32% of the total patients enrolled), although surgical mortality (37.5%) was better than with medical therapy alone (52.5%), it was significantly higher than in the younger group (23.0%) [64]. In patients aged 80 to 84 years, however, surgical intervention carries no advantage over medical therapy for type A aortic dissection (both mortality rates were 45.5%) [64]. On the other hand, for acute type B aortic dissection, elderly patients (age ≥ 70 years) with hypotension/shock had the highest risk of death (56%), followed by any branch vessel involvement (28.6%) or presence of periaortic hematoma (10.5%) [67]. Nevertheless, without any of these three risk factors, the mortality rate among elderly patients with acute type B aortic dissection, treated conventionally, was extremely low (1.3%) [67]. A review of the world literature indicated that medical treatment is even favorable in octogenarians with intramural hematoma of the thoracic aorta [68].

In short, it is clearly evident that patient selection plays a crucial role in determining surgical outcome for patients with acute aortic dissection [65–67]. An Italian group of surgeons suggested that surgical intervention for type A acute aortic dissection in octogenarians is unlikely to reverse a fatal outcome [69], which indeed raised some important moral, ethical, and practical concerns [70]. Although acceptable surgical results can be achieved in some selected octogenarians following hypothermic circulatory arrest for repairing thoracic aorta [71–73], increased incidence of perioperative stroke has also been recognized in this particular patient population [71–73]. There is a general consensus now that compared to younger patients with acute aortic dissection or thoracic aortic aneurysm, the clinical characteristics, management, and outcome are significantly different in the elderly [64–73]. Emergency operations, especially in those elderly patients with preoperative disorder of vital organ systems, may increase the possibility of adverse outcome dramatically [65, 66, 69, 71].

Conversely, recent experiences have confirmed the technical feasibility and clinical safety of endovascular stent-graft treatment in type B aortic dissection [74–77]. Combined data from the EUROSTAR (the European Collaborators on Stent Graft Techniques for Thoracic Aortic Aneurysm and Dissection Repair) and UK Thoracic Endograft registries involved 131 patients with aortic dissection [77]. Among them, primary technical success was achieved in 89% and the overall 30-day mortality rate was only 8.4% [77]. Although the ongoing randomized INvestigation of STEnt grafts in patients with type B Aortic Dissection (INSTEAD) trial may provide further insights into this developing and exciting subject [78], it is clear that endoluminal stent-graft treatment represents a promising alternative to medical therapy and surgical repair, particularly with the prospective of better survival and less complications in the elderly patient population.

Conclusion

The aging of the population and improvements in outcomes after cardiovascular procedures have resulted in a growing demand for complex surgical intervention in this group of patients. This inevitable challenge is present not only in industrialized

societies but also in many developing countries. With earlier referral, careful preoperative evaluation, strategic planning, and the continuing efforts to optimize surgical techniques as well as CPB management [79], operative mortality and morbidity following primary or reoperative cardiovascular interventions are expected to fall even in this high-risk patient subset. Although ethical issues are important to consider in the elderly cohort with increased surgical risk, the surgeon's decision should be based on the available evidence and be justified by the best interest of the individual patient. Last but not least, many new techniques and technologies are rapidly evolving (such as endovascular stent-graft treatment for life-threatening thoracic aortic diseases) which may become the preferred initial therapy, particularly for elderly patients.

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Intensive Care Unit Arrhythmias

S.M. Hollenberg

Introduction

Arrhythmias are common in the intensive care unit (ICU) and represent a major source of morbidity and increased length of stay. Arrhythmias are most likely to occur in patients with structural heart disease. The inciting factor for an arrhythmia in a given patient may be a transient imbalance, often related to hypoxia, infection, cardiac ischemia, catecholamine excess (endogenous or exogenous), or an electrolyte abnormality. Management includes correction of these imbalances as well as medical therapy directed at the arrhythmia itself.

The physiologic impact of arrhythmias depends on ventricular response rate and duration. Bradyarrhythmias may decrease cardiac output due to heart rate alone in patients with relatively fixed stroke volumes, and loss of an atrial kick may cause a dramatic increase in pulmonary pressures in patients with diastolic dysfunction. Similarly, tachyarrhythmias can decrease diastolic filling and reduce cardiac output, resulting in hypotension, in addition to producing myocardial ischemia. Clearly, the impact of a given arrhythmia in a given situation depends on the patient's cardiac physiology and function. Similarly, urgency and type of treatment is determined by the physiologic impact of the arrhythmia as well as by underlying cardiac status.

The purpose of this chapter is to provide an update regarding current concepts of diagnosis and acute management of arrhythmias in the ICU. A systematic approach to diagnosis and evaluation will be presented, followed by consideration of specific arrhythmias.

Diagnosis of Arrhythmias

Basic Principles

The first principle in managing arrhythmias is to treat the patient, not the electrocardiogram (EKG). Accordingly, one must first decide whether the problem is an arrhythmia or an artifact and whether the cardiac rhythm is sufficient to account for the patient's problem.

The next step is to establish the urgency of treatment. Clinical assessment includes evaluation of pulse, blood pressure, peripheral perfusion, and the presence of myocardial ischemia and/or congestive heart failure. If the patient is unconscious or hemodynamically unstable in the presence of a tachyarrhythmia other than sinus tachycardia, prompt cardioversion is indicated. If the patient is stable, there is time to establish the rhythm diagnosis and decide upon the most appropriate course of

treatment. Bradyarrhythmias produce less diagnostic challenge and treatment options are relatively straightforward.

The goals of antiarrhythmic therapy depend on the type of rhythm disturbance. The initial goal for the treatment of tachyarrhythmias in the critical care unit is to slow the ventricular response (or to increase it in the case of a bradyarrhythmia). The next goal is to restore sinus rhythm, if possible. If sinus rhythm cannot be restored, prevention of complications becomes an issue.

Evaluation of Bradycardias

A comprehensive description of the diagnosis of arrhythmias is beyond the scope of this chapter. A 12-lead EKG with a long rhythm strip and a previously obtained 12-lead EKG for comparison are ideal; If a previous EKG is not available, a systematic approach using a current 12 lead EKG is essential.

For evaluation of bradycardias, the first step is to locate the P wave. P-waves are often best seen in leads II and V_1. Normal p-waves are upright in leads II, III, and aVF, and may be biphasic in leads II and V_1. Ectopic atrial and junctional rhythms often present with negative P-waves in leads II, III, and aVF. The next step is to establish the relationship between the P-wave and the QRS complex. If there are more P-waves than QRS complexes, then AV block is present. If there are more QRS complexes than P-waves, the rhythm is likely an accelerated junctional or ventricular rhythm. If the relationship of the P-wave and QRS is 1:1, then measurement of the PR interval can yield useful diagnostic clues.

Management of Bradycardias

Sinus Node Dysfunction

Bradycardias associated with sinus node dysfunction include sinus bradycardia, sinus pause, sinoatrial block, and sinus arrest. These disturbances often result from increased vagal tone [1]. If bradycardia is transient and not associated with hemodynamic compromise, no therapy is necessary. If bradycardia is sustained or compromises end-organ perfusion, therapy with antimuscarinic agents, such as atropine, or beta agonists, such as ephedrine, may be initiated. Transcutaneous or transvenous pacing may be necessary in some cases.

Patients with a combination of bradycardia with paroxysmal atrial tachycardias due to preexisting conduction system disease can be challenging to manage pharmacologically. In these cases, insertion of a temporary pacemaker may allow the administration of rate-lowering agents.

Heart Block

The most common cause of acquired chronic atrioventricular (AV) heart block is fibrosis of the conducting system. Although pre-existing conduction system disease is a risk factor for the development of complete heart block, no single laboratory or clinical variable identifies patients at risk for progression to high degree AV block [2]. In first-degree AV block there is prolongation of conduction time of the atrial impulses to the ventricles, with a PR interval greater than 200 msec. In second-degree AV block, conducted atrial beats are interspersed with non-conducted beats.

Second-degree AV block is divided into Mobitz type I (Wenckebach) and Mobitz Type II block. In Mobitz I block, the PR interval lengthens progressively until the P wave fails to conduct. In most cases the block occurs at the AV node. Mobitz I block can occur in healthy individuals, the elderly and in patients with underlying heart disease. In Mobitz type II AV block the PR interval remains constant until a P wave fails to conduct. Mobitz II block occurs below the AV node, and thus is more dangerous since it is much more likely to progress to complete heart block. In third-degree AV block none of the atrial impulses are conducted to the ventricles. The escape rhythm, whether junctional or ventricular, is generally regular.

Asymptomatic bradyarrhythmias do not carry a poor prognosis and in general no therapy is necessary [3]. If organ perfusion is compromised, or hemodynamic instability occurs, one or two doses of atropine (1.0 mg i.v.) may be tried, but pacing may be required. Pacing may also be useful in patients with bradycardia-tachycardia ("sick sinus") syndrome, in whom treatment for tachycardia results in symptomatic bradycardia.

Conduction abnormalities, either transient or permanent, can complicate acute myocardial infarctions. Conduction abnormalities associated with an acute inferior myocardial infarction usually result from AV nodal ischemia, are transient, and carry a low mortality rate. Conduction abnormalities in association with an acute anterior myocardial infarction, however, represent extensive necrosis of the infranodal conduction system and the myocardium, and are associated with high in-hospital mortality [4]. The ACC/AHA/NASPE recommended guidelines for temporary pacing in patients with an acute myocardial infarction are shown in Table 1.

Table 1. Recommendations for temporary transvenous pacing after an acute myocardial infarction. Adapted from [49]

Class I
1. Asystole
2. Symptomatic bradycardia
3. Bilateral bundle branch block (alternating BBB or RBBB with alternating LAFB/LPFB, any age)
4. New or indeterminate-age bifascicular block (RBBB with LAFB or LPFB, or LBBB) with first-degree AV block
5. Mobitz type II second-degree AV block
Class IIa
1. RBBB and LAFB or LPFB (new or indeterminate).
2. RBBB with first-degree AV block
3. LBBB, new or indeterminate.
4. Incessant VT, for atrial or ventricular overdrive pacing.
5. Recurrent sinus pauses (greater than 3 seconds) not responsive to atropine.
Class IIb
1. Bifascicular block of indeterminate age.
2. New or age-indeterminate isolated RBBB.
Class III
1. First degree heart block.
2. Type I second-degree AV block with normal hemodynamics.
3. Accelerated idioventricular rhythm.
4. BBB or fascicular block known to exist before AMI

RBBB: right bundle branch block; LBBB: left bundle branch block; LAFB: left anterior fascicular block; LPFV: left posterior fascicular block; AMI: acute myocardial infarction.

Evaluation of Tachyarrhythmias

The first step in the evaluation of the critically ill patient with a tachyarrhythmia is to assess hemodynamic stability. If hemodynamics are compromised due to the arrhythmia, cardioversion should be performed unless pharmacologic treatment is immediately successful. However, before proceeding with cardioversion, one should consider whether the arrhythmia is in fact the basis for the deterioration in hemodynamics.

The next step in evaluation is to determine whether the arrhythmia is supraventricular or ventricular in origin. First, one examines QRS width. A narrow QRS complex (<0.12 seconds) indicates a supraventricular tachycardia (SVT). One should try not to rely solely on a rhythm strip from one monitor lead for diagnosis, as there can be variability in QRS width depending on which lead is examined. A 12-lead EKG is more useful, and may also identify Q waves indicative of prior myocardial infarction or other abnormalities. Comparison with a previous EKG can be useful as well, to identify pre-existing bundle branch block, for example.

Carotid sinus massage and other maneuvers that increase vagal tone, slows AV conduction time and increases refractoriness, and this can aid in the diagnosis through demonstration of P waves or interruption of a re-entrant supraventricular tachycardia. Intravenous adenosine (6 mg bolus, with a second dose of 12 mg 1 to 2 minutes later if there is no response) can also be used for this purpose. The effects are more pronounced when given through a central venous line, in which case the dosage is usually halved. Responses to vagal maneuvers or adenosine are listed in Table 2. Side effects include bronchospasm, proarrhythmia (a 2.7% incidence of induction of atrial fibrillation has been reported) [5], and also ventricular tachycardia (VT) and fibrillation [6], as well as bradycardia including asystole; these effects are usually transient because the half-life of adenosine is only 6 to 10 seconds.

VT can be diagnosed using some clinical and EKG clues. VT is approximately four times more common than SVT with aberrancy [7]. VT is much more common in patients who have a history of myocardial infarction or heart failure. Circulatory collapse is more common with VT than SVT, but patients with VT may maintain a normal blood pressure. A careful review of medications is important to exclude iatrogenic causes of VT.

A QRS width of more than 0.14 seconds with right bundle branch block (RBBB) or 0.16 seconds during left bundle branch (LBBB) block favors VT [8]. Comparison of QRS morphology during the tachycardia with the morphology of ventricular premature beats in sinus rhythm can be helpful. Marked left axis deviation (-60° to -120°) may indicate a ventricular origin of the arrhythmia. Other diagnostic clues suggestive of VT are fusion and capture beats, but these are seen in only 20-30% of

Table 2. Differentiation of tachycardias by response to vagal maneuvers

Arrhythmia	Response to vagal maneuvers/adenosine
Sinus tachycardia	Gradual slowing with resumption of the tachycardia
AVNRT	Abrupt termination or only very transient slowing
Atrial fibrillation/flutter	Increased AV block briefly with slowed ventricular response rate
Multifocal atrial tachycardia	Increased AV block briefly with slowed ventricular response rate
Ventricular tachycardia	Usually no response

AV: atrioventricular; AVNRT: AV nodal re-entrant tachycardia.

cases of VT [9]. Fusion beats, a hybrid of the supraventricular and ventricular complexes, occur when two impulses, one supraventricular and one ventricular, simultaneously activate the same territory of ventricular myocardium. The implication is that the wide complexes are ventricular. Capture beats are occasional beats conducted with a narrow complex, and such beats rule out fixed bundle branch block. AV dissociation is diagnostic of VT, but is present in less than 50% of cases of VT and is difficult to identify at faster heart rates.

It is better to err on the side of overdiagnosis of VT. In a study analyzing adverse events incurred by patients with VT misdiagnosed as SVT and given calcium channel blockers [10], many of the patients decompensated promptly and some required resuscitation, despite the fact that all study patients were hemodynamically stable when first seen in VT.

It is also noteworthy that ST segment depression during SVT lacks specificity in predicting ischemia. In one series of 100 patients with SVT, associated ST segment deviation was only 51% specific (with a positive predictive value of only 6%) for significant angiographic coronary artery disease or scintigraphic evidence of ischemia [11].

It is useful to divide SVTs into regular and irregular rhythms, as this narrows the differential diagnosis and therapeutic options. Regular narrow complex SVT include sinus tachycardia, atrioventricular node reentrant tachycardia (AVNRT), AV reentrant tachycardia (AVRT), ectopic atrial tachycardia, and atrial flutter with fixed conduction.

Management of Regular Narrow Complex Tachycardias

Sinus Tachycardia

Sinus tachycardia often occurs as a response to a sympathetic stimulus, such as hypoxia, vasopressors, inotropes, pain, hypovolemia, or hyperthyroidism. Treatment focuses on identifying and trying to correct the underlying cause. If ischemia is the cause and treatment is warranted, beta-blockers are the first treatment option. However, it is worth considering that the sinus tachycardia may be an appropriate hemodynamic response to hypotension, hypovolemia, or low cardiac output; if this is the case, overzealous use of beta-blockers can reduce cardiac output, with potentially disastrous consequences.

AV Nodal Reentrant Tachycardia

AVNRT typically occurs with sudden onset at a heart rate of 140–180 beats per minute. It is more prevalent in females and is not usually associated with structural heart disease. AVNRT involves dual AV nodal pathways and re-entry. Typical AVNRT is initiated by a premature atrial contraction that conducts antegrade down a slow AV pathway with a short refractory period and then retrograde up a fast AV pathway with a long refractory period (which had been refractory to antegrade conduction when the premature beat occurred but has now recovered) (Fig. 1).

The key to treatment is to block AV conduction. Acute treatment may include Vagal maneuvers and intravenous adenosine may terminate the re-entrant cycle, or other AV nodal blockers, such as non-dihydropyridine calcium-channel blockers, beta-blockers, and digoxin may be used [12]. Preventive therapy usually entails medications that suppress the initiating premature atrial contractions, with beta-

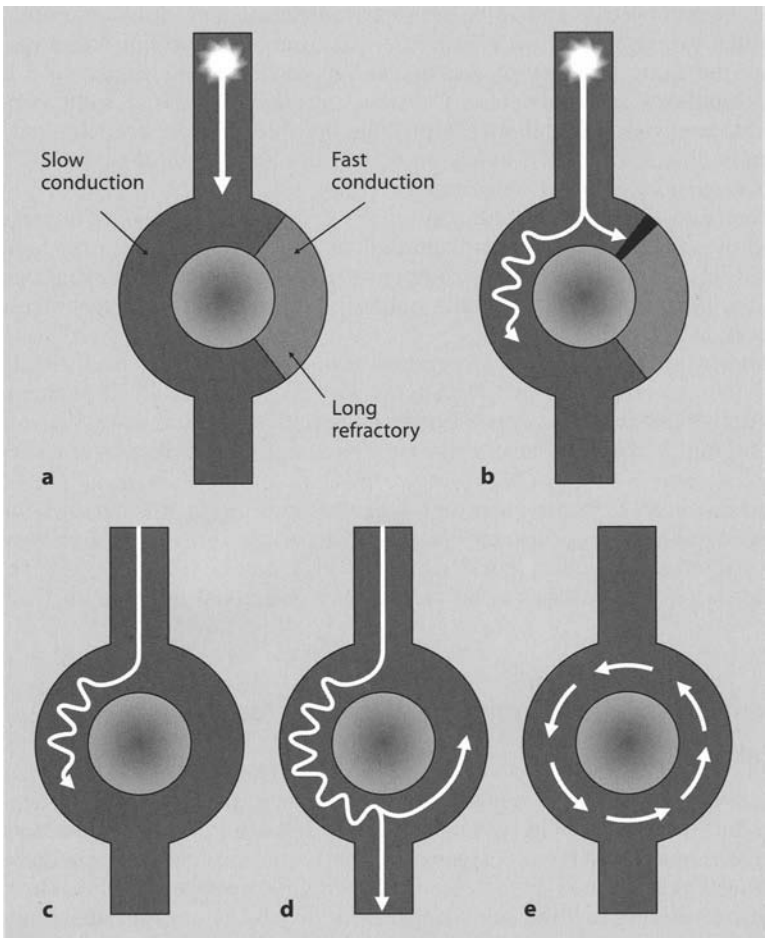

Fig. 1. **a** Atrioventricular (AV) node demonstrating dual pathways: a slow pathway with a short refractory period and a fast pathway with a long refractory period. **b** A premature impulse conducts down the slow pathway while the fast pathway is still refractory to conduction. **c** As the impulse conducts down slow pathway, the fast pathway recovers. **d** The impulse goes up fast pathway and also conducts to the ventricle. **e** The impulse cycles around the AV node, completing the re-entrant circuit.

blockers as the first choice. Catheter ablation of one of the pathways is another option for recurrences refractory to medications.

AV Re-entrant Tachycardia (Wolff-Parkinson White Syndrome)

AVRT using an accessory bypass tract occurs in 0.1 to 0.3% of the general population. The accessory pathway bypasses the AV node and can activate the ventricles prematurely in sinus rhythm, producing the characteristic delta wave. The diagnosis of Wolff-Parkinson White syndrome (WPW) is reserved for patients with both preexcitation and tachyarrhythmias. In AVRT, conduction can go down the bypass tract and back up the AV node, producing a wide QRS complex (antidromic) or down the

AV node and back up the bypass tract, producing a narrow QRS complex (orthodromic). AVRT should be suspected in any patient whose heart rate exceeds 200 bpm. Atrial fibrillation (AF) is a potentially life-threatening arrhythmia in patients with WPW syndrome, as it can generate a rapid ventricular response with subsequent degeneration into ventricular fibrillation. This is important, as one third of patients with WPW syndrome have AF [13].

Adenosine should be used with caution in any young patient suspected of having WPW as it may precipitate AF with a rapid ventricular response rate down an antegrade accessory pathway. Procainamide, ibutilide, and flecainide are preferred agents, since they slow conduction through the bypass tract. The long-term treatment of choice for symptomatic patients is radiofrequency catheter ablation of the accessory pathway.

Management of Irregular Narrow Complex Tachycardias

Irregular narrow complex SVT includes atrial fibrillation, multifocal atrial tachycardia, atrial flutter with variable block, and sinus tachycardia with frequent premature atrial complexes.

Atrial Flutter and Fibrillation

Atrial flutter is a macro-re-entrant arrhythmia identified by flutter waves often best seen in the inferior leads, at 250 to 350 bpm. Patients often present with two-to-one AV conduction with a ventricular rate of 150 bpm, although the AV conduction ratio can change abruptly. Acute treatment consists of AV-nodal-blocking drugs for rate control. If the patient becomes clinically unstable, low energy DC-synchronized cardioversion (50–100 joules) has a success rate of 95 to 100% [14]. Intravenous ibutilide converted about 75% of patients to sinus rhythm in clinical trials, but prolongs the QT interval, and can provoke sustained polymorphic VT in 1–2% of cases. Ibutilide should not be used in patients with a prolonged QT_c interval (greater than 420 msec) [15, 16]. If a temporary or permanent pacemaker with an atrial lead is in place, atrial overdrive (burst) pacing can sometimes restore sinus rhythm via overdrive suppression.

Atrial fibrillation is the most common narrow complex tachyarrhythmia in the ICU [17]. The prevalence of atrial fibrillation in the general population increases exponentially with age [18]. The most important risk factors for development of AF in the general population are structural heart disease (70% in Framingham study over 22 year follow-up), hypertension (50%) [19], valvular heart disease (34%) [20], and left ventricular hypertrophy.

The three goals of therapy for atrial fibrillation are to control the rate, to restore and maintain normal sinus rhythm, and to prevent complications. Pharmacologic agents for acute rate control include beta-blockers, non-dihydropyridine calcium channel blockers, and digoxin.

Beta-blockers provide more effective rate control than calcium channel blockers at rest and during exercise [21]. The intravenous medication most often used is metoprolol given at 2.5–5 mg i.v. over 1–2 minutes every 5–10 minutes for a total of 15 mg as blood pressure tolerates. Esmolol, 0.5 mg/kg bolus, then 0.05 mg/kg/min infusion, is an alternative with a more rapid onset and offset, which can be useful in unstable patients.

Non-dihydropyridine calcium channel blockers (diltiazem and verapamil) are also effective AV nodal blockers. Verapamil may have more negative inotropic properties than diltiazem and thus may induce hypotension in patients with left ventricular dysfunction and borderline blood pressure [22]. Diltiazem is available in i.v. form and is commonly used as a continuous infusion at a rate of 5–15 mg per hour.

Digoxin controls ventricular response through a centrally mediated vagal mechanism and by direct action on the AV node. It controls resting heart rates in patients who do not have increased catecholamine levels, but is less effective in the ICU. Intravenous digoxin begins to slow the heart rate in 30 minutes [23].

Hemodynamically unstable patients with atrial fibrillation require emergent cardioversion without waiting for prior anticoagulation, and those with acute heart failure or ischemia should be considered for urgent cardioversion. Electrical cardioversion may be more effective when the defibrillator pads are placed in an anterior/posterior orientation to direct the current through the atria.

For other patients, cardioversion carries a stroke risk, even if the duration of atrial fibrillation is less than one week [24]. Due to delay between resumption of organized atrial electrical activity and of organized mechanical contraction, there can be delay between cardioversion and embolic events ranging from 6 hours to 7 days [25]. Anticoagulation with i.v. heparin should be considered if atrial fibrillation persists for greater than 48 hours. The stroke risk in non-anticoagulated patients taken as a whole is about 2% per year (0.05% per day), but individual factors modulate that risk. The risk factors for stroke are heart failure, hypertension, age >75 years, diabetes, prior history of transient ischemic attack or stroke, and female gender [26].

Post-operative atrial fibrillation is common, especially after cardiac surgery, when the incidence is 25 to 40% of patients, with peak onset on day two [27, 28]. There are numerous risk factors for postoperative atrial fibrillation, with advanced age being the most important. Rate control should be initiated, but atrial fibrillation often runs a self-correcting course in this setting, with resumption of sinus rhythm in more than 90% of patients by 6–8 weeks after surgery, and so cardioversion is not always necessary [29]. Immediate cardioversion should be performed in patients with recent onset atrial fibrillation accompanied by symptoms or signs of hemodynamic instability resulting in angina, myocardial ischemia, shock, or pulmonary edema without waiting for prior anticoagulation.

Antiarrhythmic agents may be chosen to reduce the risk of recurrence of atrial fibrillation. The choice of an antiarrhythmic agent depends on the clinical setting. Propafenone may be used in patients without structural heart disease, although other agents may be more effective. Sotalol can be used for adrenergically mediated atrial fibrillation. Amiodarone is recommended as the first line drug in patients with structural heart disease, with dofetilide as an alternative. Class IC antiarrhythmic agents (flecainide, encainide, moricizine) should be avoided in patients with coronary heart disease due to the increased mortality shown in the Cardiac Arrhythmia Suppression Trial (CAST) [30, 31].

Multifocal Atrial Tachycardia

Multifocal atrial tachycardia is an irregular atrial tachycardia diagnosed by identification of three or more P wave morphologies and PR intervals. Multifocal atrial tachycardia is most often associated with hypoxia in the setting of pulmonary disease, but may occasionally be due to use of theophylline, metabolic derangements, and end-stage cardiomyopathy. Treatment consists of correcting hypoxia by treating

underlying pulmonary disease and/or correcting electrolyte abnormalities [32]. AV nodal blockers are sometimes useful to control the ventricular response in the interim.

Ventricular Tachycardia

VT can be monomorphic or polymorphic, sustained or non-sustained. Sustained VT is defined as persisting for longer than 30 seconds; non-sustained VT has at least 3 or more ventricular beats but lasts less than 30 seconds. Differentiation of VT into monomorphic and polymorphic varieties is useful because they occur in different settings and respond differently to treatment. Polymorphic VT, in turn, can be divided into that with a long QT interval (torsades de pointes) and that without QT prolongation, which is an ischemic rhythm. Three or more distinct episodes of ventricular tachycardia or fibrillation within a 24 hour period is termed ventricular storm.

Non-sustained VT is fairly common following a myocardial infarction. Prognosis is dependent upon the timing of onset of VT in relation to the incident myocardial infarction. Non-sustained VT occurring during the first 48 hours of myocardial infarction is most likely related to myocardial reperfusion and has no prognostic significance. However, non-sustained VT occurring more than 1 week after myocardial infarction doubles the risk of sudden cardiac death in patients with preserved left ventricular (LV) function [33]. Evaluation for recurrence of ischemia is appropriate, as is assessment of LV performance. The risk of sudden cardiac death is increased more than five-fold in patients with LV dysfunction (ejection fraction less than 40%) [34].

Monomorphic Ventricular Tachycardia

Sustained monomorphic VT is a re-entrant rhythm that usually occurs from a fixed substrate rather than acute ischemia; it most commonly occurs more than 48 hours after a myocardial infarction, or in the setting of cardiomyopathy. Initial management of sustained monomorphic VT with a history of structural heart disease depends on its rate, duration, and the patient's hemodynamic status. Unstable VT is an indication for prompt defibrillation. Hemodynamically stable patients with a risk of imminent circulatory collapse may be treated with an antiarrhythmic such as i.v. amiodarone. Amiodarone can be given as a 150 mg i.v. bolus over 10 minutes followed by an infusion of 360 mg (1 mg/min) over six hours, and then 540 mg (0.5 mg/min) over the remaining 18 hours. Bradycardia and hypotension can result from i.v. amiodarone, in which case the rate of the infusion should be decreased. Current ACLS guidelines consider lidocaine and i.v. procainamide alternative choices, although lidocaine is more effective in VT due to ischemia than that due to post-infarction scar. Lidocaine is administered by i.v. bolus of 0.5 to 0.75 mg/kg, followed by continuous infusion at 1 to 4 mg/min. Procainamide is administered at 20 mg/min i.v. for a loading dose of 17 mg/kg, then continued as an infusion at 1 to 4 mg/min. The infusion should be stopped if the patient becomes hypotensive or the QRS widens by 50% above baseline. The most serious side effects of procainamide are hypotension and proarrhythmia (most commonly torsades de pointes), both of which increase in frequency in patients with renal insufficiency because of decreased excretion.

Recurrent monomorphic VT is an indication for i.v. antiarrhythmic drug therapy, with either amiodarone, lidocaine, or procainamide. Enthusiasm for the use of chronic antiarrhythmic agents to prevent ventricular arrhythmias was considerably dampened after CAST, which showed an increase in mortality in patients receiving flecainide or encainide in patients with coronary artery disease [30]. There has been concern that other antiarrhythmic agents could have the same proarrhythmic effects. Available data suggest that amiodarone and sotalol are the most effective antiarrhythmic drugs for preventing sustained VT.

Clinical trials comparing insertion of automated implantable cardioverter defibrillators (AICD) to antiarrhythmic drug therapy have generally shown a benefit for AICD placement, particularly in high-risk patients with decreased ejection fraction or inducible sustained VT [35, 36]. The MADIT-II (Multicenter Automatic Defibrillator Implantation-II) trial demonstrated that prophylactic placement of an ICD in patients with LV ejection fraction (LVEF) $\leq 30\%$ after myocardial infarction improved survival [37]. The timing of ICD implantation however, is uncertain. In the recent DINAMIT (Defibrillator in Acute Myocardial Infarction Trial) study, placement of an ICD immediately after a myocardial infarction did not reduce all-cause mortality [38]. and analysis of MADIT-II demonstrated that patients with a remote myocardial infarction (at least 18 months previous) benefited greatly from the ICD, whereas those with a more recent myocardial infarction (less than 18 months) did not [39]. Data from the SCD-Heft (Sudden Cardiac Death-Heart Failure) trial also showed a survival benefit in patients with either an ischemic or a non-ischemic cardiomyopathy and EF $< 35\%$ after implantation of an ICD compared to amiodarone [40]. Due to the outcomes of these trials, referral for ICD implantation is recommended for survivors of sudden cardiac death and patients with a previous myocardial infarction and LVEF of less than 35%.

Polymorphic Ventricular Tachycardia

Polymorphic VT with a normal QT interval is considered to be an ischemic rhythm that typically degenerates into ventricular fibrillation (VF). It is almost never asymptomatic and thus direct current synchronized cardioversion is the initial recommended treatment. Polymorphic VT with a normal corrected QT (QTc) is a more ominous sign than monomorphic VT in patients with myocardial ischemia. Medications that might predispose to ischemia, such as inotropes or vasopressors, should be stopped or tapered, if possible, and beta-blockers started if blood pressure permits. Intra-aortic balloon pumping may be useful as a supportive measure, but revascularization is usually required. If withdrawal of vasopressors is contraindicated on a clinical basis, intravenous infusion of lidocaine or amiodarone should be initiated.

Torsades de Pointes

Torsades de pointes ('twisting of the points') is a syndrome comprised of polymorphic VT and a prolonged QTc interval (by definition ≥ 460 msec). This may be due to various medications, including procainamide, disopyramide, sotalol, phenothiazines, quinidine, some antibiotics (erythromycin, pentamidine, ketoconazole), some antihistamines (terfenadine, astemizole), and tricyclic antidepressants. Other etiologies include hypokalemia, hypocalcemia, subarachnoid hemorrhage, congenital prolongation of the QTc interval, and insecticide poisoning [41]. A key to treatment is

correction of any exacerbating factors and normalization of electrolyte disturbances, particularly hypomagnesemia, hypocalcemia, and hypokalemia. Magnesium (1–2 grams i.v.) should be given to all patients, without the need to check a level beforehand. Other potential treatments may include overdrive pacing, isoproterenol, or atropine to increase heart rate and thus shorten QTc.

Electrical Storm

The definition of electrical storm is more than three distinct episodes of VT/VF within a 24-hour period [42]. In patients with ventricular arrhythmias requiring ICD placement, the incidence of ventricular storm ranges from 10 to 30% with the first episode occurring at an average of 133 ± 135 days after implantation [43, 44]. An identifiable precipitating factor (hypokalemia, myocardial ischemia, or prior exacerbation of acute heart failure) was found in only 26% of the patients.

Evaluation should include measurement of serum electrolytes, obtaining an EKG, and investigation for ischemic heart disease, which may include coronary angiography. Proarrhythmia due to antiarrhythmic drugs that slow conduction velocity prominently, such as flecainide, propafenone, and moricizine, should be excluded [45, 46]. Treatment for proarrhythmia consists of hemodynamic support until the drug is excreted.

While exacerbating factors (acute heart failure, electrolyte abnormalities, proarrhythmia, myocardial ischemia, and hypoxia) are corrected, repeated doses of intravenous amiodarone should be given, even if the patient is already on oral amiodarone [47]. Deep sedation can help reduce sympathetic activation. Mechanical ventilatory support and i.v. beta-blockers can be used in conjunction, but i.v. amiodarone is the pharmacologic treatment of choice for this condition. If pharmacologic therapy and antitachycardia pacing are unsuccessful, electrophysiology mapping guided catheter ablation can be considered, although this is often difficult in unstable patients [48]. The prognosis of patients with electrical storm after ICD implantation is poor, with a 2.4-fold increase in the risk of subsequent death, independent of ejection fraction. The risk of sudden cardiac death is greatest three months after an electrical storm.

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Diastolic (Dys)Function in Sepsis

D.J. Sturgess, T.H. Marwick, and B. Venkatesh

Introduction

Sepsis is a clinical syndrome that results from the systemic response of the body to infection [1]. It is a serious clinical problem, accounting for substantial morbidity and mortality. The majority of these patients die of refractory hypotension and of cardiovascular collapse [2].

The hemodynamic consequences of sepsis are complex and wide ranging. These consequences can result from absolute or relative decrease in central blood volume [3], altered left ventricular (LV) [4, 5] and right ventricular (RV) function [6], and severe peripheral vasodilation [7]. The etiology of these cardiovascular abnormalities is complex but appears to be mediated by a circulating factor(s) [8].

Research regarding the cardiovascular manifestations of sepsis has tended to focus upon the evaluation of systolic performance. However, diastolic dysfunction is increasingly appreciated as a contributor to morbidity and mortality in other clinical settings [9]. Diastolic dysfunction can impact adversely on ventricular filling. However, the impact of sepsis upon diastolic function is incompletely understood.

The principal aim of this chapter is to review current methods of assessing diastolic function in the critically ill patient and examine the evidence regarding the impact of severe sepsis and septic shock upon ventricular diastolic function.

Definition of Diastole

The challenge of conceptually dividing diastolic from systolic ventricular function is highlighted by the number of definitions in the cardiac literature. The traditional definition of diastole refers to the period of the cardiac cycle from the end of ventricular ejection until the onset of ventricular tension development during the subsequent beat [10]. An alternative defines systole by the myocyte contraction-relaxation cycle and diastole refers to the remainder of the cardiac cycle [11]. However, since the traditional definition is more widely used clinically, it will be accepted here. Thus, diastole normally consists of isovolumetric relaxation, early diastolic rapid filling, diastasis (slow filling), atrial contraction (see Figure 1).

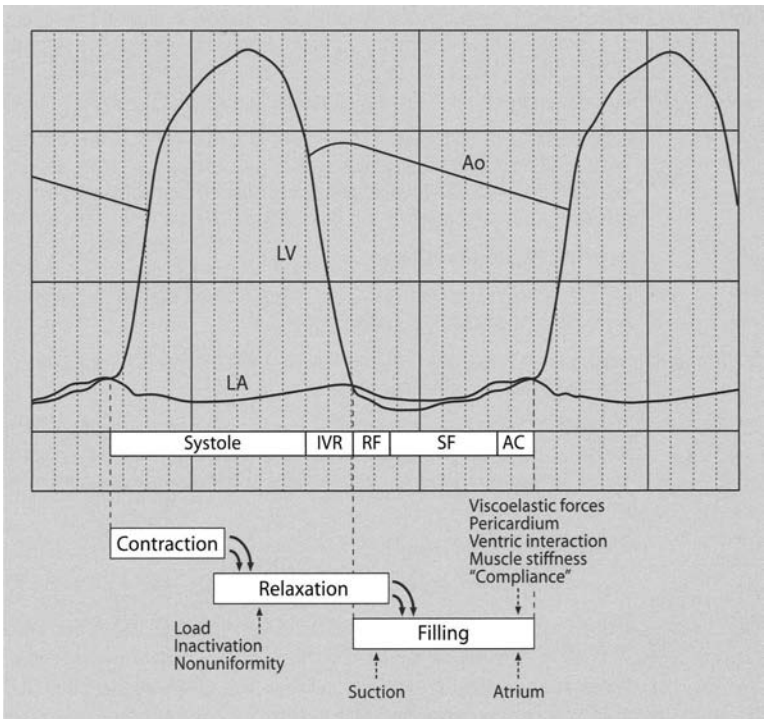

Fig. 1. Diastolic filling of the left ventricle. Left ventricular (LV), left atrial (LA) and aortic (Ao) pressures are represented on the same axes. Under classic definitions the cardiac cycle is divided into systole and four phases of diastole: isovolumetric relaxation (IVR), rapid filling (RF), diastasis or slow filling (SF) and atrial contraction (AC). An alternative approach of dividing the cardiac cycle into contraction, relaxation and filling is also presented, along with a number of determinants of diastolic function which are indicated by arrows. From [21] with permission.

Evaluation of Diastolic Function

No single index reliably differentiates normal from abnormal diastolic function. Therefore, comprehensive evaluation of diastolic function relies upon measurement of a number of indices (Table 1). Although diastolic function is a complex interplay of numerous components, the most clinically relevant determinants of ventricular filling include ventricular relaxation, stiffness, and filling pressures. These determinants may be assessed either at cardiac catheterization or by echocardiography.

Ventricular relaxation is the result of a series of energy-consuming steps that result in a decline in myocardial tension [12]. It consists of the isovolumetric relaxation and early diastolic filling periods [13]. Classically, relaxation has been described by invasive measures such as the maximum rate of pressure decline (-dP/dt) and the time constant of relaxation (tau or τ) [11].

Non-invasive measures, such as those performed during echocardiography, are more readily performed in the intensive care unit (ICU). These include Doppler evaluation of mitral valve inflow such as isovolumetric relaxation time (IVRT), peak E wave velocity, E/A ratio, E/time velocity integral (VTI), and the E-wave decelera-

Table 1. Abbreviations and description of commonly used indices of diastolic function.

Abbreviation	Description
-dP/dt	Maximum rate of pressure decline during the ventricular relaxation phase.
τ or tau	Time constant of relaxation. The value of τ is calculated as the inverse gradient of the linear relationship between the natural log LV diastolic pressure versus time. Thus, it is the time taken for LV diastolic pressure to fall to approximately two-thirds of its original value. A higher value of τ is consistent with slowing of the relaxation phase.
IVRT	Isovolumetric relaxation time.
E	E wave velocity. The peak rate of LV filling in early diastole as measured by pulsed wave Doppler of the mitral valve inflow.
E/A	E wave to A wave ratio. The ratio of the peak rate of LV filling in early diastole (E) to that during atrial contraction (A).
E/VTI	The ratio of peak E wave velocity to E wave velocity time integral (VTI). This variable measures peak filling rate normalized to mitral stroke volume. VTI refers to the area under the curve of Doppler velocity versus time and is a measure of flow during that period.
DT	E wave deceleration time.
E'	Peak velocity of the mitral annulus in early diastole as measured by tissue Doppler.
E/E'	The use of E' to adjust the E velocity for the effects of relaxation, thus yielding an estimate of ventricular filling pressure
Vp	Propagation velocity of early diastolic flow into the LV as measured using color M-mode echocardiography (color flow propagation).
E/Vp	The use of Vp to adjust the E velocity for the effects of relaxation, thus yielding an estimate of ventricular filling pressure
dV/dP	Change in cavity pressure for a given change in ventricular volume (stiffness). Indices of ventricular stiffness often incorporate cardiac dimensions as surrogates of ventricular volume.
dP/dV	Compliance (the reciprocal of stiffness).
E/LVEDV	Normalized peak filling rate. The ratio of E to LV end diastolic volume.
S	The systolic (S wave) component of pulmonary vein flow as measured by pulsed wave Doppler. Note that atrial filling from the pulmonary veins normally occurs throughout the cardiac cycle.
D	The diastolic (D wave) component of pulmonary vein flow.
durAr	The duration of flow reversal into the pulmonary veins during atrial contraction.

tion time. However, these variables tend to be influenced by a number of interrelated properties, including heart rate, filling pressure, ventricular systolic function, and ventricular stiffness [14].

Novel echocardiographic techniques include tissue Doppler imaging (TDI) and color flow propagation. TDI is an echocardiographic technique that directly measures myocardial velocities [15]. E' (pronounced 'E prime') correlates with invasively measured τ [16]. Propagation velocity (Vp) has also been shown to correlate with τ [17]. These new techniques are promising, in that they are potentially less preload dependent than other echocardiographic approaches [9].

Ventricular stiffness, a term used to describe the passive viscoelastic properties of the ventricular chamber, is determined by the material properties of the myocardium (myocardial stiffness), the extent of myocardial relaxation, ventricular geometry (including shape and wall thickness), and extracardiac factors [18]. These extracardiac factors include pericardial restraint, ventricular interaction and intrathoracic pressure.

One method of evaluating stiffness uses invasive pressure-volume loops. Examination of the relationship between diastolic pressure and volume allows determination of the change in cavity pressure for a given change in ventricular volume (dV/dP) [19].

The relationship between passive ventricular volume and pressure is curvilinear, with increasing ventricular stiffness (reduced compliance) at higher ventricular volumes [19]. In order to accurately describe the shape and position of the passive pressure-volume curve, it is crucial to obtain data through a wide range of passive diastolic pressures and volumes and to account for transmural (rather than intraventricular) pressure [18]. This is impractical at the bedside of critically ill patients. Certain echocardiographic variables, such as mitral valve inflow and pulmonary venous flow are influenced by ventricular stiffness, but do not directly quantify it.

Ventricular filling pressures are often estimated and used in the management of critically ill patients [20]. Measurements of ventricular filling pressures include ventricular end diastolic pressure and atrial pressures. Direct assessment of LV filling pressures requires left heart catheterization, which is infrequently performed in the ICU. Left atrial pressure is more commonly estimated as pulmonary artery occlusion pressure (PAOP) from an indwelling pulmonary artery (Swan-Ganz) catheter. Right sided pressures are often assessed in the ICU with central venous or pulmonary artery catheters.

There are a number of echocardiographic variables that offer information regarding ventricular filling pressures. The mitral valve inflow velocity profile and pulmonary venous flow provide an assessment of ventricular filling pressures. Well-characterized patterns of mitral valve inflow have been related to invasive measures of diastolic function [21]. Doppler evaluation of pulmonary venous flow appears to complement information derived from assessment of mitral valve inflow [9]. Increased left atrial pressures are associated with reversal of flow into the pulmonary veins during atrial contraction. This reversal of flow tends to increase in velocity and duration relative to mitral A wave flow duration with worsening diastolic properties [9]. Another indicator of elevated left atrial pressure is increased left atrial volume [9]. E velocity is dependent upon filling pressure and ventricular relaxation. The use of the E/Vp ratio is one method of adjusting the E velocity for the effects of relaxation, thus yielding an estimate of ventricular filling pressure [9]. Another estimate of LV filling pressure uses E' to adjust for the effects of relaxation, yielding the E/E' ratio. E/E' has been shown to be an estimate of LV filling pressure in a variety of clinical settings including hypertrophic cardiomyopathy [22], sinus tachycardia [23], atrial fibrillation [24], post-cardiac transplant [25], and critical illness [26, 27].

Diastolic (dys)Function in Sepsis

Parker et al. highlighted the clinical significance of diastolic dysfunction in sepsis as early as 1984, when they demonstrated an association between ventricular stiffness and outcome in patients with septic shock [28]. It has not been possible to deter-

mine the exact prevalence of abnormal diastolic function in sepsis. This is largely because of differences in study methodology and the lack of consensus regarding the characterization of abnormal diastolic function. Each of these limitations reflects the complexity of evaluating diastolic function. Nonetheless, evidence suggests that septic shock is commonly associated with diastolic dysfunction and that this represents a spectrum that includes isolated diastolic dysfunction, as well as combined diastolic and systolic impairment [29].

Data from animal models

Even under experimental conditions, data regarding diastolic function in sepsis appears inconsistent. For instance, although many animal models of sepsis have demonstrated decreased diastolic compliance [30, 31], others have revealed increased diastolic compliance [32, 33]. The different findings may result from differences in fluid administration. The impact of fluid resuscitation was highlighted by Zhong et al. who studied endotoxemic guinea pigs [34]. LV diastolic compliance following endotoxin administration was decreased in the absence of fluid resuscitation, but increased in animals that received generous crystalloid resuscitation. This effect appeared to be modulated by a mechanism independent of ventricular tissue hydration.

Human volunteers

Endotoxemia in humans has been associated with increased LV compliance. Suffredini et al studied the effects of endotoxin in healthy volunteers [35]. Following endotoxin administration and volume loading, LV ejection fraction (LVEF) decreased, while LV end-diastolic and end-systolic indices increased. Filling pressures before and after fluid loading (including central venous pressure (CVP) and PAOP) were not significantly different between endotoxin and control groups. However, by five hours following intervention, the increase in PAOP was associated with an 18% increase in LV end-diastolic volume (LVEDV) in the endotoxin group compared to a 0.6% decrease in the control group.

Critically ill patients

As already mentioned, there is significant overlap between different determinants of diastolic function. This is particularly significant because different methods for evaluating diastolic function can provide information that is relevant to a number of inter-related processes. However, in order to aid conceptualization, an attempt will be made to discuss the impact of sepsis upon diastolic function in terms of ventricular relaxation, stiffness, and filling pressures.

Human data on diastolic dysfunction primarily based on evaluation of ventricular relaxation

Although there are scant data regarding the impact of sepsis upon ventricular relaxation, current evidence is consistent with delayed relaxation. Jafri et al. [36] observed that Doppler parameters of LV filling were abnormal in a cohort of septic patients with or without shock. Diastolic filling variables and heart rate were similar in septic patients with or without shock. Compared with controls, septic patients demonstrated an abnormal pattern of diastolic filling as evidenced by increase in

peak atrial velocity, decreased E/A ratio, increased atrial filling fraction and prolongation of atrial filling period as a function of the diastolic filling period. This is consistent with delayed relaxation and decreased LV end diastolic compliance.

Poelart et al. [29] studied 31 ventilated patients with persistently vasopressor-dependent (>48 hours) septic shock. Invasive hemodynamics were obtained concomitantly with transesophageal echocardiography (TEE). Measurements included LV end-systolic and end-diastolic areas, early and late filling parameters and systolic and diastolic filling characteristics of the right upper pulmonary vein. Each Doppler measurement was characterized by maximal flow velocity and VTI. Post-hoc analysis of Doppler flow characteristics supported the concept that septic shock can be associated with a continuum of LV pathophysiology, ranging from apparently normal, through isolated diastolic dysfunction, to combined systolic and diastolic dysfunction. The small numbers of patients in each of these subsets, potential confounding variables (such as age, atrial contractile function, and loading conditions) and the post-hoc separation prevented any further conclusions about diastolic function.

Munt et al. [5] studied LV diastolic filling patterns in 24 septic patients. Transthoracic pulsed wave Doppler echocardiography was used to measure peak filling rate normalized to mitral stroke volume (E/VTI). E/A ratio and deceleration time were chosen as secondary variables. Although patients with a history of cardiac disease were excluded from the study, the absence of a control group makes it difficult to know whether sepsis induced an abnormality of LV relaxation in non-survivors, or whether there was preexisting diastolic dysfunction in this subgroup. Furthermore, no account was made for the potential impact of systolic function upon diastolic filling.

Human data on diastolic dysfunction primarily based on evaluation of left ventricular stiffness

A landmark paper by Parker et al. [28] reported combined hemodynamic and radionuclide cineangiographic findings in 20 patients with septic shock. All patients were initially resuscitated with intravenous fluids to a PAOP of 12–15 mm Hg, then vasoactive agents were added as required. A control group of 32 critically ill patients who were not shocked and had negative blood cultures demonstrated normal LVEF; however, no ventricular volume data were reported for the controls. Survivors (n=13) demonstrated initially high mean LV volumes (LV end-systolic and end-diastolic volume indexes) that recovered to normal values over the next 7–10 days. In contrast, non-survivors (n=7) had normal mean LV volumes that were unaltered with time. The same group of investigators subsequently reported similar results from a study of 54 patients with blood culture positive septic shock [37]. Fourteen of the patients had been included in the previous report [28]. Data regarding LV volumes and pressures in patients with septic shock are presented in Table 2.

A study by Ognibene et al. [38] combined hemodynamic measurements and radionuclide angiography before and after volume infusion in 56 patients within 24 hours of admission to the ICU. Three groups were defined: control group, sepsis without shock group, and septic shock group. The pre-volume infusion PAOP was significantly higher in septic shock patients compared to controls. Similarly, there was a trend toward higher pre-volume infusion left ventricular end-diastolic volume index (LVEDVI) in the septic shock group. However, this may have been due to pre-enrollment aggressive fluid resuscitation in patients with septic shock.

Table 2. Left ventricular end diastolic volume index (LVEDVI) and pulmonary artery occlusion pressure (PAOP) in patients with septic shock. TEE – transesophageal echocardiography; TTE transthoracic echocardiography; NS – not studied

Reference	Year	Investigation	Sample Size	LVEDVI (mL/m^2)	PAOP (mmHg)
Parker et al [28]	1984	hemodynamic and radionuclide	13 (survivors)	159 ± 29	13.7 ± 1.6
			7 (non-survivors)	81 ± 9	10.6 ± 1.5
Ognibene et al [38]	1988	hemodynamic and radionuclide	21	109 ± 7.2	9.6 ± 0.5
Schneider et al [39]	1988	hemodynamic and radionuclide	18	95 ± 5.8	10.0 ± 0.9
Parker et al [37]	1989	hemodynamic and radionuclide	33 (survivors)	122 ± 8	11.7 ± 0.8
			21 (non-survivors)	99 ± 9	12.8 ± 1.0
Parker et al [40]	1990	hemodynamic and radionuclide	22 (survivors)	145	13.7
			17 (non-survivors)	124	14
Jardin et al [41]	1994	Hemodynamic and TTE	32	66 ± 18	13 ± 3
Jardin et al [42]	1999	TTE	34 (survivors)	75.3 ± 20.1	NS
			56 (non-survivors)	64.9 ± 25	NS
Vieillard-Baron et al [43]	2001	TEE	40	61 ± 17	NS

Human data on diastolic dysfunction primarily based on evaluation of right ventricular stiffness

There are conflicting data regarding the impact of sepsis upon RV diastolic function. It is difficult to determine the relative contributions of fluid management, increased pulmonary vascular resistance (potentially resulting from acute lung injury [ALI] and acute respiratory distress syndrome [ARDS] associated with sepsis) and septic cardiomyopathy.

In addition to their previous work, Parker et al. [40] have performed serial hemodynamic and radionuclide angiographic studies on 39 patients with blood culture positive septic shock. Septic shock was demonstrated as a biventricular phenomenon. This was characterized by depression of both ventricular ejection fractions and simultaneous dilation of both ventricles. Survivors (n = 22) initially demonstrated severe abnormalities, but both ventricles returned toward normal during recovery. Non-survivors demonstrated less severe abnormalities initially; however, these abnormalities did not significantly improve on subsequent evaluation. Changes in RVEDVI followed the same direction as changes in LVEDVI in the majority (n = 28) of patients. Data regarding RV volumes and pressures in patients with septic shock are presented in Table 3.

Vieillard-Baron et al. [43] documented minor RV dilatation (as defined by RVEDA-LVEDA ratio) in 13 out of 40 patients with septic shock, whereas RV size was normal in 27 patients. Another study by Vieillard-Baron et al. [44] recently evaluated 83 TEE examinations performed on 30 patients with vasopressor dependent

Table 3. Right ventricular end diastolic volume index (RVEDVI) and central venous pressure (CVP) in patients with septic shock.

Reference	Year	Investigation	Sample Size	RVEDVI (mL/m^2)	CVP (mmHg)
Kimchi et al [6]	1984	hemodynamic and radionuclide	25	98.2 ± 48	11.2 ± 4.6
Schneider et al [39]	1988	hemodynamic and radionuclide	18	114 ± 8.5	8.1 ± 0.08
Parker et al [40]	1990	hemodynamic and radionuclide	22 (survivors)	124	9.5
			17 (non-survivors)	120	9.8

septic shock. Amongst their findings, the diastolic size of the RV was judged normal in 70 examinations and moderately dilated in the remaining thirteen. No patient exhibited major dilation at any time.

Human data on diastolic dysfunction primarily based on evaluation of filling pressures
Filling pressures are often used as therapeutic goals in the resuscitation of septic patients. Therefore, there are limited clinical data regarding the direct impact of sepsis upon filling pressures. A well described finding is that patients with sepsis demonstrate dissociation between filling pressures and EDV [6, 45].

Prognostic Significance of Diastolic Function

As already noted, Parker et al. [28] described an association between diastolic function and mortality in patients with septic shock. It was proposed that non-survivors did not demonstrate LV dilation and, therefore, were unable to maintain stroke volume and cardiac output [46]. The prognostic significance of diastolic function has also been demonstrated in echocardiographic studies. For instance, Munt et al. [5] studied associations between mortality and LV diastolic filling patterns in 24 septic patients. All examinations were performed in hemodynamically stable patients within 24 hrs of the diagnosis of sepsis. In a multivariate analysis, only E wave deceleration time and APACHE (Acute Physiology and Chronic Health Evaluation) II score were independent predictors of mortality.

Our group recently studied mortality in a cohort of 94 critically ill patients (including thirty with sepsis) who had transthoracic echocardiography supplemented by tissue Doppler assessment of E/E' [47]. No association was demonstrated between tissue Doppler variables and outcome. However, LV volumes demonstrated significant associations with hospital mortality (LVESV hazard ratio 2.3 [$p = 0.039$]; LVEDV hazard ratio 2.2 [$p = 0.031$]. Multivariate analysis demonstrated LVESV and APACHE III as independent predictors of mortality in this cohort.

Controversies and Difficulties in Assessing Diastolic Function

Accurate evaluation of diastolic function in severe sepsis and septic shock is difficult for a number of reasons. To begin with, sepsis potentialy affects loading conditions and contractility of the ventricle. In turn, many indices of diastolic function are

affected by these alterations in loading and contractility. Due to the interdependence of many diastolic processes and the influence of systolic function, comprehensive assessment is necessary to prevent incorrect conclusions.

Most available methods for evaluating diastolic function have important limitations. This has resulted in a lack of consensus regarding reference standards. For instance, it has been proposed that the combination of hemodynamic and cineangiographic data to calculate ventricular volumes may artefactually overestimate ventricular volumes [43]. On the other hand, two-dimensional echocardiography can underestimate ventricular volumes [48]. Also, many of the invasive techniques that have contributed to the understanding of diastolic function in cardiac patients are impractical or inappropriate in the ICU environment. Novel non-invasive techniques, such as tissue Doppler, color flow propagation, and three-dimensional echocardiography promise to contribute to our understanding of diastolic function in patients with severe sepsis and septic shock. Furthermore, it is yet to be determined whether non-invasive estimates of filling pressures (such as E/E' or E/Vp) will provide useful therapeutic or prognostic information in septic patients or whether they can be used in the diagnosis of ARDS (instead of invasive measurements of filling pressures).

Most studies of septic patients are necessarily performed after initiation of hemodynamic support, including active fluid management. Valid control groups are difficult to construct, in that it is unlikely that controls have been exposed to comparable therapies or interventions. As a result, it is difficult to differentiate the relative contributions of septic processes and resuscitation to observed pathophysiology. Additional difficulties arise in attempting to quantify the impact of illness severity or pre-existing cardiac disease.

Based upon current evidence, it is possible to make a limited number of conclusions regarding the impact of sepsis upon diastolic function. First, sepsis potentially affects the diastolic function of both ventricles. Second, the effect of sepsis upon diastolic function constitutes a spectrum of pathophysiology ranging from insignificant to severe dysfunction. This heterogeneity contributes to difficulty in defining robust therapeutic targets. The reliability of CVP and PAOP as surrogates of preload has been questioned in this respect.

Comprehensive evaluation of diastolic function is challenging in the setting of critical illness. In this regard, the increasing availability of safe, non-invasive bedside techniques such as echocardiography will facilitate further research. Further research is warranted and it is hoped that the resulting developments will contribute to improved outcomes from severe sepsis and septic shock.

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Autonomic Dysfunction: A Relevant Component in Multiple Organ Dysfunction Syndrome

H. Schmidt, U. Müller-Werdan, and K. Werdan

Introduction

In our article "We need to know more about autonomic dysfunction in our critically ill patients" [1] in the 1999 issue of this Yearbook, we postulated that autonomic dysfunction may have a relevance beyond just being an epiphenomen in these patients, and listed the questions to which answers may prove or disprove this hypothesis:

- Is the impairment of autonomic function caused by sepsis or multiorgan failure?
- If so, can a blunted autonomic function predict mortality in multiple organ dysfunction syndrome (MODS)?
- Is the autonomic function in MODS confounded by age or application of drugs?
- Can our pharmacological treatment strategies improve autonomic dysfunction?

Now, eight years later, we have gained many new insights into the intriguing interplay of cardiovascular reflexes that mediate the autonomic dysfunction seen in MODS and sepsis, so that we can now answer at least some of the aforementioned questions.

Assessment of Autonomic Function in the Critically Ill

Insights into the autonomic nervous system enable us to understand better the pathogenesis and symptoms of many intensive care unit (ICU) disorders, like MODS, sepsis, and cardiogenic shock. Moreover, assessment of the impaired autonomic nervous system also provides the physician in the ICU with a potent tool for evaluation of disease prognosis and for the introduction of new therapeutic strategies.

A major task of the autonomic nervous system is the fine tuning of the cardiorespiratory interplay to maintain appropriate oxygen delivery to the tissues. The physiological interplay of cardiovascular and ventilatory mechanisms in regulating the oxygen delivery to the tissues is well known and described [2, 3], but our knowledge of the cardiorespiratory reflex behavior in ICU-relevant disease states is limited.

Methods for Assessing Autonomic Function

Heart rate variability

Heart rate and rhythm are strongly influenced by the undulating sympathetic and parasympathetic tone. Information concerning heart rate variability is most abundant for heart diseases. Depressed heart rate variability predicts mortality and arrhythmic complications after acute myocardial infarction. Measurement should be

performed one week after such an event, preferably using time domain measurements (see below), which provide better prognostic information than frequency domain measurements [4].

Two methods of analysis have been developed to evaluate the sympathetic/parasympathetic influence on variations of heart rate. Time domain measurements are simple to use, but generally require a longer period of recording (24 hours). Short time recordings (5–20 min) can be used for a spectral analysis of heart rate variations (frequency domain measurements), providing four main components for evaluation: High frequency (HF, 0.15–0.4 Hz), low frequency (LF, 0.04–0.15 Hz), very low frequency (VLF, 0.003–0.04 Hz) and ultra-low-frequency (ULF, <0.003 Hz) [4]. Efferent vagal activity mainly contributes to the HF component. LF, expressed in normalized units, is a marker reflecting sympathetic or both vagal and sympathetic activity.

Heart rate variability is a promising tool for the evaluation of severity of illness in MODS and sepsis [5–9], and can be measured reliably and safely. It reflects the coupling of cardiorespiratory reflexes, which essentially form the basis of interorgan communication, and, thereby, allows decoupling of the heart from other organs like the lung and the brain to be identified.

Non-linear analysis of heart rate variations

Under physiological conditions, the heart behaves in a non-linear manner [5–8]. This means that techniques evaluating the complexity of a non-linear system will be required for future precision analyses. Methods that appear to be more appropriate for describing the complex phenomenon of heart rate regulation than the conventional methods for determining heart rate variability include power-law correlation and de-trended fluctuation analysis [10, 11].

Baroreflex sensitivity

The autonomic system has been shown to trigger sudden cardiac death after myocardial infarction. The analysis of vagal reflexes has significant prognostic value which is independent from the left ventricular ejection fraction (LVEF) or ventricular arrhythmias. Impairment of baroreflex sensitivity adds to the prognostic value of heart rate variability as obtained by time-domain analysis [12, 13].

Baroreflex sensitivity characterizes the ability of the autonomic nervous system to augment vagal, and decrease sympathetic, activity after a sudden increase in blood pressure. For the assessment of this parameter, traditional techniques exist, while new methods record spontaneous baroreflex function [14]. Commonly used in the clinical setting is the invasive phenylephrine bolus method (summary in [1]). This technique evaluates the lengthening of the heart interval after increasing the systolic blood pressure [1].

Non-invasive methods for the calculation of baroreflex sensitivity include the sequence technique, the α-index technique, and the transfer function technique [14–16].

Chemoreflex sensitivity

Stimulation of the chemoreceptors produces both respiratory and cardiovascular effects and elicits a sympatho-excitatory response. Therefore, respiratory chemoreflex sensitivity (peripheral hypoxic, peripheral hypercapnic, and central hypercapnic) can be distinguished from cardiac chemoreflex sensitivity. The described methods provide a helpful tool for identification of the pathogenesis of several disorders,

such as chronic heart failure, sudden cardiac death, or acute respiratory distress syndrome (ARDS), assessing the influence of the sympathetic nervous system on the cardiorespiratory system [1].

Chemoreflex sensitivity is presently assessed by a hypoxic stimulus, with most data obtained in patients with advanced chronic heart failure. Chua et al. [17–19] reported augmented hypoxic and central hypercapnic chemoreflex sensitivity in patients with chronic heart failure, eliciting an enhanced ventilatory response. Patients with chronic heart failure also have an exaggerated hypoxic chemoreflex sensitivity which attenuates the baroreflex sensitivity. Hyperoxia ameliorates the autonomic function: Chemoreflex sensitivity decreases, whereas baroreflex sensitivity is enhanced [17–19]. An increased ventilatory hypoxic chemoreflex sensitivity was reported by Chua et al. [17–19] in patients with stable chronic heart failure and was accompanied by an increased incidence of non-sustained ventricular tachycardias.

The cardiac chemoreflex sensitivity is a parameter that can be calculated either as the ratio between heart interval alterations and the decrease in arterial oxygen tension or the lengthening of the heart interval versus the increase in venous oxygen tension [20]. Respiration impairs the assessment of this parameter via activation of the pulmonary stretch receptors. Therefore, it should be obtained either under controlled breathing or be corrected for this effect [20]. Cardiac chemoreflex sensitivity allows the evaluation of cardiorespiratory interaction, which is otherwise difficult to quantify in ICU patients. This issue is discussed in more detail in the next section.

Autonomic Dysfunction and the Role of Disease Severity

The development of MODS is associated with a systemic inflammatory response syndrome (SIRS) and an inappropriate release of inflammatory mediators leading to subsequent cell damage of parenchymatous organs. Since autonomic dysfunction forms a part of severe SIRS, disturbances of the neurally-mediated organ interactions in sepsis and in non-infectious inflammatory response syndrome may well contribute to the development of MODS [5]. Godin and Buchman [5] proposed this concept of an 'uncoupling' of neurally-mediated organ interactions in MODS and sepsis: Bacterial toxins and sepsis mediators can potently alter the neural reflexes and cytokine pathways and thus cause a defect in interorgan communication, thereby advancing single organ dysfunction into MODS. This hypothesis is supported by observations that a decreased heart rate variability, which characterizes autonomic dysfunction and a loss of autonomic balance, occurs in sepsis and MODS and has been demonstrated to predict a several fold increase in the mortality of critically ill patients [21–23]. A reduction in heart rate variability, correlating with the severity of illness, has been described in septic patients [24].

Cardiac chemoreflex sensitivity is another tool for the assessment of inter-organ communication in chronic heart failure and in patients after myocardial infarction [25–29].

The aforementioned examples show that cardiac chemoreflex sensitivity contains potentially useful prognostic and pathophysiologically relevant information. Nevertheless, the application of a hypoxic method is problematic in critically ill patients with MODS and acute lung injury (ALI), because of their reduced tolerance to a hypoxic stimulus with the potential hazard of induction of arrhythmias. The application of oxygen is less hazardous but it has the disadvantage of producing only a

small change in heart rate by deactivation of the peripheral chemoreceptors [22]. In addition, an influence of spontaneous heart rate fluctuations on the calculation of the cardiac chemoreflex sensitivity cannot be excluded. The method for calculating the cardiac chemoreflex sensitivity, introduced in our recent study [22], overcomes the disadvantages of the other methods since it uses activation and deactivation of the peripheral arterial chemoreceptors and, thus, characterizes the dynamic response pattern in a region enclosing the resting state.

We also demonstrated that cardiorespiratory interactions reflected by cardiac chemoreflex sensitivity can be safely determined in critically ill patients with septic and non-septic MODS by the measurement of 'hyperoxic cardiac chemoreflex sensitivity' [22]. The cardiac chemoreflex sensitivity is related to the severity of illness: The more severe the MODS, the more the cardiac chemoreflex sensitivity is blunted. This study [22] did not show a significant relationship to age.

It is now proven that the chemoreflex sensitivity has a significant prognostic value in chronic heart failure and that it is related to the severity of illness in this disease entity [22, 26–29]. The assessment of chemoreflex sensitivity is also applicable to patients who have been rescued from sudden cardiac death because of ventricular fibrillation after myocardial infarction. This cohort of patients had significantly lower chemoreflex sensitivity in comparison to patients with myocardial infarction who did not have to be resuscitated [29].

Autonomic Dysfunction and Severity of MODS

Our recent study [22] shows that the impairment of cardiac chemoreflex sensitivity is related to the APACHE II score. This may suggest that cardiac chemoreflex sensitivity may characterize one aspect of MODS: In this clinical condition, the cardiorespiratory system is disturbed and patients may, therefore, not be able to vary the heart rate to hyperoxic stimulation as in healthy subjects. According to these results, it seems that the more severe the MODS, the more the heart and lung coupling is impaired.

Godin and Buchman [5] introduced heart rate variability and approximate entropy, a measure characterizing dynamic-nonlinear coordination, as another parameter for quantification of the inter-organ communication in MODS. They revealed a reversible attenuation of these parameters in healthy subjects, 3–4 hours after intravenous infusion of endotoxin [30]. Administration of endotoxin can also elicit an attenuation of the beating rate variability of spontaneous contracting cardiomyocytes, whereas tumor necrosis factor (TNF)-α did not have this effect [31]. Tibby et al. [32] showed that an increased MODS severity was associated with a loss of heart rate variability.

Neuromuscular dysfunction such as encephalopathy, polyneuropathy, or myopathy, is seen in up to 70% of septic patients [33, 34] and can prolong the weaning process [35, 36]. The decrease in cardiac chemoreflex sensitivity is correlated with a fall in the Glasgow Coma Scale (GCS) [22]. This could be due to more pronounced critical illness neuromuscular dysfunction in severe MODS, which also affects the neural cardiorespiratory reflex arcs. Importantly, the patients did not receive any muscle relaxants on the day of cardiac chemoreflex sensitivity assessment.

Autonomic Dysfunction in MODS

Since autonomic dysfunction forms part of severe SIRS, disturbances of the neurally-mediated organ interactions in sepsis and in non-infectious inflammatory response syndrome may well contribute to the development of MODS. A basic feature of the healthy human body is continuous communication between all vital organs through signals of the autonomic nervous system. Godin and Buchman [5] proposed a concept of 'uncoupling' of these neurally-mediated organ interactions in MODS and sepsis. Intact parasympathetic activity – the 'anti-inflammatory cholinergic reflex' – seems to be a precondition to fully suppress the overwhelming inflammatory response in sepsis and MODS [37–40].

Our recent study [41] complements the knowledge on autonomic dysfunction in ICU patients by providing clinical evidence that there is not merely a reduction in a single component of autonomic function in MODS but a real impairment of the total, complex spectrum of autonomic function as described by heart rate variability, baro- and chemoreflex sensitivities.

Moreover, this attenuation has prognostic implications. A simple heart rate variability variable, derived from continuously recorded electrocardiograms (EKGs), predicted 28-day mortality as effectively as one of the 'gold standards', the APACHE II score, in the evaluated cohort. Neither sedation nor catecholamine administration correlated significantly with the reduction in autonomic function. Mechanical ventilation may have affected short term variables of heart rate variability but not other parameters, including the standard deviation of all N-N intervals (SDNN), the standard deviation of the average of N-N intervals for each 5-min period over 24 hr (SDANN), baro- and chemoreflex sensitivities. The degree of autonomic dysfunction is equally pronounced in young, middle-aged and older MODS patients with the exception of baroreflex sensitivity (which is more blunted in older patients) [41].

Reduced vagal tone (baroreflex) after myocardial infarction has a significant prognostic value independent of LVEF or ventricular arrhythmias [42]. Baroreflex sensitivity in patients with chronic heart failure is depressed and the chemoreflex sensitivity upregulated [26–28]. Hennersdorf et al. [43] described chemoreflex sensitivity as a sensitive marker for prediction of fatal arrhythmic events in survivors of sudden cardiac arrest.

In intensive care medicine, decrease in heart rate variability has been mainly described in pediatric patients with MODS. Toweill et al. [44] analyzed hemodynamic signals during sepsis and septic shock in 30 pediatric patients. They hypothesized that uncoupling occurs between the autonomic and cardiovascular system during sepsis and septic shock, and they also found an attenuation in heart rate variability in sepsis versus septic shock. Biswas et al. [45] evaluated heart rate variability in 15 pediatric patients admitted to hospital with acute traumatic brain injury and found marked autonomic dysfunction in patients with an intracranial pressure >30 mmHg (LF/HF ratio attenuated) and a cranial perfusion pressure <40 mmHg (again LF/HF ratio blunted). Patients who were later classified as brain dead had a markedly lower LF/HF ratio. Korach et al. [46] have suggested that a decrease in the LF/HF ratio can even be used as a diagnostic test for sepsis (in a mixed cohort of medical and neurological patients, $n=41$).

Sedation, Mechanical Ventilation, Catecholamines and Autonomic Function

It might be suspected that blunted inter-organ communication is artificially induced by sedation, catecholamines, and mechanical ventilation. Indeed, several anesthetics are reported to affect heart rate variability and baroreflex sensitivity [47, 48]. Non-depolarizing neuromuscular blocking agents may impair chemoreflex sensitivity [49]. Nevertheless, in our recent study [41], none of the MODS patients received neuromuscular blocking agents while autonomic function was tested. The study showed no significant differences in the assessed variables of autonomic function between sedated and non-sedated patients [41]. Sixty-four percent of the MODS patients received catecholamines. None of the variables differed between patients who did receive and those who did not receive catecholamines. The presence of mechanical ventilation was not significantly related to SDNN, SDANN, LF/HF, or baro- or chemoreflex sensitivities, but was related to a lower proportion of interval differences between two normal R-R intervals that were in excess of 50 ms in length (pNN50), root mean square of successive R-R differences (rMSSD), lnHF, lnLF and lnVLF. Korach et al. [46] found that the use of catecholamines, sedation, and mechanical ventilation were not associated with a decrease in heart rate variability variables (LF/HF ratio). Since autonomic function is reduced in relation to the severity of MODS [22], it can be speculated, in accordance with the 'uncoupling' hypothesis [5], that the effect of MODS is a more prominent factor for reducing autonomic function than sedation, catecholamine administration, or mechanical ventilation. The differences in heart rate variability between groups with and without mechanical ventilation may be induced by different respiratory regimes applied to the ventilated patients.

Autonomic Function and Age

It has been well established that in healthy subjects heart rate variability declines with age [4, 50, 51]. An attenuated heart rate variability has been shown after myocardial infarction and in chronic heart failure [4, 52, 53]. A recent study [41] showed a decrease in heart rate variability variables in MODS patients but no differences among the three age groups.

Baroreflex sensitivity in healthy subjects is attenuated with age, but in the sick elderly, disease can further potentiate this reduction [54]. Our recent study [41] of MODS patients showed no measurable differences between the younger and middle-aged patients and between the younger and older age group (younger: <40 years, middle-aged: 40–60 years, and older patients: >60 years). The only difference shown was for middle-aged vs. older MODS patients. This indicates that even for baroreflex sensitivity, where age has an effect, MODS is the most important factor and dominates the impairment.

There are few data describing chemoreflex sensitivity in healthy subjects [20]. Hence, it is difficult to differentiate between age and disease effects for changes in chemoreflex sensitivity. There is some evidence that an age effect seems to play a significant role in MODS patients [55].

In summary, the results of the aforementioned studies suggest that the decrease in autonomic function is mainly attributed to severity of disease, which superimposes upon potential age effects. Hopefully, autonomic function may recover by effective treatment of MODS not only in the younger, but also in the elderly, patient.

Autonomic Function and Survival

A reduction in heart rate variability is a predictor of mortality and fatal arrhythmic events after myocardial infarction [4]. Our recent study [41] aimed to find a simple set of variables from the continuously recorded EKG which would predict 28-day mortality; the lnVLF fulfilled these criteria best (Fig. 1). Kleiger et al. [52] first described that a decrease in SDNN <50 ms after a myocardial infarction is associated with a 5.3 times higher risk for mortality than a SDNN >100 ms. The UK Heart study [56] revealed a 9-times higher risk in overall mortality for patients with chronic heart failure and a SDNN <50 ms in comparison to patients with an SDNN >100 ms. In the Autonomic Tone and Reflexes After Myocardial Infarction (ATRAMI) trial [42], a SDNN <70 ms combined with a reduced baroreflex sensitivity (<3 ms/mmHg) was associated with a 7.3-times higher relative risk for cardiac death after myocardial infarction. Thus, the overall variability of heart rate variability (SDNN) seems to be the most accurate predictor of mortality for cardiac patients.

The VLF power spectrum contains rhythms from a lot of physiological variables, such as hormones, temperature, vasomotion [4, 57], and depends on parasympathetic outflow [58]. Nevertheless, there remains some controversy regarding the interpretation of VLF. Yien et al. [59] found, in a cohort of 52 medical and neurological patients, that the predicted outcome based on VLF and LF in heart rate and blood pressure correlated positively with the APACHE II score. The VLF on the first postoperative day was also the strongest predictor of length of stay in the ICU after abdominal aortic surgery ([60], n=106 postoperative patients).

The finding that lnVLF was the strongest predictor of mortality in MODS patients and had similar accuracy as the APACHE II score for prediction of survival in cardiac MODS patients emphasizes the relevance of these variables of autonomic function.

Moreover, there is some evidence that blunted heart rate variability can be ameliorated by adequate therapy (Fig. 2). Hence, adequate therapy might restore autonomic function and, thus, improve survival in MODS according to the 'uncoupling hypothesis' [5].

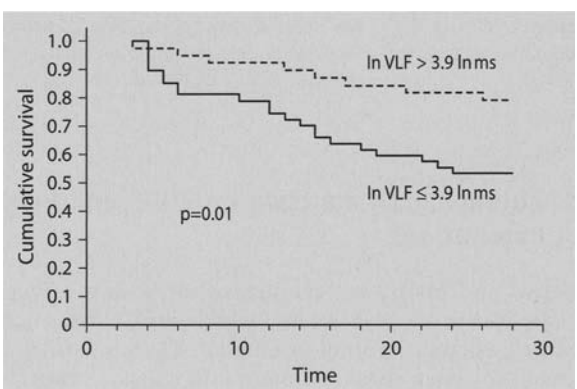

Fig. 1. Kaplan-Meier-survival curve for 28-day mortality using the heart rate variability variable, VLF, in a cohort of MODS patients (n=85). The dashed line indicates values above, and the solid line below, the cut-off point (lnVLF >3.9 lnmsec); the p value derived from the Mantel-Cox model was 0.01. The hazard ratio for 28-day mortality was 2.9 (95% CI 1.3–6.6). Adapted from [41]

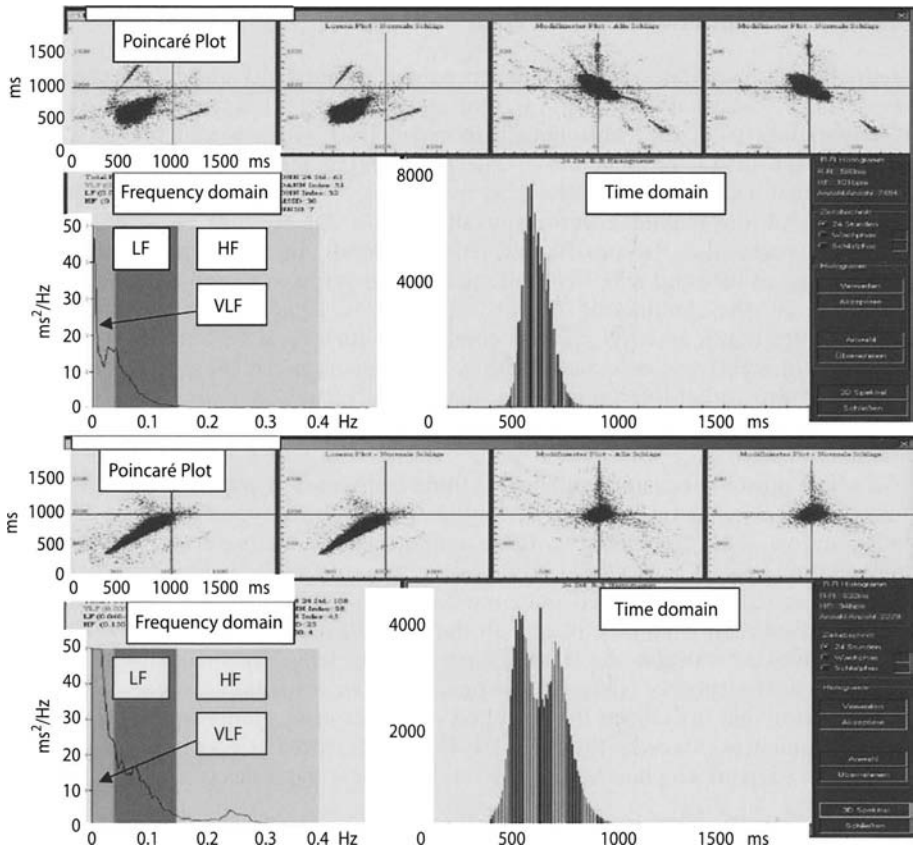

Fig. 2. Adapted screenshots of time domain and frequency domain analysis of heart rate variability in a 24-year old patient with sepsis and an APACHE II score of 25 at admission to hospital (upper panel) and after recovery (40 days later in the outpatient department, lower panel). The frequency domain diagram is separated into areas for VLF (very low frequency power, 0.003 to 0.04 Hz), LF (low frequency power, 0.04–0.14 Hz) and HF (high frequency power, 0.15 to 0.4 Hz) which are indicated on the abscissa. The ordinate depicts the power spectral density. The time domain chart illustrates how many RR-intervals (abscissa) of a certain length were detected (number on ordinate). The Poincaré plot [4] illustrates the length of the current RR-interval vs that of the adjacent.

Autonomic Dysfunction in MODS and Possible Therapeutic Implications

Sepsis and MODS are characterized by an overwhelming flooding of the organism with mediators and toxins, which might influence neural connections at several stages: afferent, central, or efferent. There may also be a diminished reactivity of the organ to reflex stimuli. Taking into consideration the interference of mediators and toxins with cardiac cellular signal transduction, blunted or dysfunctional cellular responses possibly contribute to decreased reflex responses of the target organ, leading to an impairment in the autonomic balance.

3-Hydroxy, 3-methylglutaryl coenzyme A (HMG-CoA)-reductase inhibitors ('statins') are known to have, in addition to their cholesterol-lowering properties, an effect on autonomic function by preserving parasympathetic tone [61]. Moreover, they have anti-inflammatory actions: Almog et al. [62] have recently shown that prior therapy with statins may be associated with a reduced rate of severe sepsis and ICU admission in patients admitted with presumed or documented acute bacterial infection.

We [63] recently analyzed the mortality data of 40 patients with MODS (inclusion criteria: ICU admission, APACHE II score ≥ 20 at admission) who were receiving statin therapy and of 80 age- and sex-matched MODS patients without statin therapy. All baseline characteristics including clinical and demographic data, scores (APACHE II, sequential organ failure assessment [SOFA], simplified acute physiology score [SAPS] II), chronic diseases, and laboratory test results were assessed within 24 hours of admission. All data were obtained from the patients' charts and subsequently computerized. An independent observer checked the patients' charts concerning statin therapy and recorded the duration of statin administration. Statin administration was managed according to routine protocols used in the ICU by the study-independent staff.

We found no differences in age, height, weight or distribution of the sexes between the statin and the non-statin groups. The APACHE II score and the SOFA score were not significantly different between the groups and cholesterol levels were comparable in both groups at admission. During the 28-day period analyzed there was a higher number of deaths in the group without statin treatment compared with the statin group [63]. These results suggest that patients under statin treatment who develop MODS may have a lower 28-day-mortality compared with MODS patients of equally pronounced disease severity who are not receiving statins. The parasympathetically mediated variables, pNN50 and lnVLF, were better preserved in the statin group compared with the non-statin group (lnVLF 4.1 ± 1.4 vs. 3.2 ± 1.3, $p = 0.02$; lnpNN50 0.09 ± 1.8 vs. 1.2 ± 1.8, $p = 0.049$). Hence, statin therapy may potentially influence short-term mortality in MODS patients by restoring parasympathetic tone and reducing inflammatory response in MODS via a cholinergic anti-inflammatory pathway [37–40].

Hackam et al. [64] have recently shown that the use of statins in cardiovascular patients is associated with a diminished risk of subsequent sepsis. These authors analyzed the incidence of sepsis for 141,487 patients older than 65 years who received statins and found a lower incidence of sepsis in patients receiving statins.

To our knowledge, there are to date three major studies focusing on statin treatment in patients with bacteremia. Liappis et al. [65] retrospectively reviewed the records of patients who were admitted because of bacteremic infection with Gram-negative bacilli and *Staphylococcus aureus* and found a reduced mortality in the group treated with statins. Almog et al. [62] found that prior statin therapy may be associated with a reduced rate of severe sepsis and ICU admission in a cohort of patients with pneumonia, urinary tract infection, and cellulitis (APACHE II score 11.1 +/– 7.2). Kruger et al. [66] assessed the association between statin administration and mortality among 438 bacteremic patients and found a significant reduction in mortality and death attributable to bacteremia in treated patients.

The cohort of patients enrolled in our study [63], differed significantly from the aforementioned studies with respect to the patient population under investigation: We assessed a cohort of ICU patients with MODS who were much sicker (APACHE II score 29.2 +/– 6.2 in the statin group) than those in the studies by Liappis et al.

[65] and Almog et al. [62]. Kruger et al. [66] described a pooled hospital mortality of 21.2% (93/438) in the assessed group of patients. Liappis et al. [65] observed hospital mortality rates of 6% (statin group) and 28% (non-statin group). Almog et al. [62] reported a 7.5% 28-day mortality rate. Analyzing the data of our study [63], a higher overall hospital mortality rate (60%) due to a more pronounced severity of illness was documented.

The mechanisms behind the clinical phenomenon are complex; we speculate that two major mechanisms might contribute:

a) Statins are able to modulate inflammatory responses and coagulation processes during septic episodes [67, 68].
b) Intact vagal activity seems to be a prerequisite to prevent a spillover of pro-inflammatory products into the circulation [37–40]. We have recently shown that MODS is characterized by a strongly suppressed vagal activity [41].

Conclusion

The research initiatives of recent years enable autonomic dysfunction to be considered as an integrated component of MODS. Moreover, this feature can predict mortality in MODS, and pharmacological treatment approaches to improve autonomic dysfunction have been started.

Nevertheless, several unanswered questions remain:

- Which cellular mechanisms can induce the autonomic dysfunction seen in MODS?
- Do MODS patients benefit from an amelioration of autonomic dysfunction?
- Can the pharmacological approaches to improve survival also be verified in prospective studies?

Finding answers to these questions will prove an intriguing and encouraging field of research for the next few years.

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Hemodynamic Monitoring

The Use of Hemodynamic Monitoring to Improve Patient Outcomes

J. Wilson, M. Cecconi, and A. Rhodes

Introduction

Hemodynamics is the physiology concerned with movements of blood and the forces involved in the circulation [1]. Hemodynamic monitoring involves the study of this physiology, with various forms of technology to understand these forces and the movement of blood, and put them into a clinical context that can be assessed and used to direct therapy. The main function of these hemodynamic forces is to transport substrates to, and clear metabolites from, the cells in order to allow adequate cellular function. The assessment of hemodynamics must, therefore, also take into account the metabolic status of the cell in particular in relation to its supply of oxygen. A relative lack of oxygen at the cellular level is known as tissue hypoxia. The identification and correction of tissue hypoxia remains one of the central facets of any protocol that aims to resuscitate patients from shock conditions. This is because tissue hypoxia has both pathological relevance *in vitro* [2] and an association with poor outcome [3]. When monitoring the circulation, therefore, an estimate must be made of the adequacy of the circulation with respect to the likelihood of there being underlying tissue hypoxia. With most currently available monitors for routine practice it is impossible to assess tissue hypoxia at either a local or a cellular level. An extrapolation is, therefore, made from a number of globally measured parameters that can provide an estimate of the likelihood of underlying disturbance. Clinicians can then use this information to direct therapeutic decisions in order to benefit their patients.

Key Variables that are Measured

Resuscitation from shock always entails attempting to increase a patient's tissue oxygen delivery (DO_2) to an amount that is considered appropriate to reverse the shock by alleviating tissue hypoxia and ensuring aerobic respiration at the tissue level. This theory is supported by a number of observations that demonstrate that acutely ill patients often have a level of oxygen utilization that is pathologically dependent on their DO_2 [4]. Any reduction in DO_2 leads to a further reduction in oxygen consumption and, therefore, tissue hypoxia. Resuscitation protocols are, therefore, aimed at increasing DO_2 to a level whereby tissue hypoxia disappears and cellular function returns to normal. The key component of DO_2 is the cardiac output. Many hemodynamic monitors, therefore, contain the technology to measure and monitor cardiac output and or its key determinants – stroke volume and heart rate. At the bedside, these variables are then manipulated to levels that have either been prede-

termined to be associated with a good outcome [5–6] or more often to a situation where the patient's clinical condition is improving on an individualized basis [7].

Key Concepts

There are a number of tests that have been described for the effective use of invasive hemodynamic monitoring procedures and these revolve around two main principles. First, is the measurement of the physiological variable reproducibly accurate, and second, if the physiological variable is known, can knowledge of that measurement be used to improve outcome in the patient population [8–9]? Thus, these tests must include answers to the following questions to be of value in treating sick patients:

1. The information received improves the accuracy of diagnosis, prognosis, and/or treatment based on known physiological principles.
2. The parameter can be reliably and safely measured under typical conditions.
3. The information received cannot be acquired from less invasive and less risky monitoring.
4. Interventions exist that can influence the monitored variable.
5. The changes in diagnosis and /or treatment result in improved patient outcomes (morbidity and mortality).
6. The changes in diagnosis and /or treatment result in more effective use of health care resources.

These tests can be combined to provide us with a number of key concepts that are of utmost importance when evaluating the differing technologies available for hemodynamic monitoring.

- *Primum non nocere* (first do no harm). Whichever technology or modality of monitoring the circulation is used, safety of the patient should be paramount. The use of monitoring should not add to the burden of morbidity suffered by the patient.
- The type of monitoring is dependent on the environment in which it is to be used. The invasiveness or sophistication of any given monitoring device must be tailored to the clinical environment. Although pulmonary artery catheterization may be reliably performed in the operating room or the intensive care unit (ICU), it is not so easily performed in the ward or emergency room setting.
- Hemodynamic monitoring should be undertaken at a time when clinical outcomes can be influenced. Prevention is better than cure. Improving carriage of oxygen to the cells is important before irreversible cell damage has occurred. Once irreversible cell damage has occurred then, no matter how much DO_2 is increased, published evidence suggests that further benefit will not accrue [6, 7].
- No monitoring therapy will improve any patient outcome unless linked to a relevant clinical protocol or therapeutic target. This is especially important as many clinical studies have demonstrated that the use of hemodynamic monitoring without an associated protocol has no benefit to patient outcomes [10–12], while studies assessing treatment protocols early on in disease processes have demonstrated efficacy in terms of reducing both morbidity and mortality [5–7]. Clinicians must be aware that the use of invasive monitoring equipment

will always carry the potential risk of harm to the patient and that they are thus duty bound to ensure that the monitoring equipment is used to direct therapy.

- Avoidance of circutrauma. There are many examples cited in the literature where overzealous use of logical and physiologically intuitive treatment strategies has ultimately been shown to be detrimental [13]. It is important not to follow the same path with hemodynamic monitoring and resuscitation. Care must be taken as over enthusiastic resuscitation has the potential to cause harm [14] but at the same time inadequate resuscitation may lead to the demise of the patient. The monitored variables should be used in a fashion that is proven to cause benefit [15]. It is important to realize that rational use of these tools can limit resuscitation as well as promoting it. It is possible to cause pulmonary edema with the overuse of intravenous fluids without some form of break that can be achieved by the sensible monitoring of preload, just as easily as it is to allow hypovolemia with tissue hypoxia from inadequate resuscitation.

Available Technologies

Central Venous Pressure Monitoring

The central venous pressure (CVP) is often used a marker of preload. In this respect it is used as an estimate of right atrial pressure (RAP). The RAP approximates to the right ventricular end-diastolic pressure (RVEDP) which is related through the ventricular compliance to the end-diastolic volume (EDV). Consequently, it is important to understand the relationship between EDP and EDV. In patients with normal ventricular compliance, an initial increase in EDV will not alter the EDP until a certain point. After that point, the increase in volume is coupled to an increase in pressure. Patients with decreased compliance have their ventricular function curve shifted to the left. It is difficult to determine this compliance clinically. Fluid challenges can be used to help differentiate between patients with low preload and low compliance and patients with high preload and normal compliance. In patients with a low preload, a fluid challenge will not alter the CVP reading or it may do for just a few minutes but then will rapidly return to the pre-fluid infusion state. Patients with a high preload and normal compliance will dramatically increase the CVP reading in response to the same fluid challenge. Isolated values of CVP are of limited value as compliance varies both from patient to patient and with time in the same patient. Despite the low specificity of CVP to accurately delineate preload, it can be used to help assess the fluid status of patients by assessing the response to a fluid challenge.

The Pulmonary Artery Catheter

The pulmonary artery catheter (PAC) is currently the gold standard method of monitoring of circulatory dysfunction. It was first described as a diagnostic tool in 1945 and was introduced to clinical practice following the work of Swan and Ganz in the 1970s. The catheter allows measurement of RAP, mean pulmonary artery pressure (MPAP), the pulmonary artery occlusion pressure (PAOP), the cardiac output, and the mixed venous oxygen saturation (SvO_2) which is a measure of the balance between oxygen supply and demand. The left ventricular end-diastolic pressure (LVEDP) can be estimated from the PAOP [16], which can be used to help assess the volume status of the patient.

The catheter estimates the cardiac output using the thermodilution technique. Injection of a cold fluid bolus into the right atrium results in a transient decrease in the blood temperature in the pulmonary artery, which is sensed by a thermistor at the tip of the catheter. The mean temperature reduction is inversely proportional to the cardiac output (derived from the modified Stewart Hamilton equation). Modern technologies allow this to be performed on a continuous basis by the utilization of a warming coil attached to the PAC. New fast acting thermistors in the tip of the PAC allow continuous assessment of the RVEDV and ejection fraction. A major criticism of the use of PAC is the labor-intensive nature of use, which requires access to a large vein (internal jugular, subclavian or femoral) through which a catheter sheath is introduced using the Seldinger technique. The catheter, with a 1.5 cm distal balloon, is advanced through the sheath and floated through the right atrium, right ventricle, into the pulmonary artery, and then finally into the wedged position. Continuous monitoring of pulmonary artery or wedge pressure during this procedure ensures correct positioning of the catheter. There is a suggestion that because of this insertion technique, the PAC is overly invasive and may complicate patients' clinical course.

Arterial Pulse Pressure Techniques

The history of pulse contour techniques dates back more than 100 years and is based on obtaining continuous cardiac output by the analysis of the arterial waveform. In 1899, Otto Frank developed the Windkessel (air chamber) model to simulate the heart-vessels interaction [17]. This model comprised a circuit in which fluid was pumped in tubes through chambers. The tubes were completely fluid-filled but the chambers contained some air. As the fluid was not compressible, the behavior of the air was thought to mimic aortic distension, or compliance, in blood vessels. Frank also deduced that the stroke volume could be calculated from the change in pressure. In 1904, Erlanger and Hooker proposed a correlation between stroke volume and change in arterial pressure and suggested there was a correlation between cardiac output and the arterial pulse contour [18]. This eventually led to the development of algorithms relating the arterial pulse contour and cardiac output; only with the recent advent of computer technology has it been possible to develop these algorithms to a level useful for clinical practice. These technologies offer the ability to monitor cardiac output (and, therefore, stroke volume) on a near real time basis. They do so by extracting data from arterial pressure lines, which are routinely used in the critically ill population. They are, therefore, described as minimally invasive devices when compared to the PAC. There are at present four companies marketing technologies that utilize these principles for the measurement and monitoring of cardiac output. Each of these technologies has distinct differences from its competitors which must be understood before being able to fully evaluate the device. What is consistent for all these devices, however, is the recognition that accurate data only comes from appropriate use of arterial pressure lines. If the arterial trace is damped or hyperresonant, then meaningless data will be derived [19]. A novel and potentially interesting by-product of these new devices is the ability to get volumetric information regarding the pre-load status of the patient. By analyzing the kinetics of the thermo/indicator-dilution curve, additional parameters can be derived that may enable clinicians to better understand the volemic state of their patients. Although of interest, these variables have yet to be rigorously tested in clinical trials to prove their efficacy in terms of improving relevant patient outcomes.

Other Minimally Invasive Devices for the Measurement of Cardiac Output

There are a variety of other technologies that are marketed for the monitoring of cardiac output. These include techniques that utilize the Doppler theory [20], the Fick principle through the re-breathing of carbon dioxide [21], or bio-impedance techniques [22]. The most popular of these techniques seem to be the technologies that utilize the Doppler theorem to assess blood velocity and, therefore, flow. This technique can be performed either from a suprasternal or a transesophageal route. The Doppler sensor can be shone across the aorta (either the arch or the descending portions) to assess blood velocity. This measure can then be changed to flow by correcting for aortic cross sectional area (either measured or calculated via an algorithm). All of these techniques have been validated to a certain extent in specific clinical situations, although their validity in all clinical environments is not as robust as with the previously described technologies.

Evidence Based Practice for the Use of Hemodynamic Monitoring

An appraisal of the current evidence for hemodynamic monitoring needs to focus on three main areas. The first is whether there are any data either for or against hemodynamic monitoring in the critically ill patient; the second area is whether there is any guidance to help us choose between different technologies; and the third area is surrounding the use of these devices within goal-directed therapies.

Evidence for or against Hemodynamic Monitoring

Much of the available evidence for or against hemodynamic monitoring is based around the PAC. Intuitively clinicians assumed that the extra information provided by this device would enable them to improve outcome for their patients; however, a number of observational studies suggested that the use of the PAC was associated with a worsening outcome [15, 23, 24]. There have now been three randomized controlled studies assessing this question [10–12]. All have been on a limited number of patients with the hypothesis being to study whether the PAC (without an associated treatment algorithm) has any influence on mortality. The answer in each of these studies was that the use of the PAC, without using it to target specific therapeutic endpoints, conferred no benefit to the patient. Conversely the opposite was also noted: that the use of the PAC did not confer any disadvantage to the patients. The salient point from all of these studies was the understanding that a monitoring technology, on its own, will seldom influence patient outcome (either beneficially or detrimentally). It is more important to use the information correctly in an evidence based protocol (see later). It is also possible that the use of these devices may limit resuscitation and, therefore, prevent deleterious or potentially injurious therapies being administered to patients. This, however, is very difficult to study and has never been (and may never be) proven.

Evidence For or Against an Individual Technology

There is a plethora of data describing the validation of individual hemodynamic monitors. It is beyond the scope of this article to go into all of these papers, but suffice to say, it is vitally important to understand how and when each device works

and the problems and pitfalls of each tool. It is also worth noting that the validation studies have all been performed in a tightly controlled research setting, and it is highly unlikely that any device will perform to the same level of accuracy in the uncontrolled clinical environment [25].There is only one study in the literature that has randomized patients between differing technologies [12]. The UK PAC-Man study randomized 802 patients to either receive the PAC or any other form of cardiac output monitoring. There was no significant differences in morbidity or mortality between these two groups (hazard ratio 10.6, 95% confidence intervals 0.9–1.26). This suggests that if cardiac output is to be used in a clinical protocol it is probably less important how it is measured than how it is used.

Goal-Directed Use of Cardiac Output Monitoring Technologies

There are many studies assessing clinical protocols in the context of randomized controlled trials. Most of these have shown utility and improved outcome for patients [5–7], a few have shown no effect [26], and only one has shown harm [14] (Table 1). Furthermore with all of these studies it is very important to note that the outcome was dependent on the early recognition by clinicians of tissue hypoxia developing in their patients and the use of cardiovascular monitoring to target goal-directed therapy. In the study where the goal-directed therapy caused harm [14], the patients were admitted to the ICU long after tissue hypoxia had progressed to tissue death and the patients were then over enthusiastically treated with extremely large doses of dobutamine. The poor outcome was perhaps inevitable and was not caused by the monitoring technique but by the lateness in instituting therapy in this criti-

Table 1. Studies assessing clinical protocols of goal-directed therapy in randomized controlled trials.

Year	Author	Patients	When	Target	Where
1993	Boyd [5]	Shoemaker high risk surgical criteria	Preoperative until 24 hours after surgery	$DO_2I > 600$ ml/min/m^2	ICU
1994	Hayes [14]	Established critically ill Shoemaker high risk surgical criteria, sepsis, respiratory failure, trauma	Within 24 hours of admission	$CI > 4.5$ l/min/m^2, $DO_2I > 600$ ml/min/m^2, $VO_2I > 170$ ml/min/m^2	ICU
1995	Gattinoni [26]	Shoemaker high risk surgical criteria, sepsis, respiratory failure, trauma	Within 48 hours for 5 days	$CI > 4.5$ l/min/m^2 or $SvO_2 > 70\%$	ICU
2001	Rivers [7]	Severe sepsis and septic shock	On admission for 6 hours before ICU admission	$ScvO_2 > 70\%$, CVP 8–12 mmHg, MAP >65 mmHg, Hct >30%	ER
2005	Pearse [6]	High risk surgical patients	Immediately after surgery for 8 hours protocol	$DO_2I > 600$ ml/min/m^2	ICU

DO_2I: oxygen delivery index; CI: cardiac index; VO_2I: oxygen consumption index; SvO_2: mixed venous oxygen saturation; $ScvO_2$: central venous oxygen saturation; CVP: central venous pressure; MAP: mean arterial pressure; Hct: hematocrit; ICU: intensive care unit; ER: emergency room

cally ill group of patients. It is important to recognize that all of these protocols are not technology specific. It is the measured variable that is being targeted that is important in the context of a specific disease setting. Although it has not been studied, it is highly likely that any validated technology that works in that environment, could have been used to direct the therapy with the same beneficial effects.

Conclusion

Hemodynamic monitoring has evolved considerably over the last 30 years. It is now widely accepted that bedside clinical examination and routine hemodynamic observations are not sufficient to evaluate the adequacy of either resuscitation or the metabolic status of a patient. These monitoring tools allow us to understand the physiology of the circulation at the bedside and, thereby, to direct therapies appropriately. It beholds us to use the information we get in a sensible, and where available, an evidenced based way. Doubts concerning the use of the PAC have driven the development of newer less invasive devices. These devices can be as accurate as the PAC and are often easier to use. This does not necessarily mean they are better than the old technology, though, and clinical utility does require outcome based studies to be performed. Many of the newer devices offer exciting and novel new variables that could be targeted as endpoints for resuscitation. Before there are good data to support this practice, however, much caution should be advocated. The most important point to recognize about modern hemodynamic monitoring, is not that we can measure variables such as cardiac output, it is that we then know what to do with the information. The combined use of appropriate monitoring together with a clinical goal-directed protocol has consistently been shown to improve outcome and is not only beneficial to patients but can also reduce costs and improve the overall utilization of healthcare resources [6, 27, 28].

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Using Mathematical Models to Improve the Utility of Quantitative ICU Data

S. Zenker, G. Clermont, and M.R. Pinsky

Introduction

Intensive care medicine is one of the areas of medicine most closely linked to applied physiology. Furthermore, it has a long tradition of being the forefront of advanced physiologic measurement technologies. The associated volume of quantitative data about a patient's physiologic status, therapy, together with the output of off-line analyses, creates an information overload that profoundly reduces efficient and effective information processing. To a certain extent, this disconnection is a reason for the slow progress in utilizing such information across patients and hospital systems to improve patient care, perhaps most prominently evidenced by the failure of the physiologically valuable information provided by pulmonary artery catheterization to improve outcome in the critical care setting [1, 2]. In fact, for newer and more advanced monitoring equipment, evaluations of utility and ability to fit into proven treatment protocols is often lacking. Although the difficulty in translating the increased amount of available patient-specific information into patient benefit may in part be due to the lack of adequate therapeutic options, where clear benefit is known, actual translation of this information into practice is a primary barrier to improving patient care.

A fundamental reason for this somewhat surprising and seemingly contradictory situation of increased sophistication of monitoring and decreased efficiency of utilization of the resulting data may be the lack of focus on understanding the relationship between monitored physiological variables and the determinants of recovery from critical illness. As we have stated in the past [3], for most intensive care unit (ICU) treatments, the fundamental rationale is the restoration of perceived normal physiological status independent of understanding the process of disease or its interaction with therapies. Since the limitations of this empiric physiological approach are increasingly being recognized, novel approaches may be called for to further improve outcomes in critical care.

One approach that has gained purchase in recent years is what we call 'functional' monitoring [4]. The underlying principle of this approach is to obtain measurements that are more directly related to key determinants of outcome than traditional physiologic variables. For example, measures of preload do not predict responsiveness to volume loading, but measures of preload responsiveness using the exact same monitoring devices do [4, 5]. Furthermore, once functional measures are defined as the key processes to assess, novel monitoring devices percolate to the top of the potential monitoring options. For example, technologies are available to measure sublingual CO_2 ($PslCO_2$) and tissue oxygen saturation (StO_2) that may provide more direct measures of tissue health as a key determinant of outcome, than measures of cardiac output.

A conceptually different approach is to apply mathematical models to make better use of the available quantitative data, including functional monitoring data, by selective extraction of meaningful information from the massive amounts of available clinical data. Additionally, these approaches may eventually help to quantify and predict the relationship between measurements, patient status, and outcome, thus enabling optimization of therapy by iterative protocolized care.

Quantitative Information and How to Use it

The very intensity of intensive care monitoring results in a concentration of the highest data density present in all medical environments. Some of these data are observational and qualitative in nature, such as the nurses' and physicians' observations regarding patient status. However, the majority of data is quantitative, traditionally consisting of a steadily growing number of often high resolution, time-series measurements. These include data streaming of hemodynamic parameters, clinical laboratory results, ventilation related parameters, and a number of other physiologic variables, like temperature, and derived parameters such as electrocardiogram (EKG) waveform analysis. In recent years, medical imaging technology, such as ultrasound and radiological technology, has increasingly become available at or close to the bedside in a critical care setting. These technologies, in principle, are all capable of providing quantitative information about a patient's physiologic status.

While the amount of quantitative information has grown, there has been little change in how the critical care physician utilizes this information. Since a human decision maker is inherently limited in the amount of data he or she can process, decisions are either based on the evaluation of brief sections of the complete time series data, or on a reduced dataset. Accordingly, a large amount of information contained in the original high-resolution time series data is lost in such an approach. Recent research is increasingly trying to reduce this loss of information by extracting additional, physiologically meaningful and easily interpretable information from the already available measurements. For example, by quantifying pulse pressure or stroke volume variation in arterial pressure measurements during positive-pressure ventilation, one may predict volume responsiveness [6–8].

Making better use of already available measurements certainly is desirable, both with respect to avoiding the possible risk to the patient introduced by new, possibly invasive monitoring equipment, and to limiting the already extreme resource utilization in intensive care medicine. The close relationship of such approaches to a model-based approach to intensive care data analysis will be discussed below. However, to make meaningful therapeutic decisions, it is insufficient to simply measure physiologic states. The pathway to successful therapy based on physiological monitoring is to understand their relationship to recovery from disease.

For the traditionally measurable physiologic variables, like blood pressure and pulse rate, this relationship can be very complicated, and our understanding of it remains limited. A possible solution to this dilemma may be to monitor functional parameters that are more directly related to key determinants of response to therapy and outcome [9].

Functional Physiologic Monitoring

In the attempt to develop monitoring techniques that would provide more directly meaningful information about tissue health, and could thus potentially serve as therapeutic target variables the improvement of which would hopefully lead to an improvement in outcome. Most prominent of these in recent years have been the measurement of tissue CO_2 and StO_2.

The relationship of tissue CO_2 levels with tissue perfusion has been known for several decades [10]. Only recently, however, have practical devices been developed that allow for the non-invasive bedside measurement of tissue CO_2 levels through the sublingual mucosal surface as $PslCO_2$. This technique has been shown to closely correlate with both local and systemic blood flow in various relevant scenarios [11–14]. The key feature of this technology that may justify its classification as 'functional monitoring' is that the readings may directly reflect microcirculatory blood flow, which is the key determinant of tissue health, as opposed to monitoring systemic blood flow, which may be misleadingly high through functionally irrelevant shunting mechanisms despite insufficient tissue perfusion at the microcirculation level in critical disease, resulting in potentially high clinical utility both for diagnosis and guiding of therapy [15–18]. Additionally, such monitoring may have potential in the pre-hospital and battlefield settings, since it is non-invasive and may have dynamic response characteristics in the detection of functionally relevant hemodynamic fluctuations comparable to more invasive measurements [19].

Another non-invasive technique that may have functional capabilities is the spectroscopic measurement of StO_2. Here, near infrared spectroscopy is used to quantify the amount of oxygenated and deoxygenated hemoglobin in the tissue (typically muscle) microcirculation [20]. This method, in combination with non-invasively applicable perturbations, such as temporary arterial or venous occlusion, may yield information about functionally relevant disturbances of both oxygen utilization and supply, with potential applications to critical illness [21–24]. There are also indications of its ability to identify the severity of disease and predict outcome in both laboratory and clinical settings [25–30]

While the technologies discussed above show promise both in obtaining a more easily interpretable estimate of the patient's physiologic status and may help to predict outcome, their value as therapeutic targets remains to be demonstrated, and will possibly be subject to similar limitations as the traditionally available physiologic measurements. While clearly providing additional insight, it seems unlikely that the complexity of physiological interactions that determine outcome in critical illness will be reflected by single, albeit functional measurement, closely enough to justify guiding therapy based on these alone. However, one can address these limitations directly by using mathematical modeling that embraces the inherent complexity of the interactions between various traditional and functional variables, disease state, and response to therapy.

The role of mathematical models has mostly been restricted to aiding data interpretation by deriving parameters from raw measurements that are more easily interpretable by the physician. However, mathematical models may be even more powerful when applied in other ways that have this far only rarely been successfully implemented in clinical practice. Recent developments in both affordable computing power and analytical as well as numerical methods may make these approaches feasible in the foreseeable future, as described below.

Mathematical Modeling as an Aid to Data Interpretation

The simplest mathematical model routinely used in the daily practice of intensive care medicine may be the electric circuit analog that gives rise to the oversimplified concept of total peripheral resistance from analysis of pressure and flow data. Using Ohm's Law, the circulation is presumed to behave like an electrical circuit where resistance can be defined as the driving pressure (arterial pressure either absolute or minus central venous pressure) divided by flow (cardiac output). A more complex example of model based data interpretation is 'pulse contour analysis' used to estimate cardiac output [31]. In this approach, a simplified mathematical representation of the vascular system coupled to the heart is used to derive changes in cardiac output from changes in the invasively or non-invasively measured arterial pressure waveform assuming a defined or estimated central arterial stiffness or elastance. A large number of specific incarnations of this basic idea have been proposed, with some implementations now having reached the maturity and stability to be marketed as out-of -the-box solutions for less invasive monitoring of cardiac output in the ICU such as Pulsion's PiCCO, LiDCO's LiDCOplus, and Edward's FloTrac devices. Unlike vascular resistance, the mathematical constructs required to derive cardiac output estimates from the arterial pulse pressure are too complex to routinely perform manually at the bedside. As a final example, we mention techniques to assess heart rate variability, which itself allows unique insights into autonomic control of cardiovascular function.

The robustness of the derived parameters is a function of the implicit assumptions made in constructing the mathematical model, as well as the specific mathematical techniques used. Many of the techniques that estimate cardiac output from arterial pressure pulse or heart rate variability from the cardiac event time series analyze the data by assuming that the underlying process can be described using a simple mathematical model characteristic for the particular analysis technique. For example, the frequency domain quantification of heart rate variability is often based on the Fourier transformation of the R-R interval signal by modeling the temporal variability as a sum of (possibly phase shifted) sinusoids of varying frequencies. The underlying assumption of signal stationarity is often violated in practical applications. More recent techniques like wavelet transforms may achieve similar results under weaker assumptions, leading to potentially more robust results [32, 33]. Thus, one may not assume that, just because a device reports a derived parameter common to another device, its accuracy and robustness will be similar without specific knowledge of the technical details of the analysis.

Mathematical Modeling: What can we Learn from Physics and Engineering?

While the application of mathematical models to data interpretation in critical care has already proven its usefulness, the potential of mathematical modeling is markedly underutilized as compared to the current practice in most of the natural sciences and engineering. Several centuries ago, the level of quantitative understanding of physical phenomena was in many ways similar to our current understanding of disease processes, in that it was largely qualitative. While serving as a basis for building mental models of reality, the predictive power of such qualitative models, which can be considered their ultimate test of validity, was naturally limited. Only

with the advent of mathematical physics, ushered in by the work of Isaac Newton, did true testable prediction become feasible. Since then, the interaction between theoretical physics (building mathematical models of physical phenomena) and experimental physics has been extraordinarily fruitful. This benefit is three fold. First, mathematical models can help to understand processes underlying observed phenomena, and help to verify whether a hypothetical mechanism can indeed explain the observed behavior of a system. Second, mathematical models ('theories') can predict previously unobserved phenomena, which can then be verified in experiments, thus deepening our understanding of the underlying laws of nature and improving our assessment of a given model's validity. And third, once validated for certain scenarios, mathematical models can also be used to perform virtual experiments that would be too costly or outright impossible to perform in reality. Validated mathematical models have made many of today's technological achievements possible by informing both the design process of technological artifacts and being utilized in real time control of technological processes.

The most desirable applications of mathematical modeling for the practice of medicine would probably be:

a) aiding the understanding of complex physiologic mechanisms;
b) performance of virtual experiments that would be costly, unethical, or impossible in reality; and,
c) the prediction of future system behavior, and, closely related, the control of physiologic processes (optimization of therapy).

While application 'a' has met with numerous successes in the past, substantial difficulties still have to be overcome before applications listed in 'b' and 'c' approach bedside applicability in the critical care setting.

Mathematical Modeling can help to Understand Mechanisms

Perhaps the most developed of the above mentioned applications of mathematical models to medicine is the application to the understanding of physiological mechanisms. While the observation of a spontaneous variability in heart rate is quite old, and its identifiable spectral components were quickly associated with functional components of the autonomic nervous system, some uncertainty remained with respect to the exact mechanisms by which the high frequency component of heart rate variability is linked to the parasympathetic branch of the autonomic nervous system, while the low frequency component is linked to both sympathetic and parasympathetic activity [34, 35]. The development of mathematical models has helped to understand how cardiopulmonary interactions as well as resonance mechanisms in the relevant physiological control loops may explain the observed phenomena [36]. With application to intensive care, we have recently been able to show how the clinical observation of a relationship between various clinical indicators of sepsis severity and outcome and the low-frequency power of heart rate variability may at least partly be explicable as a saturation phenomenon on the basis of the non-linear characteristics of the baroreflex feedback loop [37], leading to a physiologically accurate correlate of the previously claimed 'sympathetic failure' in a situation of severely elevated sympathetic activity.

Specifically, there are two interacting mechanisms that may contribute to the weakening of low frequency power in sepsis: The reduction of gain in the effector branch of baroreflex through vasodilatory effects of inflammation, which has been

shown theoretically to contribute to reduced low frequency power [36], and saturation of the sigmoidal non-linearity in the central component of the baroreflex feedback loop in the hemodynamically stressed condition of sepsis.

Mathematical Modeling may help to Perform otherwise 'Impossible' Experiments *in silico*

Once a valid mathematical model of the physiologic processes of interest has been developed and validated in a defined environment, it can be utilized to explore system responses under a wide range of conditions inexpensively and without doing harm to study subjects, whether they are humans or animals. These investigations may take the form of simulated laboratory experiments, where a mathematical model incorporating both animal physiology and experimental setup may allow to evaluate effects of variations of both properties of the animals and parameters of the experimental protocol, or even of simulated clinical trials for the *virtual* evaluation of therapeutic strategies [38].

To illustrate the use of virtual experiments to supplement real life experimental work, we explored the effects of the inevitable inter-animal variability in physiological response on outcome in an animal model of hemorrhage [39]. Figure 1 shows the calibration results of a mathematical model designed to replicate the animal study of hemorrhagic shock *in silico* for a survivor and a non-survivor animal, top panels showing actual measurements for each animal, bottom panels simulation results after manual parameter adjustment. The mathematical model describes both the animal's physiology and the experimental procedure, which consisted of repeated bleeding episodes triggered by the animal's recovery to a blood pressure of 40 mmHg, and resuscitation triggered by its final cardiovascular decompensation. Potentially, a complete experiment can be simulated from one set of initial conditions, allowing one to theoretically explore effects of alterations in physiological parameters and initial conditions (fitness of the host), treatments and their temporal relationship to the physiological response to the insult and outcome.

After we verified our ability to simulate the actual experimental physiological data in a qualitative fashion, we strove to assess the model behavior under differing conditions, starting with virtual experiments that are simpler than the real-life protocol. Figure 2 shows an example where outcome (blood pressure after approx. 17 hours) is shown as a function of the bleeding rate and the total volume bled in a single constant-rate bleeding expressed as a fraction of total blood volume when using the parameter set from the survivor pig in Figure 1 as a starting point. The survivable total blood loss shows a threshold behavior, with bleeding rate affecting this threshold only at low bleeding rates when slower compensatory mechanisms like inter-compartmental volume shifts start to matter. Figure 3 shows the dependency of outcome under a single bleed of fixed volume and rate on the tissue sensitivity to hypotension, as expressed as the midpoint of a sigmoidal damage rate/pressure relationship, and on the strength of the sympathetic response, as expressed by a scaling factor on the peripheral effector sites. As can be seen, qualitative behavior in this scenario is as expected, with higher sensitivity to hypoperfusion, and lower sympathetic response range being associated with a lower survivability.

Subsequently, this model was used to simulate the real-life experimental protocol. The same plot of dependency of survival on hypotension sensitivity and baroreflex

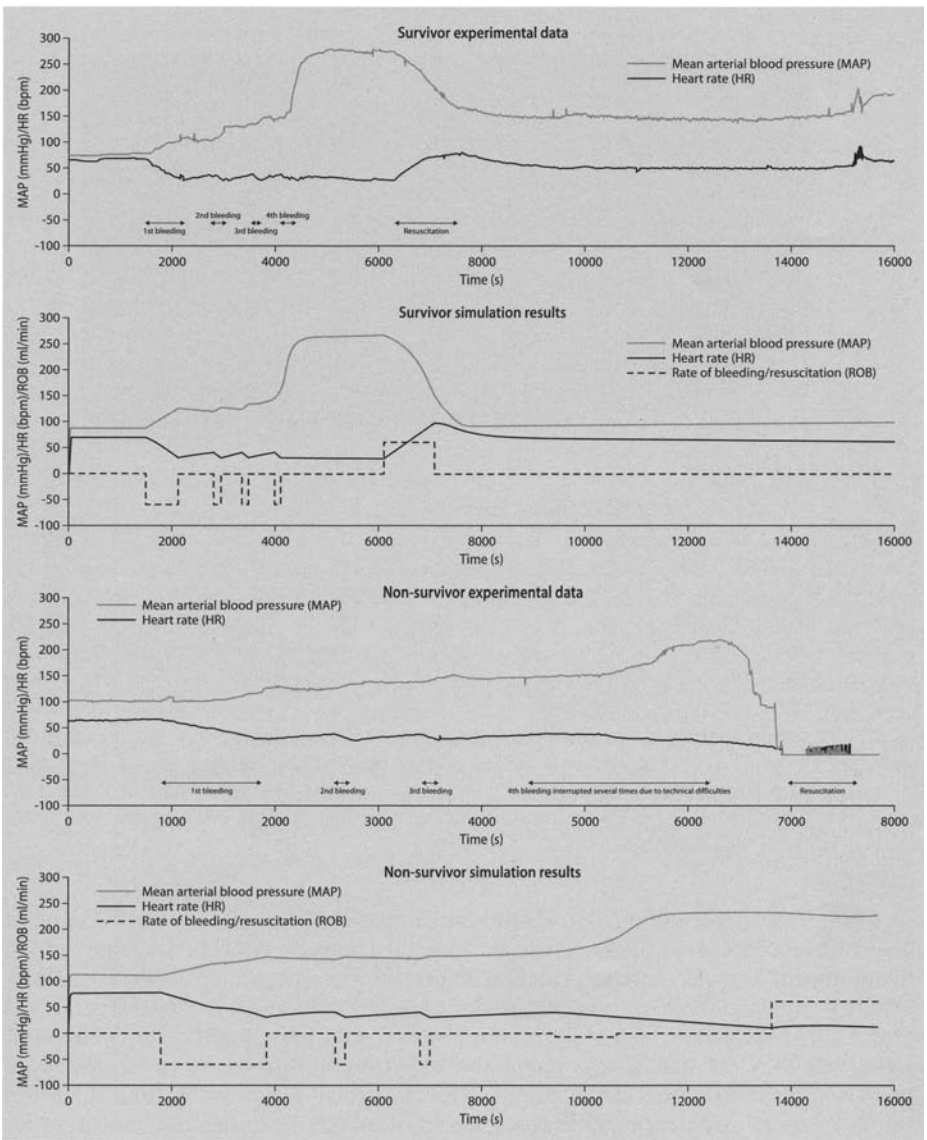

Fig. 1. Example calibration results for mathematical model of hemorrhagic shock experimental protocol and physiology in a pig model. Top panels show actual experimental data (arterial blood pressure and heart rate, as well as beginning and end of controlled bleeding episodes) for a surviving and non-surviving animal. Bottom panels show simulation results after parameter adjustment. Note that heart rate does not drop in the simulation when the non-surviving animal dies (blood pressure falls and stays low, in agreement with the experiment) since cardiac rythmogenesis is represented in a very simplified fashion in the current model.

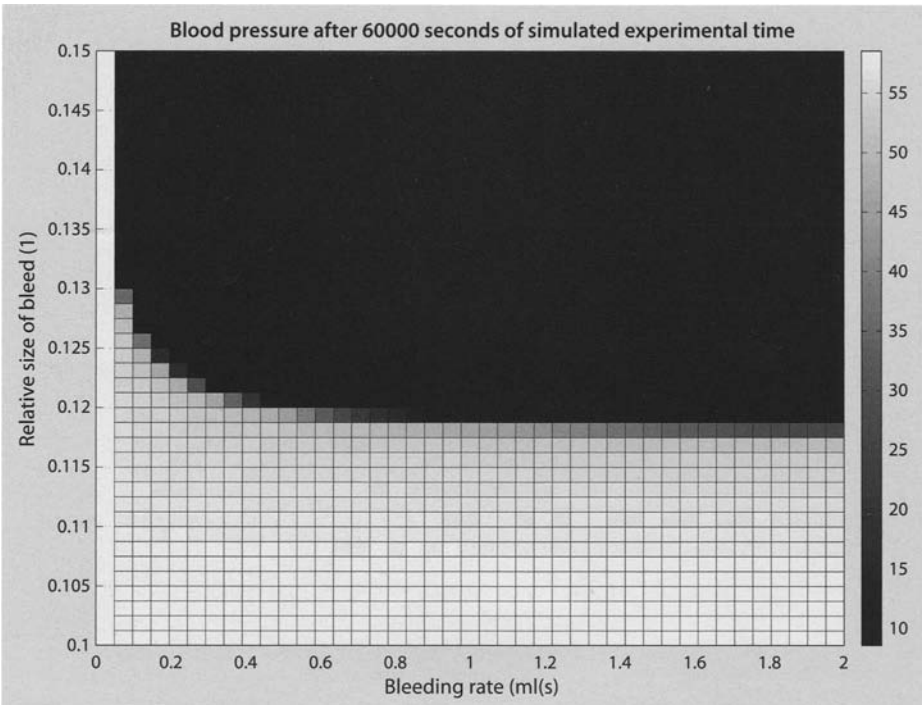

Fig. 2. Simulated dependency of outcome represented by arterial blood pressure on the total bleeding rate (x-axis) and the relative bleeding volume (y-axis) of a single constant rate bleeding episode when using the survivor parameter set from Figure 1. Each rectangle represents one simulated experiment, tone encodes blood pressure after 60,000 seconds of simulated experimental time, with lighter tones corresponding to higher blood pressures.

response range depicted in Figure 3 for the simple single constant rate bleeding is shown in Figure 4 for the real-life experimental protocol. As can be seen, a non-trivial dependency of outcome on variations in parameters is observed. While definitive interpretation of these simulation results awaits further experimental and theoretical validation, a key insight gained using this only qualitatively calibrated model was that the use of this particular experimental protocol, which bases its decisions on the response of the animal, may introduce a non-trivial, and for some regions of states and parameters somewhat paradoxical, dependency of outcome on the properties of the individual animal. More fit animals led to good responsiveness that may in fact lead to longer shock duration and thus worse outcome (Fig. 4). These findings are relevant to the trauma literature wherein previously healthy trauma victims often present with compensated shock but carry a worse prognosis because of delayed resuscitation owing to the false sense of security that mentation and a normal blood pressure give the acute health care providers. In this setting, the modeling effort helped to understand some of the dynamic properties of the experimental protocol in its interaction with the animals' physiology, and, thereby, aided the ongoing improvement of the real life laboratory experiments and clinical practice.

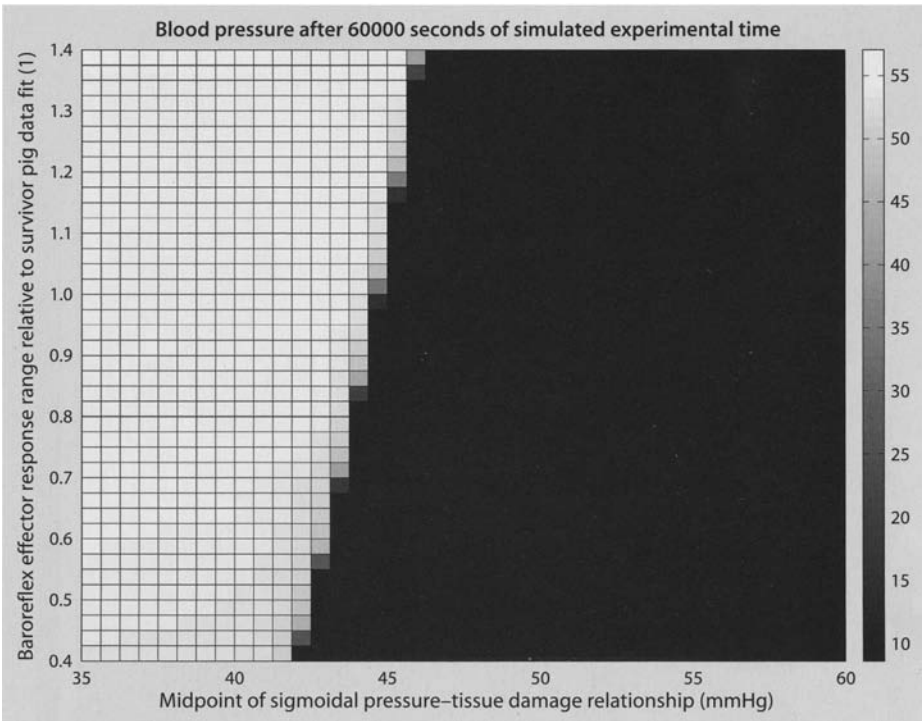

Fig. 3. Simulated dependency of outcome on the midpoint of the sigmoidal rate of tissue injury/arterial pressure relationship, and thus effectively tissue sensitivity to hypotension (x-axis) and the baroreflex effector response range (y-axis) of a single constant rate bleeding episode when using the survivor parameter set from Figure 1 as reference. Each rectangle represents one simulated experiment, tone encodes blood pressure after 60,000 seconds of simulated experimental time, with lighter tones corresponding to higher blood pressures.

Mathematical Modeling for Prediction and Selection of Therapeutic Strategies

The application of mathematical models to predict future developments in the individual patient and evaluate and optimize available therapeutic strategies with respect to outcome is the area of application with the largest potential benefits, since it could potentially support an approach to therapeutic decision making that is, at the same time, more individualized and more quantitative than the current paradigm of evidence-based medicine. Unfortunately, this area of application is also the most challenging and least developed. Current day implementations are mostly limited to small physiologic subsystems, oftentimes based on essentially empirical linearized representations of the underlying physiology.

An example of the application of control theory to therapy is the closed-loop blood glucose control with infusion pumps, where the dysfunctional physiological control loop is replaced by a system consisting of a glucose sensor, a computer that makes the insulin dosing decisions, and a pump that injects insulin [40]. Other examples of model-based control include the closed-loop control of blood pressure and opioid dosing during surgery [41, 42].

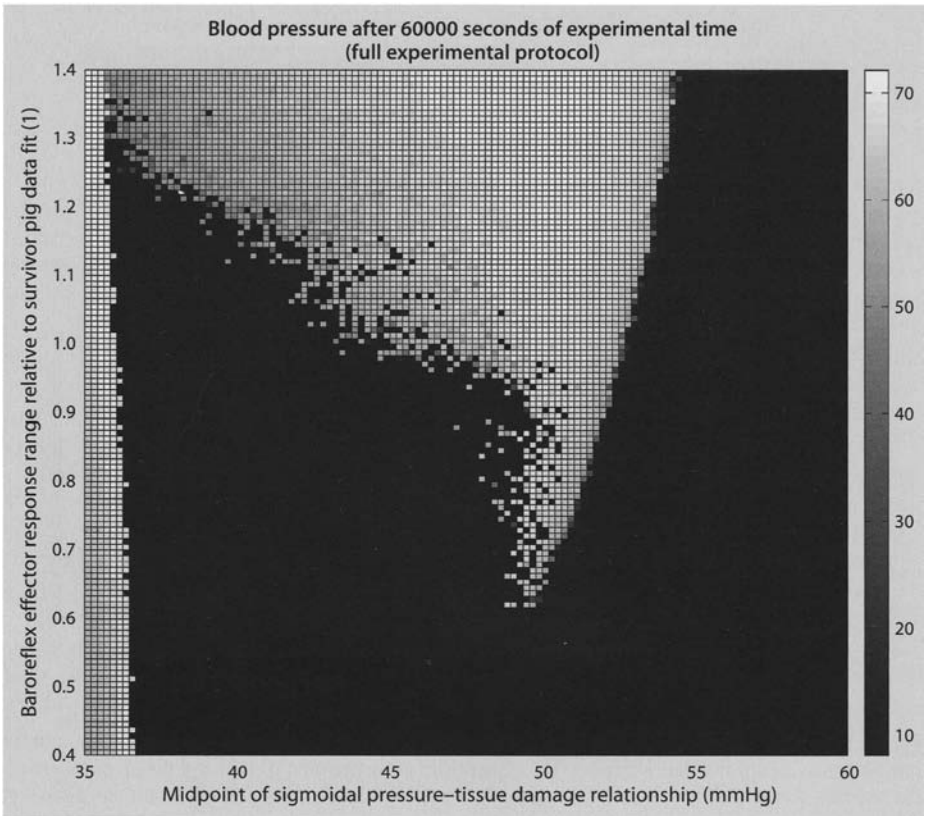

Fig. 4. Simulated dependency of outcome on the midpoint of the sigmoidal rate of tissue injury/arterial pressure relationship, and thus effectively tissue sensitivity to hypotension (x-axis) and the baroreflex response range (y-axis) of the full experimental protocol, when using the survivor parameter set from figure 1 as reference. Each rectangle represents one simulated experiment, tone encodes blood pressure after 60000 seconds of simulated experimental time, with lighter tones corresponding to higher blood pressures (here, a higher number of $120 \times 120 = 14400$ simulations was run to better resolve the structure at the boundaries of survival/death).

While this type of application can increase efficiency by reducing work load on care personnel while at the same time minimizing the possibility of human error, it seems doubtful whether the achievable increase in control precision over the level an experienced ICU nurse can achieve will create more than an incremental improvement in outcome, although that incremental improvement may be large.

A more comprehensive approach would seem necessary to obtain further improvements in outcome over unassisted human decision making. Such an approach would need to exploit the ability of mathematical models to incorporate arbitrary amounts of quantitative data as well as, in principle, an unlimited number of complex physiologic interactions into its current estimate of patient state and its prediction of future developments, and the effects of therapeutic options. Specifically, this would involve making a best estimate of model parameters and states based on all available data, to then use the dynamic mathematical model to propa-

gate the estimated system state into the future, and predict effects of available therapeutic interventions. Realizing such an interactive approach would markedly improve the usefulness of continuous, high precision measurements of physiologic variables in the ICU environment. Regrettably, some fundamental obstacles will have to be overcome first before such an approach is realized.

The most important obstacle is the lack of validated, quantitatively correct models describing sufficiently large parts of the relevant physiology of critically ill patients. This, however, does not represent a fundamental problem since it seems reasonable to assume that the human body as an, albeit extremely complex, physical system, should be amenable to mathematical description. The increasing amount of mechanistic insight into the body's functioning generated by the basic sciences can serve to define the structural framework of relevant mathematical models. The complex, hierarchical structure of this organism, which spans several orders of magnitude in spatial scales alone, may necessitate an at least partially stochastic description.

This insight leads to the next fundamental obstacle. When applying mathematical models to physiologic processes, it has traditionally been attempted to estimate a single set of parameters and/or system states of the model from available experimental data that represent a 'best fit', which, when using a least squares approach, corresponds to a maximum likelihood estimate. Unfortunately, there is often more than one solution equally compatible with the actual observations when using mathematical models complex enough to represent significant parts of our knowledge of the structure of the underlying physiologic processes (a phenomenon termed 'ill-posedness of the inverse problem'). Although this plurality defines real life as well, it does complicate decision support algorithms that presumably focus on single point goals. Still, this 'ill-posedness' is a reflection of clinical reality. We should, therefore, develop ways to quantify these multiple solutions. At the very least, such solutions would allow us to get a quantitative handle on the present amount of uncertainty about a patient's status based on our currently available information on physiology, bedside monitoring, and known treatments. To achieve this goal, we have recently proposed a methodology based on a Bayesian approach to probabilistic reasoning that derives density estimates in parameter and state space from the available observations and an underlying deterministic or partially stochastic model of pathophysiology [43]. Using this approach the patient's status is described by a multidimensional and potentially multi-modal probability distribution, with concentrations of probability density corresponding to possible differential diagnoses. Subject to the availability of valid models, this approach would not only allow one to quantify the current uncertainty about patient status, but would also enable one to explore which additional observations (diagnostic interventions) could most effectively contribute to decreasing uncertainty about patient status. Additionally, propagation of state densities would allow for prediction and evaluation of therapeutic options while fully reflecting the uncertainty present, thus giving the physician an idea about the quality of the data he or she is basing their decisions on, which, in terms of patient safety, appears as an absolute prerequisite. This methodology could, in principle, be validated in controlled clinical trials, allowing for an approach to medical decision making that would satisfy the criteria of evidence-based medicine, while taking into account all known information about the individual patient at the same time.

Conclusion

Functional physiological monitoring is a promising approach to making more directly interpretable measurements. The application of mathematical models to the critical care setting will allow one to both more accurately assess the patient's physiologic status based on all available quantitative and qualitative information and infer individually optimal therapeutic strategies. This approach has the potential to become a powerful tool for improving outcomes and optimizing resource utilization in the critical care setting. However, most of its possibilities are still far from being realized, and only proof-of-concept examples have reached the maturity to allow beside application. Turning the conceptual framework outlined above into a useable aid for day-to-day decision making in the ICU of the future will require coordinated and highly interdisciplinary efforts from many scientific disciplines, including, but not limited to, medicine, physics, biology, and mathematics. We believe, however, that the eventual results, both in terms of improvements of care and in the deepening of our understanding of pathophysiologic processes relevant to critical illness, may well justify the investment.

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The Meaning of Hemodynamic Monitoring in Patients with Shock: Role of Echocardiography

A. Vieillard-Baron

Introduction

The development of noninvasive devices to manage hemodynamics in patients with shock is directly prompted by the results of recent studies in the intensive care unit (ICU), and during the perioperative period, which demonstrated the inability of an invasive approach, based on right heart catheterization, to improve prognosis [1]. Some authors have suggested that these results were largely due to inaccurate use of right heart catheterization, without clear goals or protocol [2]. However, previous studies have demonstrated that optimization of cardiac output and mixed venous oxygen saturation (SvO_2) with clear endpoints also fails to improve prognosis [3]. So, the lack of efficacy is inherent in the device. In 1985, Eugene Robin suggested that using right heart catheterization in patients with shock led physicians to give fluids plus diuretics whatever the wedge pressure [4]. In 2003, François Jardin claimed that we were going to move from the "age of oil lamps" to the "age of electricity" [5]. In fact, we are going to change our practices in the management of shock, from an invasive and quantitative approach of hemodynamics to a non-invasive one, more functional and especially qualitative, mainly thanks to the use of echocardiography. This leads us to think about the meaning of hemodynamic monitoring.

What is Monitoring?

Monitoring should be a diagnostic aid and guide treatment. So, in hemodynamics, monitoring should help us to determine the cause of shock, i.e., hypovolemia, left or right ventricular failure, vasoplegia, pericardial effusion, and so guide the treatment, i.e., infusion of fluids, inotropic drugs, vasoconstrictive drugs, or fluid removal. This is especially true in septic shock, where most of these causes can be associated, making it essential to have a monitoring device capable of assessing all of them independently. Martin Tobin also emphasizes that 'good' monitoring should measure relevant variables, provide interpretable data, be easy to implement, and not cause harm (M. Tobin, post-graduate course on ICU monitoring, Congress of the American Thoracic Society, San Diego, 2006). Once again, in hemodynamics, this does not seem to correspond to an invasive approach, but rather to a non-invasive one using echocardiography.

How to 'Monitor' Hypovolemia?

The parameters recorded by an invasive approach have not been found to be relevant to this issue. Figure 1 reports the maximum and minimum values of cardiac filling pressure observed in fluid responsive patients in three recent studies [6–8]. As demonstrated, a central venous pressure (CVP) and a pulmonary artery occlusion pressure (PAOP) as high as 18 and 17 mmHg, respectively, could be associated with a fluid-responsive status in shock patients. Cardiac index (CI), a parameter commonly measured by right heart catheterization, is frequently in a normal range at baseline before volume expansion in true hypovolemic patients receiving mechanical ventilation [9], and frequently not significantly different between patients who do and do not respond to fluids [8]. However, it is true that coupling CI and PAOP variations following a fluid challenge could be used to assess fluid requirement. An increase in CI, associated with a slight increase in PAOP, suggests the presence of hypovolemia, whereas the absence of a significant increase in CI, associated with a marked increase in PAOP, demonstrates the uselessness of volume expansion. But, in this situation, physicians risk the deleterious effects of a useless volume expansion several times a day, such as cardiac overload, pulmonary edema, and impairment in oxygenation.

Recently, echocardiography has been reported to accurately identify patients who need fluids, providing the errors made using an invasive approach are not repeated. This means not evaluating cardiac filling pressures, as previously proposed [10], but using alterations in some cardiac function parameters induced by tidal ventilation [11]. The best is probably to examine the venae cavae and their respiratory diameter variations [12]. Whereas the superior vena cava, visualized by a transesophageal approach, partially or totally collapses at each insufflation in the case of hypovolemia [8], the inferior vena cava, visualized by a subcostal approach, significantly increases in diameter (Fig. 2) [13]. From the concept of 'fluid challenge', we now pass to the concept of 'fluid responsiveness', which is totally adapted to the use of echocardiography.

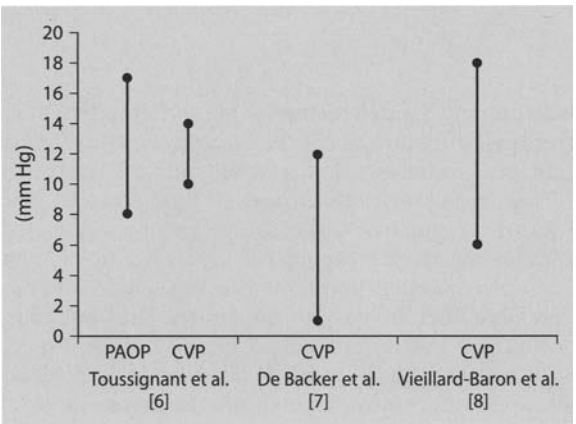

Fig. 1. Maximum and minimum values of central venous pressure (CVP) and pulmonary artery occlusion pressure (PAOP) in patients who responded to fluids, as reported in three recent studies in critically ill patients [6–8].

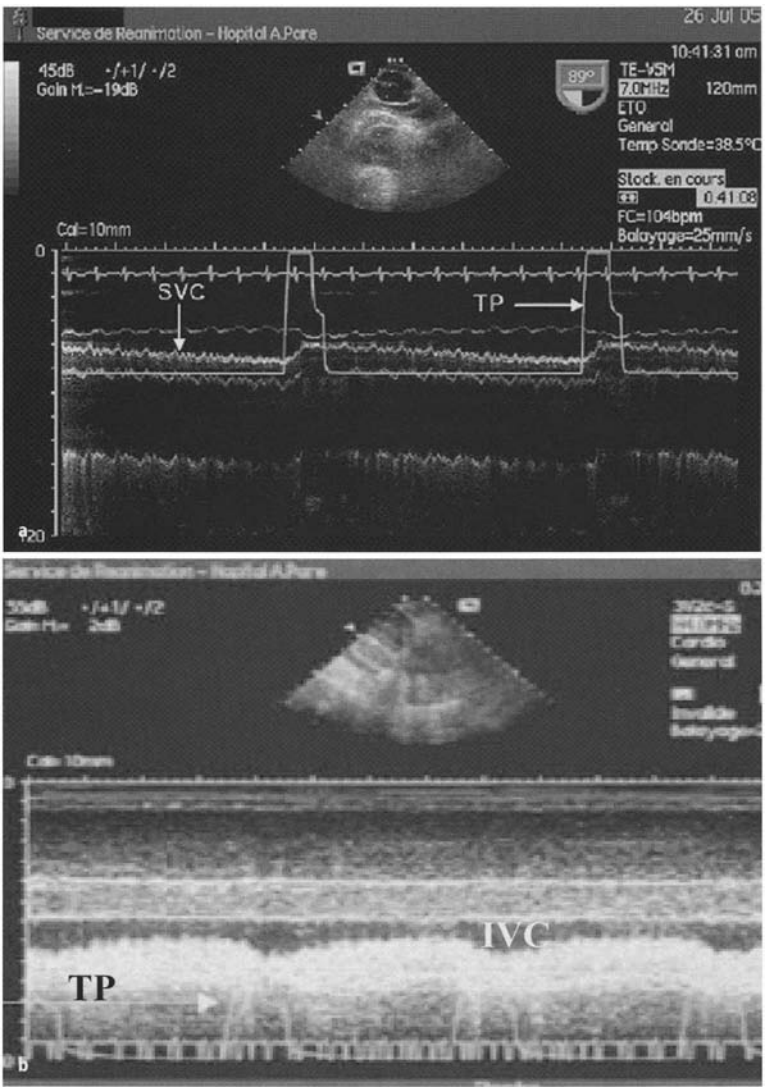

Fig. 2. Panel **a** represents a cyclic collapse of the superior vena cava (SVC) at each insufflation in a hypovolemic patient. Panel **b** represents significant increase in inferior vena cava (IVC) diameter at each insufflation in another hypovolemic patient. TP: tracheal pressure.

How to 'Monitor' Left Ventricular Failure?

Using right heart catheterization, left ventricular (LV) systolic dysfunction is also classically assessed by the comparison between PAOP and CI. LV failure is diagnosed when a high PAOP is associated with a low CI. Whereas this is true in very simple clinical situations, such as pure cardiological situations, it is not relevant in more complex ones, as in the ICU. For example, LV failure is common in septic shock, and may frequently require infusion of an inotropic drug [14]. But this failure is associ-

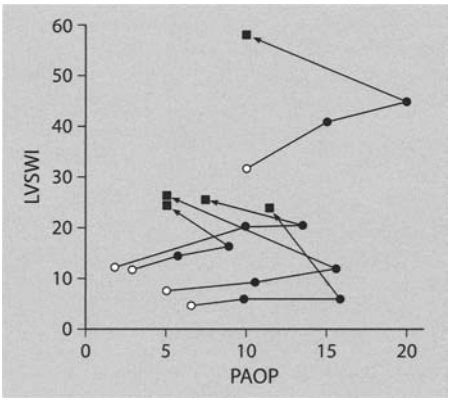

Fig. 3. Relationship between pulmonary artery occlusion pressure (PAOP) and left ventricular systolic work index (LVSWI) in five patients with septic shock. In most of these patients, this relationship suggested hypovolemia (open circle) at baseline, although most of them were fluid nonresponders, as demonstrated by the first step (first closed circle) and the second step (second closed circle) of volume expansion. Finally, dobutamine infusion restored a normal relationship (closed square), suggesting the presence of severe systolic LV dysfunction.

ated with a low or normal PAOP [15], even after resuscitation. Right heart catheterization is, therefore, inaccurate for this diagnosis, as illustrated in Figure 3, and this probably explains why LV systolic dysfunction has long been markedly underestimated in this situation.

Echocardiography does not require an algorithm combining measurements of cardiac filling pressures and CI, but directly visualizes segmental and global LV contractility. Whereas measurement of LV volumes, to calculate LV ejection fraction (LVEF), is classically recommended in assessment of LV systolic function, we recently demonstrated by a transesophageal route the accuracy of a qualitative approach, which permitted non-echocardiographers to separate patients with severe and moderate LV failure from those without such failure [16].

How to 'Monitor' Right Ventricular Failure?

Clearly, most parameters used with right heart catheterization to assess right ventricular (RV) function are actually indicators of the status of the pulmonary circulation, such as pulmonary vascular resistance (PVR), and pulmonary artery pressure (PAP). The assumption is that impairment of these parameters, reflecting damage to the pulmonary circulation, may suggest that the right ventricle tolerates poorly these effects. However, since the famous paper of Zapol and Snider [17], it is well recognized that, in mechanically ventilated patients, PVR strongly depends on flow: An increase in CI induces a decrease in PVR, and a decrease in CI induces an increase in PVR. This follows the recruitment and derecruitment of pulmonary capillaries crushed by a positive alveolar pressure. We have also demonstrated that, in patients with severe acute respiratory distress syndrome (ARDS), an elevated systolic PAP does not predict RV tolerance [18]. In some cases, a slight increase in PAP may be enough to induce RV failure, whereas in other situations the right ventricle is able to adapt to a marked increase in PAP. Previous studies proposed assessing RV function directly by measuring RVEF, using fast thermistance catheters [19]. Once again, this suggestion turned out to be inaccurate, especially in mechanically ventilated patients [20], and in patients with significant pulmonary hypertension [21]. Finally, we demonstrated that RV fractional area contraction, a surrogate for RVEF, measured by echocardiography, did not differ significantly among patients with and without acute cor pulmonale (Fig. 4) [22].

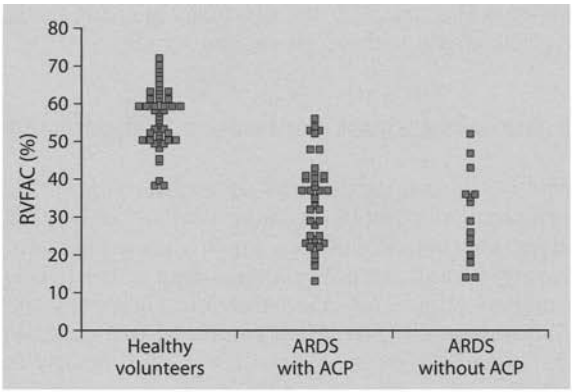

Fig. 4. Right ventricular fractional area contraction (RVFAC) in three groups, healthy volunteers, and patients with acute respiratory distress syndrome (ARDS) with and without acute cor pulmonale (ACP). Note the large overlap of values.

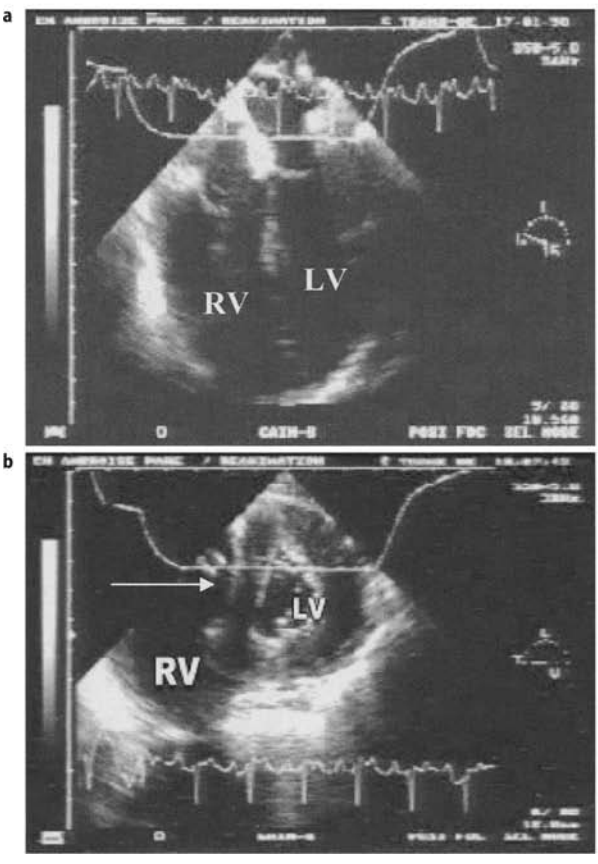

Fig. 5. Acute cor pulmonale in a shock patient ventilated for acute respiratory distress syndrome related to varicella pneumonia. The right ventricle (RV) was severely dilated (panel **a**), whereas paradoxical septal motion (arrow) was present (panel **b**). LV: left ventricle.

Echocardiography may directly visualize the right ventricle, and so quickly assess its function. Because of its properties, i.e., a 'passive conduit' that in a normal situation ejects blood into a low-pressure circulation, a failing right ventricle dilates markedly, which is very easy to assess with echocardiography. Moreover, echocardiography is also able to detect acute cor pulmonale, a situation not so rare in critically ill

patients (Fig. 5) [22]; the diagnosis is easily made by the association of a dilated right ventricle with septal dyskinesia [23].

But What about Continuous Hemodynamic Monitoring?

One of the major criticisms of skeptics regarding the use of echocardiography for hemodynamic monitoring in critically ill patients is that it cannot be done continuously. This is true, but then which kind of elaborate continuous monitoring has previously had an impact on monitoring in the ICU? None, and surely not right heart catheterization. This is illustrated in Figure 6, which shows the results of a study by Gattinoni et al. [24]. When compared to a control group, the mortality was exactly the same in a group where CI was continuously monitored and optimized, and in another group in which SvO_2 was also continuously monitored and optimized [24]. The second message of this study was that, after initial resuscitation, CI and SvO_2 were already within the normal range of values [24].

One of the objectives of hemodynamic monitoring, perhaps the most important, is to evaluate the risk for organ hypoperfusion, and then to correct it. In the literature, low blood pressure and metabolic acidosis in critically ill patients seem to evaluate this risk accurately. Low blood pressure is easily detected because most of our seriously ill patients have an arterial catheter, and so blood pressure is continuously recorded. In a recent study, Varpula et al. demonstrated that a strong independent factor of mortality was the time during which a patient had a mean arterial pressure (MAP) less than 60 mmHg [25]. Metabolic acidosis can be diagnosed by repeated measurements of arterial base excess. Estenssoro et al. reported a significantly lower base excess in non-survivors in the ICU, and, more interestingly, a lack of improvement during the first days of treatment [26]. Finally, by coupling continuous monitoring of blood pressure and 'semi-continuous' monitoring of base excess, physicians may estimate the risk of organ hypoperfusion. These tests can be used for screening, and echocardiography, which is quickly performed if the screening test is positive, may then be used to detect hypovolemia, RV failure, or LV failure.

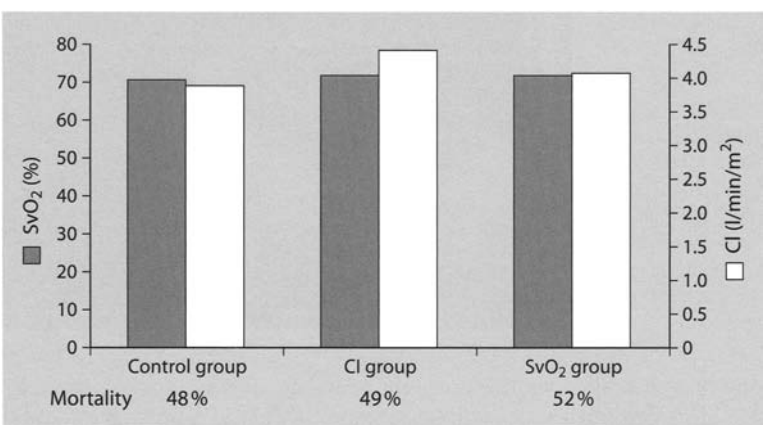

Fig. 6. Schematic representation of the study by Gattinoni et al. [24]. The mortality rate did not differ according to the group, or to the optimization of cardiac index (CI) or mixed venous oxygen saturation (SvO_2). After initial resuscitation, CI and SvO_2 were within normal ranges in the control group.

Conclusion

Development of a new type of more functional and qualitative monitoring, mainly based on the use of echocardiography, calls for a complete change in the way we think about hemodynamic diagnosis. Use of algorithms, which couple CI and filling pressure, should be abandoned, and physicians need only describe what they see on the screen of the echocardiograph to give adequate treatment, providing they have performed echocardiography in high-risk situations for organ hypoperfusion, such as low blood pressure or persistent metabolic acidosis.

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Transpulmonary Thermodilution for Advanced Cardiorespiratory Monitoring

F.J. Belda, G. Aguilar, and A. Perel

Introduction

Since the introduction of the pulmonary artery catheter (PAC) into clinical practice in the 1970s, this device has been considered to be the gold standard for cardiac output measurement and advanced hemodynamic monitoring. Nevertheless, in the last 10 years, its risk-to-benefit ratio has become a subject of controversy. One recent meta-analysis on the impact of the PAC in critically ill patients [1] has presented conclusive results showing that the PAC does not bring any clinical benefit, although its use does not prolong hospital length of stay or increase the mortality rate, as was previously claimed by Connors et al. [2]. Another recent prospective multicenter study on 1041 critical patients came to the same conclusions as the meta-analysis [3]. Finally, in a randomized trial comparing hemodynamic management guided by a PAC with hemodynamic management guided by a central venous catheter (CVC), using an explicit management protocol in 1000 patients with established acute lung injury (ALI), PAC-guided therapy did not improve survival or organ function, but was associated with more complications than the CVC-guided therapy. The authors concluded that these results, when considered with those of previous studies, suggested that the PAC should not be routinely used for the management of patients with ALI [4]. The negative results of the PAC studies have led to a gradual decrease in the use of this monitoring modality. In fact, a survey in Germany in 2006 showed that, in a population of 3877 critically ill patients, less than 15% of patients with the criteria of severe sepsis or septic shock were monitored with a PAC [5].

However, a recent retrospective study in a population of 53,000 critically ill trauma patients demonstrated that the use of the PAC, in contrast to conventional hemodynamic monitoring, was associated with a decreased mortality rate [6]. Proponents of the PAC still claim that it is a powerful tool that suffers from gross misinterpretation of data and from routine use without any specific indication, and that there is currently no evidence from randomized, controlled trials that any diagnostic or monitoring tool used in intensive care patients improves outcomes [7].

As a result of the decline in the use of the PAC, we have been witnessing the development of less invasive techniques of hemodynamic monitoring in recent years [8]. These 'less invasive monitoring techniques', which are capable of monitoring cardiac output, include transesophageal echocardiography (TEE), Doppler ultrasonography, thoracic bioimpedance, partial rebreathing of CO_2, pulsed dye densitometry, lithium dilution, pulse contour analysis, and transpulmonary thermodilution. An experimental study that evaluated the reliability of cardiac output measurement by partial rebreathing of CO_2, pulmonary arterial thermodilution, transpulmonary thermodilution, and Doppler ultrasonography, used the periaortic flow as the refer-

ence measurement for cardiac output and concluded that only the pulmonary artery thermodilution (r=0.93) and the transpulmonary thermodilution (r=0.95) could be considered as interchangeable with the method of reference, even in situations of hemodynamic instability [9].

However, although many studies have dealt with the accuracy of cardiac output measurement by various new monitoring technologies, monitoring cardiac output by itself is frequently insufficient for the complex hemodynamic management of critically ill patients. The PiCCO monitor (Pulsion, Germany), which uses the transpulmonary thermodilution technique, offers complete hemodynamic monitoring, including an integrated pulse contour method for continuous cardiac output measurement, while other measured and derived parameters enable the simultaneous estimation of the cardiac preload, afterload, contractility, and extravascular lung water (EVLW) at the bedside. The monitoring of cardiac output by means of transpulmonary thermodilution is considered to be minimally invasive since it requires only an arterial (thermistor-tipped) catheter and a central venous pressure (CVP) line.

Technical Considerations with Transpulmonary Thermodilution

The transpulmonary thermodilution technique begins with the injection of an ice-cold (<8 °C) or ambient temperature (<24°) bolus of saline [10] through a temperature sensor placed in a central venous line. The change in temperature of the injectate is sensed by a thermistor that is embedded in the femoral (or axillary) arterial catheter (catheter 5F, 20 cm long). However, the technique has been recently validated using longer catheters (4F, 50 cm long) placed in the radial artery with the thermistor tip located at the axilary artery level [11]. The direct axillary or radial artery approaches serve as alternatives to the femoral route in those patients in whom femoral cannulation is contraindicated or is technically complex (aortic-femoral bypass, femoral arteriopathy, morbid obesity, etc).

The cardiac output is calculated by the analysis of the thermodilution curve in the usual way using the Stewart-Hamilton algorithm. From this, preload indices (intrathoracic blood volume (ITBV) and global end-diastolic volume (GEDV), and EVLW are calculated. The continuous measurement of cardiac output by the pulse contour method of the PiCCO is based on Wesseling's method, which determines the area underneath the systolic portion of the arterial pulse. An initial cardiac output has to be measured for the calibration process, in which the aortic impedance is calculated by dividing the cardiac output by the area under the systolic portion of the pulse contour. The calculated impedance is used for the continuous derivation of the cardiac output from the arterial pressure waveform.

The measurement of cardiac output using this techique has been validated by several clinical studies summarized in a recent paper [12]. Additionally, other studies appear to confirm the fact that the continuous cardiac output measurements from pulse contour analysis are accurate, remain reliable during significant hemodynamic changes, and are not influenced by the use of drugs that change the blood pressure and/or the systemic vascular resistance [12]. In addition, the use of intra-aortic balloon counterpulsation does not contraindicate the use of the PiCCO monitor, since hemodynamic information from transpulmonary thermodilution is not affected whilst on this device [13]. It has also been shown that the precision of cardiac output, ITBV, and EVLW measurements are maintained during continuous veno-venous therapies of renal replacement [14] and during hypovolemic shock [15].

Hemodynamic Monitoring by Means of Transpulmonary Thermodilution

Cardiac Output

Acute circulatory failure is often due to a fall in blood pressure and/or cardiac output, because both pressure and flow are major determinants of organ function. However, hypotension can be due to a low cardiac output, but may also result in systemic vasodilation. In this sense, the measurement of cardiac output might be useful to differentiate between high and low flow states and, therefore, to discriminate between patients who will benefit from vasopressors (high cardiac output and low arterial pressure) and those who will benefit from fluids and/or inotropes (low cardiac output). In addition the measurement of cardiac output is important to identify those patients whose low flow state cannot be identified by clinical examination alone.

Preload

The evaluation of preload in low flow states is the most useful tool to identify those patients who would benefit from volume loading. Although cardiac filling pressures (CVP and pulmonary artery occlusion pressure [PAOP]) are often still used for the assessment of preload, these parameters have been repeatedly shown to reflect preload poorly. The reasons for the inadequacy of filling pressures to assess preload include erroneous readings from the pressure waveforms, discrepancies between measured pressures and transmural pressures (especially at high levels of positive end-expiratory pressure [PEEP] or with dynamic hyperinflation) [16], and simply because the physiological relation between the ventricular end-diastolic pressure and its volume depends on the distensibility and compliance of the cardiac chambers (e.g., high PAOP due to left ventricular hypertrophy, high CVP due to *cor pulmonale*, etc.).

Several other parameters have been proposed to evaluate preload at the bedside. These include the right ventricular end-diastolic volume (RVEDV) measured by a special PAC; the left ventricular end-diastolic area (LVEDA) measured by echocardiography; the ITBV evaluated by the double indicator dye-cold dilution technique; and more recently the GEDV obtained by transpulmonary thermodilution. It has been demonstrated that the changes in the GEDV induced by a volume load have a good correlation with changes in stroke volume and, therefore, in cardiac output [17]. This is consistent with the physiological relationship between preload and stroke volume. Both ITBV and GEDV have been shown to be more reliable indicators of preload than the cardiac filling pressures [18, 19]. In contrast with the measurement of the RVEDV, the determination of the GEDV does not require a PAC. Compared with the echocardiographic measurement of the LVEDA, the GEDV is not dependent on operator skills or on the presence of a 24 hour echo service, and its measurement can be repeated easily with each cardiac output determination at the bedside.

Nevertheless, both GEDV and ITBV must be interpreted along with the patient's clinical status and other hemodynamic variables. Sakka and Meier-Hellmann [20] published a case report of a patient with pulmonary embolism in whom the ITBV was low, the CVP high, and the cardiac output did not increase after a volume load. The authors suggested that in these patients, other causes that can reduce the central blood volume (for example, pulmonary embolism, tension pneumothorax, etc) should be ruled out.

Prediction of the Response to a Fluid Challenge

One of the most frequent challenges for the intensivist is the prediction of the response of the cardiac output to fluid loading. This is especially true since most studies show that only 50 % of critically ill patients respond favorably to fluid loading, and since fluid loading may have a detrimental effect on pulmonary and other organ function. Because the slope of the relationship between preload and stroke volume depends on ventricular contractility, the isolated evaluation of ventricular preload is not enough to predict the response to volume loading [21]. Although volumetric indicators of preload are useful in the prediction of the response to volume expansion when their values are high or low, they are not conclusive when they are in the intermediate range [21]. A series of dynamic parameters have, therefore, been proposed in order to predict the hemodynamic effects of fluid loading mainly in mechanically ventilated patients, using the influence of the positive pressure breath on the stroke volume [22]. In sedated patients on mechanical ventilation, the intrathoracic inspiratory positive pressure produces a stroke volume variation (SVV). The SVV informs us about the sensitivity of the heart to a potential volume load. In the same way, because the pulse pressure (systolic less diastolic blood pressure) is directly proportional to the left ventricular stroke volume, variations in pulse pressure (PPV) induced by ventilation are well correlated with those in stroke volume, and consequently can predict the response to a volume load (Fig. 1) [23]. The PiCCOplus monitor automatically calculates the PPV and the SVV on a beat-to-beat basis using the pulse contour analysis. As with the PPV, the SVV has been demonstrated to be a precise predictor of volume responsiveness in patients submitted to neuro- [24] and cardiac surgery [25]. However, it is important to remember that the PPV and SVV are affected by the size of the tidal volume. For example, it has been shown recently that the SVV may show values compatible with hypovolemia even during hypervolemic situations when very high ventilatory tidal volumes (>15 ml/kg) are employed [26]. Reuter et al. [27] demonstrated the validity of these parameters also in patients with an open thorax following midline sternotomy. In a later study in a similar patient population, it was demonstrated that "responders" to a volume load became "non-responders" after sternotomy [28]. According to the authors, opening of the thorax may cause the heart to function on the plateau portion of the Frank-Starling curve, turning these patients into "non-responders".

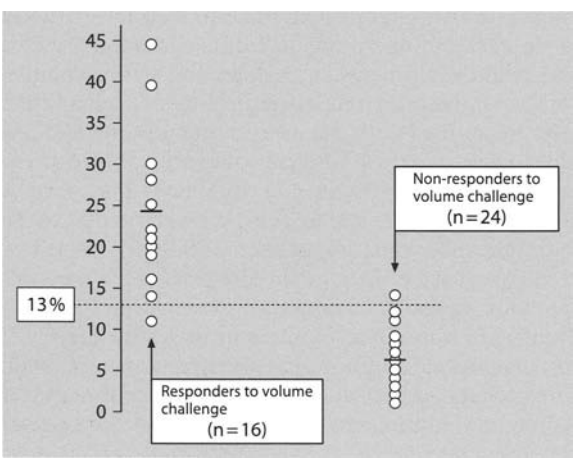

Fig. 1. Variations in pulse pressure (PPV) due to mechanical ventilation. PPV induced by the ventilation correlated well to stroke volume variations (SVV), and consequently can predict the response to a volume load. Patients with a PPV above 13% will have a good response to volume loading. From [50] with permission.

Evaluation of Cardiac Contractility/Function

In low flow states, evaluation of cardiac contractility/function can be useful to identify those patients who may benefit from the administration of inotropic agents. The precise evaluation of cardiac contractility at the bedside is not straightforward because, amongst other reasons, it is dependent on preload and/or afterload. The ventricular ejection fraction is the parameter most frequently used to evaluate the ventricular function, being the ratio of the stroke volume to the ventricular end-diastolic volume. Transpulmonary themodilution measures the GEDV, which constitutes the blood volume of four cardiac chambers at the end of diastole [12]. Therefore, the quotient between the stroke volume and one-fourth of the GEDV can estimate the global ejection fraction (GEF) of the heart. This parameter is calculated automatically by the PiCCOplus and can be used to identify patients with ventricular dysfunction. In addition, the PiCCOplus monitor provides a continuous assessment of left ventricular contractility by measurement of the dP/dtmax, which is derived from the maximum speed of the arterial pressure curve during the ejection phase. A good correlation has been demonstrated between the dP/dtmax estimated from the pressure curve of the femoral artery and that obtained directly from the left ventricle [29].

Respiratory Monitoring by Means of Transpulmonary Thermodilution

Detection of Pulmonary Edema

Although chest radiography and arterial blood gases are the main components of the international definition of ALI and acute respiratory distress syndrome (ARDS), these parameters have been demonstrated to be of little value in the identification of patients with pulmonary edema [30]. Because of this, several techniques have been proposed to evaluate lung edema (EVLW) in humans. The double indicator dye, cold dilution technique has been one of the most frequently used methods for this purpose in critically ill patients [31], and stands in contrast to other techniques that cannot be performed at the bedside (computerized axial tomography, magnetic nuclear resonance, positron emission tomography). However, this technique is not frequently used nowadays because it is cumbersome and costly, and because it has been suggested that although the technique is useful to evaluate interstitial edema in cases of indirect ALI/ARDS, it is less accurate in cases of direct ALI/ARDS [32].

The transpulmonary thermodilution technique, which is based on the injection of cold solution only (single indicator), is much simpler, yet offers similar results in the measurement of EVLW when compared with both the double indicator technique [33] and the 'gold standard' gravimetric method [34]. Additionally, the EVLW calculated by the PiCCO has been used, along with the PAOP, as a reference parameter for validation of lung ultrasound for the diagnosis of pulmonary edema [35]. This EVLW measurement has been shown to be able to detect even small increases (10–20 %) in EVLW, indicating the presence of incipient edema in the absence of other clinical and diagnostic signs [36].

Monitoring EVLW can also be useful as a guide for fluid therapy, especially in patients with increased pulmonary microvascular permeability (e.g., sepsis). In view of the recent findings that fluid restriction and negative fluid balance may improve the evolution of ALI/ARDS [37], EVLW measurement may have special importance as it can be used to identify those patients with high EVLW who would benefit from

such a therapeutic approach. In other words, the measurement of EVLW could be the response to the current controversy between the 'dry or wet' therapy of patients with ARDS [38]. It is, however, important to note that the benefit of fluid restriction must be balanced against the risk of possible hemodynamic deterioration. By measuring EVLW simultaneously with cardiac output, preload (GEDV) and fluid responsiveness (PPV and SVV), the PiCCO monitor is capable of guiding fluid therapy and solving therapeutic dilemmas.

Theoretically, the measurement of EVLW may be less accurate in patients undergoing pulmonary resection (lobectomy, pneumonectomy), due to the changes in gas volume and pulmonary blood flow that occur during and after these surgical procedures. However, Roch et al. [39] and Kuzkov et al. [40] have found that both the double indicator dilution technique and transpulmonary thermodilution may be useful for EVLW monitoring after pneumonectomy, although, when compared with lung gravimetry, both methods, and especially transpulmonary thermodilution, tended to overestimate the measurement of EVLW under these circumstances [39]. Clinical studies have not yet been performed to test the prognostic value of EVLW measurement in these patients.

Calculation of the Pulmonary Vascular Permeability

By definition, EVLW may increase with hydrostatic (cardiogenic) pulmonary edema or in edema due to disturbances in pulmonary vascular permeability. In the former, the increase in EVLW stems from an increase in pulmonary blood volume (PBV) and pressure, leading to a low ratio of EVLW to PBV. However, in the presence of permeability pulmonary edema, the EVLW to PBV ratio is expected to be much higher. By being able to measure both parameters, the PiCCO monitor offers an automatic calculation of the EVLW and the PBV, termed the pulmonary vascular permeability index (PVPI). This parameter may be useful not only to discriminate between cardiogenic and permeability pulmonary edema, but also to evaluate the effects of several illnesses and treatments on pulmonary vascular permeability. In this way, Morisawa et al. [41] suggested that the PVPI can be useful in determining the origin of ALI/ARDS. This study demonstrated that the PVPI, ITBV, and EVLW values were significantly higher in direct lung injury (aspiration, pneumonia) than in indirectly induced ALI/ARDS (e.g., sepsis).

Two recent studies from the same group examined the correlation of EVLW and PVPI with the lung injury score (LIS) and produced discordant results. In the first study, by Groeneveld et al. [42], EVLW was measured by the double dye-dilution technique in 16 patients after major vascular surgery, and PVPI was found to be significantly higher in patients with an LIS>1 compared to those with an LIS equal to or lower than 1. In the second study, Verheij et al. [43] studied 67 patients after cardiac and major vascular surgery. Using the same technique, they concluded that the changes in both PVPI and EVLW were not correlated to the LIS. Comparisons of the PVPI with the LIS should be interpreted with caution, because the parameters included in the LIS may not be the most appropriate for the evaluation of pulmonary injury, and are confounded by objective and subjective multi-factorial factors (e.g., lung compliance depends on lung recruitment, applied-PEEP can be very variable, and the evaluation of the affected lung quadrants depends on the quality of the X-ray image).

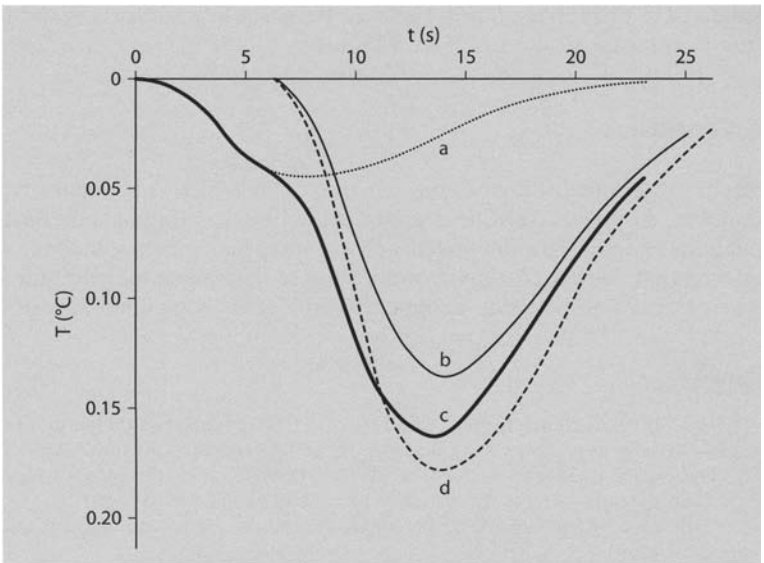

Fig. 2. Intracardiac right to left shunt detection by transpulmonary thermodilution. The cold indicator goes through the foramen ovale and reaches the arterial temperature sensor much more rapidly creating a characteristic 'hump' in the curve. a: 'shunt' curve; b: normal curve; c: effective curve (a+b); d: curve in absence of 'shunt'. From [50] with permission.

Causes of Hypoxemia

Arterial hypoxemia is mainly due to ventilation-perfusion mismatch and/or intrapulmonary shunting. Occasionally arterial hypoxemia may be caused by an anatomic intra-cardiac right-to-left shunt across an open foramen ovale which is present in 20–34% of autopsies in the general population [44]. The prevalence of intracardiac right to left shunt is around 25% in patients with pulmonary hypertension [45], ARDS [46], and positive pressure ventilation [47], while in liver cirrhosis its incidence can be as high as 70% [48]. The occurrence of such a shunt may also increase with the application of PEEP [49]. Color-Doppler and contrast echocardiography can be used to diagnose an intracardiac right to left shunt [50] but are not routinely used in patients with ARDS. However, a right to left shunt can be easily identified by simple observation of the thermodilution curve, since part of the cold indicator goes through the foramen ovale and reaches the arterial temperature sensor much more rapidly, creating a characteristic 'hump' in the curve (Fig. 2). The early diagnosis of such a shunt can have important therapeutic implications, such as the administration of inhaled nitric oxide (NO) [49] or reduction of the PEEP level [50]. The efficacy of these therapeutic maneuvers can be seen immediately by the disappearance of the two-phase morphology of the thermodilution curve.

Last but not least, the transpulmonary thermodilution technique may help in the prediction of potential PEEP-induced hemodynamic instability. Although the application of PEEP may improve gas exchange, it can, nevertheless, also cause a decrease in the cardiac output, preventing the expected benefits of oxygen delivery. These cardiovascular adverse effects of the PEEP cannot usually be predicted by conventional static hemodynamic parameters. However, the presence of high PPV and SVV values has good correlation with the percentage of reduction in cardiac output in

response to the application of PEEP and is an indication for a very cautious application or increase in the PEEP level [50].

Conclusion

The transpulmonary thermodilution technique, which is the mainstay of the PiCCO monitoring system, constitutes a minimally invasive, simple and effective monitoring method, which offers integrated and comprehensive hemodynamic and pulmonary information. Such a combined monitoring of hemodynamic and pulmonary parameters is essential for decision-making and problem-solving in the care of the critically ill.

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Using Heart-Lung Interactions for Functional Hemodynamic Monitoring: Important Factors beyond Preload

J.C. Kubitz and D.A. Reuter

Introduction

The basic mechanism underlying functional preload indices, such as stroke volume variation (SVV), pulse pressure variation (PPV), or systolic pressure variation (SPV), is that mechanical ventilation induces cyclic alterations in ventricular filling and, in consequence, in stroke volume and cardiac output. This phenomenon is most easily recognized in clinical practice as periodical variations in the arterial pressure signal. Based on the understanding of the Frank-Starling-relationship, i.e., the relation of cardiac preload and stroke volume, the ventilation-synchronous variations of cardiac output, or the indices named above, which serve as surrogates, allow assessment of left ventricular (LV) filling, and, more importantly the evaluation of the steepness of the patient-individual LV function curve [1]. The usefulness of these functional preload indices in assessing cardiac preload and in predicting whether a patient will respond to fluid administration with an increase in cardiac output (fluid responsiveness) has been demonstrated in many studies.

In the last two decades, numerous investigations on heart-lung-interactions have not only led to the integration of these functional preload indices into clinical practice, but also to a better understanding of their interdependence from other physiological mechanisms besides preload. In addition, due to the complexity of heart-lung-interactions, it is obvious that functional preload indices must be influenced by other factors than cardiac preload, which may also probably limit, in specific clinical situations, their ability to assess preload and fluid responsiveness. Considering the physiology behind the functional preload indices, these factors can be grouped into respiratory issues and cardiovascular issues.

Respiratory issues

Tidal Volume

Augmenting tidal volumes increases lung inflation and, thereby, affects cardiac preload and afterload. It is predominantly the right ventricle that is affected. An increase in intrathoracic pressure, which is associated with an augmentation of tidal volumes impedes venous return, a) by decreasing the pressure gradient between the right atrium and the venous capacity vessels [2], and b) by compression of the vena cava due to an increased pleural pressure during inspiration [3]. Therefore, augmenting tidal volumes essentially results in a larger variation in venous return, right ventricular (RV) and, consequently, LV stroke volume during the respiratory cycle. Further, both hyperinflation and hypoinflation may increase RV afterload by differ-

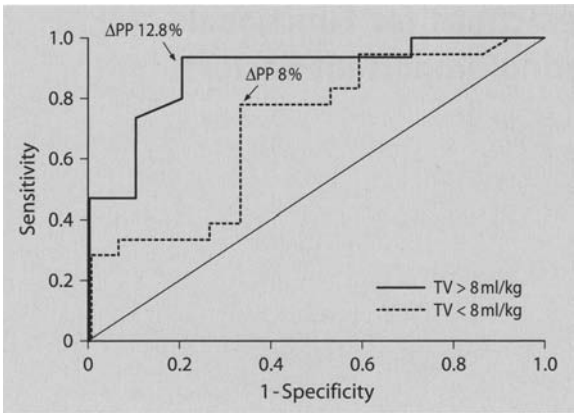

Fig. 1. Receiver operating characteristic (ROC) curve plots of pulse pressure variation (ΔPP) in patients ventilated with tidal volumes (TV) of at least 8 ml/kg (plain) and below 8 ml/kg (dashed). The best cut-off values for ΔPP were 12.8% for tidal volumes of at least 8 ml/kg and 8% below it. From [5] with permission.

ent mechanisms. Hyperinflation induces pulmonary hypertension by compressing alveolar and pulmonary vessels. Such hyperinflation may occur in only a few alveoli or in the whole lungs. Hypoinflation can increase RV afterload, too, by the well known hypoxic pulmonary vasoconstriction. LV preload, which is what we estimate with the functional preload indices, depends on RV output and the factors influencing this output as described. LV afterload, too, is altered by an increase in intrathoracic pressure. LV afterload is, in contrast to RV afterload, decreased by a reduced systolic transmural pressure.

The influence of tidal volume on functional preload indices (SVV, PPV) has been a point of ongoing discussion. An increase in tidal volume necessarily increases the change in intrathoracic pressure during the respiratory cycle and should, thereby, increase functional preload indices such as PPV and SVV. In cardiac surgery patients, it was shown that both PPV and SVV increased with tidal volumes from 5 to 15 ml/kg body weight both before and after fluid loading [4]. This increase in PPV and SVV correctly reflects fluid responsiveness, as venous return and LV filling decrease with augmentation of tidal volume, but the intravascular volume status may not change. However, at low tidal volumes, the change in intrathoracic pressure during the respiratory cycle may be too small, so that PPV and SVV may loose their usefulness as markers of fluid responsiveness, as reported by De Backer and colleagues for tidal volumes <8 ml/kg of body weight (Fig. 1) [5].

Positive End-expiratory Pressure

Whereas augmenting tidal volumes increases the degree of change in intrathoracic pressure during the respiratory cycle, the application of positive end-expiratory pressure (PEEP) constantly increases pleural pressure and intrathoracic pressure. Thus, the ventilation-induced cyclic changes in intrathoracic pressure occur at a higher pressure level. The increase in intrathoracic pressure following the application of PEEP reduces venous return, thereby ventricular filling and, consequently, cardiac output, and this not in a cyclic fashion but constantly. Further, PEEP increases transpulmonary pressure resulting in an increased RV afterload. Both effects lead to a reduced LV filling (i.e., also to an increased fluid responsiveness of the left ventricle) with a concomitant increase in functional preload indices. In consequence, the application of PEEP results in a decrease in cardiac output in the

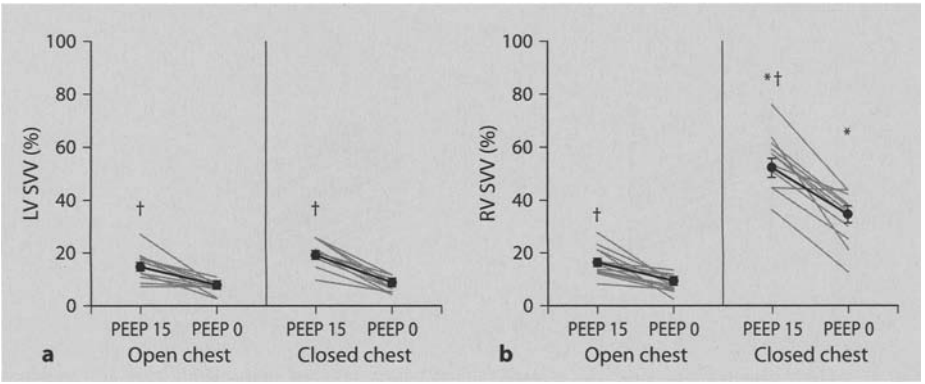

Fig. 2. Left and right ventricular stroke volume variation (SVV) during open and closed chest conditions. **a** Left ventricular SVV (LV SVV) (14 animals); **b** Right ventricular SVV (RV SVV) (12 animals). Thin lines: individual changes in SVV during ventilation without positive end-expiratory pressure (PEEP) and with PEEP 15 cmH_2O. Thick line, dots and error bars: mean value ± SEM. *$p < 0.05$, vs. open chest, same PEEP level. †$p < 0.05$, vs. no PEEP. From [9] with permission

majority of mechanically ventilated patients, except for patients with LV backward failure. However, in the absence of a decrease in cardiac output following application of PEEP, functional preload indices (SVV, PPV, SPV) will also not be affected [6, 7]. Moreover, as was shown by Michard and colleagues, PPV measured prior to the application of PEEP was strongly correlated with the reduction in cardiac index induced by this application of PEEP. Thus, these functional indices of preload may also serve as a useful tool for predicting the hemodynamic effects of PEEP [8]. In accordance with those data, it has also recently been demonstrated in an animal model that PEEP increases LV SVV, and that this effect is found during open as well as during closed chest conditions [9] (Fig. 2).

Chest and Lung Compliance

The change in intrathoracic pressure caused by a mechanical breath is dependent on chest compliance. If chest compliance is high, a given tidal volume will result in a minor change in intrathoracic pressure and, consequently, in ventricular filling as if chest compliance is low. In animals, a decrease in chest compliance induced by a pneumoperitoneum has been shown to increase SPV [10]. A more profound change in chest compliance occurs with a sternotomy; the opening of the thoracic cavity increases chest compliance tremendously. Both, in animals [9] and in patients [11] it could be shown that sternotomy led to an increase in cardiac output and a concomitant decrease in SVV, indicating an augmented ventricular filling and thus, a higher preload during open-chest conditions (Fig. 3).

Lung compliance is the important determinant for the transmission of alveolar pressure to the pleural space. The higher the compliance, the more pressure is transmitted from the alveoli to the pleural space [12]. If we apply this information at a given intravascular volume status on functional preload indices, these indices will theoretically be higher in healthy than in damaged lungs for the same alveolar pressure. This is because in the healthy lung more pressure is transmitted to the pleural space resulting in higher pleural pressures and a stronger decrease in venous return

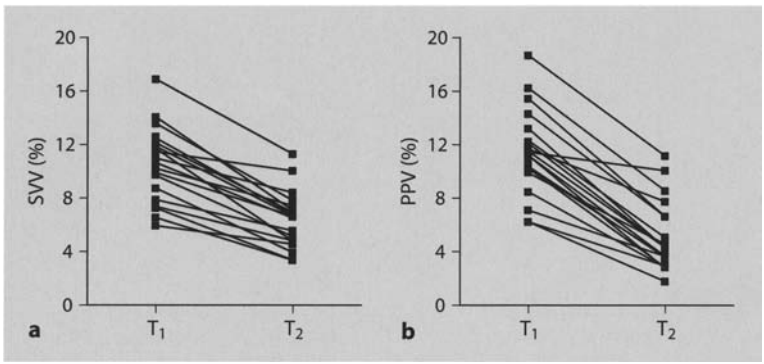

Fig. 3. a Stroke volume variation (SVV) of each patient before (T1) and immediately after (T2) mid-line thoracotomy and pericardiotomy. **b** Aortic pulse pressure variation (PPV) of each patient before (T1) and immediately after (T2) mid-line thoracotomy and pericardiotomy. From [11] with permission

to the right ventricle. On the other hand, higher airway pressures are frequently needed to ventilate patients with reduced lung compliance, such as patients with acute respiratory distress syndrome (ARDS), so that pleural pressure will theoretically not be much different from normal lungs. Unfortunately, so far there are no experimental or clincial data describing sufficiently the relationship between lung compliance and the variation in functional preload indices.

Cardiovascular Issues

Heart Rate and Rhythm

Heart rate and heart rhythm have an influnece on ventilation-induced heart-lung interactions. A decrease in heart rate, or a lower ratio of heart beats to respiratory cycles, seem to reduce the hemodynamic consequences of heart-lung interactions; in mechanically ventilated patients receiving esmolol, for example, administration of the beta-blocker led to a reduction in the respiratory-related arterial pressure variability and the systolic pressure variation [13] (Fig. 4). Beta-blockers seem to suppress the autonomic, sympathetic response to lung inflation which usually causes cardiac acceleration (respiratory sinus arrhythmia). It has to be noted that massive hyperinflation will reduce heart rate due to high intrathoracic pressures, as known from alveolar recruitment maneuvers.

Cardiac arrhythmias affect both systolic and diastolic ventricular function and stroke volume. Depending on the nature of the arrhythmia, it will be the predominant factor determining stroke volume variation. In patients with atrial fibrillation and frequent extrasystoles, the functional preload indices will no longer reflect ventilatory-induced changes in ventricular filling. PPV has recently been reported to become a poor predictor of preload responsiveness in mechanically ventilated patients with severe arrhythmias [14]. However, so far we have insufficient data to finally decide at which grade of arrhythmia functional preload indices can no longer be used to predict fluid responsiveness.

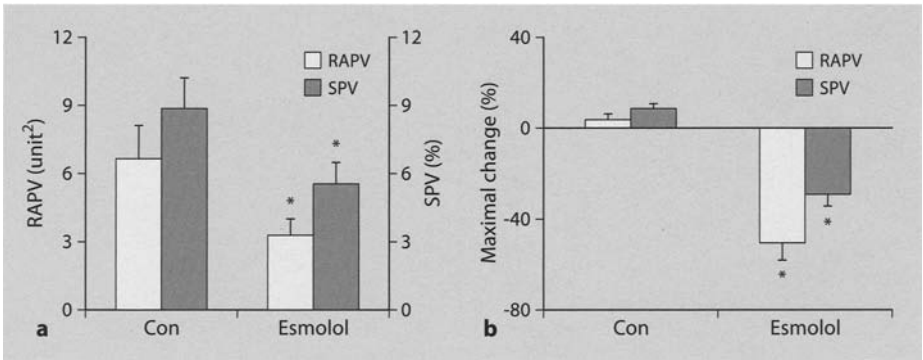

Fig. 4. Mean value (**a**) and maximal change (**b**) of respiratory-related arterial pressure variation (RAPV) and percentage systolic pressure variation (%SPV) in response to control and esmolol. Values are mean ± SEM. *$p < 0.05$ compared with saline control (Con), as determined using Student's t test ($n = 10$). From [13] with permission

Ventricular Function

The influence of ventricular dysfunction or failure on functional preload indices is different for the right and the left ventricle. In right heart failure, the right ventricle is not able to provide a sufficient output for adequate LV filling. This may be due to impaired RV contractility or increased RV afterload, which can be aggravated by the cyclic increase in transpulmonary pressure during mechanical ventilation. In the case of RV overload, LV end-diastolic volume (LVEDV) is reduced not only because of a reduced RV output but also due to a leftward shift of the interventricular septum. The LVEDV will then, at the same end-diastolic pressure, be smaller than prior to the septal shift. Further, the shape of the left ventricle may be distorted due to the septal shift resulting in reduced LV end-diastolic compliance and contractility [15]. Therefore, in a situation of RV failure with RV overloading, we would expect a large variation in parameters derived from the arterial pressure wave (SPV, PPV, SVV), while the heart is not responsive to fluid administration. This was recently clearly described by Jardin [16].

Functional preload indices, such as PPV, SPV, or pulse contour SVV, describe the steepness of the LV function curve. In comparison to a non-compromised left ventricle, the ventricular function curve of the failing left ventricle is flat. Thus, the same changes in LVEDV induced by mechanical ventilation will result in smaller LV SVVs in the failing heart compared to the non-compromised heart. However, in the presence of isolated LV dysfunction, i.e., which is not accompanied by RV failure, these functional parameters should also allow LV fluid responsiveness to be assessed. This was demonstrated in patients undergoing cardiac surgery with documented impaired LV function (ejection fraction < 0.35), where a high SVV was associated with a positive response to fluid loading. Thus, in these patients also, functional indices of preload seem to be a valuable tool to assess fluid responsiveness [17] (Fig. 5). However, in this context it seems important to differentiate whether the LV failure is a global or a regional myocardial failure. In the presence of regional myocardial failure, as, for example, in acute regional myocardial ischemia, regionally confined dyskinesias will attribute a different variation in LV stroke volume from one respiratory cycle to another. However, experimental or clinical data on this issue are lacking.

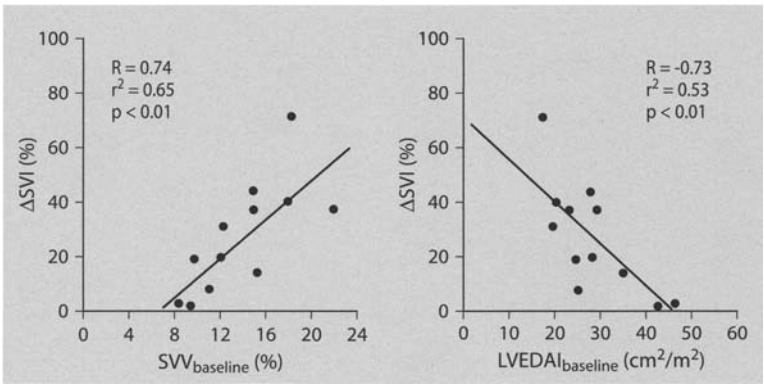

Fig. 5. Linear correlation analysis of the relation between changes in preload variables stroke volume variation (ΔSVV; left panel) and left ventricular end-diastolic area index (ΔLVEDAI; right panel) caused by volume loading and the associated changes in stroke volume index (ΔSVI). From [17] with permission

Cardiac Afterload

Cardiac afterload changes dynamically during ventricular ejection. In normal hearts, it is maximum, in terms of maximal LV wall tension, at the end of isovolumetric contraction. In patients with LV overload, as in cardiac backward failure, the maximal wall stress occurs during LV ejection, as ejection pressure increases during systole while LV volume approaches normal values [18]. Therefore, in such patients, stroke volume is theoretically more sensitive to the arterial pressure than in healthy patients and we would expect the functional preload indices to decrease if cardiac afterload increases. However, in dogs with normal cardiac function and hemorrhagic shock, the application of the vasopressor, norepinephrine, led to a decrease in PPV and SPV (Fig. 6) [19]. In this study, however, the time interval between the

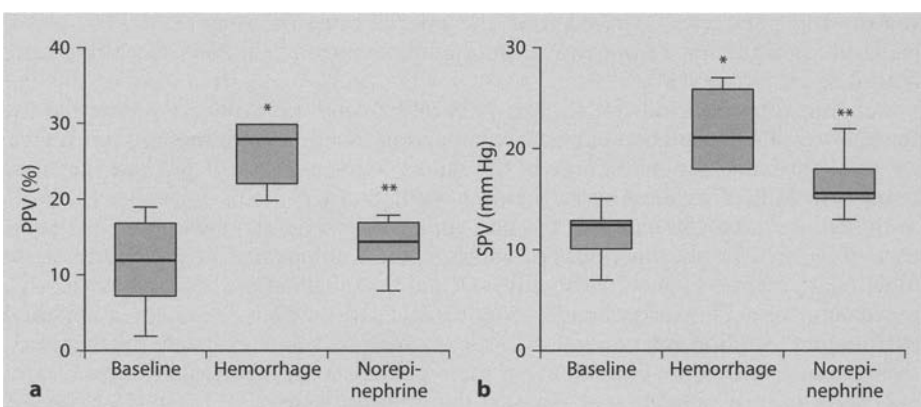

Fig. 6. Box plots showing changes in comparison with baseline in pulse pressure variation (PPV, **a**) and arterial systolic pressure variation (SPV, **b**) following hemorrhage and treatment with norepinephrine. The line in each box indicates the median. The upper and lower limits of each box indicate the 75th and 25th percentiles, respectively. The error bars above and below each box represent the 90th and 10th percentiles, respectively. *$p < 0.05$ vs. baseline; **$p < 0.05$ vs. hemorrhage. From [19] with permission

measurements without and with norepinephrine was very long. Further the positive inotropic effects of norepinephrine were not discussed. Therefore, the actual influence of an increase in the vasomotor tone on functional preload indices remains incompletely understood. However, in patients with sepsis-induced hypotension and, therefore, reduced vasomotor tone, the delta down component of SPV allowed accurate assessment of fluid responsiveness [20].

Arterial Compliance

The relationship between changes in stroke volume and changes in arterial pressure is dependent on arterial compliance. Clinical determination of arterial compliance is difficult at the present time. One proposed method is the stroke volume-to-aortic-pulse-pressure ratio (SV/PP) [21]. If arterial compliance is low, small changes in LV stroke volume will result in large changes in arterial pressure. *Vice versa*, if arterial compliance is high, large alterations in LV stroke volume will only cause minor changes in arterial pressure [7]. One would, therefore, assume that measuring LV SVV, for example, by pulse contour analysis would be superior to SPV and PPV. However, there are so far no data to confirm this hypothesis. PPV, SPV [21], and LV SVV [23] have all been reported to be valuable tools for guiding fluid therapy in septic patients, in which arterial compliance is probably altered in the course of the disease. However, whether one parameter is superior to the others has still not been conclusively determined.

Conclusion and Perspective

The interaction of mechanical ventilation and LV function is complex. Both ventilatory issues – tidal volume, PEEP, chest and lung compliance – and cardiovascular issues – heart rate and rhythm, ventricular function, cardiac afterload, arterial compliance – may affect functional preload indices. How these factors influence these indices has to be known for correct interpretation of the values derived from the arterial pressure signal and real-time continuous cardiac output devices.

In clinical situations, in which the confounding factors described above play more than a subordinate role in the generation of the functional preload indices, as, for example, during weaning from mechanical ventilation, we are still looking for the 'perfect' method to predict the hemodynamic reaction to fluid administration. Such alternatives might be, at least in part, volumetric parameters of preload [24]. The measurement of changes in aortic blood flow following a passive leg raising maneuver, which has been reported to have a high sensitivity and specificity in a critically ill population including patients with spontaneous respiratory efforts and arrhythmias [22], may be of particular interest. In addition, the recently proposed systolic variation test [25, 26] may in the future provide further helpful information on fluid responsiveness. However, further data are necessary to confirm these first stimulating results.

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Diagnosis of Central Hypovolemia in a Spontaneously Breathing Patient

N. Airapetian, J. Maizel, and M. Slama

Introduction

Volemia is the total blood volume of the body (plasma and cells) and is normally situated in the range of 65 to 75 ml/kg. Hypovolemia is a very frequent clinical situation in intensive care. Two types of hypovolemia are distinguished: Absolute and relative hypovolemia. Absolute hypovolemia is defined as a reduction in total circulating blood volume [1, 2], which may be related to blood loss (hemorrhage) or plasma loss (gastrointestinal, renal, cutaneous, extravasation into interstitial tissues). Relative hypovolemia is defined as an inadequate distribution of blood volume between the central and peripheral compartments (venodilatation or during positive pressure ventilation).

Absolute and relative hypovolemia result in a reduction in systemic venous return, causing a reduction in the stroke volume responsible for decreased cardiac output [3, 4], as the Frank-Starling principle defines a relationship between an increase in stroke volume and an increase in end-diastolic ventricular pressure (or ventricular preload) (Fig. 1). Hypovolemia is responsible for decreased left ventricular (LV) filling, leading to a reduction in preload and, therefore, a reduction in stroke volume according to the Frank-Starling relationship [3, 4].

The decreased cardiac output can be responsible, first, for orthostatic hypotension and then for permanent hypotension, shock, and, finally, multiple organ failure (MOF). The pathophysiological consequences of the decreased cardiac output secondary to hypovolemia are reduction of arterial oxygen delivery, a limited increase

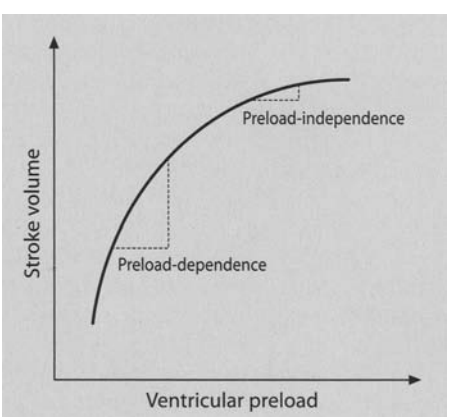


Fig. 1. Frank Starling relationship

in oxygen extraction, and switching of the cell to anaerobic metabolism when the critical oxygen delivery threshold is reached. This switching to anaerobic conditions results in the production of lactate and protons, with a risk of organ dysfunction [5]. The endothelium is the first target of tissue hypoperfusion resulting in a loss of its structural properties responsible for capillary leakage and an alteration of its anticoagulant properties [6, 7].

The diagnosis of hypovolemia can be very easy in an obvious clinical and laboratory context (gastrointestinal losses, hemorrhage, signs of extracellular dehydration, low blood pressure, low venous pressure, high serum proteins, high hematocrit). However, the diagnosis is often difficult in the intensive care unit (ICU) where patients have several interrelated diseases (sepsis, heart disease, lung disease).

Blood volume can be evaluated by dilution techniques using an intravascular indicator, but these techniques are not routinely used in intensive care. However, cardiac, pulmonary or central blood volumes can be estimated or measured invasively (Swan-Ganz catheter, PICCO, etc.) [2] or by echocardiography [8–12]. The most important aspect for the intensive care physician is to determine whether LV preload is optimal, allowing the ventricle to maintain the largest possible stroke volume in order to optimize cardiac output and ensure adequate oxygen supply to the organs. Finally, the intensive care physician is faced with the following question: "Will volume expansion increase cardiac output in this patient?" Various indices able to predict, in a given situation, that volume expansion will increase cardiac output can guide the intensive care physician.

Predictive Indices of Fluid Responsiveness

Benefits and Risks of Volume Expansion

The expected benefit of volume expansion is an increase in venous return, an increase in stroke volume, cardiac output, blood pressure (systolic, mean, and pulse pressure), and an increase in tissue oxygen delivery. The rapidity with which these objectives are achieved during the management of hypovolemia constitutes a decisive prognostic element in terms of morbidity and mortality especially in septic shock [13].

Inappropriate fluid resuscitation that does not improve the patient's cardiac output exposes the patient to the inherent risks of volume expansion *per se*, independent of the type of solution used. The increase in hydrostatic pressure induced by volume expansion, by inducing transfer of fluid to the extravascular sector, can be responsible for pulmonary edema [14]. Fluid extravasation from the vascular sector to the interstitial sector predisposes to the development of diffuse peripheral edema that can compromise tissue oxygenation [15]. The other complications related to volume expansion are cerebral edema (especially in a context of neurological intensive care) [16], and disorders of serum sodium [17], serum potassium, and/or serum chloride [18]. In a context of uncontrolled hemorrhage, volume expansion can accentuate bleeding by increasing arterial or venous blood pressure [19]. Specific risks inherent to the type of fluid resuscitation solution are hyperglycemia in the case of glucose solution, and anemia and clotting disorders due to hemodilution. Blood-derived products carry a risk of transmission of infectious agents and colloid solutions can have allergic risks [20].

Volume expansion is a therapeutic procedure for which the indication must be considered in any situation of hemodynamic instability. Although the expected clini-

cal benefit of volume expansion varies according to the clinical setting, it is essentially secondary to the increased cardiac output induced by volume expansion. When cardiac output is not increased, there is a risk of all of the complications described above. It is, therefore, important to identify criteria predictive of the efficacy of volume expansion.

Clinical and Laboratory Indices

Certain clinical settings, such as shock secondary to traumatic lesions, are clearly indicative of the presence of hypovolemia. However, in intensive care, the clinical setting is usually not sufficient to reliably predict the benefit of volume expansion. In the absence of a clinical context highly suggestive of hypovolemia (such as shock with external bleeding), the presence of documented hypotension, tachycardia, or signs of tissue hypoperfusion are not sufficient to confirm the diagnosis of hypovolemia, as they are very non-specific. Conversely, the presence of satisfactory blood pressure or peripheral edema does not exclude a potential benefit of volume expansion [21, 22].

Modifications of laboratory parameters reflecting the metabolic and visceral consequences of the circulatory abnormalities induced by hypovolemia are usually observed. The abnormalities most commonly observed are functional renal insufficiency and hyperlactatemia. However, they are not absolutely specific and can be observed in all forms of shock. The simple finding of a high blood urea in an intensive care patient provides no information about its organic or functional origin. Similarly, hyperproteinemia or elevations in hemoglobin concentration or hematocrit are also very non-specific.

Static Hemodynamic Criteria Predictive of Fluid Responsiveness

A static criterion is a parameter measured under a single ventricular loading condition. A single determination of this parameter is expected to more or less reliably estimate the preload of one or both ventricles. This estimation of preload can be used to evaluate the probability of responsiveness to ventricular filling from Starling's law by estimating that the lower the preload the higher the probability of response to volume expansion.

Many static indices of ventricular preload have been developed and analyzed in the intensive care setting: Pulmonary artery occlusion pressure (PAOP), central venous pressure (CVP), right ventricular (RV) end-diastolic volume (RVEDV) measured by a rapid thermistance catheter [1, 2, 23]. Many other indices, such as LV end-diastolic diameter, surface area, or volume obtained by echocardiography; Doppler indices derived from mitral flow (E/A ratio), pulmonary venous flow, tissue Doppler (E/Ea ratio), color-coded Doppler (E/Vp ratio) have also been proposed [2, 24]. However, although these indices provide a measure of preload, the optimal preload to ensure the greatest stroke volume remains unknown. Furthermore, none of these indices has been shown to be sufficiently discriminative to differentiate patients likely to benefit from volume expansion from those unlikely to benefit from this treatment [1, 2, 23, 25].

Dynamic Criteria Predictive of Fluid Responsiveness

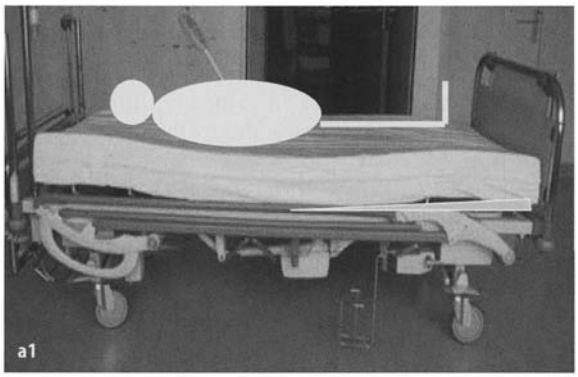
New, so-called 'dynamic' indices have been introduced based on animal and clinical studies [8–10, 12, 26–32]. They are essentially indicated in critically ill, intubated, and ventilated intensive care patients. These criteria are described as dynamic because, by plotting two points on the Starling curve, they try to determine whether the patient is situated on the ascending portion of this curve (where any variation of preload induces a variation of stroke volume or preload dependence situation) or on the plateau portion (where a variation of preload is not accompanied by variation of stroke volume or preload independence situation) (Fig. 1). Several approaches can be used to determine on what portion of the preload/stroke volume relationship the ventricle is functioning in order to establish the diagnosis of preload dependence or independence.

During mechanical ventilation, variations in intrathoracic pressures induced by insufflation decrease the biventricular preload and decrease the stroke volume when the ventricles are functioning in the ascending portion of the Frank-Starling relationship [25]. This is reflected by respiratory cyclic variations in stroke volume and, therefore, in the blood pressure curve during mechanical ventilation. The presence of these cyclic variations therefore indicates that any variation in preload would induce a variation in stroke volume, and that volume expansion would increase stroke volume and cardiac output (preload-dependence situation). On the other hand, the absence of these variations indicates that any variation in preload would not modify stroke volume and that consequently volume expansion would not induce a significant increase of cardiac output (preload-independence situation) (Fig. 1). Analysis of the respiratory variability of LV stroke volume during mechanical ventilation, therefore, provides a dynamic, biventricular evaluation of preload-dependence. The respiratory variability in stroke volume can be estimated by invasive or non-invasive methods: Respiratory variability in systolic blood pressure or pulse pressure using an arterial catheter, the pre-ejection period by esophageal Doppler, or aortic flow by transesophageal or transthoracic echocardiography [8–10, 12, 26–32].

A fluid challenge test can also define two points on the Starling curve to distinguish 'responders' from 'non-responders'. Although this test remains one of the most widely used diagnostic tests, it carries a risk of pulmonary edema [33].

Recent publications have proposed the passive leg raising test as an alternative to the fluid challenge test to predict preload-dependence [33, 34] (Fig. 2). This maneuver mobilizes about 300 ml of blood from the lower limbs to the intrathoracic compartment and reproduces the effects of volume expansion. It is reversible and devoid of any risks of volume expansion. This test usually consists of raising both legs to an angle of 45° in relation to the bed [33, 34].

Most studies performed on mechanically ventilated patients have demonstrated the superiority of dynamic indices over static indices to predict fluid responsiveness.



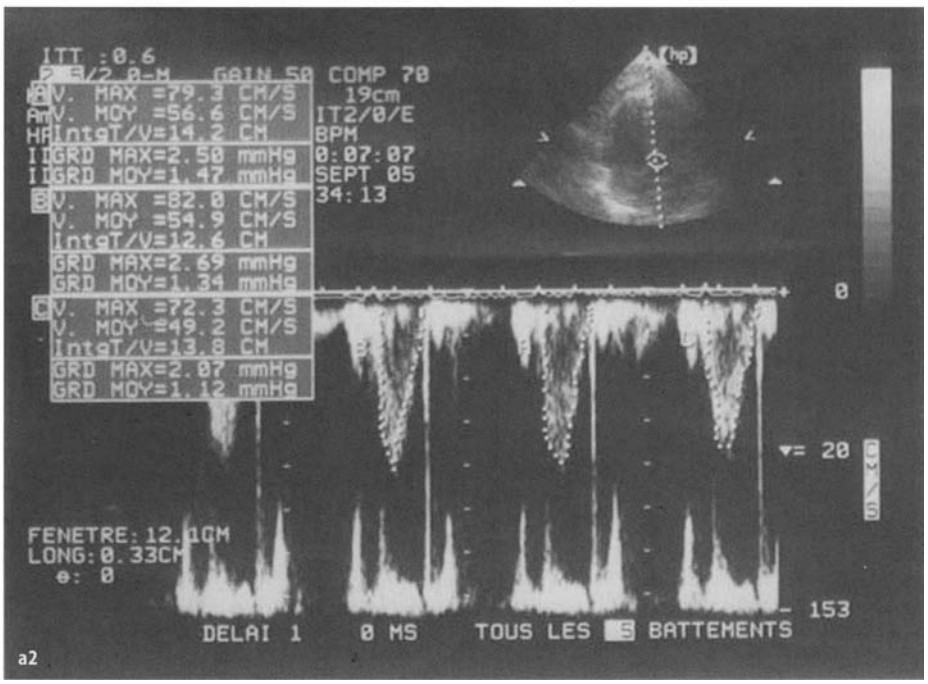
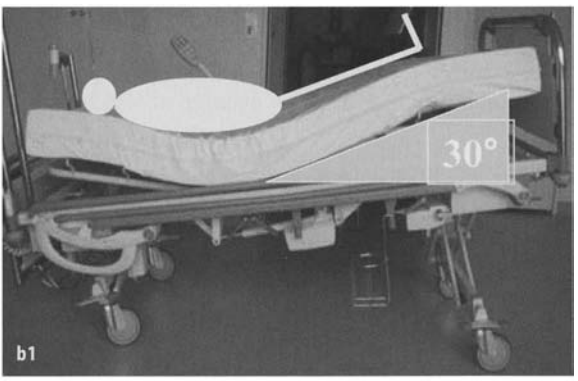
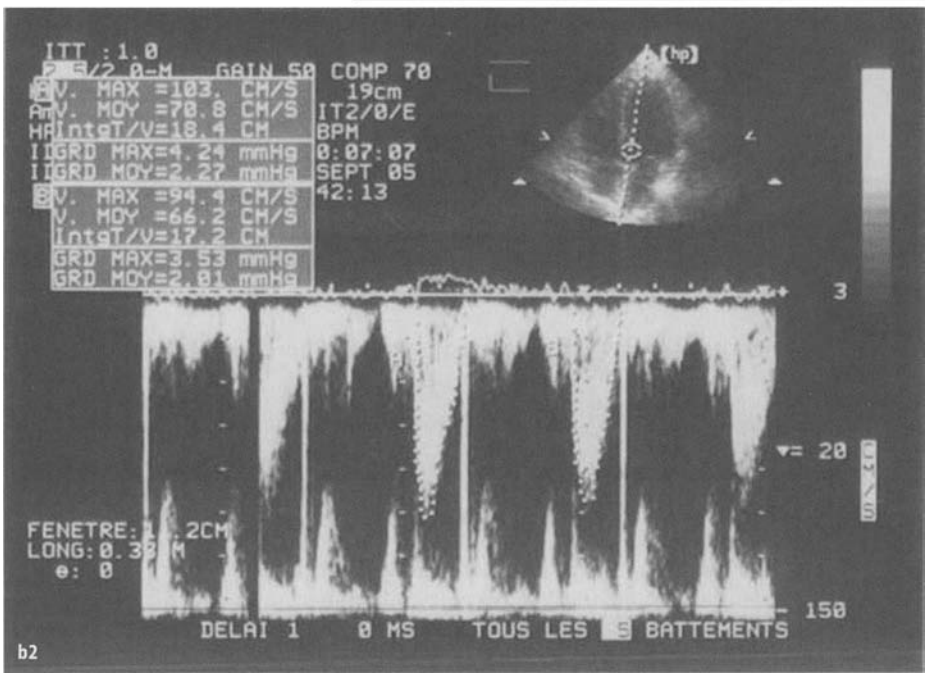


Fig. 2. a1, b1 Leg raising. **a2, b2** Aortic blood flow recorded using pulsed Doppler at baseline (**a2**) and during passive leg raising (**b2**).





Predictive Indices of Fluid Responsiveness in Spontaneously Breathing Patients

Few studies have evaluated fluid responsiveness in spontaneously breathing patients without mechanical assistance. Most predictive indices of fluid responsiveness (static and dynamic) have been validated on sedated, mechanically ventilated patients [8, 9, 12, 26–29, 32, 33, 35, 36]. In 2002, Michard and Teboul [2] found twelve studies on predictive indices of fluid responsiveness that had been published since 1966. Most patients were mechanically ventilated (84%) and the overall fluid responsiveness rate was 52%.

In 2005, Coudray et al. [1] reviewed eight prospective studies and three review articles studying predictive indices of fluid responsiveness in spontaneously breathing

patients. One study was conducted on 44 healthy subjects [37]. Most of these studies used the thermodilution technique to measure cardiac output and stroke volume. Most published studies were conducted on mixed populations comprising spontaneously breathing patients (an average of 37.5%) and mechanically ventilated patients.

Heart-lung Interaction

The pathophysiology of cardiorespiratory interactions has been less extensively studied in spontaneously breathing patients. In sedated, ventilated patients, during insufflation, the increased intrathoracic pressure and transpulmonary pressure (alveolar pressure – pleural pressure) result in a reduction in the preload and an increase in RV afterload. These two phenomena lead to a reduction in RV stroke volume at the end of insufflation. This reduction is responsible, 2 to 3 cardiac cycles later (pulmonary transit time), for a reduction in LV preload and LV stroke volume during exsufflation. These phenomena are even more marked when the ventricles are in a preload-dependence situation [25].

On the other hand, in spontaneously breathing patients, on inspiration, the reduction in intrathoracic pressure and the increase in transpulmonary pressure are responsible for an increase in RV preload and a more moderate increase in RV afterload, respectively. The increase in the RV stroke volume at the end of inspiration, 2 to 3 cardiac cycles later, results in an expiratory increase in LV preload and stroke volume. These phenomena are even more marked when the ventricles are in a preload-dependence situation [1, 25].

Static Predictive Indices of Fluid Responsiveness in Spontaneously Breathing Patients

In a study by Kumar et al. [37] on healthy subjects, static indices of ventricular preload (CVP, PAOP, diastolic blood pressure [DBP], LVEDVI, RVEDVI), and cardiac performance indices (cardiac index, stroke volume index) were measured before and after 3 liters of normal saline loading. This study demonstrated the absence of a correlation between changes in CVP ($r=0.22$, $p=0.49$), PAOP ($r=0.29$, $p=0.26$) and variations in cardiac performance indices (cardiac index, stroke volume index). Similarly, no correlation was observed between baseline measurements of static indices and variations in cardiac performance indices after fluid loading.

Most static indices have been studied on series predominantly composed of intubated patients. Wagner and Leatherman [38] and Schneider et al. [39] showed that DBP, measured by right heart catheterization, was significantly lower in the group of fluid responders. However, the majority of patients in these studies were mechanically ventilated (94% and 67%). Other studies comprising mechanically ventilated and spontaneously breathing patients failed to demonstrate the value of DBP in predicting fluid responsiveness [40, 41].

Similarly, Coudray et al. reviewed five studies on mixed populations of patients (mechanically ventilated and spontaneously breathing) and demonstrated the absence of a correlation between the initial PAOP and fluid responsiveness [1].

More recently, Heenen and colleagues [42], in a study on 21 patients with spontaneous breathing movements (9 subjects on mechanical ventilation and 12 with a face mask), assessed the value of various static (PAOP, DBP) and dynamic indices (pulse pressure variation [PPV], ΔDBP) to predict fluid responsiveness. Four patients received colloid volume expansion (500 ml) and the other 17 received crystalloids

(an average of 1 liter). The cardiac index was increased by more than 15% in 9 subjects (i.e., 43%). No correlation was demonstrated between PAOP ($r^2=0.15$; $p=0.08$) and DBP ($r^2=0.16$; $p=0.08$) measured at baseline and the variation of the cardiac index (ΔCI) and no correlation was observed between respiratory variations in right atrial pressure (RAP) and ΔCI. The authors compared the predictive values of the various indices (static and dynamic). The areas under the curve (AUCs) for static indices were significantly greater than those of dynamic indices (0.73 ± 0.13 for PAOP, $p<0.05$ versus PPV and 0.69 ± 0.12 for DBP, $p<0.05$ vs PPV). Despite this significant difference between AUCs, the value of static indices to predict fluid responsiveness was low due to the absence of a correlation between ΔCI and the values of static indices.

In spontaneously breathing patients, static indices are, therefore, poorly predictive of fluid responsiveness. However, most studies were performed either on populations predominantly composed of mechanically ventilated patients or on intubated subjects with spontaneous breathing movements.

Dynamic Predictive Indices of Fluid Responsiveness and Passive Leg Raising

Few published studies have evaluated dynamic indices in patients with spontaneous breathing movements. In 1999, Magder and Lagonidis [43] published a study based on 28 spontaneously breathing patients on the first postoperative day after cardiac surgery, several hours after extubation. All patients had a Swan-Ganz catheter in place. They were randomized to two groups to receive either 150–400 ml of saline or 100 ml of 25% albumin. The authors also tested whether respiratory variations in DBP were predictive of fluid responsiveness. A >1 mmHg reduction in DBP measured at the base of the 'a' wave was considered significant. Thirteen patients presented respiratory variations in DBP and the cardiac index was increased by more than 250 ml/min in ten of these patients. This increase in cardiac index was greater in the group receiving albumin.

In 2006, Heenen et al. [42] in the study already cited, unexpectedly demonstrated the superiority of static indices over dynamic indices to discriminate between responders and non-responders to volume expansion.

A fluid challenge test constitutes a widely used diagnostic test, which, when combined with measurement of stroke volume or cardiac output, is able to discriminate responders from non-responders. However, fluid challenge is associated with certain risks, particularly pulmonary edema. The passive leg raising test has been proposed as an alternative to fluid challenge (Fig. 2). Passive leg raising is a postural maneuver, which reversibly reproduces the effects of volume expansion [33, 34].

One of the first studies on passive leg raising was published in 2002. Boulain et al. [33] studied the hemodynamic effects of passive leg raising in 15 sedated and mechanically ventilated intensive care patients with acute circulatory insufficiency. In this study, passive leg raising induced a significant increase in PPV measured at the radial artery, PAOP, and stroke volume. The intensity of the effect of passive leg raising on stroke volume was correlated with the effect of passive leg raising on pulse pressure ($r=0.77$; $p<0.001$). Volume expansion was performed with 300 ml of macromolecular solution over 20 minutes. The effects of volume expansion on stroke volume were correlated with those of passive leg raising on pulse pressure ($r=0.84$; $p<0.001$).

In another study, Monnet et al. [34] showed that when passive leg raising induced an increase in aortic flow of more than 10%, it was predictive of an increase in aor-

tic flow of more than 15% in response to volume expansion (sensitivity 97%, specificity 94%). The patients included in this study presented signs of acute circulatory insufficiency (hypotension, tachycardia, oliguria, mottled skin). Volume expansion was performed with 500 ml of isotonic saline over 10 minutes. Esophageal Doppler was used to measure aortic flow. Thirty-seven (52%) of the 71 patients included in this study responded to volume expansion. In this study, 22 subjects had spontaneous breathing movements (spontaneous breathing mode with inspiratory assistance). The authors showed that respiratory cyclic variations in pulse pressure of $\geq 12\%$ in response to the passive leg raising test were predictive of an increase in aortic flow by more than 15% in response to volume expansion (sensitivity 88% and specificity 93%).

Conclusion

Most studies performed on sedated and mechanically ventilated patients have demonstrated the superiority of dynamic indices in predicting fluid responsiveness. However, very few published studies have been conducted in spontaneously breathing patients (not intubated, not ventilated), and most studies were performed on intubated subjects with spontaneous breathing movements. The results of these studies showed a potential value of dynamic indices. However, validation of these indices requires further studies, comprising larger patient populations and comparative studies with static indices.

The passive leg raising test and the growing use of echocardiography in intensive care are two interesting fields of investigation in order to define a reliable index to predict fluid responsiveness.

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Assessment of Fluid Responsiveness in Spontaneously Breathing Patients

J.-L. Teboul, B. Lamia, and X. Monnet

Introduction

Assessment of volume responsiveness is an important issue in patients with spontaneous breathing activity. The difficulty in predicting the response to fluid infusion in this population of patients is variable and depends on the clinical situation. Three different scenarios must be distinguished:

- The first scenario refers to patients admitted to the emergency room for evident acute blood losses or body fluid losses. The diagnosis of hypovolemia is almost certain and the presence of clinical signs of hemodynamic instability (hypotension, tachycardia, oliguria, mottled skin, altered mental status, etc) strongly suggests that a positive hemodynamic response to volume resuscitation will occur, although these signs lack sensitivity. The degree of hypotension, of tachycardia, and of oliguria is important for estimating the degree of hypovolemia and hence the degree of urgency for initiating volume resuscitation.
- The second scenario refers to patients admitted to the emergency room with a high degree of suspicion of septic shock. In this situation, cardiac preload is always inadequate since relative as well as absolute hypovolemia are always present in the early phase of septic shock. The study by Rivers et al. [1] emphasized the importance of volume resuscitation in the first hours of management in this category of patients. There is no need to search for sophisticated parameters to predict volume responsiveness since a positive hemodynamic response is always present at this stage. Rather, there is a need to define parameters that can indicate whether volume infusion should be either continued or stopped because of no further expected efficacy (see the third scenario). There is also a need to define indicators of lung intolerance; however, this is not the subject of the present chapter.
- The third scenario refers to patients hospitalized in the intensive care unit (ICU) who experience hemodynamic instability that requires urgent therapy. In these patients, volume responsiveness is not guaranteed since they have already been volume resuscitated and continuation of volume infusion carries risks of pulmonary edema. In spontaneously breathing patients either without an endotracheal tube or making inspiratory efforts while receiving mechanical ventilation, prediction of volume responsiveness can be a difficult challenge. In these conditions, indices of volume responsiveness that use heart-lung interaction, such as respiratory variations in arterial pressure or in stroke volume and derived indices, are no longer reliable.

Static Markers of Cardiac Preload as Predictors of Volume Responsiveness

From the Frank-Starling relationship (ventricular preload vs stroke volume), the response to volume infusion is more likely to occur when the ventricular preload is low than when it is high. Hence, markers of ventricular preload have been suggested for predicting volume responsiveness. This issue has been extensively discussed in a recent review article by Coudray et al. [2]. Therefore, in the present chapter, we will only give a summary of the main parameters.

Cardiac Filling Pressures

Historically, ventricular filling pressures, namely central venous pressure (CVP) or right atrial pressure (RAP) for the right ventricle, and pulmonary artery occlusion pressure (PAOP) for the left ventricle, have been first proposed as parameters to guide volume resuscitation.

Central venous pressure and right atrial pressure

These two parameters are assumed to reflect the right ventricular (RV) filling pressure. A few studies have addressed the question of whether CVP or RAP can predict volume responsiveness in critically ill patients [3, 4]. In most of these studies, a limited number of patients with spontaneous breathing activity were included. In two studies [5, 6], RAP was lower before volume infusion in patients who responded to fluid infusion (in terms of an increase in cardiac output) than in non-responders. However, a small percentage of patients were studied while breathing spontaneously (6% and 33%, respectively). In addition, the study by Wagner and Leatherman [6], reported a weak correlation between pre-infusion RAP and changes in stroke volume induced by fluid infusion. For example, a fluid-induced increase in stroke volume by 25% was observed for a pre-infusion RAP value of 2 mmHg as well as for a value of 13 mmHg [6]. Conversely, a RAP value of 11 mmHg was associated with a fluid-induced increase in stroke volume ranging from 10 to 45% [6]. In other studies including patients with spontaneous breathing activity, there was no relationship between the initial RAP and the response to volume infusion [7–11]. All these studies, therefore, suggest that RAP is a poor predictor of volume responsiveness for the general population of critically ill patients including those who exhibit spontaneous breathing activity, although the total number of patients included in the available studies is quite low.

Pulmonary artery occlusion pressure

The PAOP is the pressure obtained after inflating the distal balloon of a pulmonary artery catheter in a large branch of the pulmonary artery. Since a static column is created between the inflated balloon and the venous site where the blood flow resumes, PAOP is assumed to reflect the pressure in a large pulmonary vein and, thus, the left atrial pressure and eventually the left ventricular (LV) end diastolic pressure (LVEDP) [12]. Obtaining a meaningful measurement can be a difficult challenge in dyspneic patients who experience large swings in intrathoracic pressure. In order to limit the influence of the intrathoracic pressure on PAOP measurements, it is recommended that PAOP be measured at the end-expiratory period. However, in patients receiving mechanical ventilation who exhibit inspiratory efforts, the end-expiratory period is not always easy to identify [13].

Provided that reliable measurements are obtained, PAOP is considered as a measure of LV preload, although preload is assumed to fit better with end-diastolic volume than with end-diastolic pressure of the ventricle [12]. A few studies have addressed the question of whether PAOP can predict volume responsiveness in critically ill patients [3, 4]. Six studies, in which 16% [14], 24% [8], 33% [5], 36% [9], 54% [7], and 57% [10] of the patients were breathing spontaneously, showed no relationship between the pre-infusion PAOP and the hemodynamic response to fluid. In one study, in which just 6% the patients were breathing spontaneously, the pre-infusion PAOP value was lower in responders but a weak correlation was found between PAOP and the increase in stroke volume induced by volume infusion ($r^2=0.33$); no cut-off value was found to discriminate responders from non-responders [6]. Finally, in one study, the pre-infusion PAOP value was surprisingly higher in the patients who subsequently responded to fluid infusion [15]. No clear explanation was given by the authors of this study, which included only four patients who were breathing spontaneously [15].

In summary, there is no evidence that the pre-infusion PAOP can predict volume responsiveness in critically ill patients, although the total number of patients with spontaneous breathing activity included in the available studies is less than 80.

End-diastolic Ventricular Dimensions

Since ventricular end-diastolic dimensions are considered as better indicators of cardiac preload than filling pressures, measurements of ventricular dimensions have also been proposed for predicting fluid responsiveness.

Right ventricular end-diastolic volume

The RV end-diastolic volume (RVEDV) can be evaluated at the bedside by fast response pulmonary artery catheters. In two studies, in which 16% [14] and 31% [15] of patients were breathing spontaneously, Diebel et al. reported lower values of RVEDV index (RVEDVI) in responder than in non-responder patients and suggested that a beneficial hemodynamic effect of volume expansion was likely when the RVEDVI was below 90 ml/m^2 and very unlikely when the RVEDVI was greater than 138 ml/m^2. However, when the RVEDVI ranged between 90 and 138 ml/m^2, which is probably the most frequent occurrence in critically ill patients who are already resuscitated, no cut-off value could be proposed to discriminate responder and non-responder patients [14, 15]. In two other studies, in which 6% and 24% of patients were breathing spontaneously, no significant difference was observed between responders and non-responders with respect to the baseline value of the RVEDVI [6, 8].

In two older studies, including 54% [7] and 33% [5] of patients breathing spontaneously, RVEDV was calculated after assessing the RV ejection fraction (RVEF) using cardiac scintigraphy and after measuring cardiac output using thermodilution. In these studies, no significant difference was observed between responders and non-responders with respect to the pre-infusion value of RVEDV [5, 7].

Left ventricular end-diastolic dimensions

Cardiac scintigraphy and echocardiography are used to estimate LV end-diastolic volume (LVEDV) and LV end-diastolic area (LVEDA). Two old studies including patients with spontaneous breathing indicated that LVEDV was of poor value for predicting volume responsiveness [5, 7]. To our knowledge, no study has examined

the significance of LVEDV or LVEDA obtained with echocardiography in patients who breathe spontaneously. It should be remembered that in deeply sedated patients receiving mechanical ventilation, the LVEDA was reported to be unreliable for assessing volume responsiveness [16–19].

Global end-diastolic volume

The global end-diastolic volume (GEDV) is obtained by the transpulmonary thermodilution method (PiCCO monitoring system). Regarding the prediction of volume responsiveness, no study using this parameter has been performed in patients breathing spontaneously. It must be remembered that in patients receiving mechanical ventilation, the GEDV was lower in volume responders than in non-responders and that the lower the pre-infusion GEDV, the more likely the positive hemodynamic response [20]. However, GEDV values in responders and non-responders overlapped and no cut-off value could identify responders and non-responders with high sensitivity and specificity values [20].

Why do Static Markers of Preload Fail to Predict Volume Responsiveness?

Markers of preload are not always accurate measures of cardiac preload

This is particularly the case in the following situations:

- In the presence of mitral stenosis or mitral insufficiency, PAOP can be higher than the LVEDP. The value measured just before the 'v' wave upslope must be taken as an estimate of LVEDP in the case of mitral insufficiency.
- In the presence of intrinsic positive end-expiratory pressure (PEEP), PAOP differs from LV filling pressure, even when the measurement is made during the end-expiratory period [21].
- Even when PAOP reflects the LV filling pressure, it can still be a poor marker of LV preload in the case of reduced LV compliance where achieving high filling pressures can be necessary to maintain optimal levels of LV preload [22].
- In the presence of tricuspid regurgitation, thermodilution RVEDV measurements can be erroneous [23].

Assessment of preload is not assessment of preload responsiveness

More generally, there is a physiological reason explaining why even the most accurate static marker of preload will never be a reliable predictor of preload-responsiveness. Indeed, the slope of the Frank-Starling curve depends on the systolic cardiac function. In this regard, a given value of preload can be associated with preload-dependence and hence with volume responsiveness in normal conditions (steep part of the Frank-Starling curve) or with preload-independence and hence with absence of volume responsiveness in the case of decreased cardiac contractility (flat part of the Frank-Starling curve).

Summary

Static markers of preload like CVP, RAP, PAOP, RVEDV, LVEDV, and GEDV are not accurate predictors of volume responsiveness in spontaneously breathing patients as they are in patients receiving mechanical ventilation without exhibiting inspiratory efforts [3, 4, 24]. Importantly, even if an accurate static measure of preload were available, it would not be possible to use it to reliably predict volume responsiveness. However, the following important points should be stressed: First, the available clini-

cal studies that have addressed the issue of volume responsiveness are scarce and did not include a lot of patients with spontaneous breathing activity. In addition, the specific behavior of those patients was not distinguished from the behavior of patients without spontaneous breathing activity. Therefore, the results of these studies must be interpreted with caution. Second, in the available studies, patient selection for fluid challenge and the method used to perform the fluid challenges (volume and type of fluid, duration of the trial, definition of fluid response) were quite heterogeneous. Third, in addition, in these studies, almost all the patients were studied after they had already been resuscitated, such that the values of preload markers were rarely low before the volume challenge was done. Thus, one cannot exclude that low values of CVP and/or PAOP and/or other markers of cardiac preload, potentially measured in non-resuscitated shocked patients, may still be associated with a positive hemodynamic response to volume loading. For example, in the study by Michard et al. [20] (in patients receiving mechanical ventilation), the rate of positive response to fluid was markedly higher (77%) in the subgroup of patients with low GEDV index values (<610 ml/m^2) compared to the subgroup of patients with intermediate GEDV index values (between 610 and 815 ml/m^2) and the subgroup of patients with high GEDV index values (>815 ml/m^2) (rates of response: 43% and 23%, respectively). Interestingly, the four patients with a GEDV index <500 ml/m^2 responded to volume loading while all five patients with a GEDV index >950 ml/m^2 did not respond to volume loading [20]. Similar findings with RAP as a static measure of preload have been recently reported by Heenen et al. [10]. On the other hand, in the available studies, volume challenges were rarely done in patients with high values of CVP and/or PAOP at baseline, mainly for ethical reasons. Thus, one cannot exclude that high values of markers of preload are associated with the absence of a hemodynamic response to volume loading. For example, in the study by Michard et al. [20], the percentage of positive response to fluid was very low in the subgroup of patients with high GEDV index values (>850 ml/m^2). Similarly, in the study by Heenen et al., high values of pre-infusion RAP were associated with a low rate of positive response to volume administration [10]. Although the markers of preload do not appear to be good predictors of volume responsiveness, most available studies have shown that these markers did increase after volume challenge in responders as well as in non-responders. This finding suggests that these static indexes are valuable tools to confirm that the volume infused actually reaches the cardiac chambers, and, therefore, that these indexes do inform about changes in cardiac preload [2].

Dynamic Parameters

Arterial Pressure and Stroke Volume Respiratory Variation

Numerous studies have demonstrated that dynamic indexes, such as respiratory variation in arterial pressure and in stroke volume, are valuable for predicting volume responsiveness in patients receiving mechanical ventilation, provided that they do not experience any active breathing efforts and/or cardiac arrhythmias and that they do not receive too low a tidal volume [3, 4]. Therefore, the findings of these studies cannot be extrapolated to patients receiving mechanical ventilation while exhibiting inspiratory efforts or to patients who breathe spontaneously without any mechanical support. To our knowledge, only three studies have investigated whether or not arterial pulse pressure variation (PPV) was predictive of volume responsiveness in such

groups of patients. In a study by Monnet et al, a PPV $\geq$ 12% predicted volume responsiveness with a sensitivity of 88% and a specificity of 93% in the subgroup of deeply sedated patients receiving mechanical ventilation [25]. By contrast, PPV had no predictive value in the subgroup of patients with ventilator triggering [25]. This latter important conclusion has been recently confirmed in the study by Heenen et al. [10]. In a study that included non-intubated patients, a PPV value >12% was shown to reliably predict a beneficial response to volume infusion in the case of quiet spontaneous breathing [11]. However, that study also showed that PPV was not valuable for predicting volume responsiveness when its baseline value was less than 12% and/or when patients were dyspneic [11].

Inspiratory Decrease in Right Atrial Pressure

During a normal spontaneous inspiration, the intrathoracic pressure decreases and this will eventually result in a decrease in RAP relative to the extrathoracic vessel compartment and, thus, in an increased venous return to the left atrium and an increased RV stroke volume if the right ventricle is preload-dependent. As a result, an increase in LV stroke volume will be expected with a time delay of a few heart beats, provided that the left ventricle is also preload-dependent. Magder et al. [9, 26] hypothesized that when the right ventricle is preload-independent, not only will the right and subsequently the left stroke volume not increase with volume loading, but the RAP will also not decrease during normal inspiration. In their first study, the authors included a heterogeneous population of patients: 36% of them experienced total spontaneous breathing and 64% of them received mechanical ventilation but were able to breathe spontaneously after disconnection from the ventilator [9]. The decrease in RAP was measured during a spontaneous inspiration (after a short disconnection from the ventilator in those receiving mechanical ventilation). An inspiratory decrease in RAP (ΔRAP) of at least 1 mmHg was predictive of a positive response to volume loading with positive predictive values of 77% [26] and 84% [9] and negative values of 81% [26] and 93% [9].

To our knowledge, this index is not widely used in clinical practice. One of the major problems that limits its use is that the patient must generate a sufficiently deep decrease in intrathoracic pressure for a correct interpretation. In the two studies by Magder et al., this was ensured by a decrease in PAOP by at least 2 mmHg during inspiration after postulating that the decrease in PAOP reliably reflects the decrease in intrathoracic pressure [9, 26]. Therefore, the use of ΔRAP requires the insertion of a pulmonary artery catheter, which obviously represents a true limitation.

Contrary to the results of Magder et al. [9, 26], Heenen et al. reported that ΔRAP was not predictive of volume responsiveness in patients with spontaneous breathing activity with or without mechanical support [10]. However, this issue is still a matter of debate [27].

Passive Leg Raising

Description and Interpretation

Passive leg raising is a maneuver that transiently and reversibly increases venous return by shifting venous blood from the legs to the intrathoracic compartment [28, 29]. Passive leg raising (45° elevation) results in an increase in right [30] and left

[31] ventricular preload. Passive leg raising can, therefore, mimic the effects of fluid loading and has been proposed for a long time as a first line therapy of hypovolemic shock ('autotransfusion' effect). The way in which passive leg raising alters preload is probably by an increase in the mean systemic pressure, the driving force for venous return, due to the gravitational shift of venous blood from unstressed to stressed volume. This mechanism is probably of major importance during passive leg raising in patients receiving mechanical ventilation because the volume of blood enclosed by the thoracic and splanchnic beds is already stressed by positive airway pressure and these vascular compartments are less compliant than when mechanical ventilation is not used [32]. In these conditions, the increase in mean systemic pressure with passive leg raising is expected to be higher in mechanically ventilated patients than in non-mechanically ventilated patients. However, the effects of passive leg raising on cardiac output are variable [30, 33–35], probably depending on the degree of leg elevation and on the existence of cardiac preload reserve. In this regard, Boulain et al. [36] reported, in deeply sedated patients receiving mechanical ventilation, that the increase in stroke volume induced by passive leg raising occurred only in patients who increased their stroke volume in response to a subsequent 300 ml volume infusion. In patients who did not respond to volume loading, passive leg raising did not change stroke volume despite increases in RAP and PAOP. Thus, passive leg raising, which was able to increase cardiac preload in all the studied patients, increased stroke volume only in those with cardiac preload-dependence. Hence, passive leg raising can be proposed as a test to detect fluid responsiveness in critically patients. It is interesting to note that in the above-mentioned clinical study, the changes in PAOP induced by passive leg raising were immediately and fully reversible when the patients' legs were laid down [36]. This finding suggests that passive leg raising may help in predicting individual fluid responsiveness while avoiding the hazards of unnecessary fluid loading. Since it does not require any analysis of respiratory changes in stroke volume or its surrogates, passive leg raising is potentially usable in patients experiencing spontaneous breathing activity or arrhythmias.

Clinical Use

Changes in arterial pulse pressure

Theoretically, the best marker of the hemodynamic response to passive leg raising as a predictor of the hemodynamic response to fluid loading would be a significant increase in stroke volume. Because arterial pulse pressure is directly proportional to LV stroke volume and assuming that arterial compliance is not altered by passive leg raising, an increase in pulse pressure during passive leg raising should indicate an increase in stroke volume and thus a positive response to fluid infusion. However, in a study by Boulain et al. [36], the correlation between passive leg raising-induced changes in radial pulse pressure and fluid loading-induced changes in stroke volume was only fair. This observation was likely because changes in radial pulse pressure may not reflect changes in aortic pulse pressure owing to the potential occurrence of complex changes in pressure wave propagation and reflection during change in blood flow induced by passive leg raising. In addition, a given change in stroke volume in different patients with different aortic compliance may be reflected by different changes in aortic pulse pressure.

Changes in 'pulse contour' cardiac output

A more direct measurement of stroke volume during passive leg raising should be more relevant to detect volume responsiveness. Because of the short period of this test (less than 1 minute), the thermodilution method is not appropriate even in its automatic and semi-continuous mode. Indeed, this method takes at least 10 minutes to completely detect a given change in cardiac output [37]. A beat-to-beat basis for measuring stroke volume should be a better approach for tracking rapid changes of stroke volume induced by passive leg raising. Technologies using pulse contour analysis – like the PiCCO system – would be appropriate for that purpose. To our knowledge, no study has yet been published on the effects of passive leg raising on PiCCO stroke volume for the prediction of fluid responsiveness. In a preliminary study performed in patients with spontaneous breathing activity and/or arrhythmias, the increase in pulse contour cardiac index induced by passive leg raising correlated nicely with the increase in pulse contour cardiac index induced by subsequent volume loading ($r=0.62$, $p<0.05$) while the passive leg raising-induced increase in pulse pressure correlated only weakly with the fluid-induced increase in pulse pressure ($r=0.40$ $p=0.03$) [38]. An increase in cardiac index $>12\%$ during passive leg raising predicted fluid responsiveness (defined by a fluid-induced increase in cardiac index $\geq 15\%$) with a sensitivity of 70% and a specificity of 92% (Fig. 1), values significantly better than those reported for the prediction of volume responsiveness by passive leg raising-induced increase in pulse pressure (50% and 80%, respectively) [38].

Changes in aortic blood flow

An alternative beat-to-beat based monitoring method is the esophageal Doppler. Recent technologic developments allow continuous measurement of both the descending aortic blood velocity (Doppler method) and the diameter of the descending aorta (time-motion echographic transducer). Therefore, it is now possible to monitor blood flow in the descending aorta.

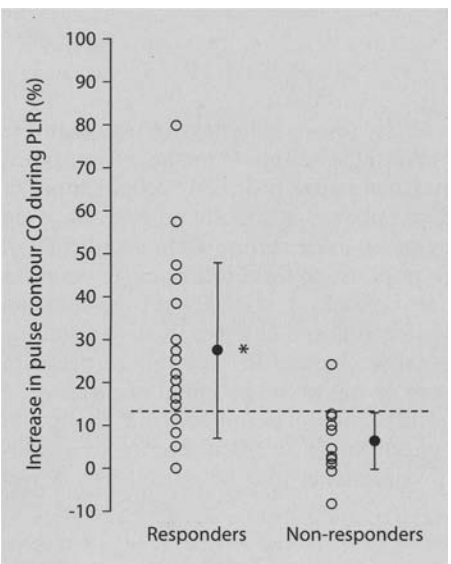

Fig. 1. Representation of individual values of percent changes in pulse contour cardiac output (CO) in response to passive leg raising (PLR) in responders to volume challenge and in non-responders. Responders to volume challenge were defined as patients who increased their pulse contour cardiac output by more than 15% after receiving fluid infusion. An increase in pulse contour cardiac output by 12% during passive leg-raising was the best cut-off value for discriminating non-responders and responders to volume challenge (see text).

In a study performed in 71 mechanically ventilated patients considered for volume expansion, we hypothesized that the increase in descending aortic blood flow in response to passive leg raising would predict fluid responsiveness [25]. Thirty-one patients had spontaneous breathing activity and/or arrhythmias. In 37 patients (responders), the aortic blood flow increased by $\geq 15\%$ after fluid infusion. A passive leg raising-induced increase in the aortic blood flow $\geq 10\%$ predicted fluid responsiveness with a sensitivity of 97% and a specificity of 94% (area under the receiver operating characteristic [ROC] curve: 0.96 ± 0.02). These excellent predictive values were quite similar in the group of patients with spontaneous breathing activity (area under the ROC curve: 1.00 ± 0.00) and in the group of deeply sedated patients (area under the ROC curve: 0.91 ± 0.06) [25]. Interestingly, a passive leg raising-induced increase in pulse pressure $\geq 12\%$ predicted volume responsiveness with significantly lower sensitivity (60%) and specificity (85%) (area under the ROC curve: 0.75 ± 0.06) [25]. This only fair prediction was similar in the group of spontaneously breathing patients (area under the ROC curve: 0.69 ± 0.13) and in the group of deeply sedated patients (area under the ROC curve: 0.74 ± 0.09) [25]. This study suggests that measuring changes in aortic blood flow rather than pulse pressure during passive leg raising is a more robust indicator of preload-responsiveness in a general population of mechanically ventilated patients including those with spontaneous inspiratory efforts and/or arrhythmias.

Conclusion

In spontaneously breathing patients (with or without mechanical ventilation), the prediction of volume responsiveness can be a difficult challenge, in particular in those who have already been resuscitated in the preceding hours or days and in whom continuation of fluid infusion carries risks of pulmonary edema. In these cases, static markers of cardiac preload are generally in the normal range and are rarely helpful for determination of volume responsiveness. Since absolute measures of preload cannot be used effectively to assess volume responsiveness, more dynamic tests need to be employed to improve the utility of these measures [39]. Because of the presence of spontaneous breathing, the indices of volume responsiveness that use heart-lung interactions, such as respiratory variation in arterial pressure and in stroke volume are no longer reliable. Careful analysis of the hemodynamic consequences of passive leg raising using real-time aortic blood flow monitoring may be helpful for predicting the beneficial effects of volume administration. In the most difficult cases, a fluid challenge strategy can still be applied provided that clinicians carefully follow the recommended rules in terms of the type of fluid, rate of infusion, clinical end-points, and safety limits in order to minimize the risks of fluid overload [40].

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Passive Leg Raising

X. Monnet, C. Richard, and J.-L. Teboul

Introduction

Passive leg raising involves the elevation of the lower limbs from the horizontal plane. It was used as an empiric rescue therapy for acute hypotension long before intensive care units (ICUs) were created. The hemodynamic effects of passive leg raising have been progressively elucidated. In view of its simplicity, there is renewed interest in passive leg raising as a means of predicting fluid responsiveness in the critically ill.

Hemodynamic Effects of Passive Leg Raising

During elevation of the legs to 45°, gravity causes a translocation of venous blood from the legs toward the intrathoracic compartment. A study conducted in healthy subjects using a radionuclide method, showed that the volume transferred during this postural maneuver was 150 ml [1], but no other study has addressed this particular physiological issue. The transfer of blood from the legs operates through the splanchnic venous network and likely increases the mean circulatory pressure, i.e., the driving pressure of the systemic venous return toward the right atrium [2]. Passive leg raising thus increases right ventricular (RV) preload [3]. If the increase in RV preload is sufficient to increase the RV output – which is generally the case -this results in an increase in left ventricular (LV) filling and preload. In this regard, passive leg raising has been reported to induce significant increases in the pulmonary artery occlusion pressure (PAOP) [4–6] as well as in the LV end-diastolic volume (LVEDV) [7, 8], under various hemodynamic conditions.

Although passive leg raising has been demonstrated to increase cardiac preload parameters in previous studies, it did not increase cardiac output in all the studied patients [8, 9]. Indeed, in response to an increased left cardiac preload, cardiac output should increase only in patients with cardiac preload dependency, according to the Frank-Starling relationship [8, 9]. Moreover, Wong and colleagues found that the passive leg raising-induced increase in cardiac output of healthy subjects was 7% in normal conditions, but increased to 11% after a 500 ml blood withdrawal [10]. Hence, passive leg raising can be considered as a 'self-volume challenge' that could be used to assess fluid responsiveness.

Passive leg raising has the advantage of a short time delay during which it exerts its hemodynamic effects and a complete reversibility. In a study in critically ill patients, we measured blood flow in the thoracic aorta by means of esophageal Doppler monitoring during a passive leg raising maneuver [11]. The changes in aortic blood flow – an estimate of cardiac output – occurred within the first 30 seconds

in all the 71 patients included in this study [11]. Additionally, when the patients' legs were lowered, cardiac output returned to its baseline value within a few seconds: as the original body posture was restored, the changes in cardiac output completely vanished [11]. Thus, passive leg raising should be considered as a reversible 'self-volume challenge', testing the volume response without administering fluid.

The time during which passive leg raising is sustained must also be considered. Some studies performed in healthy subjects found that the increase in cardiac output induced by passive leg raising disappeared after a few minutes although the legs were maintained elevated longer [12]. This may be due to redistribution of venous blood or to lung sequestration of the blood transferred toward the intrathoracic compartment. While the effects of a short duration passive leg raising vanish rapidly after down-tilt, it must be acknowledged that some degree of pulmonary sequestration could appear if passive leg raising is prolonged for minutes and the volume translocated toward the great veins could progressively fall. In such conditions, the translocation of blood toward the intrathoracic compartment would not be completely reversible [12, 13]. To summarize, for correct assessment of the hemodynamic response to passive leg raising, one must be able to observe its effects in a short time. In particular, one must choose a technique of hemodynamic monitoring that is able to track the rapid and transient changes in cardiac output induced by passive leg raising.

How to Perform Passive Leg Raising?

A major advantage of the passive leg raising maneuver as a 'self-volume challenge' is its easiness to perform. To avoid any risk of gastric regurgitation, caution must be kept to down-tilt the trunk of the patient at the horizontal level and not lower; passive leg raising is not a Trendelenburg maneuver. Passive leg raising and Trendelenburg maneuvers may have different hemodynamic effects [14], since with the Trendelenburg position, an unknown amount of venous blood is sequestrated in the head compartment while another amount from the lower body compartment is transferred toward the thorax. Furthermore, the baroreceptor stimulation observed during a head-down tilt may not occur during passive leg raising. More importantly, gastric regurgitation and ensuing aspiration pneumonia may occur with the Trendelenburg position.

In practice, passive leg raising should be performed simply by means of the automatic system of the patient's bed (Fig. 1). If the patient is managed in the semi-

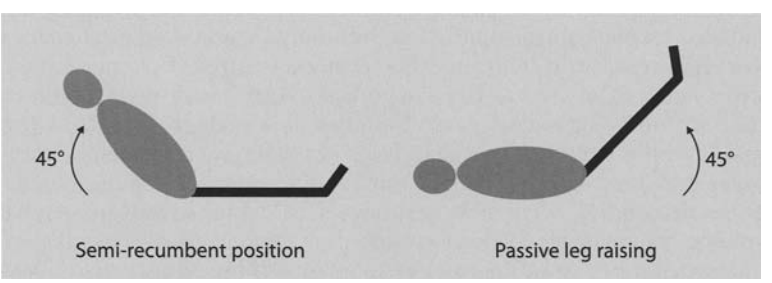

Fig. 1. How to perform passive leg raising? An automatic pivot of the bed allows the patient to be transferred from the semi-recumbent position to a passive leg raising posture with ease and without inducing pain. The lower limbs are raised at a 45° angle while the patient's trunk is tilted down to a supine position. Thus, the angle between the trunk and the lower limbs remains unchanged (135°).

recumbent position at 45° as recommended [15], passive leg raising only consists of pivoting the bed, without changing the angle between the trunk and the lower limbs. In this case, the trunk is lowered to the horizontal position while the lower limbs are tilted upwards to 45°. Compared to leg elevation performed by manually moving the patient's legs and holding them in position, automatic leg elevation avoids any clinician effort and enables the passive leg raising posture to be maintained for the time (around one minute) necessary to assess its full hemodynamic effects. A second great advantage of this technique is that it avoids any discomfort for the patient. Discomfort or pain may occur due to skin contact, body manipulation, or hip flexion and may induce sympathetic stimulation, which would have cardiovascular effects potentially leading to an erroneous interpretation of the hemodynamic effects of the passive leg raising. When performing passive leg raising using the automatic system of the electrical bed, we did not observe any significant increase in heart rate, and presumably there was no other significant sympathetic activation, even in patients who did not receive any sedation or analgesia [11].

Prediction of Fluid Responsiveness by Passive Leg Raising in the Critically Ill

The Issue of Predicting Fluid Responsiveness

Not all patients with circulatory failure respond to fluid infusion by a significant increase in cardiac output [16]. Indeed, in about half of the critically ill patients who are considered for fluid therapy, the heart is working on the upper and flat part of the curve describing the Frank-Starling relationship [16]: In such patients, any increase in cardiac preload cannot result in an increase in cardiac output and the patient is considered as a 'non-responder' to fluid infusion. If administered, volume could exert harmful rather than beneficial effects in such patients, such as an increase in lung water, worsening of gas exchange and lung compliance, increase in tissue edema, RV dilation with left shift of the interventricular septum. The need to avoid such deleterious effects of a volume infusion which would not increase cardiac output, has stimulated an interest in how to define predictors of preload responsiveness. Static measures of cardiac preload of any sort do not reliably predict a patient's response to fluid administration [17] and assessment of fluid responsiveness must rather be based on the response to dynamic tests which induce transient changes in cardiac preload [18].

For this purpose, a first method is to analyze the respiratory variation in hemodynamic signals [16]; changes in intrathoracic and transpulmonary pressures induced by mechanical ventilation induce cyclic and regular changes in cardiac preload that result in significant cyclic changes in stroke volume in the case of preload dependency. Thus, it has been hypothesized that such cyclic changes of stroke volume (or of its surrogate) could be taken as a marker of preload-responsiveness in mechanically ventilated patients [16]. Accordingly, respiratory variation of arterial pulse pressure [19], of subaortic outflow [20], of arterial pulse contour [21, 22], and of the descending aortic flow [23] have been demonstrated to be reliable markers of volume responsiveness. However, the predictive value of these respiratory variation indexes may be lost in the cases of spontaneous breathing activity or arrhythmias. In such frequent situations, the variation in stroke volume may not be due to concomitant changes in cardiac preload but rather to the heterogeneity of the intrathoracic pressure variation or of the cardiac cycle length. Accordingly, we demonstrated that in patients with spontaneous breathing activity, the respiratory variation of arterial

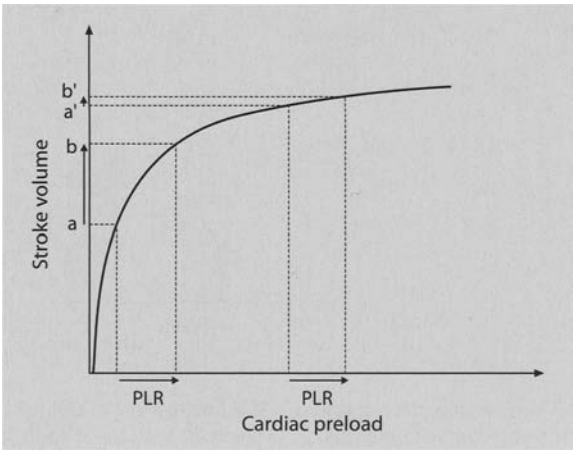

Fig. 2. Passive leg raising (PLR) acts like a 'self-volume challenge'. Passive leg raising allows estimation of which part of the Frank-Starling curve the patient's heart is working on. If the increase in cardiac preload induced by passive leg raising induces significant changes in stroke volume (from a to b), the heart can be supposed to work on the initial part of the curve and the patient will likely respond to fluid infusion. Conversely, if the same changes in cardiac preload during passive leg raising do not significantly change stroke volume (from a' to b'), the heart is likely preload independent and fluid should not be administered.

pulse pressure was of poor specificity for predicting fluid responsiveness [11] and this has been confirmed by others [24].

Since passive leg raising induces a transient increase in cardiac preload, it is considered as another method for predicting the part of the Frank-Starling relationship on which the patient's heart is actually working: On the initial, steep part of the curve, where passive leg raising may induce large changes in cardiac output, or on the upper, flat part, where passive leg raising is supposed not to induce any change in cardiac output (Fig. 2). The issue has thus emerged as to which estimate of cardiac output or stroke volume is most accurate at assessing the effects of passive leg raising. Due to the short-term effects of passive leg raising, a real-time cardiac output monitoring technique would be particularly appropriate for rapidly tracking the transitional hemodynamic changes related to passive leg raising. In this regard, the automatic, semi-continuous measurement of cardiac output by thermodilution is not suitable for assessing the effects of passive leg raising.

Effects of Passive Leg Raising on Arterial Pulse Pressure

Boulain et al. [5] performed passive leg raising in critically ill patients with acute circulatory failure who were sedated and receiving mechanical ventilation. These authors observed that passive leg raising-induced increases in arterial pulse pressure – taken as a surrogate of stroke volume – correlated significantly with the changes in cardiac index induced by subsequent fluid loading. However, the correlation between the passive leg raising-induced changes in pulse pressure and the volume-induced changes in cardiac index was only fair, maybe because the arterial pulse pressure is far from the best estimate of stroke volume. Indeed, pulse pressure depends not only on stroke volume, but also on arterial compliance and is influenced by complex propagation/reflection of the arterial waveform along the arterial tree.

Effects of Passive Leg Raising on the Aortic Blood Flow

Another beat-by-beat estimate of stroke volume can be provided by esophageal Doppler. By means of a small-caliber probe located in the esophagus, this minimally invasive monitoring device measures the blood velocity in the descending thoracic aorta.

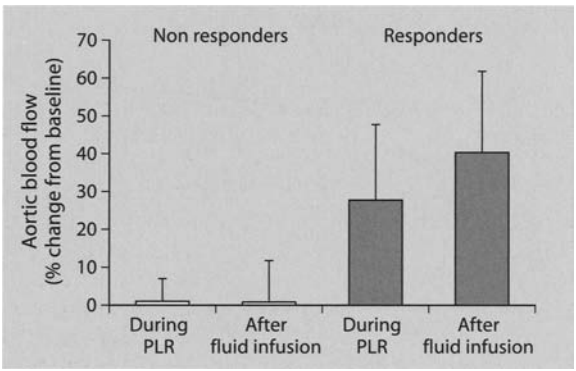

Fig. 3. Prediction of fluid responsiveness by passive leg raising (PLR) and esophageal Doppler in the critically ill. In 71 critically ill patients with acute circulatory failure, the aortic blood flow was measured by esophageal Doppler during a passive leg raising test and after fluid infusion [11]. In patients who did not respond to fluid infusion (non-responders, n = 34, open bars), passive leg raising did not alter the aortic blood flow significantly before fluid infusion. Conversely, in patients who responded to fluid infusion by an increase in aortic blood flow ≥ 15% (responders, n = 37, full bars), the fluid-induced changes in aortic blood flow were preceded by a significant increase in aortic blood flow during passive leg raising.

Simultaneous measurement – or estimation – of the aortic diameter at the same level allows calculation of the aortic blood flow, which has been demonstrated to correlate with cardiac output [25]. Moreover, esophageal Doppler monitoring devices are able to reliably track changes in cardiac output with various hemodynamic interventions [23, 26, 27].

In 71 mechanically ventilated patients with acute circulatory failure, we measured aortic blood flow during passive leg raising and during a subsequent fluid infusion of 500 ml saline [11]. In 37 patients, fluid infusion induced an increase in aortic blood flow greater than 15%, defining a positive fluid response. In these volume responders, a passive leg raising maneuver performed before fluid infusion significantly increased aortic blood flow by 28 ± 21% while passive leg raising did not alter aortic blood flow in non-responders (Fig. 3). Moreover, an increase in aortic blood flow greater than 10% during passive leg raising allowed a positive fluid response (increase in aortic blood flow ≥ 15%) to be predicted with a sensitivity of 97% and a specificity of 94% [11]. Our results were confirmed by a recent similarly designed study [28]. In our study, the passive leg raising-induced changes in arterial pulse pressure were of poorer predictive value for fluid responsiveness than the passive leg raising-induced changes in aortic blood flow, suggesting that the latter is a more direct estimate of cardiac output; if passive leg raising increased pulse pressure by ≥ 12%, the ensuing response to volume expansion could be predicted with a sensitivity of 60% and a specificity of 85% only and the receiver operating characteristic (ROC) curve analysis confirmed the superiority of aortic blood flow over pulse pressure for assessing the hemodynamic response to passive leg raising.

Importantly in our study, we specifically identified a subgroup of patients with spontaneous ventilator triggering or with arrhythmias [11]. As expected, the respiratory variation of arterial pulse pressure, which was also calculated, was not reliable for predicting the hemodynamic response to volume in this category of patients (see above). In contrast, the response of aortic blood flow to passive leg raising remained an excellent predictor of fluid responsiveness [11] in this subgroup of patients with inspiratory efforts or arrhythmias. Moreover, passive leg raising exerts its effects on cardiac preload over a period that includes numerous respiratory, and many more cardiac, cycles. Thus, passive leg raising appears to be able to resolve the crucial problem of predicting fluid responsiveness in the large population of patients who are arrhythmic or who do not receive deep sedation.

Effects of Passive Leg Raising on Cardiac Output Measured by Pulse Contour Analysis

The automatic analysis of the contour of the systemic arterial waveform provides a beat-by-beat estimation of stroke volume. Since it is a more direct estimate of cardiac output than pulse pressure, it has been logically tested for measuring the hemodynamic effects of passive leg raising with a view to assessing fluid responsiveness. As observed with esophageal Doppler, the passive leg raising-induced increase in the pulse contour cardiac index reliably predicted a positive response to fluid loading. In a preliminary clinical study, we observed that an increase in cardiac index $\geq 12\%$ during passive leg raising predicted fluid responsiveness (defined by a fluid-induced increase in cardiac index $\geq 15\%$) with a sensitivity of 70% and a specificity of 92% [29]. Interestingly, all the patients in this study had spontaneous breathing activity or arrhythmias, which confirms the particular interest of passive leg raising in such conditions. Whether other techniques measuring beat-by-beat stroke volume and cardiac output, such as echocardiography or thoracic bioimpedance, could similarly predict fluid responsiveness by observing the effects of passive leg raising is quite likely but remains to be demonstrated.

Conclusion

It has now been well demonstrated that the main hemodynamic effect of passive leg raising is to increase cardiac preload by shifting blood from the lower limbs toward the intrathoracic compartment. Passive leg raising can be used at the bedside as a 'self volume-challenge', reversible and easy-to-perform. This very simple postural maneuver has been demonstrated to be a valuable tool for predicting fluid responsiveness: the response of estimates of stroke volume to a short passive leg raising maneuver is correlated to the response of cardiac output to a subsequent fluid administration. Thus, passive leg raising can be considered as one of the tools of the functional hemodynamic monitoring concept [18]. Interestingly, this dynamic method remains fully reliable in patients with spontaneous triggering of the ventilator or with arrhythmias, conditions where prediction of fluid responsiveness cannot be provided by the respiratory variation of hemodynamic signals.

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Extravascular Lung Water Measurement

B. Maddison, T. Best, and R.M. Pearse

Introduction

Extravascular lung water (EVLW) is the term used to describe water within the lungs but outside the pulmonary vasculature. Excessive EVLW volume is a common and serious feature of critical illness. However, clinical assessment of the extent of pulmonary capillary leakage is difficult and inconsistent [1, 2]. Traditional methods of reducing EVLW volume include the use of loop diuretics and vasodilator drugs. The choice of these interventions is very much at the discretion of the clinician; pharmacological therapy is titrated to achieve a subjective clinical improvement rather than a quantitative EVLW volume target. The critically ill patient may also require fluid resuscitation to correct hypovolemia and to maintain oxygen delivery to the major organs. However, in the presence of increased pulmonary capillary permeability or impaired myocardial function, the administration of large volumes of intravenous fluid is associated with a significant risk of pulmonary edema. Effective fluid resuscitation, therefore, involves a fine balance between the harmful effects of inadequate tissue oxygen delivery on the one hand and excessive EVLW volume on the other.

In a large retrospective analysis of 373 critically ill patients, EVLW volume was found to be significantly greater in non-survivors and proved to be an independent predictor of mortality [3]. The use of EVLW volume measurement to guide clinical management during critical illness has also been shown to reduce the duration of mechanical ventilation and intensive care unit (ICU) stay [4, 5]. While it seems logical to include EVLW volume measurement in the routine monitoring of the critically ill patient, there is considerable uncertainty regarding the validity of the technology available for this purpose. The aim of this chapter is to describe the alternative approaches to EVLW volume assessment and discuss the merits of each.

Gravimetric Studies

The gravimetric method of EVLW volume measurement is a research technique first described by Hemingway in 1950 and later adapted by Pearce [6, 7]. This method was widely used in the 1950s and '60s to evaluate the physiology and pharmacology of pulmonary edema formation. EVLW volume is calculated from a number of post-mortem measurements which allow the estimation of the wet:dry weight ratio of lung (Table 1). This technique has recently been described in detail by Rossi et al. [8]. The gravimetric technique is considered to be the 'gold standard' to which new methods of EVLW volume measurement should be compared. However, this method is limited both by complexity and the fact that only a single, post-mortem, measurement is possible.

Table 1. Formulae used in the gravimetric determination of EVLW. Qwt: weight of added water; Ww_b: wet weight of blood; Wd_b: dry weight of blood; Fw_b: fraction water of blood; Ww_h: wet weight homogenate; Wd_h: dry weight homogenate; Fw_h: fraction water of homogenate; Ww_s: wet weight supernatant; Wd_s: dry weight supernatant; Fw_s: fraction water supernatant; Hct: hematocrit; Qb: residual blood content in lungs; Qr: red cell mass of lung; Qh: total weight of homogenate; Hb_s: hemoglobin concentration in supernatant; Hb_b: hemoglobin concentration in blood; EVLW: extravascular lung water.

[1] $Fw_s = \frac{Ww_s - Wd_s}{Ww_s}$

[2] $Fw_h = \frac{Ww_h - Wd_h}{Ww_h}$

[3] $Qr = Qh \times \frac{Hb_s}{Hb_b} \times \frac{Fw_h}{Fw_s} \times Hct$

[4] $Qb = Qr + \left[Qr\left(\frac{1 - Hct}{Hct}\right)\right]$

[5] $Fw_b = \frac{Ww_b - Wd_b}{Ww_b}$

[6] $EVLW = Qh \times Fw_h - Qb \times Fw_b - Qwt$

Indicator Dilution

History of Indicator Dilution

The indicator dilution technique was originally developed as a method of cardiac output measurement. The most significant contributions to our understanding of this technique were made in the 1920s by Stewart and Hamilton [9, 10]. Following this early work, the role of indicator dilution continued to develop. In 1955, the first measurements of pulmonary blood volume in man were performed by Kunieda and Fujimoto using sequential injections of Evans blue dye [11]. This work was subsequently repeated using other indicators including indocyanine green and radioiodinated human serum albumin [11]. The double indicator dilution method of EVLW volume measurement was first performed with radioisotopes by Chinard and Enns in a series of experiments in the 1950s [12]. This work eventually led to the development of a bedside technique, which was introduced in the 1980s.

Principles of Indicator Dilution

The ideal indicator should be stable, non-toxic and easily measured. The indicator should distribute uniformly throughout the physiological compartment under investigation but not be lost from the system during passage from the point of injection to the site of detection. The indicator should then dissipate rapidly to avoid any error resulting from recirculation. For the purposes of intrathoracic blood volume (ITBV) and EVLW volume measurement, the most important criteria are as follows:

1. Motion of the indicator is representative of the motion of the test fluid
2. Indicator volume is small enough not to alter the distribution of the test fluid
3. The indicator completely leaves the test system
4. Flow of the test fluid is constant during the period of evaluation
5. The whole test compartment is in a dynamic equilibrium allowing the indicator to penetrate the entire compartment and leave within the duration of a single circulation

Perhaps not surprisingly, the perfect indicator does not exist although a number of substances have been used for this purpose. The most common practical disadvan-

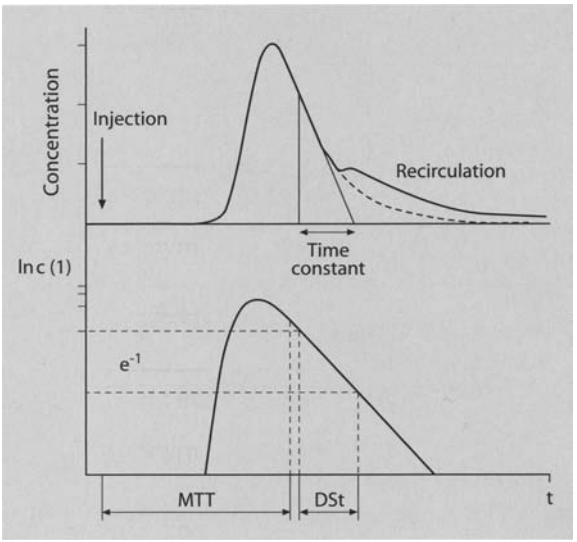

Fig. 1. Relationship between concentration and time for an indicator injected as a bolus into a central vein and measured in a major artery. MTT: mean transit time; DSt: downslope time.

tages include poor stability, difficulties with measurement and recirculation or accumulation. Although the difficulties with recirculation may be overcome by extrapolation of the concentration-time curve (Fig. 1), accumulation of the indicator usually limits the maximum number of accurate measurements. Consequently, most indicators are only suitable for laboratory studies.

There are two principles of indicator dilution which are particularly relevant to the measurement of EVLW volume, pulmonary blood volume, and ITBV. The first principle was reported by Stewart in 1921 [10]. This describes the relationship between cardiac output (CO), the volume throughout which an indicator is distributed (V), and the mean time taken for the indicator to pass from the point of injection to the point of detection mean or mean transit time (MTT):

$$V = CO \times MTT$$

The second principle, described by Newman in 1951, explains the relationship between cardiac output, the volume of the largest chamber (C) in the path of the indicator, and the rate constant of the decay curve of the semi-logarithmic plot of indicator concentration against time, which has also been termed downslope time (DSt) [13]:

$$C = \frac{CO}{DSt}$$

The conformation of the transpulmonary indicator dilution curve is therefore influenced by the various intrathoracic compartments (Fig. 2). The volume of some of these compartments may, therefore, be estimated through the use of indicators with different properties.

Double Indicator Dilution Technique

Although, this technique has been performed with various pairs of indicators, the general principle is the same with each. One indicator should diffuse into and out of

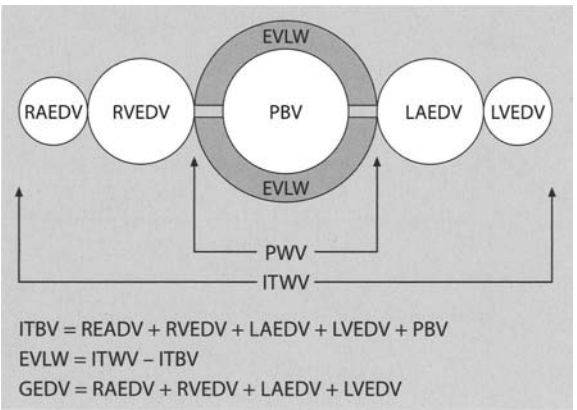

Fig. 2. Relationship between the intrathoracic compartments that influence measurements taken from the transpulmonary indicator dilution curve. For the purposes of thermal indicator dilution, pulmonary and intrathoracic water volumes are regarded as equal to the equivalent thermal volume. RAEDV: right atrial end-diastolic volume; RVEDV: right ventricular end-diastolic volume; PWV: pulmonary water volume; LAEDV: left atrial end-diastolic volume; LVEDV: left ventricular end-diastolic volume; EVLW: extravascular lung water volume; ITWV: intrathoracic water volume.

the EVLW compartment within the duration of one circulation time. In clinical practice, a cold saline thermal indicator is generally used for this purpose. The second indicator must distribute throughout the ITBV compartment but not diffuse into the EVLW compartment. This non-diffusible indicator is often strongly bound to plasma proteins, e.g., indocyanine green. Both indicators should be injected simultaneously to avoid any error due to discrepancies in cardiac output. According to Stewart's equation, the difference in mean transit time of the two indicators will allow the estimation of the difference in distribution volumes which in turn represents EVLW volume.

At present, the most widely used double indicator method of EVLW volume measurement is that of indocyanine green-thermal double indicator dilution. For the purposes of thermal indicator dilution, pulmonary and intrathoracic water volumes are regarded as equal to the equivalent thermal volume. Measurements made using this technique appear to correlate well with those made using the gravimetric method in dogs [14, 15]. However, in a more detailed study, Roch et al. compared the two techniques in pigs randomized to either a direct or indirect precipitant of acute lung injury (ALI) [16]. The methods compared well during indirect ALI but the correlation was not as strong for comparisons made on the direct model. In a more unusual study, the indocyanine green-thermal double indicator dilution technique was compared to gravimetric studies in nine human organ donors [17]. This study reported good linear association and reproducibility between the two techniques, although one subject was excluded because the indocyanine green-thermal double indicator dilution technique grossly underestimated EVLW volume. Subsequent macroscopic examination revealed significant regional lung injury in this patient. Studies have also compared a related double indicator dilution technique with gravimetric measurements [18, 19]. In these studies, a deuterium oxide or heavy water (^2H_2O) indicator was used in preference to a cold saline thermal indicator whilst indocyanine green was used as the non-diffusible indicator. Once again these data suggest a good correlation under baseline conditions. However, at higher values, the double indicator dilution technique was again found to underestimate EVLW volume. It would appear that the indocyanine green-thermal double indicator dilution technique consistently underestimates EVLW volume at higher values. This error may result from the re-distribution of pulmonary blood flow to relatively

spared regions of lung. As a result, areas of high EVLW volume may be under-represented leading to a falsely low measurement.

Devices have been produced commercially to allow indocyanine green-thermal double indicator dilution at the bedside. However, the clinical use of this equipment has proved unpopular, not least because of the financial cost of indocyanine green. These monitors have now been withdrawn from production, although consumables are still available on a limited basis for one device (COLD-Z, Pulsion Medical Systems). A small number of centers still conduct clinical research using this equipment.

Single Indicator Dilution technique

Estimation of EVLW by the transpulmonary single thermal indicator dilution technique was first proposed around 25 years ago [20]. Intrathoracic thermal volume is calculated using a cold saline indicator in an identical fashion to the double indicator dilution technique described above. However, rather than using a second, diffusible, indicator to measure ITBV, this value is calculated by using additional mathematical analysis of the thermal indicator dilution curve. By applying the principle described by Newman et al. [13], it is possible to calculate the volume of the single largest mixing chamber in the course of an indicator, in this case this is the pulmonary water volume. By subtracting pulmonary water volume from intrathoracic water volume, global end diastolic volume (GEDV) may be calculated. An important assumption is then made that the relationship between ITBV and GEDV is constant [21]. This allows the calculation of ITBV and, therefore, EVLW (Table 2).

There are several potential sources of error associated with this approach to EVLW volume measurement. In common with the indocyanine green-thermal double indicator dilution technique, equilibration of the saline bolus with body temperature may result in significant loss of indicator along the pathway between the injection point in the superior vena cava and the measurement point in the femoral artery. If the tip of the femoral artery catheter is not at the level of the diaphragm, part or all of the volume of the abdominal aorta will be included in the measurement. This will result in a falsely high estimate of ITBV and, therefore, a falsely low measurement of EVLW volume. The assumption that the relationship between GEDV and ITBV is constant regardless of clinical circumstances should also be questioned. Although studies support the existence of a relationship between these two variables, this is affected by changes in cardiac output and circulating volume [8, 22, 23].

Three studies have compared the transpulmonary single thermal indicator dilution technique to the gravimetric method [8, 24, 25]. As might be expected, these

Table 2. Calculation of extravascular lung water (EVLW) volume using the transpulmonary thermal indicator dilution technique. CO: cardiac output; MTT: mean transit time; DSt: downslope time; PTV: pulmonary thermal (water) volume; GEDV: global end-diastolic volume; ITTV: intrathoracic thermal (water) volume; ITBV: intrathoracic blood volume

[1] $ITTV = CO \times MTT$	[4] $ITBV = 1.25 \times GEDV$
[2] $PTV = \frac{CO}{DSt}$	[5] $EVLW = ITTV - GEDV$
[3] $GEDV = ITTV - PTV$	

studies all suggest a reasonable correlation between the two techniques. However, this technique consistently overestimates EVLW volume when compared to the gravimetric method. Where performed, Bland-Altman analysis indicates significant bias and limits of agreement between the two techniques [8, 25].

An early comparison between the transpulmonary single thermal and indocyanine green-thermal double indicator dilution suggested a very poor correlation between the two techniques [26]. However, a subsequent study in a larger population of critically ill patients, suggested reasonable agreement between the corresponding values of ITBV and EVLW volume [21]. A number of studies suggest that the accuracy of EVLW volume estimation by the single thermal indicator technique may be improved by adapting the mathematical relationship between ITBV and GEDV [8, 22, 23]. Michard et al. evaluated a range of clinical factors that may influence EVLW volume measurements made by transpulmonary single thermal indicator dilution [27]. When compared with the indocyanine green-thermal double indicator dilution technique, the reliability of measurements was not affected by height, weight, cardiac output, or the dose of vasoactive agents. However, EVLW volume (as measured by double indicator dilution), PaO_2/FiO_2 ratio, tidal volume, and positive end-expiratory pressure (PEEP) significantly affected the agreement between the two techniques.

The transpulmonary thermal single indicator dilution method of EVLW volume measurement is a practical bedside method of EVLW volume measurement. However, despite the prognostic significance of the data provided by this technique [28, 29], there remains some doubt regarding the accuracy of EVLW volume measurement by this method.

Radiological Techniques

Chest Radiography

Pulmonary edema is often diagnosed through clinical examination and chest radiography. Studies have identified a group of patients with abnormal chest radiographs and normal lung water measurements, illustrating the difficulty in distinguishing excess lung water from pulmonary infiltrates or regions of atelectasis [1]. Halperin et al. evaluated the portable chest radiograph against the indocyanine green-thermal double indicator dilution technique in 12 patients admitted to intensive care with a diagnosis of respiratory failure [2]. They concluded that the chest radiograph allowed diagnosis of excessive EVLW in every case but only when EVLW volume reached a threshold of 35% greater than normal. However, the absence of any sign of pulmonary edema on the chest radiograph almost ruled out excessive EVLW volume. Even as a non-quantitative method, the chest radiograph remains a poor indicator of pulmonary edema because interpretation may be influenced by a range of confounding factors.

Computed Tomography

Whilst computed tomography (CT) may generate excellent images of thoracic structures, the quantification of EVLW volume is more difficult because the associated changes are non-specific. The computation of gas and tissue lung volumes from CT densities is based on several assumptions:

1. A linear correlation between physical density and attenuation of electromagnetic radiation.
2. The approximation of the physical density of non-aerated lung tissue (including lung parenchyma, blood, and water) to the physical density of water.
3. Any CT volume unit identified in the lung consists only of gas and lung tissue as opposed to infective cellular debris, pus, etc.

In patients with ALI, quantitative CT measurements of lung edema appear to correlate well with those made using the indocyanine green-thermal double indicator dilution method [30]. Unfortunately, because of the risks associated with radiation exposure and transfer of critically ill patients, in addition to financial costs and limited availability, it is impractical to make repeated EVLW volume measurements by CT.

Magnetic Resonance Imaging

Magnetic resonance imaging (MRI) allows detailed three-dimensional imaging of tissue without the use of ionizing radiation. Knowledge of the signal produced by proton density and T1 weighted images allows calculation of the relative proton density within tissue. Because lung parenchyma contains insignificant amounts of fat and other hydrogen-bound complexes, the proton density measured by MRI principally reflects water content. Early attempts at proton MRI of the adult lung have proved challenging. The low proton density of the lung resulted in a poor signal whilst blood flow, cardiac pulsation, and respiration caused considerable artifact. However, recent improvements in MRI technology allow more rapid image acquisition and electrocardiograph (EKG)-respiratory gating. This allows co-ordination of stationary image capture within the cardiac and respiratory cycles with a substantial improvement in the clarity of the images acquired. MRI may, therefore, be used in the assessment of a variety of lung conditions including pulmonary edema [31]. Qualitative and quantitative assessments of lung water content have been performed using MRI in both the normal adult lung [32, 33] and animal models of pulmonary edema, where comparisons have been made with the gravimetric technique [34, 35]. The use of contrast agents has allowed measurement of total intravascular volume and the simultaneous determination of total lung water using a multi-spin-echo sequence. This provides a measurement of EVLW volume which also correlates well with the gravimetric technique [36].

An alternative approach to the radiological imaging of lung water is the use of sodium MRI [37]. This approach utilizes the distribution of sodium ions to identify EVLW. The concentration of sodium ions in extracellular fluid is greater than ten times that of intracellular fluid. The majority of sodium ions within the lung will, therefore, be situated within plasma, the interstitium or the alveoli. The use of contrast agents allows the plasma signal to be suppressed. Generation of an extravascular lung sodium image is then possible and this indicates the presence and distribution of EVLW. The signal intensity appears to correlate well with EVLW volume measured by the gravimetric method [37]. However, at present it seems unlikely that either sodium MRI or the more basic MRI assessment of pulmonary edema will be integrated into routine clinical practice.

Ultrasonography

Ultrasonography is considered a poor technique for imaging the lung because ultrasound waves are reflected by air within the lung, creating reverberation artifact [38]. However, this technology may still be used to provide an indication of lung water content. The 'comet-tail' is a form of artifact which arises when there is a marked difference in acoustic impedance between an object and its surroundings [39]. In the presence of excessive EVLW volume, the comet-tail sign may be seen to originate from water-thickened interlobular septa and fan out across the lung surface. This sign may be detected with either radiological or cardiac ultrasound equipment.

In one study of critically ill patients, multiple comet-tail artifacts were identified by ultrasonography in the lungs of 86 out of 92 patients with radiographic evidence of ALI, cardiogenic pulmonary edema, or exacerbation of chronic interstitial lung disease [40]. Further work suggests that the comet-tail score has a linear relationship with a chest radiographic EVLW score [41] and may be useful in distinguishing pulmonary edema from other forms of lung disease [42]. In a study of 20 cardiac surgical patients, the comet-tail score was found to have a positive linear correlation with EVLW volume determined by transpulmonary thermal indicator dilution, as well as pulmonary artery occlusion pressure (PAOP) and chest radiograph lung water score [43]. However, whilst chest ultrasound may be a simple and quick method of EVLW volume assessment, this approach does not appear to have any advantage over chest radiography in terms of diagnostic accuracy.

Ultrasound Velocity and Electrical Impedance Dilution

Krivitski et al. have described a novel technique whereby EVLW volume may be estimated by ultrasonographic blood velocity measurement and electrical impedance dilution [44]. In an animal study, changes in systemic arterial sound velocity and electrical impedance during the intravenous injection of hypertonic and isotonic sodium chloride solution were used to calculate lung permeability and provide an estimate of EVLW volume [44]. These measurements were comparable to those made using the gravimetric technique. This technique has also been used to monitor changes in EVLW volume during hemodialysis [45]. Although the indexed measurements were similar to those from the animal study, they do not seem to be consistent with clinical changes associated with hemodialysis.

Other Techniques

Positron Emission Tomography

Positron emission tomography (PET) is a nuclear imaging technique that involves the use of radioactive isotope markers which emit positrons during the process of spontaneous decay. Emitted positrons, having annihilated electrons, produce gamma rays that are emitted and detected to generate a three-dimensional image. Continuous intravenous infusion of water labeled with O^{15} and subsequent inhalation of C^{11} labeled carbon monoxide allows the quantitative measurement of total thoracic water and ITBV. EVLW volume is then determined by subtraction. This technique correlates well with both the indocyanine green-thermal double indicator dilution and gravimetric techniques in dogs [46].

Dual-isotope Technique

Iodine^{131} labeled iodo-antipyrine and ^{99m}Tc labeled erythrocytes have been used to measure EVLW volume in dogs with ALI and verified against the gravimetric technique with good correlation [47].

Impedance Plethysmography

Changes in ITBV and EVLW volume can be monitored by impedance plethysmography. Accumulation of lung water leads to a decrease in internal thoracic impedance which is measured non-invasively using cutaneous electrodes placed over the right lung [48]. This technique has been successfully used to anticipate the onset of clinically evident pulmonary edema by between 30 and 60 minutes.

Conclusion

Although lung water measurement may have a beneficial effect on the outcome of critical illness, there appears to be no consensus regarding the optimal method of EVLW volume assessment. Clinical evaluation of lung water is subjective and unreliable. Radiological techniques may provide a useful insight but for reliable assessment transfer to the CT or MRI scanner is required. At present, the only practical approach to quantitative lung water monitoring in the critically ill is transpulmonary indicator dilution. The transpulmonary double indicator dilution technique appears to provide valid clinical data but is no longer commercially available. The transpulmonary single thermal indicator dilution technique is in widespread use, but the accuracy of this approach is unclear.

Although these conclusions lead us to question the validity of routine EVLW volume measurement, recent developments in the management of EVLW volume suggest otherwise [49]. Clinical management strategies to improve EVLW volume may lead to improved survival. There is, therefore, a need to develop and evaluate new approaches to EVLW volume measurement at the bedside.

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Intravenous Fluid Therapy

Fluid Management in Sepsis: Colloids or Crystalloids?

G. Marx, T. Schuerholz, and K. Reinhart

Introduction

Sepsis and septic shock are associated with both a relative and an absolute intravascular volume deficit [1]. The absolute volume deficit occurs with fever, and includes perspiration and increased insensible loss, vomiting, diarrhea, and volume loss by drains or sequestration. The relative volume deficit is due to vasodilatation, venous pooling, and alterations in the endothelial barrier. The functional disturbances induced by sepsis are reflected by increased blood lactate concentrations, oliguria, coagulation abnormalities, and altered mental state.

Inflammatory cascading reactions, including a variety of mediators that occur in sepsis, induce increased microvascular permeability and capillary leakage which, in turn, result in interstitial fluid accumulation, loss of protein and tissue edema [2]. In this situation, hypoalbuminemia frequently occurs as a result of transcapillary loss and impaired hepatic synthesis of albumin resulting in reduced intravascular colloid osmotic pressure (COP), which further compromises the ability to preserve intravascular volume [3]. Sepsis and septic shock are, therefore, characterized by a reduction in cardiac preload and cardiac output resulting in arterial hypotension associated with impaired tissue perfusion and organ oxygenation causing organ dysfunction.

In this clinical situation, fluid resuscitation is essential for restoration and maintenance of an adequate intravascular volume in order to improve tissue perfusion and nutritive microcirculatory flow [4]. The recognition of the degree of hypovolemia is of utmost importance. Failure to identify the extent of fluid deficit in this situation is an error resulting in low cardiac output state and multiple organ dysfunction or failure. Circulatory stability following fluid resuscitation in the septic patient is usually achieved at the expense of tissue edema formation, which may significantly influence vital organ function [5].

The risk of edema has been used to discredit each type of fluid [6]. Because crystalloid fluid distributes primarily in the interstitial space, edema is an expected feature of crystalloid fluid resuscitation. However, edema is also a risk with colloid fluid resuscitation, especially in the presence of increased microvascular permeability, as colloids do not remain in the intravascular compartment and the leakage of macromolecules might result in an increase of interstitial oncotic pressure and the expansion of the interstitial compartment. On the other hand, advocates of colloid therapy in sepsis argue that by maintenance of an increased COP, fluid is retained in the intravascular space, even in the presence of increased permeability [7].

Fluid therapy in sepsis is aimed at restoration of intravascular volume status, hemodynamic stability, and organ perfusion. The type of fluid resuscitation, crystal-

loid or colloid, in sepsis remains an area of intensive and controversial discussion [8]. Despite its clinical relevance and ongoing discussion for decades there has been a striking lack of studies investigating the optimal fluid strategy and including sufficient numbers of patients.

In four meta-analyses comparing the effects of crystalloids and colloids on patient outcome, either no clear difference between crystalloids and colloids [9–11], or a slight benefit of crystalloids [12], has been found [12]. However, in the meta-analysis of Velanovich [12] there was a 12.3% difference in mortality rate in trauma patients in favor of crystalloids and for non-trauma patients, there was a 7.8% difference in mortality rate in favor of colloid treatment. Cook and Guyatt pointed out in one editorial that "...original research may be more likely to advance this field than will additional meta-analyses. Finally, participation in well-designed clinical trials represents the optimal approach to resolving continued controversies in patient care [13]". Since 2001, several experimental and clinical studies have addressed the issue of fluid resuscitation in sepsis which we will discuss in this chapter.

Fluid Resuscitation in Experimental Sepsis

Evidence from experimental models can make an important contribution to understanding the underlying pathophysiological phenomenon in fluid replacement strategies [14]. van Lambalgen and colleagues reported, in a rodent endotoxin model, a decrease in plasma volume after infusion of a crystalloid solution and an increase after the administration of gelatin [15]; the authors demonstrated no difference in the degree of capillary leakage between septic rats treated with normal saline or gelatin. Morisaki and colleagues, however, did not find a difference between infused crystalloid or colloid solutions in the maintenance of plasma volume using a hyperdynamic sepsis model in sheep [16]. Furthermore, using the same model, Morisaki and colleagues investigated the effects of colloid and crystalloid fluid infusion for 48 h on microvascular integrity and cellular structures in the left ventricle and gastrocnemius muscle [16]. Despite similar circulatory response and increased organ blood flows, septic sheep treated with pentastarch (molecular weight [MW] 63–264 kDa) had greater capillary luminal areas with less endothelial swelling and less parenchymal injury than septic sheep treated with Ringer's lactate infusion, in both muscle types. In accordance, there are more data indicating a beneficial effect of colloid solutions in sepsis under well-defined experimental conditions. It has even been suggested that a particular hydroxyethyl starch (HES) solution called pentafraction (MW 120–280 kDa), containing a selected category of medium weight molecules, compared to pentastarch may reduce capillary leakage by a direct sealing effect [17]. This hypothesis implies that appropriately sized HES molecules might act as plugs and seal or even restore microvascular integrity at capillary-endothelial junctions. This suggestion has been supported mainly by laboratory investigations using ischemia-reperfusion models [18–22]. During sepsis using a porcine fecal peritonitis model, less pentafraction was required in comparison to pentastarch to prevent hemoconcentration [23] and pentafraction was associated with less hepatic and pulmonary structural damage [24]. In an established porcine septic shock model [25] we used ^{51}Cr to determine red blood cell volume and plasma volume because this technique has been shown to be accurate even in septic shock with increased microvascular permeability [26], whereas using a plasma-indicator may be associated with a marked overestimation of the plasma volume due to an increased transcapillary

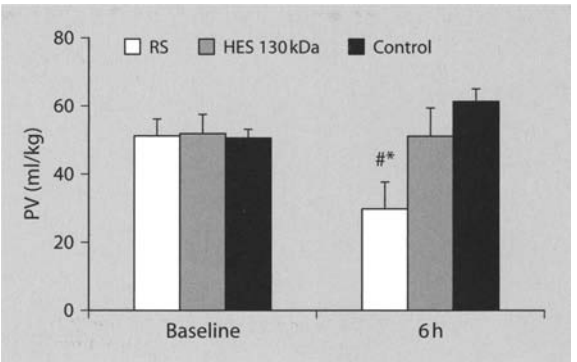

Fig. 1. Levels of plasma volume (PV) at baseline, and 6 h after induction of experimental sepsis in pigs [29]. RS: Ringer's solution; HES 130 kDa: 6% hydroxyethyl starch 130/0.4. All values are given as mean ± SD. * $p \leq 0.05$ versus control group; # $p \leq 0.05$ RS versus HES 130 kDa

loss of the indicator [27]. We were able to show that in this model HES and gelatin solutions maintained plasma volume suggesting the intravascular persistence of artificial colloids in the presence of capillary leakage [25]. In addition, use of Ringer's solution was associated with an increased formation of platelet-derived microvesicles compared to use of artificial colloids [28]. These results suggest that the intravascular activation of platelets in experimental sepsis may be enhanced using the crystalloid, Ringer's solution.

A HES solution with a low MW (130 kDa) has been developed with the aim of improving the pharmacokinetic effects while preserving the efficacy of the volume effect. Using our porcine septic shock model, we tested the effects of HES 130 kDa and a crystalloid regimen with Ringer's solution on plasma volume maintenance as well as on systemic hemodynamics [29]. We found that it was possible in the presence of marked capillary leakage not only to maintain plasma volume and COP by HES 130 kDa but also as a consequence to preserve systemic oxygenation and hemodynamics (Fig. 1). Neither was the case using Ringer's solution.

In our study, animals receiving HES 130 kDa showed a significantly higher cardiac output, oxygen delivery, and mixed venous oxygen saturation (SvO_2) than those receiving Ringer's solution. Thus, these global parameters for tissue oxygenation indicate beneficial effects of HES 130 kDa compared to Ringer's solution. Furthermore it was not possible to prevent respiratory acidosis and arterial hypoxia in the Ringer's solution group by the preset ventilatory pattern. The underlying reasons for this remain speculative: Ringer's solution may increase tissue edema compared to hyperoncotic HES 130 kDa. One effect of such edema would be to retard oxygen uptake by increasing the distance from blood vessels to the mitochondria; this in turn, potentially could reduce functional capacity and contribute to the development of multiple organ failure (MOF) [30]. The basic components of the different solutions may be important as well. Recently, it has been shown that a crystalloid solution, in which lactate was substituted by ethyl pyruvate, ameliorated intestinal hyperpermeability in rats [31]. Pyruvate probably serves as an endogenous scavenger of reactive oxygen species (ROS), which have been implicated in the pathogenesis of sepsis. In a murine model of lethal endotoxemia, treatment with a Ringer's ethyl pyruvate solution instead of a Ringer's lactate solution prolonged survival and blunted the release of interleukin (IL)-6 [32].

Investigating the effects of HES 130/0.42 and HES 200/0.5, we demonstrated that it was possible in our porcine septic shock model to significantly attenuate systemic capillary leakage by HES 130/0.42 in comparison to HES 200/0.5 [33] (Fig. 2). Both

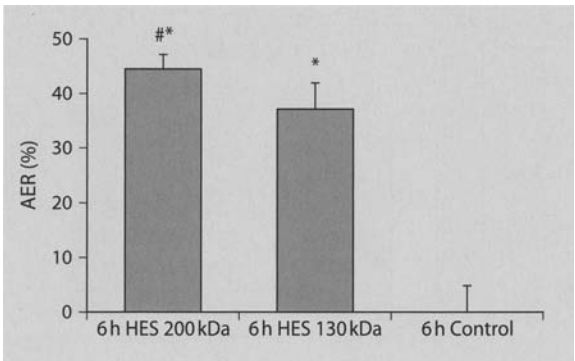

Fig. 2. Albumin escape rate (AER) 6 h after induction of experimental sepsis in pigs and administration of 6% hydroxyethyl starch 200/0.5 (HES 200 kDa), 6% hydroxyethyl starch 130/0.4 (HES 130 kDa), or control [33]. All values are given as mean ± SD. $P \leq 0.05$ versus control group; # $p \leq 0.05$ HES 200/0.5 versus HES 130/0.42. From [33] with permission

solutions were similar effective in maintaining systemic hemodynamics and oxygenation. Attenuation of systemic capillary leakage is in line with a higher plasma volume and a less pronounced positive fluid balance in the HES 130/0.42 group compared to the HES 200/0.5 group, although it needs to be stressed that the latter two differences were not statistically significant. Our result of reduced macromolecular leakage after HES 130/0.42 treatment is furthermore in agreement with other experimental observations demonstrating prevention of capillary leak syndrome by specific HES preparations in post-ischemic and septic conditions [20, 34]. Recently, Hoffmann et al. demonstrated, in a normotensive endotoxic model in hamsters, an attenuation of macromolecular leakage using HES 130/0.42 compared to crystalloid resuscitation [34]. Collis et al. showed in an *in vitro* model using cultured umbilical vein and arterial cells that pentafraction compared to albumin inhibited lipopolysaccharide (LPS)-stimulated von Willebrand factor (vWF) release in a dose-dependent manner but not endothelial E-selectin and neutrophil CD11b/CD18 expression, suggesting an inhibition of endothelial cell activation by pentafraction [35]. Recently, Lv et al. reported that HES 200/0.5 in septic rats may downregulate hepatic inflammatory mediator production and that these anti-inflammatory effects may be induced by inhibition of nuclear factor κB (NF-κB) and activator protein (AP)-1 [36]. In line with our results, Holbeck et al. demonstrated in an *in vivo* model using cat skeletal muscle that HES 200/0.5 had no direct effect on albumin microvascular permeability [37].

The mechanism by which HES 130/0.42 attenuates capillary leakage is not known. Transvascular macromolecular transport involves convective (i.e., by large pores) and diffusive (i.e., paracellular transport through intercellular junctional pathways or via small pores) forces. Regulation of paracellular transport is associated with actin-based systems that link cells by cadherins, proteins that are important for tight junction formation. It has been shown *in vitro* that vascular cellular adhesion molecule-1 (VCAM-1), upregulated during sepsis, induces an increase in permeability by modulating cadherin function through the production of ROS [38]. The transport of solutes across the microvascular walls depends, in part, on mechanical pressure or shear stress forces, plasma and interstitial protein concentration, wall thickness, and perivascular barriers to albumin diffusion [39]. It has been speculated, on the basis of experimental work, that the presence of surface binding proteins, the charge of subendothelial matrix proteins, and the surface charge may be important [40]. The loss of negative endothelial charge in sepsis due to an increased protein extravasation has been demonstrated in a hyperdynamic sepsis model in rats [41].

One might speculate that the less altered pharmacokinetics of HES 130/0.42 compared to HES 200/0.5 when administered repeatedly [42] might be a potential reason for the differences in the albumin escape rate demonstrated in our study. It appears that the differences in degree of substitution, mean MW, and concentration may be important for specific effects on capillary leakage syndrome and microcirculation [43]. HES 130/0.42 provides a relatively small range of distribution of the mean MW as well as of the degree of substitution [34]. Comparing HES 200/0.5, which accumulates only slightly after repetitive administration, with 6% HES 130/0.42, a faster elimination of HES 130/0.42 did not lead to a reduction in clinical efficacy. In patients undergoing cardiac surgery, Gallandat Huet et al. [44] reported similar HES volume requirements for hemodynamic stabilization (2466 ± 516 ml for 200/0.5 versus 2550 ± 561 ml for HES 130/0.4) in both groups until 16 hours after the end of surgery. Jungheinrich et al. found that infusion volumes of 2000 ± 424 for HES 200/0.5 and 2035 ± 446 for HES 130/0.4 were equally effective for hemodynamic stabilization up to the first postoperative day in patients undergoing orthopedic surgery [45]. Several studies have documented comparable courses of COP for HES 200/0.5 versus HES 130/0.4 [44, 45]. Jungheinrich et al. [45] showed a significantly lower *in vivo* MW in the HES 130/0.4 group accompanied by lower HES plasma concentrations postoperatively, as expected from the comparison of pharmacokinetics of the two HES types. COP depends on the concentration of oncotically active molecules, not on the HES concentration *per se*. For example, in the case of an *in vivo* molecular weight for HES 130/0.4 of about half the value for HES 200/0.5 and a difference in HES concentration of a factor of two at a certain time point, the number of oncotic active molecules in similar plasma volumes, and hence contribution to total COP, will be similar [46]. Thus, HES 130/0.42 may be more efficacious for volume expansion and even the differences in albumin escape rate might be explained by these pharmocodynamic differences between HES 200/0.5 versus HES 130/0.42.

On the other hand, the release of endotoxin in sepsis activates leukocyte-endothelial cell adhesion, capillary leakage, and changes in vascular micro-hemodynamics [47]. Hoffmann et al. demonstrated a reduction in endotoxin-induced leukocyte-endothelial cell interaction in endotoxemic hamsters using HES 130/0.42, thereby ameliorating endothelial damage [34]. Lang et al. demonstrated recently that human serum albumin preparations show modest intrinsic non-thiol-dependent anti-inflammatory properties *in vitro*, a phenomenon that was not observed with HES 200/0.5 [48]. The binding of neutrophil-derived myeloperoxidase to bovine aortic endothelial cells, a mediator of multiple oxidative and nitric oxide (NO)-consuming reactions, was also enhanced by HES 200/0.5 [48]. Increased NO production through inducible NO synthase (iNOS) activity was shown to decrease the expression of tight junction proteins and decrease tight junction localization in endotoxemic mice [49]. These effects were associated with gut epithelial barrier dysfunction as evidenced by increased ileal mucosal permeability. Hence, there is experimental evidence that different HES solutions may have different effects on sepsis-induced microcirculatory disorders: HES 200/0.5 may further microvascular permeability and according to Hoffmann et al. [34] one might speculate that HES 130/0.42 may have some effects on the inflammatory process, which might contribute to its beneficial effects on sepsis-induced microcirculatory disorders.

In summary there is a limited but encouraging body of experimental evidence suggesting beneficial effects of colloid resuscitation, especially with 6% HES 130/0.42, in short term models of sepsis.

Fluid Resuscitation in Clinical Sepsis

Major advances in the research of fluid resuscitation have been achieved recently. The Saline versus Albumin Fluid Evaluation (SAFE) trial including 6997 critically ill patients in Australian intensive care units (ICUs) demonstrated that the use of 4% albumin or normal saline for fluid resuscitation resulted in similar outcomes after 28 days [50]. This was an adequately powered study to provide reliable estimates of the relative treatment effects of different resuscitation fluids. Inclusion criteria were rather non-specific, thus almost all hypovolemic patients were eligible. Patients who had undergone cardiac surgery or liver transplantation and those with burns were excluded. The primary outcome was also reported in six pre-defined subgroups, including severe sepsis, trauma, and acute respiratory distress syndrome (ARDS). Both groups were well matched at baseline. It is noteworthy that the patients randomized to 4% albumin received significantly less study fluid in the first three days after inclusion compared to those receiving normal saline, resulting in a greater positive fluid balance in the latter group. The two groups had similar 28-day mortalities: albumin 20.9% and saline 21.1% (RR 0.99; 95% CI 0.91–1.09). There was no difference in survival time, the number of patients developing MOF, ICU or hospital stay, days of mechanical ventilation, or days of renal replacement therapy. Subgroup analysis in the patients with trauma and brain injury revealed an excess of deaths with albumin use (mortality for albumin 24.5%, mortality for saline 15.1%; RR 1.62; 95% CI 1.12–2.34; $p=0.009$). Comparing the 28-day mortality in patients with severe sepsis, the authors revealed a mortality of 30.7% for albumin and 35.3% for saline (RR 0.87; 95% CI 0.74–1.02; $p=0.009$). This study demonstrated powerful evidence that, with the exception of trauma patients with associated brain injury, it is safe to give 4% albumin as well as saline to a heterogeneous group of critically ill patients.

Incorporating the results and data of the recruited 6997 patients from the SAFE study, the Cochrane reviewers had to remove the earlier suggestion that administration of albumin is associated with an increased risk of death [51]. They concluded against the use of albumin on the basis of cost; that in view of the absence of any evidence of a mortality benefit from albumin and the increased cost of albumin compared to alternatives such as saline, it would seem reasonable that albumin should only be used within the context of well concealed and adequately powered randomized, controlled trials. In an interesting meta-analysis, Vincent et al. determined the effect of albumin administration on morbidity defined as the incidence of complications, including death in acutely ill hospitalized patients [52]. The authors identified 71 randomized, controlled trials including 3782 patients comparing the administration of albumin with that of crystalloid, no albumin, or lower-dose albumin. Patients in the included trials experienced a total of 3,287 complications, including 515 deaths and 2,772 cardiovascular, gastrointestinal, hepatic, infectious, renal, respiratory, and other complications. Albumin significantly reduced overall morbidity, with a risk ratio of 0.92 (CI 0.86–0.98). Control group albumin dose significantly affected the incidence of complications ($p=0.002$). In 32 trials with no albumin administered to the control group, the risk ratio was 0.77 (CI: 0.67–0.88) compared with 0.89 (CI: 0.80–1.00) in 20 trials with control patients receiving low-dose albumin and 1.07 (CI, 0.96–1.20) in 19 trials with control group patients receiving moderate-dose albumin.

In a randomized, controlled, non blinded trial including patients with cirrhosis and spontaneous bacterial peritonitis, treatment with albumin significantly improved

outcome in terms of morbidity and mortality [53]. Renal impairment developed in 33% of the patients in the control group but in only 10% of those in the albumin group. The in-hospital mortality rates were 28% and 6%, respectively, and at three months, the mortality rates were 41% and 22%. Yet again, the methodology has been questioned, especially the monitoring of fluid therapy and the potential lack of fluid administration in the control group [54].

In a prospective clinical study, septic patients were randomized to fluid resuscitation with albumin 5% or HES 10% 260/0.5 with a study endpoint of pulmonary artery occlusion pressure (PAOP) of 15 mmHg [55]. There were no differences in resulting hemodynamic status between the groups. Hankeln and colleagues compared, in a cross-over study, the effects of HES 10% 200/0.5 and lactated Ringer's solution on hemodynamics and oxygen transport in critically ill patients, of whom 50% were septic [56]. Following HES administration these investigators found a significant improvement in cardiac index and oxygen transport variables, which could not be achieved by the lactated Ringer's solution. In septic patients receiving HES 10% 200/0.5 over 5 days, splanchnic perfusion assessed by gastric intramucosal pH (pHi) measurements could be preserved, whereas the pHi decreased in patients receiving albumin 20% indicating worsened splanchnic perfusion [57]. In another prospective, randomized study in patients with sepsis, administration of HES 10% 200/0.5 resulted in a lower plasma concentration of adhesion molecules compared to administration of 20% albumin [58].

Thus, a body of short-term studies support the hypothesis that the administration of HES might be beneficial for hemodynamic stabilization in patients with severe sepsis or septic shock. In comparison with gelatin, however, treatment with HES did not show a survival benefit but a higher rate of renal failure [59]. Recently, a randomized, multicenter German study, the Efficacy of Volume Substitution and Insulin Therapy in Severe Sepsis (VISEP) trial, investigated whether volume resuscitation with either modified Ringer's lactate (n=275) or 10% HES 200/0.5 (n=262) would have an effect on the morbidity and mortality of patients with severe sepsis/septic shock [60]. It has to be stressed that this work has only been published as an abstract so far. HES administration was limited to 20 ml/kg/day, and further volume therapy in the HES group was allowed with modified Ringer's lactate. Between 0–96 hours, hemodynamic goals were applied according to the algorithm introduced by Rivers et al. [61]: mean arterial pressure (MAP) >70 mmHg, central venous pressure (CVP) >8 mmHg, central venous oxygen saturation ($ScvO_2$) >70%. Primary study endpoints were 28-day mortality and mean sequential organ failure assessment score (SOFA) score. At the time of the first interim analysis with 537 patients, enrollment was suspended because of differences in the frequency of acute renal failure and renal replacement therapy. Administration of 10% HES 200/0.5 resulted in significantly faster normalization of CVP, but not of MAP and $ScvO_2$. There was no difference in 28-day mortality. Multivariate analysis showed that adverse renal effects were associated with the cumulative dose of HES. The authors concluded that therapy with 10% HES 200/0.5 cannot be recommended in patients with severe sepsis/septic shock because 10% HES 200/0.5 is associated with development of acute renal failure in a dose-dependent fashion.

Recently, it was shown that large amounts of HES aggravate macrophage enzyme release in patients with impaired renal function. This can result in an acquired lysosomal storage disease [62]. Further adverse effects associated with the use of HES solutions are effects on clotting [63], and dose-dependent tissue deposition in many tissues but especially in the reticuloendothelial system [64]. This effect has been

associated with MOF and death [65, 66]. Taking the results of the VISEP study and the previous study by Schortgen et al. [59], there is evidence from two large randomized trials that use of 10% HES 200/0.5 and 6% HES 200/0.6–0.66, respectively compared with Ringer's lactate or gelatin, is associated with acute renal failure in patients with severe septsis. In view of the recent data, the safest options for colloidal fluid resuscitation in sepsis for early hemodynamic stabilization appear to be modified fluid gelatin and albumin used in combination with crystalloids or crystalloids alone.

Whether 6% HES 130/0.4 can be used safely in septic patients needs to be elucidated. There is some experimental evidence suggesting differences between HES 130 and HES 200 [33], but this remains to be tested in adequately powered long-term (90 days) clinical studies.

Conclusion

In conclusion, based on the evidence available, achieving adequate volume loading in septic patients seems more important than the type of fluid used [67, 68]. Administration of albumin is safe in septic patients, but not cost-effective in comparison with crystalloids or artificial colloids. Colloid administration may restore hemodynamic stability in patients with severe sepsis more rapidly than crystalloids. 10% HES 200/0.5 and 6% HES 200/0.6–0.66 are associated with an increased incidence of acute renal failure and need for renal replacement therapy in severe septic patients and should not be used in this group of patients. Higher cumulative doses of HES have been reported to result in life-threatening organ dysfunction and increased mortality. Colloids have various non-oncotic properties that may influence vascular integrity, inflammation, and pharmacokinetics. The clinical relevance of these properties is unknown.

As so often, further research is needed to elucidate the effects of different fluid types in sepsis. Additionally, this research must take into account the physicochemical properties of the various colloids. Last, but not least, the paucity of high quality, well powered, long-term, randomized controlled trials needs to be emphasized in order to address further clinical research questions on fluid resuscitation in sepsis.

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Balanced Volume Replacement Strategy: Fact or Fiction?

J. Boldt

Introduction

Adequate volume restoration in the hypovolemic patient appears to be essential to stave non-compensatory, irreversible shock and subsequently to avoid development of multiple organ dysfunction syndrome. Vigorous optimization of the circulation is a prerequisite in the management of such patients. This maneuver is aimed at guaranteeing stable macro- and micro-hemodynamics, while avoiding excessive fluid accumulation in the interstitial tissue. The choice of fluid for this purpose engenders considerable controversy and there is still a dispute over the beneficial and adverse effects of each fluid type.

What is 'Standard Therapy' Today?

Today's correction of hypovolemia is based on either exclusively using crystalloids or using a combination of crystalloids and colloids. When crystalloids are given, normal saline is often preferred because it is isotonic and cheap. However, substantial alterations in acid-base balance develop in patients in whom large volumes of saline solution are infused. This effect has been described as 'hyperchloremic acidosis' [1, 2]. Little information exists as to the clinical importance of this type of acidosis:

- It may impair end-organ perfusion (e.g., splanchnic perfusion [3]).
- It may interfere with cellular exchange mechanisms [4].
- In animal experiments, hyperchloremic acidosis was associated with a reduction in renal blood flow (by vasoconstriction) and a negative effect on glomerular filtration rate [4].
- In healthy volunteers, in whom 50 ml/kg normal saline was infused, metabolic acidosis developed. In these patients, time to first urination was significantly increased [5].
- Negative consequences of hyperchloremic acidosis on organ function have been elucidated in some studies: In patients undergoing abdominal aortic aneurysm repair, lactated Ringer's solution (total dose 6,800 ml) or normal saline (total dose 7000 ml) was used for volume replacement in a double-blinded fashion [6]. Only the normal saline-treated patients developed hyperchloremic acidosis; they needed significantly more blood products than the Ringer's lactate patients.
- Scheingraber et al. [7] studied 24 patients scheduled for elective lower abdominal gynecologic surgery. Approximately 6,000 ml of either normal saline or

Table 1. Electrolyte concentration of plasma and some plasma substitutes

	Plasma	0.9 NaCl	Ringer	RL	HES (6%, 130/0.4)
Na^+ mmol/l	140	154	154	131	154
K^+ mmol/l	4.2	–	4.0	5.4	–
Ca^{++} mmol/l	2.35	–	2.7	1.8	–
Magnesium mmol/l	0.9	–	–	0,5	–
Phosphate mmol/l	1.25	–	–	–	–
Cl^- mmol/l	103	154	163	112	154
Bicarbonate/anion	35	–	–	28	–
Na^+/Cl^--ratio	1.36	1.0	0.94	1.17	1.0
Osmolality (mosmol/l)	295	308	324	280	308

RL: Ringer's lactate; HES: hydroxyethyl starch

Ringer's lactate were infused over 2 hrs. Normal saline-treated patients showed a smaller (not significant) urine output (approx. 700 ml) than Ringer's lactate-treated patients (approx. 1100 ml).

Almost all colloids (e.g., albumin, hydroxyethyl starch [HES], dextrans) are prepared in saline solution containing non-physiological high concentrations of sodium (154 mmol/l) and chloride (154 mmol/l) (Table 1), which may result in acid-base derangements ('unbalanced colloids'). Thus, use of considerable amounts of these colloids may be associated with hyperchloremic acidosis: Acute normovolemic hemodilution (aim: hematocrit 22%) in patients undergoing gynecologic surgery using either (unbalanced) 5% albumin or (unbalanced) 6% HES 200/0.5 resulted in metabolic acidosis in both groups [8]. A dilution of extracellular bicarbonate or changes in strong ion differences and albumin concentration may be explanations for this type of acidosis. Others found decreases in base excess after use of standard unbalanced high molecular weight HES solution but not with albumin [9].

New Approaches to Volume Therapy

Colloids have been shown to be more effective for correcting intravascular volume deficits and subsequently for improving systemic and microcirculatory hemodynamics than crystalloids [10, 11]. Recently, there has been increasing interest on another aspect with respect to treating hypovolemia: Plasma-adapted, balanced solutions have been reported to possess considerable advantages compared to conventional, unbalanced plasma substitutes [3, 12, 13].

Most of the fluids used for resuscitation or for correction of hypovolemia do not meet the criteria of an 'ideal' volume replacement strategy. Volume therapy with HES has become an established approach to correct hypovolemia under a variety of conditions in several countries. It is generally suspected, however, that HES significantly alters plasma coagulation and platelet function, leading to an increased bleeding tendency [14, 15]. HES with a high mean molecular weight (MW) and a high molar substitution (e.g., Hetastarch: Mw 450 kDa, molar substitution 0.7) diminished factor VIII related antigen and factor VIII ristocetin cofactor more than HES with lower MW and lower molar substitution [6, 7]. Platelet function abnormalities were also more commonly associated with infusion of high MW HES [16, 17].

One way to improve safety of HES with regard to coagulation is to modify the solvent. Not only the physico-chemical characteristics of the HES preparation may affect platelet function, the electrolyte composition of the solvent of the HES preparation may also influence platelet function [18]. Hextend® is a first generation, high molecular weight HES (weight average MW approximately 670 kDa) with a high molar substitution (0.75) that is dissolved in a physiologically 'balanced' solution containing 143 mmol/l Na^+, 124 mmol/l Cl^-, 28 mmol/l lactate, 2.5 mmol/l Ca^{++},3 mmol/l K^+, 0.45 mmol/l Mg^{++}, and 5 mmol/l glucose [12, 19]. This specific HES preparation is reported to deteriorate coagulation significantly less than standard high-molecular weight (HMW), highly substituted HES solutions (Hetastarch) [3, 20]. Others, however, could not verify that modification of a first-generation HMW (MW >550 kDa), highly-substituted (molar substitution >0.7) HES preparation (Hextend®) eliminated the negative effects on coagulation [21]. The slowly degradable, HMW HES (MW >550 kDa) with a high molar substitution (>0.7) (Hextend®) is predisposed to exert negative effects on platelet function in a balanced solution; platelet glycoprotein IIb-IIIa availability increased significantly after hemodilution with this solution [23]. This unexpected platelet stimulating effect is unique among the currently available starches and is most likely induced by the calcium chloride dihydrate (2.5 mmol/l) contained in its solvent.

Balancing the volume replacement regimen also showed other beneficial effects aside from those on coagulation: In elderly patients undergoing elective open surgical procedures, conventional HMW-HES (Hetastarch) or a hetastarch in a balanced electrolyte and glucose formulation (Hextend®) was used [3]. Only patients treated with the conventional hetastarch developed hyperchloremic acidosis (postoperative base excess: -0.2 versus -3.8 mmol/l). Gastric tonometry indicated improved gastric mucosal perfusion with the balanced solution (Hextend®) when compared to the saline-based hetastarch [3].

Modifying the solvent of a first-generation HES with a high MW and a high molar substitution does not eliminate the problems that are generally associated with such a solution [14, 15], e.g., slow degradation, plasma and tissue accumulation, coagulation disturbances. Subsequently, a more rapidly degradable third generation HES with a lower MW (130 kDa), a lower molar substitution (0.4–0.42), and a lower C2/C6 ratio has been developed to improve safety and reduce the negative

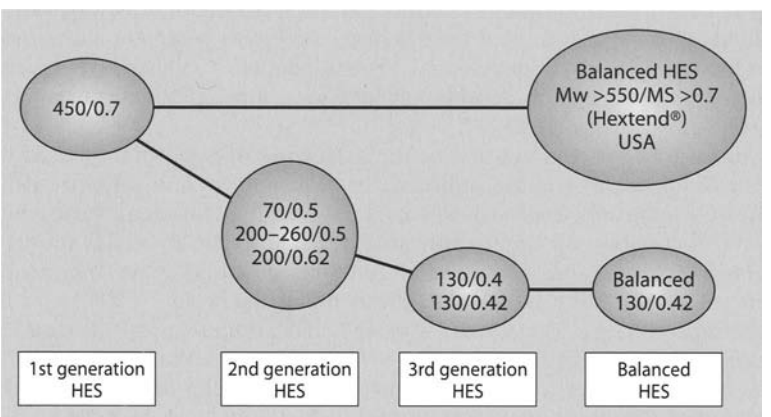

Fig. 1. Development of hydroxyethyl starch (HES) solutions since their introduction in the 1960s.

impact on coagulation [14, 23, 24] (Fig. 1). These solutions show more favorable physico-chemical properties than other HES preparations, but similar hemodynamic efficacy [25, 26]. They are, however, still dissolved in non-physiologic saline solution. Consequently, a new third generation HES solution with a low MW (130 kDa) and a low molar substitution (0.42) prepared in a plasma-adapted solution (containing 140 mmol/l Na^+, 118 mmol/l Cl^-, 4 mmol/l K^+, 2.5 mmol/l Ca^{++}, 1 mmol/l Mg^{++}, 24 mmol/l acetate, 5 mmol/l malate; B. Braun, Melsungen, Germany) has been developed [27]. This new, balanced HES solution is associated with similar hemodynamic effects to a conventional unbalanced HES preparation [27, 28]. Used in a total plasma-adapted volume replacement strategy and given in high doses, the balanced HES preparation showed more favorable effects on electrolyte concentrations and base excess: A total balanced, high-dose volume replacement strategy resulted in significantly less alterations in acid-base status (base excess, pH) and chloride concentration than a non-balanced regimen (Fig. 2). In the patients who received a non-balanced replacement strategy, infusion of the high, non-physiological amounts of sodium and chloride of the colloid and the crystalloid were not compensated for, resulting in hyperchloremic acidosis. The unbalanced volume replacement concept was associated with a base excess of <-5 mmol/l in 7 out of 15 patients and significantly elevated plasma Cl^- levels of >115 mmol/l in 14 out of 15 patients. Because base excess may also serve as an important marker to identify patients with malperfused tissues, limiting acid-base alterations by the choice of volume replacement regimen may be helpful in this context: Producing (hyperchloremic) acidosis by admin-

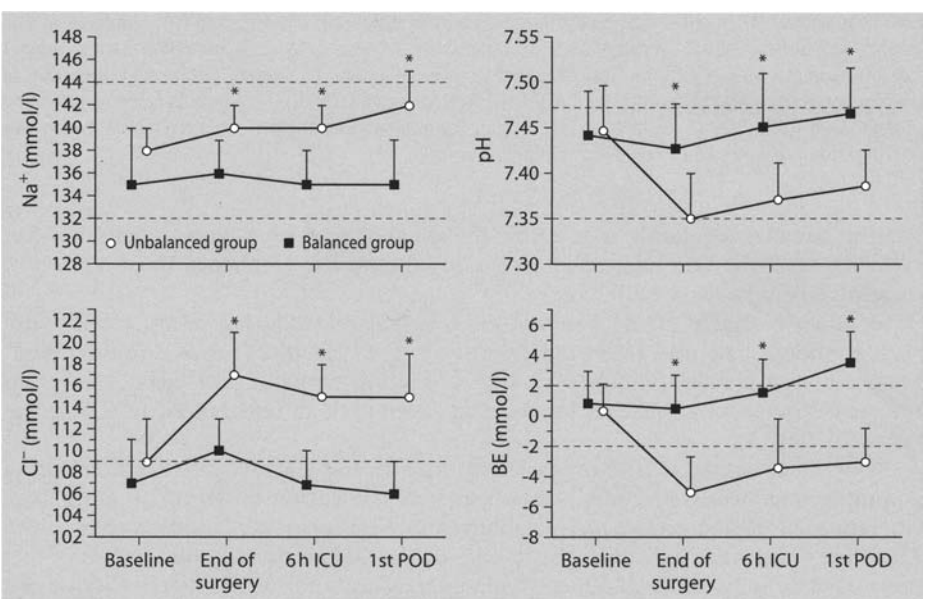

Fig. 2. Changes in Na^+, Cl^-, pH, and base excess (BE) in patients undergoing major abdominal surgery. The patients received either a plasma-adapted crystalloid (140 mmol/l Na^+, 127 mmol/l Cl^-, 4 mmol/l K^+, 2.5 mmol/l Ca^{++}, 1 mmol Mg^{++}, 24 mmol/l acetate, 5 mmol/l malate) plus a plasma-adapted (balanced) 6% HES 130/0.42 dissolved in 140 mmol/l Na^+, 118 mmol/l Cl^-, 4 mmol/l K^+, 2.5 mmol/l Ca^{++}, 1 mmol Mg^{++}, 24 mmol/l acetate, 5 mmol/l malate) or a non-balanced regimen consisting of saline solution plus a conventional 6% HES 130/0.42 (dissolved in saline solution). POD: postoperative day. Modified from [28]

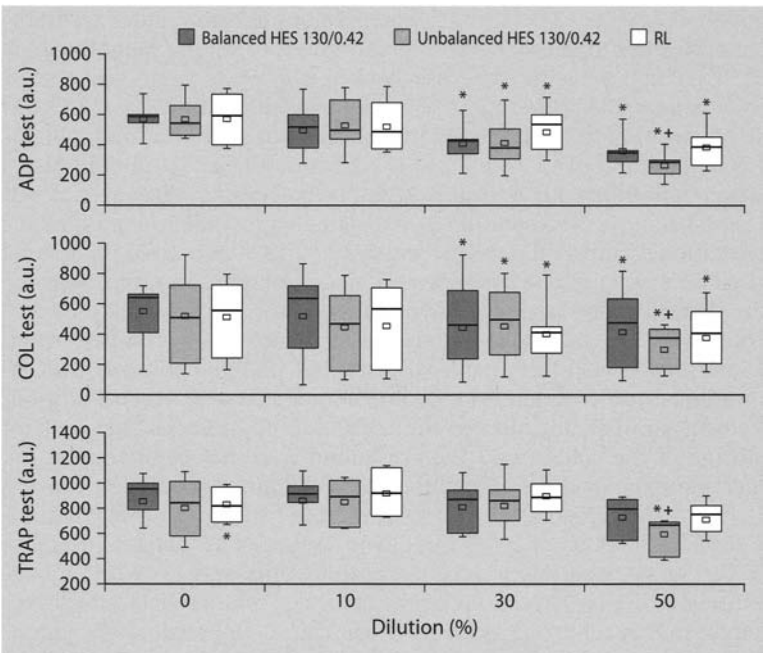

Fig. 3. In vitro studies on platelet function using whole blood aggregometry. Blood from healthy volunteers was diluted (10%, 30%, 50%) with plasma-adapted (balanced) 6% HES 130/0.42 dissolved in 140 mmol/l Na^+, 118 mmol/l Cl^-, 4 mmol/l K^+, 2.5 mmol/l Ca^{++}, 1 mmol Mg^{++}, 24 mmol/l acetate, 5 mmol/l malate; conventional 6% HES 130/0.4 (dissolved in saline solution); or Ringer's lactate (RL). Induction of platelet aggregation was performed with ADP (ADPTest), collagen (COLTest) or thrombin-receptor-activating protein (TRAPTest). Dilution by 50% resulted in significant differences in platelet aggregation between balanced and non-balanced HES solutions. Modified from [30]

istering unbalanced fluids may mask the diagnosis of perfusion deficits or may result in inappropriate clinical interventions due to the erroneous presumption of ongoing tissue hypoxia [29].

In *in vitro* studies using hemodilution, extensively diluting blood (50%) with such a modern balanced HES preparation (6% HES 130/0.42) resulted in only moderately altered platelet function (similar to dilution with Ringer's lactate), whereas the most compromised platelet function was seen with an unbalanced HES preparation [30] (Fig. 3).

In the new, balanced HES preparation, maleate and acetate were used instead of adding lactate, because lactate metabolism is dependent on a well functioning liver, whereas maleate and acetate are metabolized in other organs in addition to the liver. This effect may have an important impact in shock situations in that excessive lactate added by the fluid replacement regimen may accumulate and lactic acidosis can no longer be used as a diagnostic tool.

The value of creating a totally balanced ('plasma-adapted') fluid replacement strategy is still unclear. Avoiding hyperchloremic metabolic acidosis appears to be a generally accepted aim when managing the hypovolemic patient. In an animal model of septic shock, volume resuscitation with Hextend® (a balanced first-generation HES preparation) compared with 0.9% saline was associated with less meta-

bolic acidosis and even longer survival [31]. Whether modulation of the acid-base status by a completely balanced volume replacement strategy would beneficially influence organ function, morbidity, or even mortality in the critically ill must be evaluated in future studies. It also remains to be elucidated whether prolonged, repetitive use of such a fluid replacement concept would be of advantage compared to a non-balanced regimen.

Conclusion

Disorders of the composition of extracellular fluid occur commonly in the critically ill patient. It is imperative to continue the search for the ideal volume replacement regimen, because the requirements of a totally balanced volume replacement regimen cannot be fulfilled with the presently available plasma substitutes. Recent papers using new HES preparations dissolved in a balanced solution have shed new light on this issue. A modern third generation HES solution prepared in a balanced solution completes the idea of a plasma-adapted volume replacement strategy and may add another piece to the puzzle of finding the 'ideal' fluid therapy in the hypovolemic, critically ill patient.

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Renal Failure

Cystatin C as a Marker of Renal Function in Critically Ill Patients at Risk for or with Acute Renal Failure

A.A.N.M. Royakkers, M.J. Schultz, and P.E. Spronk

Introduction

Acute renal failure is a common complication of critical illness [1, 2]. Of all intensive care unit (ICU) admissions, 15–20% develop acute renal failure and 4–6% require some form of renal replacement therapy [3]. Causes of acute renal failure include direct renal toxicity due to medication or radiocontrast agents, hypovolemic hypotension, and shock. Acute renal failure frequently accompanies sepsis – its incidence varies from 20% in patients with moderate sepsis to >50% in patients with septic shock [2, 4]. Acute renal failure carries a high mortality rate, in particular in patients with sepsis – in patients with acute renal failure alone mortality is 45%; in patients with acute renal failure and sepsis, mortality is reported to be as high as 70% [4]. The most frequently used form of renal replacement therapy is continuous venovenous hemofiltration (CVVH), an expensive and laborious treatment. CVVH, however, permits efficient control of fluid balance and azotemia in ICU patients with acute renal failure [5].

Acute renal failure can be defined as a sudden fall in the glomerular filtration rate (GFR). The actual GFR, though, is difficult to measure in the ICU setting. In addition, GFR may change rapidly in critically ill patients. Presently, detection of acute renal failure is primarily based on an increase in plasma creatinine or urea concentration. However, there are limitations to the use of creatinine and urea for estimating GFR. Determination of residual renal function in CVVH-treated patients is further hampered by the fact that these molecules are cleared by CVVH itself. As an alternative, a proposed classification scheme for acute renal failure including criteria for plasma creatinine and urine output, has been proposed, known as the Risk of renal dysfunction, Injury to the kidney, Failure of kidney function, Loss of kidney function, and End-stage kidney disease (RIFLE) criteria [6].

At present, biomarkers of renal function in critically ill patients other than plasma creatinine and urea are seldom used. Such biomarkers, however, may be useful for detection of acute renal failure in an early stage, e.g., before evident clinical signs of kidney injury (the I– and F–criteria in RIFLE) have developed. Secondly, biomarkers of renal function may be useful to detect recovery of renal function in patients treated with continuous renal replacement therapy, such as CVVH. Cystatin C has been in use as an endogenous marker of renal function in patients outside the ICU for more than 15 years; serum cystatin C concentrations have been found to relate to early renal impairment and correlate well with (changes in) plasma creatinine. In this chapter, the literature on serum cystatin C in critically ill patients is reviewed and discussed.

Determining Glomerular Filtration Rate

There are several gold standard methods for determining GFR, including inulin clearance and isotope clearance techniques (Table 1). Unfortunately, these techniques are expensive and laborious and, therefore, not routinely used in clinical practice. Plasma creatinine and urea concentrations, as well as clearance of creatinine based on 24-hour urine collections, are frequently used indicators of GFR. However, plasma creatinine concentrations are affected by muscle mass [7] and diet [8], and vary with age and gender [9]. In addition, as plasma creatinine concentrations rise, its tubular secretion increases, leading to overestimation of GFR in patients with moderate to severe decreases in renal function [10]. Similarly, plasma urea concentrations are affected by various disease states, hepatic function, and diet [11]. An additional problem arises in patients with acute renal failure treated with continuous renal replacement therapy, such as CVVH. Indeed, creatinine and urea are removed by the hemofilter. Consequently, plasma creatinine and urea concentrations are useless for determining GFR in these patients.

A more accurate and practical indicator, preferably an endogenous marker of GFR is needed. An ideal marker of GFR is solely eliminated from the human body by glomerular filtration. In addition, this marker should be freely filtered in the glomerulus, and be neither secreted nor reabsorbed by the renal tubules. If an endogenous marker, it should be produced by the human body at a constant rate, not being influenced by disease state or medication. Furthermore, such a marker should not be removed by means of hemofiltration. Finally, ideally a biomarker of renal function should rapidly follow changes in GFR.

Table 1. Methods for determining/estimating glomerular filtration rate (GFR)

Methods	Advantage(s)	(potential) Disadvantage(s)	In practice
• inulin clearance • clearance of isotopes	• precise measurement of GFR • considered gold standard methods	• laborious and expensive	• (almost) never used in daily practice
• Cockgroft-Gault • MDRD (modification of diet in renal disease) – equation • creatinine clearance on 24-hour urine collection	• cheap and easy estimation of GFR	• influenced by several factors, including muscle mass, age, gender, diet • inaccurate with higher plasma concentrations • plasma concentrations influenced with hemofiltration	• most often used in daily practice
• serum cystatin C measurement	• produced at a constant rate • not influenced by muscle mass, age, gender, diet • serum concentrations only slightly influenced by hemofiltration	• expensive (?) estimation of GFR	• hardly used in daily practice

Cystatin C

Cystatins are a superfamily of cysteine proteinase inhibitors found in plants and animals. Cystatins comprise a group of proteinase inhibitors, widely distributed in tissues and body fluids. Cystatin C, one molecule of this family, is of interest from a medical point of view. It has a molecular weight of 13 kDa, is composed of 120 amino acids, lacks carbohydrate and has two disulfide bridges located near the carboxyl terminus (Fig. 1). The concentration of cystatin C is independent of age, sex, body mass, hydration status, and infection [12]. Cystatin C is completely filtered in the glomerulus and metabolized, but not secreted, in the tubulus. When the GFR decreases, serum cystatin C concentrations rise, even with small reductions in GFR. Cystatin C appears to be a better marker of renal function than creatinine, especially in the early phase of renal impairment. Therefore, it is advocated as an endogenous marker of GRF [13, 14]. Importantly, commercially available assays measure cystatin C in a few minutes [12]. A limitation of the use of cystatin C in the ICU is the potential influence of glucocorticosteroids and thyroid function on serum cystatin C concentration [15]. Another limitation may be costs involved with measuring cystatin C. Indeed, it is currently 15 times more expensive to measure plasma cystatin C concentrations then plasma creatinine concentrations.

Serum Cystatin C in Non-ICU Patients

Over the last decades, numerous studies have been carried out to evaluate the accuracy of serum cystatin C concentrations as a marker for GFR in non-ICU patients (over 50 studies have been published, for a complete reference list see [16]). Studies have been performed in pediatric as well as adult patients, including those at risk for or with established renal disease, transplants, and liver disease. Some studies have limitations because they examined changes of GFR in small patient groups (causing type II errors), or because of the choice of the reference standard for GFR (i.e., no golden standard method for determination of GFR was used).

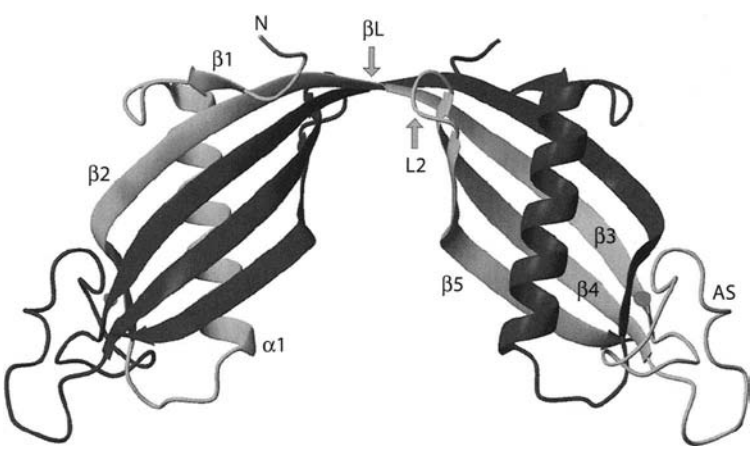

Fig. 1. Three-dimensional structure of cystatin C. From [31] with permission

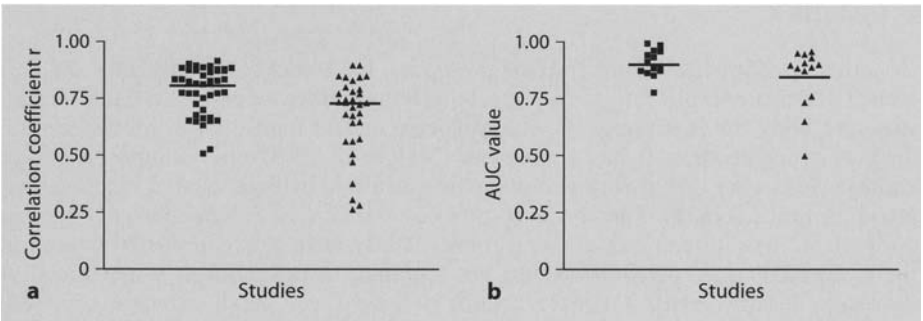

Fig. 2a. Scatter plot of correlation coefficients for the reciprocal of serum cystatin concentrations (squares) and the reciprocal of plasma creatinine concentrations (triangles) from 33 and 29 data sets, respectively; the horizontal lines represent the cumulative mean correlation coefficient of all studies. **b** Scatter plot of receiver operating characteristic (ROC)-plot area-under-the-curve (AUC) values for serum cystatin C and plasma creatinine concentrations from 14 data sets; the horizontal line represents the cumulative mean. From [16] with permission.

In a meta-analysis by Dharnidharka et al., the accuracy of serum cystatin C and plasma creatinine concentrations in relation to a reference standard of GFR were compared [16]. Combining the results of 36 data sets for serum cystatin C concentrations and 29 data sets for plasma creatinine concentrations, the overall coefficient of correlation, r, was significantly greater for the reciprocal of cystatin C concentrations (mean r = 0.816 [95% confidence interval (CI) 0.726 – 0.758]) in comparison to the reciprocal of creatinine concentrations (r = 0.742 [0.804 – 0.826]) (Fig. 2a).

Nevertheless, correlation coefficients reflect only a linear relation and may not translate into agreement or diagnostic accuracy. More meaningful tests are comparisons of sensitivity, specificity, and positive and negative predictive values. These values, however, are highly dependent on the cut-off values chosen. The cut-off values used for serum cystatin C concentrations were widely disparate in the studies, precluding meaningful analysis of these parameters. Another method for assessment of diagnostic accuracy is receiver operating characteristic (ROC) analysis. Combining the results of 11 data sets, ROC-plot area under the curve (AUC) showed a greater divergence of values for the reciprocal of creatinine concentrations than for the reciprocal of cystatin C concentrations (AUC for 1/creatinine 0.837 vs. AUC for 1/cystatin C 0.926); 95% CI did not overlap ([0.796 – 0.878] vs. [0.892 – 0.960]) (Fig. 2b) [16]. These results demonstrate the superiority of serum cystatin C concentrations over plasma creatinine concentrations with respect to GFR determination in non-ICU patients.

With the accuracy of cystatin C established, determination of its utility as a measure of GFR in clinical practice rests also with the rapidity with which serum cystatin C concentrations change with changes in renal function. In most studies, the number of cystatin C concentration measurements per patient was low, making it difficult, if not impossible, to draw conclusions with respect to this issue. However, one study nicely showed a more rapid change in serum cystatin C concentrations as compared to systemic creatinine concentrations with changes in renal function (Fig. 3) [17].

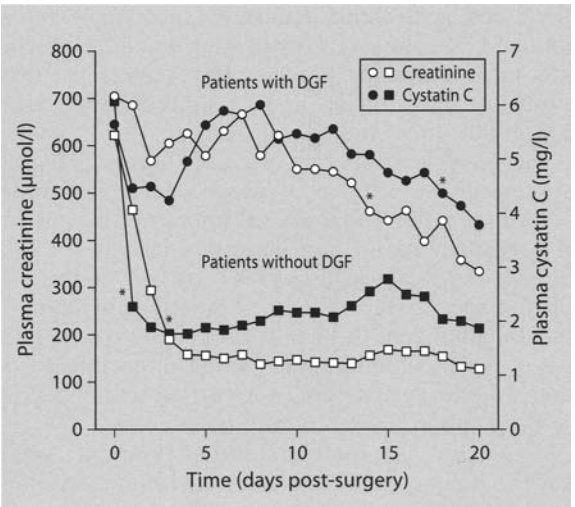

Fig. 3. Cystatin C and creatinine kinetics in renal transplant patients (N = 30). Transplant patients were separated into two groups: Normal course (absence of complications; N = 16) and delayed graft function (DGF) (N = 14), defined as requiring hemodialysis during the first 2 weeks after surgery. Values are presented as medians. * indicates first day value significantly different from the day of surgery. From [17] with permission

Serum Cystatin C for Early Detection of Acute Renal Failure in Critically Ill Patients

While the serum cystatin C concentration is considered to be a reliable marker of renal function in non-critically ill patients, its value in critically ill patients can be hypothesized to be lower. First, while the generation rate of cystatin C is reported to be stable in non-critically ill patients [18], the production of cystatin C may be influenced by critical illness itself. Indeed, several disease states may have an effect on serum cystatin C levels, including thyroid dysfunction [19, 20]. Furthermore, the use of corticosteroids has been suggested to elevate serum cystatin C concentrations [21–23]. Second, even if serum cystatin C concentrations do have an excellent correlation with plasma creatinine in critically ill patients, it is uncertain whether serial measurement of serum cystatin C concentrations affects clinical practice, and if so, influence patient outcome.

Åhlstrom et al. assessed serum cystatin C as a marker of acute renal failure in 202 ICU patients and evaluated its power in predicting survival in patients who evetually developed acute renal failure [24]. For this study, serum cystatin C concentrations, plasma creatinine concentrations, and urea concentrations were measured on admission, daily during the first 3 days, and once per 1–2 days thereafter until ICU-discharge. Acute renal failure occurred in 54 patients (27%). Serum cystatin C was a good predictor of acute renal failure in critical illness (ROC–AUC: 0.885, 0.893, and 0.901 for day 1–3 serum cystatin C concentrations, respectively) and correlated well with plasma creatinine and urea concentrations (r: 0.72, and 0.86, respectively). Serum cystatin C concentrations were not predictive of mortality, however. Importantly, this study showed that abnormal concentrations of serum cystatin C and plasma creatinine appeared in the same time frame. Furthermore, hydrocortisone (100–300 mg i.v. per day, prescribed in the majority of cases for suspected relative adrenal insufficiency due to septic shock) did not seem to affect serum cystatin C concentrations.

In a smaller study, Mazul-Sunko et al. investigated 29 critically ill septic patients [25]. In this study no correlation was found between serum cystatin C concentra-

tions and acute renal failure. Of note, however, only one sample was analyzed (obtained on the day of admission); another dissimilarity with the previous study was urine output in patients with acute renal failure: while most patients in the study by Åhlstrom et al. [24] suffered from anuria, a higher urine output was observed in this study.

Herget-Rosenthal et al. evaluated serum cystatin C concentrations in 85 patients at high risk for acute renal failure [26]. In this study, acute renal failure was defined according to the RIFLE-classification [6]. In acute renal failure by R-, I-, and F-criteria, serum cystatin C concentrations increased 1.5–2 days earlier than plasma creatinine levels; ROC–AUC was 0.82 and 0.97 on the two days before R-criteria were fulfilled. Serum cystatin C concentrations were found to be only moderate predictors of renal replacement therapy in the further course of acute renal failure, but the number of patients fulfilling the F-criteria was rather low (27 patients). Importantly, neither thyroid dysfunction, nor corticosteroid deficiency or excess seemed to affect serum cystatin C concentrations.

Le Bricon et al. compared serum cystatin C concentrations with an isotope clearance technique for GFR, plasma creatinine concentrations, 24 hour creatinine clearance, and two GFR-prediction equations (the Cockcroft-Gault creatinine clearance and the modified diet in renal disease-estimated GFR) [27]. Twenty-eight surgical ICU patients were followed for 5 days. Serum cystatin C concentrations correlated well with plasma creatinine concentrations, the Cockcroft-Gault creatinine clearance, and also with the modified diet in renal disease-estimated GFR. The sensitivity and specificity of serum cystatin C concentrations to detect a GFR <80 ml/min were 88% and 97%.

Delanaye et al. confirmed these findings in a smaller study on 14 patients admitted to a medical ICU [28]. In this study, GFR was estimated by creatinine clearance using 24-hour urine collection and the Cockcroft-Gault equation. The ability of cystatin C to detect a GFR <80 ml/min/1.73 m^2 was significantly better than that of creatinine.

Finally, Villa et al. measured serum cystatin C concentrations and 24-hour creatinine clearance in 50 critically ill patients at risk of developing acute renal failure [29]. Half of the patients developed acute renal dysfunction, only five (20%) of these 25 patients had elevated serum creatinine, whereas 76% had elevated serum cystatin C levels. This study suggests that even with small decreases in renal dysfunction, serum cystatin C concentrations may be superior to plasma creatinine to detect patients at risk of developing acute renal failure and reflect GFR changes sooner than concentrations of plasma creatinine.

In conclusion, in critically ill patients, serum cystatin C seems to be an early and efficient marker for renal dysfunction. Especially with mild reductions in GFR it is a better predictor for the development of renal failure than plasma creatinine. Of note, in some of the abovementioned studies the numbers of patients were small [25, 28]. In addition, all study subjects were admitted to one hospital and, therefore, vulnerable to a center-effect. Finally, the choice of the reference standard for GFR (i.e., no golden standard method for determination of GFR was used) makes interpretation of results difficult [24, 25]. Only one study used the RIFLE-criteria to identify acute renal failure [26]. Finally, although it has been suggested that serum cystatin C concentrations may be influenced by severity of illness, thyroid dysfunction, or the use of glucocorticosteroids [15], this was not confirmed in the studies mentioned above [24, 26].

Serum Cystatin C for Detection of Residual Renal Function During CVVH

In patients on CVVH-treatment it is difficult to determine residual renal function. Indeed, renal function is usually calculated from plasma creatinine and/or urea concentrations. Yet, creatinine and urea are removed by the CVVH-filter, and thus are useless for calculation of GFR.

If cystatin C is removed by CVVH, then a similar problem arises with the use of cystatin C as a marker for residual renal function while CVVH is applied. However, recently Baas et al. showed that clearance of cystatin C by CVVH is low [30]. These investigators studied serum cystatin C concentrations in 18 patients with oliguric acute renal failure treated with CVVH during three consecutive collection periods. Serum cystatin C concentrations were measured in blood samples taken from the afferent and efferent lines, and in corresponding ultrafiltrate samples. Serum cystatin C concentrations were 2.25 ± 0.45 mg/l in the afferent and 2.19 ± 0.56 mg/l in the efferent samples; cystatin C concentrations in corresponding ultrafiltrate samples were 1.01 ± 0.45 mg/l. The sieving coefficient of cystatin C was 0.52 ± 0.20; clearance of cystatin C was 17.3 ± 6.6 ml/min; removed quantity of cystatin C averaged 2.0 mg/hour. This quantity is less than 30% of the production of cystatin C. Therefore CVVH is unlikely to influence serum cystatin C concentrations, suggesting it can be used as a marker of residual renal function during CVVH-treatment.

Of note, as pointed out above, it has been shown that serum cystatin C concentrations rapidly decline with recovery of renal function. Indeed, serum cystatin C concentration has been shown to normalize within several days with recovery of renal function after renal transplantation; in addition, normalization of cystatin C was found to appear approximately 2 days earlier than normalization of plasma creatinine levels [17]. As yet, no such studies have been performed in critically ill patients.

Conclusion

Early detection of acute renal failure is mandatory to design or apply measures to prevent persistent anuria and consequential need for renal replacement therapy during critical illness and thereafter. Biomarkers of renal function may not only be useful for early detection of acute renal failure, but also in the recognition of recovery of renal function in patients treated with CVVH. Cystatin C is a promising biomarker of renal function in critically ill patients. Indeed, even before clinical signs of kidney injury are revealed, serum cystatin C levels rise. Notably, cystatin C is not cleared by hemofiltration, which makes it a potential marker of residual renal function during CVVH. In this way serum cystatin C concentrations may be useful in the decision when to stop CVVH.

Presently, three large multicenter studies are exploring the kinetics of cystatin C in critically ill patients at risk for acute renal failure, patients with established acute renal failure, and patients who are in need of renal replacement therapy. One randomized study is investigating the effect of using (changes in) serum cystatin C concentrations to stop CVVH on duration of CVVH-therapy and length of stay in the ICU.

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Adjustment of Antimicrobial Regimen in Critically Ill Patients Undergoing Continuous Renal Replacement Therapy

D. Kuang and C. Ronco

Introduction

Infection is a common problem in the intensive care unit (ICU). Severe sepsis and septic shock are conditions at the end of the spectrum of human response to infection. Acute renal failure is increasingly seen as part of the multiple organ dysfunction syndrome, which is the most frequent cause of death in patients admitted to the ICU. However, severe sepsis and septic shock are the primary causes of the multiple organ dysfunction syndrome. In the past decades, continuous renal replacement therapy (CRRT) has been widely employed as an extracorporeal blood purification method in the management of septic patients with or without acute renal failure in the ICU, because it offers several advantages over conventional intermitant hemodialysis and peritoneal dialysis [1].

Antimicrobial therapy poses one of the greatest challenges to the intensivist involved in the management of septic patients with persisting high mortality and morbidity rates in ICU [2]. The goal of antimicrobial prescription is to achieve effective active drug concentrations that result in clinical cure while avoiding drug-associated toxicity [3]. However, sepsis, acute renal failure, and CRRT may have profound effects on the pharmarcokinetic and pharmarcodynamic properties of various antimicrobials used in the ICU. The purpose of this chapter is to discuss the impact of these factors on the pharmacological processes and dosing adjustment of antimircobials used in critically ill patients.

Basic Pharmacological Parameters of Antimicrobial Agents: Pharmacokinetics and Pharmacodynamics

A constellation of pathophysiological changes can occur in patients with sepsis, which, along with the influence of sepsis-related organ failure and organ-supportive therapy, can complicate antimicrobial dosing. Knowledge of the pharmacokinetic and pharmacodynamic properties of the antimicrobials used for the management of sepsis is essential for selecting the antibacterial dosage regimens [2, 4].

Pharmaco*kinetics* refers to the study of concentration changes of a drug over a given time period. The important pharmacokinetic parameters include plasma protein binding (PPB), volume of distribution (V_d), clearance (Cl), half-life ($t_{½}$), peak serum drug concentration achieved by a single dose (Cmax), minimum serum drug concentration during a dosing period (Cmin), and area under the serum concentration-time curve (AUC). These factors can be used to determine whether appropriate concentrations of the antimicrobial agent are being delivered to the target area.

Pharmaco*dynamics* is the study of the biochemical and physiological effects of drugs and their mechanisms of action. The primary pharmacodynamic parameters include the time for which the serum concentration of a drug remains above the minimum inhibitory concentration for a dosing period (T > MIC), the ratio of the antibacterial Cmax to MIC (Cmax/MIC), the ratio of the AUC during a 24-hour time period to MIC (AUC_{24}/MIC), and the post-antibiotic effect. The rate and extent of the bactericidal activity of an antimicrobial agent is dependent on the interaction between drug concentration at the site of infection, bacterial load, phase of bacterial growth, and the MIC of the pathogen.

Different antimicrobial classes appear to have different types of kill characteristics on bacteria. The β-lactam group of antimicrobials has a time-dependent kill characteristic with T > MIC as the best predictor of efficacy. These agents appear to have improved antibiotic efficacy when the exposure time rather than concentrations are maximized. Other representative agents in this group include aztreonam, carbapenems, macrolides, clindamycin, and vancomycin. In contrast, aminoglycosides, metronidazole, and daptomycin have a concentration-dependent kill characteristic. More effective killing is observed with higher drug concentrations. Cmax/MIC and AUC/MIC ratios are the parameters correlating with clinical efficacy with this group of agents. Fluoroquinolones are more complex and were initially reported to be Cmax/MIC dependent, although subsequent studies have also found that AUC_{24}/MIC is important.

Impact of Sepsis on Pharmacological Characteristics of Antimicrobial Agents Based on Pathophysiological Changes

The appropriate prescription of antimicrobials requires a detailed knowledge of the pathophysiological and subsequent pharmacokinetic changes that occur throughout the course of sepsis [2]. Concomitant patient factors that may influence the pharmacological characteristics of the antimicrobials include changes in total body water, albumin and acute phase protein levels, muscle mass, blood pH, bilirubin concentration, renal, hepatic, and cardiac function.

Various endogenous inflammatory mediators are produced during the development of sepsis. These mediators may affect the vascular endothelium directly or indirectly, resulting in maldistribution of blood flow, endothelial damage, and increased capillary permeability, which cause fluid shifts from the intravascular compartment to the interstitial space. This would increase the volume of distribution of water-soluble antimicrobials. In addition, it is interesting to note that the serum concentration of albumin may change in these critically ill patients, as acute phase reactant proteins are preferentially synthesized. The binding affinity of drugs to albumin may also decrease due to uremia.

Hypotension is very common due to the inflammatory response associated with sepsis. Inotropic agents are often prescribed in septic patients who fail to respond to administration of intravenous fluids. Therefore, an increased cardiac index and renal preload can always be found. Consequently, serum creatinine and drug clearance are increased in patients with absent kidney and/or liver dysfunction.

As a serious complication, multiple organ dysfunction syndrome often occurs and results in a consequent decrease in antimicrobial clearance, which prolongs the elimination $t_{1/2}$ and may increase antimicrobial concentrations and/or lead to the accumulation of metabolites. These pathophysiological changes will reverse with

recovery from sepsis. Furthermore, since the physiology of these patients may change over a relatively short period of time, ongoing evaluations are indicated to allow timely adjustment of antimicrobial dosing.

Impact of Acute Kidney Injury on Pharmacological Characteristics and Dosage Adjustment of Antimicrobial Agents: Difference from Chronic Renal Disease

Impaired renal function may have profound effects on the pharmacokinetics and pharmacodynamics of renally excreted antimicrobials, necessitating modification of the dosage regimen in order to avoid toxicity through accumulation of the parent and/or its metabolites. The most universal pharmacokinetic equation is:

$$t_{1/2} = 0.693 \times V_d/Cl \quad \text{(Eqn 1)}$$

Since the $t_{1/2}$ is reciprocal to the clearance, an interpolation for any degree of renal impairment can be made from the extreme values for normal kidney function and anuria. Although the volume of distribution of most drugs rises slightly, these discrepancies are considerably smaller than the total volume of body fluid and as a result the influence is limited. However, great inter- and intraindividual variations of actual volumes of distribution have been described in critically ill patients with acute renal failure. A difference was also found in the PPB due to the reduction in net protein content and accumulation of various uremic toxins.

Neither the presence of renal failure nor of CRRT requires adjustment of a loading dose which depends solely on the volume of distribution. However, maintenance doses that undergo considerable renal excretion should be adapted to the reduced renal clearance. There are two approaches to adjusting drug dosage in non-dialyzed patients with impaired renal function: the Dettli rule and the Kunin rule. The Dettli rule adjusts the maintenance dosage in proportion to the reduced clearance. Alternatively, Kunin's rule is derived from the elimination $t_{1/2}$. The normal starting dose is given, and one-half of the starting dose is repeated at an interval corresponding to one $t_{1/2}$. The Dettli rule results in an AUC that is the same as in normal individuals. With the Kunin rule, the peak levels (Cmax) are identical, but the AUC and the Cmin are higher than in normal individuals [5].

Adjustment of the microbial regimen mainly depends on a precise estimate of the glomerular filtration rate (GFR) of the patient. However, if a patient is anuric or in acute renal failure, the Cockcroft-Gault equation or the Modification of Diet in Renal Disease (MDRD) equation will not give a true reflection of GFR. It is still difficult for clinicians to measure the accurate GFR in patients with acute renal failure and a non-steady-state condition. Convenient biochemical markers such as serum creatinine or blood-urea-nitrogen (BUN) are not accurate reflections of renal function due to the lag time in rapidly fluctuating renal function. Urine output may also be misleading in view of the different phases in the natural progression of acute renal failure. Among newer markers, serum cystatin C has not yet been well validated as an early and reliable GFR indicator in patients with acute renal failure.

Several pharmacotherapeutic recommendations on adjustment of antimicrobial regimens according to renal impairment are available [6–8]. However, the variation between these sources is remarkable, including drugs for which no adjustment was recommended in one source while another marked them as contraindicated in renal failure. We should clearly identify the categories of renal impairment for dose or

interval adjustment and be prudent in choosing a regimen with different recommendations in different sources for the same condition.

Nevertheless, we have to emphasize that all the recommendations currently available are based on chronic renal dysfunction and pharmacokinetic data from studies conducted among healthy volunteers. They cannot, therefore, be extrapolated readily to critically ill patients with acute renal failure due to sepsis. Consequently, drug handling in such patients remains largely unpredictable and calculations based on data in the literature only yield rough estimates of drug dosage adaptation.

Impact of CRRT on Pharmacological Characteristics and Dosage Adjustment of Antimicrobial Agents: How to Achieve an Accurate Regimen?

CRRT would have a profound effect upon the pharmacokinetics of antimicrobial agents with multiple variables affecting drug clearance. The factors governing the extent of drug removal from the extracorporeal system can be broadly classified into two major categories:

Pharmacological Factors of Antimicrobial Agent

Molecular weight

Most antimicrobial agents have a molecular weight (MW) up to 500 Da. Generally, it is easier for smaller drugs (MW <500 Da) to pass through a membrane and be removed. However, large molecular drugs, such as vancomycin at 1448 Da, can easily pass through typical high-flux membranes. Only cuprophane and some other cellulose-based membranes with small pores create a significant filtration barrier to unbound drugs.

Volume of distribution

The removal of agents with a large volume of distribution by CRRT is minimal despite efficient clearance due to the small proportion of total body drug present in the systemic circulation. Since total body water constitutes approximately 67% of the body weight, a drug that distributes well in all fluid compartments would have a volume of distribution of close to 0.7 l/kg. As a result, any drug with a volume of distribution >0.8 l/kg would likely signify tissue binding, and therefore, probably not be efficiently removed by CRRT.

Plasma protein binding

Only unbound drug present in plasma water is pharmacologically active and can be removed by extracorporeal processes. Therefore, antimicrobials with a high degree of PPB ($>80\%$) will be poorly cleared by CRRT [9]. Many factors may alter the fraction of unbound drug such as systemic pH, heparin therapy, hyperbilirubinemia, concentration of free fatty acids, relative concentration of drug, and proteins that may act as competitive displacers. Thus, the reported unbound fraction in healthy volunteers and in patients with chronic renal insufficiency may differ substantially from that in critically ill patients [10].

Fractional extracorporeal clearance

The total body clearance of an antimicrobial agent is the sum of clearances from different sites in the body which may include hepatic, renal, other metabolic pathways, and extracorporeal therapy. But extracorporeal elimination is only considered clinically significant if its contribution to total body clearance exceeds 25–30% [11]. This also explains why extracorporeal elimination is not clinically relevant for drugs with overwhelming non-renal clearance. A patient's residual renal function also needs to be taken into account for total body clearance, because significant residual renal function may reduce the fraction that is removed by extracorporeal procedures and may render extracorporeal elimination negligible.

It should be emphasized that extracorporeal elimination only replaces glomerular filtration. However, renal drug clearance includes glomerular filtration, tubular secretion, and reabsorption. Therefore, any attempt to determine the extracorporeal creatinine clearance and use the same dosage guidelines as in patients with reduced renal function cannot be recommended, especially with drugs largely eliminated by tubular secretion [9].

Drug charge

The Gibbs-Donnan effect may have a significant effect on polycationic drugs. Since large anionic molecules such as albumin do not pass through membranes readily, and retained proteins on the blood side of the membrane make the membrane negatively charged, they may partially retard the transmembrane movement of polycationic drugs (e.g., aminoglycosides). This drug charge and membrane interaction may help to explain the discrepancy between PPB and the observed sieving coefficient.

Technical Factors of Extracorporeal Blood Purification Therapies

Membrane

The surface area and the pore size of the dialytic membrane or the hemofilter are considered as two crucial factors determining the extent of drug removal. In general, the pore size of conventional dialytic membranes made up of natural substances (cellulose or cuprophane) is relatively small, permitting passage of fluid and small solutes (<500 Da) only. High-flux dialytic membranes are usually made up of biosynthetic material (polysulfone, polyacrylonitrile, polyamide) with relatively larger pore sizes (5,000–20,000 Da). Even larger pore sizes are used in hemofilters (20,000–50,000 Da).

Diffusion (hemodialysis)

The efficiency of solute removal based on diffusion in hemodialysis is determined by the concentration gradient in addition to the porosity and surface area of the dialytic membrane. Compared with convective clearance, diffusive clearance will decrease as MW increases. Due to the lower diffusive permeability, MW has a greater influence on diffusive clearance with conventional dialysis membranes than with the synthetic membranes used in CRRT [9]. In continuous veno-venous hemodialysis (CVVHD), diffusive clearance of small unbound solutes will equal the dialysate flow rate (Q_d). Dialysate saturation (S_d) represents the capacity of a drug to diffuse through a dialysis membrane and saturate the dialysate, which is calculated by dividing drug concentration in the dialysate (C_d) by its plasma concentration (C_p):

$$S_d = C_d / C_p \qquad \text{(Eqn 2)}$$

Consequently, diffusive drug clearance (Cl_{HD}) is calculated by multiplying Qd by Sd:

$$Cl_{HD} = Q_d \times S_d \quad \text{(Eqn 3)}$$

Since a higher MW decreases the speed of diffusion or a higher Q_d decreases the time available for diffusion, an increase in each of these parameters will produce a decrease in S_d [11]. S_d can theoretically be influenced by drug-membrane interactions and by protein adsorption to the membrane. When extracorporeal drug clearance is calculated, S_d can be replaced approximately by the unbound fraction. However, it should be emphasized that S_d does not remain constant, and it would be a serious mistake to use the same S_d in different Q_ds.

Convection (hemofiltration)

Convective solute removal used in hemofiltration is not affected by MW up to the cut-off value of the membrane. Continuous hemofiltration usually uses highly permeable membranes, with high cut-off values (20,000 – 50,000 Da), so the MW of antimicrobials will have little impact on drug sieving with hemofiltration. The capacity of a drug to pass through the membrane of a hemofilter is expressed mathematically as the sieving coefficient, which is the relation between drug concentration in the ultrafiltrate (C_{uf}) and in the plasma (C_p):

$$\text{Sieving coefficient} = C_{uf} / C_p \quad \text{(Eqn 4)}$$

For most antimicrobials, the sieving coefficient can be estimated by the extent of the unbound fraction (sieving coefficient $\approx$ 1 – PPB). However, the sieving coefficient is a dynamic parameter and is dependent on the age of the membrane and on the filtration fraction (Q_{uf}/Q_b).

There are two basic dilutional modes (predilution and postdilution) for the substitution fluid which may influence the efficiency of solute removal. In the postdilution mode, the convective clearance ($Cl_{post\text{-}HF}$) of an antimicrobial agent can thus be easily obtained by multiplying the ultrafiltration rate (Q_{uf}) by its sieving coefficient:

$$Cl_{post\text{-}HF} = Q_{uf} \times \text{sieving coefficient} \quad \text{(Eqn 5)}$$

However, if hemofiltration is used in predilution mode, the patient's blood is diluted with a substitute fluid prior to entry into the dialyzer. So the correction of the predilutional effect must be integrated in the clearance equation:

$$Cl_{pre\text{-}HF} = Q_{uf} \times \text{sieving coefficient} \times [Q_b / (Q_b + Q_{uf})] \quad \text{(Eqn 6)}$$

As a newer technical evolution in CRRT, high volume hemofiltration (HVHF) or pulse-HVHF are increasingly used in critically ill patients in the ICU. In order to achieve the balance between greater solute clearance and fewer associated complications, such as circuit clotting, predilution and postdilution are often used simultaneously each with a certain percent. This makes it more complicated to calculate the drug clearance.

Combination with diffusion and convection (hemodiafiltration)

In hemodiafiltration, calculation of the drug clearance during this combination therapy is extremely difficult, especially at different Q_{uf} and Q_d rates. Drug clearance with continuous veno-venous hemodiafiltration (CVVHDF) in postdilution may be estimated by calculating the convective clearance and diffusive clearance from the following equation:

$$Cl_{HDF} = Q_{uf} \times \text{sieving coefficient} + Q_d \times S_d \quad \text{(Eq 7)}$$

However, available data demonstrate that a greater overestimation will be induced if S_d is replaced by the unbound fraction, especially with a high Q_d.

Since an interaction between diffusive and convective solute transfer has been demonstrated in intermittent high-flux hemodiafiltration by protein layer formation on the blood side of the capillary, the two processes may interact in such a manner in CVVHDF that solute removal is significantly less than what is expected if the individual components are simply added together. In CVVHDF, as the presence of convection-derived solute in the dialysate decreases the concentration gradient, the driving force for diffusion, the S_d can be lowered even further. The diffusive clearance of a drug during CVVHDF is difficult to predict and will depend on its MW, Q_b, Q_d, Q_{uf}, and the membrane used.

In continuous arteriovenous hemodiafiltration (CAVHDF), Vos and Vincent [12, 13] found a close exponential correlation of a drug's diffusive mass transfer coefficient (K_{rel}) through membranes:

$$K_{rel} = K_d / K_{cr} = (MW / 113)^{-0.42} \quad \text{(Eqn 8)}$$

where K_d and K_{cr} are the diffusive mass transfer coefficients for the drug and creatinine, respectively, and 113 is the MW of creatinine.

The drug clearance of CAVHDF (Cl_{CAVHDF}) may be estimated as:

$$Cl_{CAVHDF} = Q_{uf} \times \text{sieving coefficient} + Q_d \times S_d \times K_{rel} \quad \text{(Eqn 9)}$$

When using the above equation to estimate CVVHDF clearance, Kroh et al. [14] found very good correlations between observed and estimated clearances ($y = 0.004 + 0.96x$). However, whether this method is also suitable for all antimicrobial agents has not yet been investigated.

Adsorption to membrane

Adsorption to filter membranes leads to increased drug removal from plasma and the various filters have different absorptive capacities. Some dialysis membranes, such as polyacrylonitrile (PAN), may adsorb a substantial amount of drug to their surface. For example, PAN membranes are described to have a high adsorbent capacity to bind aminoglycosides and levofloxacin. However, adsorption is a saturant process, and the influence on drug removal will depend on the frequency of filter changes [10]. Although dosing adjustment will not account for adsorption effects, using drug-adsorbing membranes for CRRT is not usually recommended [15].

High volume CRRT (HV-CRRT)

High volume CRRT (HV-CRRT), like HVHF, is increasingly used in septic patients with acute renal failure in the ICU. Nevertheless, the different effects of HV-CRRT and low volume CRRT (LV-CRRT) on the pharmacological characteristics of antibiotic removal have been understated [16]. Pharmacokinetic experiments have found that many antimicrobials exhibit two and three compartment characteristics. In standard LV-CRRT, the rate-limiting step of drug clearance has been Q_d and/or Q_{uf} because Q_b greatly exceeds Q_d or Q_{uf}. Consequently, no appreciable rebound occurs after LV-CRRT stops because drugs transfer to the central compartment at least as fast as the drug is being removed by the CRRT. On initiation of HV-CRRT, the central compartment becomes rapidly stripped of unbound drug. The rate-limiting step of any further drug removal becomes the rate at which the drug can transfer from the peripheral compartments into the center compartment for removal by HV-CRRT.

Available data indicate that an increase in Q_{uf} from 14 ml/min to 28 ml/min will decrease the sieving coefficient for drugs like vancomycin by approximately 30%. However, as Q_d increased from 8.3 ml/min up to 33.3 ml/min, a 30% decline in the sieving coefficient for vancomycin was seen with AN69 hemodiafilters. Doubling Q_d from standard low-volume flows to higher dialysate flows may result in substantially less than a doubling of solute dialytic clearance, particularly for larger solutes. Increasing Q_d (= 2,000 ml/hr) should result in decreasing S_d, but the rate of S_d decline is filter dependent. Therefore, the drug clearance calculation during HV-CRRT is rather complex, and the change in sieving coefficient and S_d should be further considered.

Adjustment of Drug Regimen

In patients with concomitant renal failure on CRRT, underdosing may lead to inadequate anti-infective therapy while overdosing may lead to unnecessary toxicity. Drug dosing adjustments during CRRT can be guided by using available drug-dosing recommendations, by measuring or estimating CRRT drug clearance, or by monitoring drug serum concentrations.

Available drug-dosing recommendations

Drug-dosing recommendations for patients with acute renal failure being treated with CRRT have not kept pace with the advances in CRRT technology and the speedy development of newer antimicrobial agents. Nonetheless, published drug-dosing recommendations for acute renal failure patients on CRRT are becoming available [7, 8, 11, 15, 17, 18]. We have searched and reviewed the recent clinical investigations and referred to some of these recommendations, then summarized the pharmacokinetic characteristics and dosing recommendations for 60 antimicrobials most commonly used in critically ill patients undergoing CRRT into a complete dosing guide (Table 1).

However, all these recommendations have unavoidable, inherent shortcomings that influence their clinical practicability. First, all these dosing recommendations are based on low Q_{uf} and Q_d with old dialysis membranes or hemofilters. Second, pharmacokinetic data are based on data obtained mainly from healthy persons or stable chronic kidney disease patients. Third, some recommendations were derived from CRRT conducted in arteriovenous mode. Fourth, the filters, Q_{uf} and Q_d, and treatment time vary considerably among these recommendations. Finally, most of these recommendations are based on very limited clinical data. Further clinical data are urgently needed to support such extrapolations, and these recommendations should not supercede sound clinical judgment [18].

It is widely recognized that the extent of drug removal during CRRT in critically ill patients with acute renal failure is dependent on numerous factors involving the patient, the illness, the drug, and the operational mode of CRRT. These parameters vary widely among different patients, or even at different moments of time in the same patient. CRRT does not always yield a stable condition, as Q_b and Q_{uf} are quite variable during the therapeutic process. Moreover, renal function and sepsis state may also improve during the course of disease with effective treatment. Therefore, it is extremely difficult and almost impossible to devise a comprehensive dosing guide for various antimicrobial agents that encompasses all of the potentially changing variables involved in CRRT for all patients. Therapy must be individualized to tailor to the needs of each patient.

Table 1. Adjustment of antimicrobial regimen in patients with acute renal failure undergoing continuous renal replacement therapy

Drug	MW (Da)	PPB	V_d (l/kg)	$T_{1/2normal}$ (h)	$T_{1/2anuria}$ (h)	Normal Dosage	Dosage adjustment on CRRT
Aminoglycoside Antibiotics							
Amikacin	585.6	0–11%	0.25–0.4	2.0–3.0	30–90	7.5 mg/kg q12h	7.5 mg/kg q24h (CVVHD/CVVH/CVVHDF: Q_d 1 l/h, Quf 1 l/h)
Gentamycin	477.6	<5%	0.26–0.4	2.0–3.0	20–60	1.7 mg/kg q8h	2.0 mg/kg q24h (CVVHD/CVVH/CVVHDF: Q_d 1 l/h, Quf 1 l/h)
Netilmicin	475.6	<5%	0.25–0.4	2.0–3.0	35–70	2.0 mg/kg q8h	2.0 mg/kg q12h (CVVHF: Q_d 0.5–1.8 l/h, Q_{uf} 100–400 ml/h, predilution, 0.6 m^2 AN69)
Tobramycin	467.5	<5%	0.26–0.4	2.0–3.0	30–60	1.7 mg/kg q8h	2.0 mg/kg q24h (CVVHD/CVVH/CVVHDF: Q_d 1 l/h, Q_{uf} 1 l/h)
Carbapenem Antibiotics							
Imipenem	299.3	13–21%	0.23	1	4	0.25–1.0 g q6h	0.5 g q6-q12h (CVVHD/CVVH/CVVHDF: Q_d 1 l/h, Q_{uf} 1 l/h, 0.9 m^2 AN69)
Meropenem	383.5	2%	0.35	1	7	0.5–1.0 g q6h	1.0 g q12h (CVVHD: Q_d 1 l/h)
							1.0 g q12h (CVVH: Q_{uf} 1–2 l/h, postdilution, 0.9 m^2 AN69)
							1.0 g q8h (CVVH: Q_{uf} 2.6 l/h, postdilution, 0.43 m^2 high-flux PS)
							1.0 g q12h (CVVHDF: Q_d 1–1.5 l/h, Q_{uf} 1–1.5 l/h, pre-/postdilution, 0.9 m^2 AN69)
Cephalosporin Antibiotics							
Cefaclor	367.8	23.50%	0.24–0.35	1	3	250–500 mg q8h	500 mg q8–12h (CVVHD/CVVH: Q_d 1.5 l/h, Quf 1.5 l/h)
Cefamandole	475.6	75%	0.16–0.25	0.5	6.0–11	0.5–1.0 g q4–8h	1.0 g q18h (CVVHD/CVVH: Q_d 1.5 l/h, Quf 1.5 l/h)
Cefazolin	454.5	84%	0.13–0.22	2	40–70	0.5–1.5 g q6h	2.0 g q12h (CVVHD/CVVHDF: Q_d 1 l/h, Quf 1 l/h)
							1.0–2.0 g q12h (CVVH: Q_{uf} 1–2 l/h)
							1.0 g q8h (CVVH: Q_{uf} 3 l/h)
Cefepime	480.6	<20%	0.71	4.6	8.1	0.25–2.0 g q8h	1.0–2.0 g q12h (CVVHD/CVVH/CVVHDF: Q_d 1 l/h, Q_{uf} 1 l/h, postdilution, 0.6 m^2 AN69)
Cefmenoxime	511.6	45–75%	0.27–0.37	1	6.0–12	1.0 g q6h	1.0 g q24h (CVVH: Q_{uf} 1 l/h)
Cefoperazone	645.7	90%	0.14	2	3	1.0–2.0 g q12h	1.0–2.0 g q24h (CVVHD/CVVH: Q_d 1.5 l/h, Q_{uf} 1.5 l/h)
Cefotaxime	455.5	37%	0.35	2	15–35	1.0 g q6h	2.0 g q12h (CVVHD/CVVHDF: Q_d 1 l/h, Q_{uf} 1 l/h)
							1.0 g q6–8h (CVVH: Q_{uf} 1–2 l/h)
							2.0 g q8h (CVVH: Q_{uf} 3 l/h)
Cefoxitin	427.4	40.75%	0.31	1	13–23	1.0–2.0 g q6–8h	1.0 g q18h (CVVHD/CVVH: Q_d 1.5 l/h, Q_{uf} 1.5 l/h)
Cefpirome	512	<10%	0.32	2	14.5	2.0 g q12h	2.0 g q8h (CVVH: Q_{uf} 3 l/h, postdilution, 0.7 m^2 high-flux PS)
Cefradine	349.4	8–17%	0.25–0.46	0.7–1.3	6.0–15	1.0–2.0 g q6h	1.0 g q12h (CVVHD/CVVH: Q_d 1.5 l/h, Q_{uf} 1.5 l/h)

Table 1. (*cont.*)

Drug	MW (Da)	PPB	V_d (l/kg)	$T_{1/2normal}$ (h)	$T_{1/2anuria}$ (h)	Normal Dosage	Dosage adjustment on CRRT
Ceftazidime	546.6	17%	0.28	2	13–25	1.0–2.0 g q8h	2.0 g q12h (CVVHD/CVVHDF: Q_d 1 l/h, Q_{uf} 1 l/h)
							1.0–2.0 g q12h (CVVH: Q_{uf} 1 l/h)
							2.0 g q8h (CVVH: Q_{uf} 3 l/h, postdilution, 0.7 m^2 high-flux PS)
Ceftriaxone	554.6	95%	0.12–0.18	6.0–9.0	12.0–24.0	0.5–1.0 g q12h	2.0 g q12–24h (CVVHD/CVVH/CVVHDF: Q_d 1 l/h, Q_{uf} 1 l/h)
Cefuroxime	424.4	50%	0.19	1.5	17	0.75–1.5 g q8h	0.5 g q8h (CVVHD/CVVH: Q_d 1.5 l/h, Q_{uf} 1.5 l/h)
Cephalexin	347.4	14%	0.35	1	16	250–500 mg q6h	0.5 g q12h (CVVHD/CVVH: Q_d 1.5 l/h, Q_{uf} 1.5 l/h)
Fluoroquinolone Antibiotics							
Ciprofloxacin	331.3	20–40%	1.9–2.8	4.4	8.7	400 mg q12h	200 mg q8–12h (CVVHD: Q_d 1–2 l/h, 0.43 m^2 AN69)
							200 mg q12h (CVVH: Q_{uf} 1 l/h, postdilution, 0.6 m^2 AN69)
							200 mg q12h (CVVHDF: Q_d 1 l/h, Q_{uf} 1 l/h, postdilution, 0.6 m^2 AN69)
							200 mg q8h (CVVHDF: Q_d 1 l/h, Q_{uf} 2 l/h, predilution, 0.6 m^2 AN69)
Enoxacin	320.3	40%	1.6	3.0–6.0	15–25	400 mg q12h	400 mg q24h (CVVHD/CVVH: Q_d 1.5 l/h, Q_{uf} 1.5 l/h)
Levofloxacin	361	24–38%	1.09–1.26	6.3	76	500–750 mg q24h	250 mg q24h (CVVHD/CVVH/CVVHDF: Q_d 1 l/h, Q_{uf} 1 l/h, postdilution, 0.6 m^2 AN69)
Moxifloxacin	401.4	47%	3.3	12	12	400 mg q24h	400 mg q 24h (CVVHD/CVVH/CVVHDF: Q_d 1 l/h, Q_{uf} 1 l/h, pre-/postdilution, 0.6 m^2 AN69)
Ofloxacin	361.4	20–25%	1.5–2.5	4.0–7.0	40–50	200–400 mg q12h	400 mg q24h (CVVH: Q_{uf} 3 l/h, postdilution, 0.7 m^2 high-flux PS)
Pefloxacin	333.4	20–30%	1.8	8.6	12.0–15.0	400–800 mg q24h	400–800 mg q24h (CVVHD/CVVH: Q_d 1.5 l/h, Q_{uf} 1.5 l/h)
Macrolide Antibiotics							
Erythromycin	734	84%	0.9	1.5	6	150–300 mg q6h	250–500 mg q12–24h (CVVHD/CVVH: Q_d 1.5 l/h, Q_{uf} 1.5 l/h)
Miscellaneous Antibiotics							
Aztreonam	435.4	55%	0.25	2	6.0–8.0	1.0–2.0 g q8–12h	2.0 g q12h (CVVHD/CVVHDF: Q_d 1 l/h, Q_{uf} 1 l/h)
							1.0 g q8h (CVVH: Q_{uf} 1 l/h)
							2.0 g q12h (CVVH: Q_{uf} 2 l/h)
							2.0 g q8h (CVVH: Q_{uf} 3 l/h)
Chloramphenicol	323.1	53%	0.9	4	3.0–7.0	12.5 mg/kg q6h	12.5 mg/kg q6h (CVVHD/CVVH: Q_d 1.5 l/h, Q_{uf} 1.5 l/h)
Clindamycin	425	60–95%	0.7	2.5	4	150–300 mg q6h	600–900 mg q8h (CVVHD/CVVH/CVVHDF: Q_d 1 l/h, Q_{uf} 1 l/h)
Colistin	1750	55%	0.34	2	7.5	2.5 mg/kg q24hr	2.5 mg/kg q48h (CVVHD/CVVH/CVVHDF: Q_d 1 l/h, Q_{uf} 1 l/h)

Table 1. (*cont.*)

Drug	MW (Da)	PPB	V_d (l/kg)	$T_{1/2normal}$ (h)	$T_{1/2anuria}$ (h)	Normal Dosage	Dosage adjustment on CRRT
Daptomycin	1619.7	92%	0.13	8	29.3	4–6 mg q24h	4–6 mg q48h (CVVHD/CVVH/CVVHDF: Q_d 1 l/h, Q_{uf} 1 l/h)
Linezolid	337.3	31%	0.6–0.8	4.4–5.5	7.0–8.0	600 mg q12h	600 mg q12h (CVVHD/CVVH/CVVHDF: Q_d 1–2 l/h, Q_{uf} 1–2 l/h, pre-/postdilution)
Metronidazole	171.2	20%	0.8	6.0–14	7.0–21	7.5 mg/kg q6h	7.5 mg/kg q24h (CVVHD/CVVH: Q_d 1.5 l/h, Q_{uf} 1.5 l/h)
Teicoplanin	1879.7	>90%	0.34–0.89	30–140	157–567	400 mg q24h	200 mg q48h (CVVHD/CVVH/CVVHDF: Q_d 1 l/h, Q_{uf} 1 l/h)
Trimethoprim	290.3	30–50%	1–2.2	11	20–50	100–200 mg q12h	100–200 mg q12h (CVVHD/CVVH: Q_d 1.5 l/h, Q_{uf} 1.5 l/h)
Vancomycin	1449.3	10–55%	0.64	6	200–250	500 mg q6h/1.0 g q12h	1.0 g q24h (CVVHD/CVVHDF: Q_d 1 l/h, Q_{uf} 1–2 l/h) 1.0 g q48h (CVVH: Q_{uf} 1–1.5 l/h)
Penicillins							
Amoxicillin	365.4	15–25%	0.37	1	5.0–20	250–500 mg q8h	1.0 g q12h (CVVHD/CVVH: Q_d 1.5 l/h, Q_{uf} 1.5 l/h)
Ampicillin (/Sulbactam 2:1)	349.4	20%	0.22	1	7.0–20	1.5–3.0 g q6h	3.0 g q8h (CVVHD/CVVHDF: Q_d 1 l/h, Q_{uf} 1 l/h) 3.0 g q12h (CVVH: Q_{uf} 1 l/h)
Azlocillin	461.5	20–46%	0.29	1.3–1.5	6	2.0–3.0 g q4h	3.0 g q24h (CVVHD/CVVH: Q_d 1.5 l/h, Q_{uf} 1.5 l/h)
Flucloxacillin	453.9	95%	0.54	1	3	1.0–2.0 g q6–8h	2.0 g q24h (CVVHD/CVVH: Q_d 1.5 l/h, Q_{uf} 1.5 l/h)
Mezlocillin	539.6	20–46	0.26	1.3	3.0–5.0	1.5–4.0 g q4–6h	2.0 g q24h (CVVHD/CVVH: Q_d 1.5 l/h, Q_{uf} 1.5 l/h)
Nafcillin	414.5	85%	0.35	1	2	1.0–2.0 g q4–6h	2.0 g q4–6h (CVVH/CVVHD/CVVHDF: Q_d 1 l/h, Q_{uf} 1 l/h)
Oxacillin	435.9	92–96%	0.19–0.33	0.5	?	0.25–1.0 g q4–6h	1.0 g q8h (CVVHD/CVVH: Q_d 1.5 l/h, Q_{uf} 1.5 l/h)
Penicillin G	334.4	6–20%	0.3	0.5	6.0–20	0.8–4.0 million U q4–6h	2.0 million U q12h (CVVHD/CVVH: Q_d 1.5 l/h, Q_{uf} 1.5 l/h)
Piperacillin (/Tazobactam 8:1)	516.5	30%	0.3	1	3.0–5.1	3.375 g q6h	2.25 g q6–8h (CVVHD, Q_d 1–1.5 l/h, 0.9 m^2 AN69) 2.25 g q4–6h (CVVH/CVVHDF, Q_d 1 l/h, Q_{uf} 1–2 l/h, postdilution, 0.7 m^2 PS)
Ticarcillin (/Clavulanate 30:1)	384.4	45–60%	0.14–0.22	2.2	11.0–17.0	3.1 g q4–6h	3.1 g q6h (CVVHD/CVVHDF: Q_d 1 l/h, Q_{uf} 1 l/h) 2.0 g q6–8h (CVVH: Q_{uf} 1 l/h)
Tetracycline Antibiotics							
Doxycycline	444.4	>90%	0.75	15–20	18–25	100 mg q24h	100 mg q24h (CVVHD/CVVH: Q_d 1.5 l/h, Q_{uf} 1.5 l/h)
Antifungal Antibiotics							
Amphotericin B lipid complex	924.1	>90%	1.7–3.9	173	173	5 mg/kg q24h	3.0–5.0 mg/kg q24h (CVVHD/CVVH/CVVHDF: Q_d 1 l/h, Q_{uf} 1–2 l/h)

Table 1. *(cont.)*

Drug	MW (Da)	PPB	V_d (l/kg)	$T_{1/2normal}$ (h)	$T_{1/2anuria}$ (h)	Normal Dosage	Dosage adjustment on CRRT
Fluconazole	306.3	12%	0.7	37	100	200–400 mg q24h	400–800 mg q24h (CVVHD/CVVHDF: Q_d 1 l/h, Q_{uf} 1 l/h)
							200–400 mg q24h (CVVH: Q_{uf} 1l/h)
Flucytosine	129.1	<10%	0.6	3.0–6.0	75–200	37.5 mg/kg q6h	37.5 mg/kg q12h (CVVHD/CVVH: Q_d 1.5 l/h, Q_{uf} 1.5 l/h)
Itraconazole, i.v.	705.6	99.80%	10	21	25	100–200 mg q12h	100–200 mg q12h (CVVHD/CVVH: Q_d 1.5 l/h, Q_{uf} 1.5 l/h)
Voriconazole, i.v.	349.3	58%	4.6	12	13.7	6 mg/kg q12h twice then 4 mg/kg q12h	6 mg/kg q12h twice then 4 mg/kg q12h (CVVHDF: Q_d 1 l/h, Q_{uf} 0.5 l/h, predilution, 0.9 m^2 AN69)
Antituberculous Antibiotics							
Ethambutol	204.3	20–30	1.6	4	20	15–25 mg/kg q24h	10–15 mg/kg q24–48 h (CVVHD/CVVH: Q_d 1.5 l/h, Q_{uf} 1.5 l/h)
Isoniazid	137.1	4–30%	0.75	1.0–4.0	1.0–17	300 mg q24h	300 mg q24h (CVVHD/CVVH: Q_d 1.5 l/h, Q_{uf} 1.5 l/h)
Rifampin	823	89%	0.9	3.5	9	600 mg q24h	600 mg q24h (CVVHD/CVVH: Q_d 1.5 l/h, Q_{uf} 1.5 l/h)
Antiviral Agents							
Acyclovir, i.v.	225.2	9–33%	0.7	2.5	20	5.0 mg/kg q8h	5.0–7.5 mg/kg q24h (CVVHD/CVVH/CVVHDF: Q_d 1 l/h, Q_{uf} 1 l/h)
Amantadine	151.2	67%	4.0–5.0	10.0–14.0	7–10 days	100 mg q12h	100–200 mg q60h (CVVHD/CVVH: Q_d 1.5l/h, Q_{uf} 1.5 l/h)
Ganciclovir	256.2	1–2%	0.47	3	30	5.0 mg/kg q12h	5.0 mg/kg q48h (CVVHD/CVVHDF: Q_d 1 l/h, Q_{uf} 0.3 l/h, postdilution)

MW: molecular weight (Da); PPB: plasma protein binding (%); V_d: apparent volume of distribution (l/kg); Q_{uf}: ultrafiltration rate; Q_d: dialysate flow rate; $T_{1/2normal}$: normal plasma half-life (h); $T_{1/2anuria}$: plasma half-life in anuric nondialyzed patients (h); CRRT: continuous renal replacement therapy; CVVHD: continuous venovenous hemodialysis; CVVH: continuous venovenous hemofiltration; CVVHDF: continuous venovenous hemodiafiltration; i.v. intravenous; ?: data not available

Estimation by mathematical equation

Making these estimations is time consuming, requiring a careful search for basic pharmacokinetic data. Drug clearance must be calculated to determine a maintenance dose. The serum concentration at steady state (C_{pss}) multiplied by the extracorporeal clearance (Cl_{EC}) provides the clinician with the amount of drug specifically removed by ultrafiltration per hour under steady-state conditions. Thereupon, one can calculate the amount of drug removed by CRRT (D_{EC}) with the following equation:

$$D_{EC} = C_{pss} \times Cl_{EC} \times T_{dur} \quad \text{(Eqn 10)}$$

where T_{dur} is the duration of CRRT.

The extracorporeal clearance can be calculated by equations 3, 5, 6, 7, and 9 according to the type of operational mode. The total amount of a drug during CRRT (D) may be calculated using the following equation including the typical anuric dose (D_{anur}) in addition to D_{EC} [19]:

$$D = D_{anur} + D_{EC} = D_{anur} + C_{pss} \times Cl_{EC} \times T_{dur} \quad \text{(Eqn 11)}$$

The drug dose during CRRT in an anuric patient may also be estimated from the following equation [15]:

$$D = D_{anur} \times [1 + Cl_{EC} / Cl_{NR} / 2^{(Interval/Halflife)}] \quad \text{(Eqn 12)}$$

where Cl_{NR} is the non-renal clearance, Halflife is the $t_{1/2}$ of the drug in an anuric non-dialyzed patient, and Interval is the dose interval in an anuric non-dialyzed patient.

At present, there is increasingly a tendency to start CRRT earlier in the course of illness, and renal replacement therapy may contribute to drug clearance. According to Dettli's equation and the related investigation by Keller et al., the estimated dose during CRRT in a patient with RRT may be [5]:

$$D_{EC} = D_n \times [P_x + (1 - P_x) \times Cl_{CRtot} / Cl_{CRn}] \quad \text{(Eq 13)}$$

where D_n is the normal dose, Cl_N is the normal drug clearance, $P_x = Cl_{NR}/Cl_N$, Cl_{CRtot} is the sum of renal and extracorporeal creatinine clearance, and Cl_{CRn} is the normal creatinine clearance.

However, complex mathematical models have been proposed, but an accurate calculable equation remains unavailable, because drug dose data in patients with acute renal failure are rare and the calculation of drug clearance in various modalities of CRRT is also complicated. Most mathematical models have only been demonstrated to be suitable for certain drugs; their application in clinical practice is still limited.

Whether it may be more appropriate to increase the drug dose or to shorten the dosing interval in critically ill patients during CRRT is dependent on the mechanisms of action and the kill characteristics of the various classes of antimicrobial agents. For concentration-dependent kill characteristic antimicrobial agents such as aminoglycosides, it is better to increase the drug dose because their antibiotic effects correlate with the Cmax. In contrast, for time-dependent kill characteristic antimicrobial agents such as β-lactam antibiotics, it is better to shorten the drug dosing interval because their antibiotic effects correlate with the T > MIC. The shorter dosing interval during CRRT may be estimated from the following equation:

$$Iv_{EC} = Iv_{anu} \times [Cl_{NR} / (Cl_{EC} + Cl_{NR})] \quad \text{(Eqn 14)}$$

where Iv_{EC} is the interval during CRRT and Iv_{anu} is the interval in an anuric patient

Drug serum concentration monitoring

Not only are pharmacokinetics and pharmacodynamics often less predictable in critically ill patients, but it has not been sufficiently documented that doses may be accurately adjusted according to current drug-dosing recommendations or available mathematical equations. Therefore, monitoring of plasma concentrations is highly recommended whenever possible, and especially for drugs with a narrow therapeutic index, such as vancomycin and aminoglycosides. Although monitoring of drug concentrations is considered reasonable to enhance optimal dosing and minimize toxic side effects, it is not readily available for all medications. The following formula is often used to estimate the dose requiring (D_{req}) to achieve the desired peak concentration (Cpeak) from the actual trough (or any) concentration (Cactual):

$$D_{req} = (C_{peak} - C_{actual}) \times V_d \times \text{Body weight} \quad \text{(Eqn 15)}$$

Conclusion

Appropriate anti-infective therapy remains essential in decreasing the persistent high morbidity and mortality rates in the ICU. Considerable data are available to demonstrate that sepsis, acute renal failure, and CRRT may each have profound effects on the pharmacokinetic and pharmacodynamic characteristics of various antibmicrobial agents commonly used in the ICU. The extent of the alteration is dependent on multiple mechanical and drug factors during the treatment of sepsis. Understanding of these interactions, fundamental pharmacological principles, and drug clearance during CRRT is important to adjust antibiotic regimens in critically ill patients. Awareness of the kill characteristics of the antibiotic in question helps determine the optimum mode of administration. Meanwhile, monitoring the drug serum concentration is still mandatory whenever clinically feasible. More pharmacokinetic simulation modeling and clinical studies are needed to provide accurate guidance on the appropriate dosage adjustment under different circumstances.

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Abdominal Pathologies

Abdominal Compartment Syndrome

M.L.N.G. Malbrain, N. Van Regenmortel, and M.L. Cheatham

Introduction

Interest in intra-abdominal hypertension (IAH) and abdominal compartment syndrome (ACS) as causes of significant morbidity and mortality among the critically ill has increased exponentially over the past decade [1, 2]. Given the prevalence of elevated intra-abdominal pressure (IAP) as well as earlier detection and appropriate therapeutic management of IAH and ACS, significant decreases in patient morbidity and mortality have been achieved in recent years.

As our understanding of the pathophysiology surrounding these two syndromes has evolved, IAP measurements have been identified as essential to the diagnosis and management of both IAH and ACS and have gained increasing prominence in intensive care units (ICUs) worldwide. The accuracy and reproducibility of the methods promoted for measuring IAP, however, have been variable [1–5]. Similarly, the threshold values used to define the presence of IAH and ACS have lacked consensus. Some use the terms IAH and ACS interchangeably, resulting in conflicting definitions, confusion, and the inability to compare the results of published clinical trials [6–9].

Given the steadily growing awareness of IAH and ACS, this book chapter will start with an overview of surveys and questionnaires on IAH and ACS followed by a summary on the recently published state-of-the-art definitions [10] and recommendations for IAH and ACS as well as standardized techniques for IAP monitoring to facilitate future research and improve patient care [9–14].

Clinical Awareness

The results of several surveys on IAH and ACS have been published [15–20], showing that there is still a general lack of clinical awareness (although better than 10 years ago) and that many ICUs never measure the IAP. When it is measured, the intravesical route is used exclusively. No consensus exists on the optimal timing of measurement or when decompressive laparotomy should be performed and there is great variation in opinions among surgeons, intensivists, and pediatricians. Table 1 summarizes the results of the different surveys.

Recently, a new survey, endorsed by the European Society of Intensive Care Medicine (ESICM) and the Society of Critical Care Medicine (SCCM) has been launched (www.wsacs.org/survey.htm). So far, there are about 1300 respondents and the goal is to reach >2500 critical care health workers. In summary, 13.6% of the respondents are still not familiar with IAH or the effects of increased IAP on organ function, while 1.3% have never heard about ACS. In total, 69.2% of the respondents

Table 1. Overview of published surveys on intra-abdominal hypertension (IAH) and abdominal compartment syndrome (ACS)

	Canada	Australia	USA	USA	UK	UK	Holland
Author [ref]	Kirkpatrick	Nagappan	Mayberry	Kimball	Ravishankar	Tiwari	Van Waes
Publication date	2006 [15]	2005 [16]	1999 [17]	2006 [18]	2005 [19]	2006 [20]	2006 (personal communication)
Respondents	Trauma physicians	ICU registrars	Trauma surgeons	SCCM members	ICU	ICU	Surgeons
Number of questionnaires	102	40	292	4538	207	222	NA
Response rate	86 (84%)	36 (90%)	292 (100%)	1622 (35.7%)	137 (66.2%)	127 (57.2%)	NA
Know ACS	100% (trauma)	92%	85%	98% (surgeon) 75% (internist-pediatrician)	98.5%	96.9% (university) 72.6% (district)	88% (university) 43% (teaching) 22% (non teaching)
Measure IAP	Screening 52%	48–83%	66%-95%	98% (surgeon) 76% (internist-pediatrician)	75.9%		
Diagnosis	Clinical (21%) IBP (95%) Stomach pressure (13%)	IBP (83%) CT (17%)	Clinical + IBP (71%) IBP alone (14%)	Clinical + IBP (70%) IBP alone (7%) Clinical alone (20%)	IBP (100%)	Clinical (68.4%) IBP (83.7%)	Bladder (stomach)
Threshold	25 mmHg + OF 34.3 mmHg – OF (8% OA)	20 mmHg (63.9%) 12 mmHg (11%)	20 cmH_2O (11%) 25 cmH_2O (22%) 30 cmH_2O (32%)	20–27 mmHg (42%) 12–19 (25% ped vs 18% surg) 8 mmHg (0%)		15–50 mmHg	25 cmH_2O 23 mmHg 20 mmHg + organ failure
Measured when?	Suspicion (66%)		When indicated		Suspicion (93%)	Abdominal distention (98.6%)	Oliguria
	Hemodynamically unstable (67%) Ventilation (75%)				Emergency laparotomy (3.8%) Fluid resuscitation (2.9%)	Oliguria (94.5%) Ventilation (72.2%)	Ventil difficulty Distention
Timing IAP					4–8hr (28%) When indicated (44.2%)		
Causes of ACS	Sepsis		Trauma		Surgery	Large bowel surgery (67%)	Peritonitis
	Fluid overload Hemorrhage					Vascular surgery (62%) Trauma (60%)	Blunt trauma Vascular GE
Treatment	OA (90%) Unable to close Bogota bag (64%) VAC (27%) Mesh (17%)			67% (pediatrician) 80% (internist) 96.4% (surgeon)			10–60% Mesh Vasuseal Bogota

ACS: Abdominal compartment syndrome; CT: Computed tomography; IAP: Intra-abdominal pressure; IBP: Intra-bladder pressure; ICU: Intensive care unit; OA: Open abdomen; OF: Organ failure; VAC: Vacuum assisted closure

believe the combination of clinical examination with IAP is the best method for diagnosing IAH, while 24.1% use IAP alone. Other methods used are abdominal CT (13.1%); abdominal perimeter measurement (10.1%), and abdominal ultrasound (7.8%). The transvesical method is most widely used (92.3%) followed by direct intraperitoneal measurement (4.2%), and the stomach (2.8%). When the bladder is used, 52.8% instil 50 ml, while 21.9% instil 100 ml and 4.3% up to 200 ml! It is, however, reassuring that already 16.2% of respondents use low instillation volumes (<25 ml) as recommended. On the other hand, 6.8% do not wait for equilibration whilst measuring IAP and 51.9% are aware of continuous IAP measuring methods. The concept of abdominal perfusion pressure (APP) is known of by 81.5% and the filtration gradient by 19.7%. Regarding normal values, 14.8% believe that the normal IAP is above 10 mmHg, while 77.1% define IAH as an IAP above 15 mmHg, and 58% define ACS as an IAP above 25 mmHg.

Definitions

Consensus definitions covering all aspects of IAH and ACS were recently developed at an international conference of experts on intra-abdominal hypertension and ACS [10] and are summarized in Table 2.

Table 2. Consensus definitions [10]

Definition 1	IAP is the steady-state pressure concealed within the abdominal cavity.
Definition 2	APP = MAP − IAP
Definition 3	FG = GFP − PTP = MAP − 2 * IAP
Definition 4	IAP should be expressed in mmHg and measured at end-expiration in the complete supine position after ensuring that abdominal muscle contractions are absent and with the transducer zeroed at the level of the mid-axillary line.
Definition 5	The reference standard for intermittent IAP measurement is via the bladder with a maximal instillation volume of 25 mL of sterile saline.
Definition 6	Normal IAP is approximately 5–7 mmHg in critically ill adults.
Definition 7	IAH is defined by a sustained or repeated pathologic elevation of IAP ≥12 mmHg.
Definition 8	IAH is graded as follows: • Grade I: IAP 12–15 mmHg • Grade II: IAP 16–20 mmHg • Grade III: IAP 21–25 mmHg • Grade IV: IAP >25 mmHg
Definition 9	ACS is defined as a sustained IAP >20 mmHg (with or without an APP <60 mmHg) that is associated with new organ dysfunction / failure.
Definition 10	Primary ACS is a condition associated with injury or disease in the abdomino-pelvic region that frequently requires early surgical or interventional radiological intervention.
Definition 11	Secondary ACS refers to conditions that do not originate from the abdomino-pelvic region.
Definition 12	Recurrent ACS refers to the condition in which ACS redevelops following previous surgical or medical treatment of primary or secondary ACS.

IAP: intra-abdominal pressure; MAP: mean arterial pressure; APP: abdominal perfusion pressure; IAH: intra-abdominal hypertension; ACS: abdominal compartment syndrome; FG: filtration gradient; GFP: glomerular filtration pressure; PTP: proximal tubular pressure

Intra-abdominal Pressure

Since the abdomen and its contents can be considered as relatively non-compressive and primarily fluid in character, behaving in accordance to Pascal's law, the IAP measured at one point may be assumed to represent the IAP throughout the abdomen [3, 4]. IAP is, therefore, defined as the steady-state pressure concealed within the abdominal cavity. IAP increases with inspiration (diaphragmatic contraction) and decreases with expiration (diaphragmatic relaxation).

Abdominal Perfusion Pressure

Analogous to the widely accepted and clinically utilized concept of cerebral perfusion pressure (CPP), calculated as mean arterial pressure (MAP) minus intracranial pressure (ICP), abdominal perfusion pressure (APP), calculated as MAP minus IAP, has been proposed as a more accurate predictor of visceral perfusion and a potential endpoint for resuscitation [21–24]. APP, by considering both arterial inflow (MAP) and restrictions to venous outflow (IAP), has been demonstrated to be statistically superior to either parameter alone in predicting patient survival from IAH and ACS [24]. A target APP of at least 60 mmHg has been demonstrated to correlate with improved survival from IAH and ACS.

Filtration Gradient

Inadequate renal perfusion pressure and renal filtration gradient have been proposed as key factors in the development of IAP-induced renal failure [24, 25]. Changes in IAP will have a greater impact on renal function and urine production than will changes in MAP. As a result, oliguria is one of the first visible signs of IAH [26].

IAP Measurement

Recent studies have shown that clinical judgment or physical examination are far from accurate in predicting a patient's IAP [27, 28]. The bladder technique has been the most widely used worldwide due to its simplicity and minimal cost [3, 4]. Recently, several methods for continuous IAP measurement via the stomach, peritoneal cavity, and bladder have been validated [29–32]. Although these techniques seem promising, further clinical validation is needed before their routine use can be recommended.

In an attempt to standardize and improve the accuracy and reproducibility of IAP measurements, it is proposed that IAP should be expressed in mmHg and measured at end-expiration in the supine position after ensuring that abdominal muscle contractions are absent and with the transducer zeroed at the level of the mid-axillary line [10]. The reference standard for intermittent IAP measurement is via the bladder with a maximal instillation volume of 25 ml of sterile saline.

Normal and Pathological IAP values

In the strictest sense, normal IAP ranges from zero to 5 mmHg [33]. Certain physiologic conditions, however, such as morbid obesity or pregnancy, may be associated with chronic IAP elevations of 10–15 mmHg to which the patient has adapted with

no significant pathophysiology. In contrast, children commonly demonstrate low IAP values [35]. The clinical importance of any IAP value must be assessed in view of the baseline steady-state IAP for the individual patient. The normal IAP is approximately 5–7 mmHg in critically ill adults [10].

Intra-Abdominal Hypertension

Pathological IAP is a continuum ranging from mild elevations in IAP without clinically significant adverse effects to substantial increases in IAP with grave consequences to virtually all organ systems in the body [7]. IAH is defined as a sustained or repeated pathologic elevation of IAP $\geq$12 mmHg [10], and can be graded in severity from I to IV (most severe). The more severe the degree of IAH, the more urgent is the need for decompression of the abdomen (either medically or surgically) with resolution of the damaging pressure.

Abdominal Compartment Syndrome

IAH clearly represents a continuum, with IAP varying from patient to patient and from moment to moment according to underlying etiologic factors, cardiac filling status, presence of organ failure, and pre-existing comorbidities (Fig. 1). Although the critical IAP that defines ACS is subject to debate, of greater importance than any one absolute IAP value is the associated development of organ dysfunction and failure [29]. In contrast to IAH, ACS should not be graded, but rather considered as an 'all or nothing' phenomenon. ACS is defined as a sustained IAP >20 mmHg (with or without an APP <60 mmHg) that is associated with new organ dysfunction/failure [10].

Classification of IAH/ACS

Primary ACS (formerly termed surgical, postoperative, or abdominal ACS) is characterized by the presence of acute or subacute IAH of relatively brief duration occurring as a result of an intra-abdominal etiology such as abdominal trauma, ruptured abdominal aortic aneurysm, hemoperitoneum, acute pancreatitis, secondary peritonitis, retroperitoneal hemorrhage, or liver transplantation. It is most commonly encountered in the traumatically injured or post-operative surgical patient.

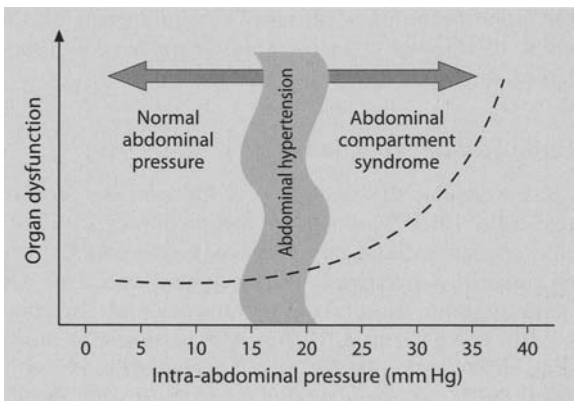

Fig. 1. Distinctions between normal intra-abdominal pressure, intraabdominal hypertension (IAH), and abdominal compartment syndrome (ACS). The shaded area illustrates that IAH may undergo shifts to the right or left depending on the clinical scenario. Adapted from [12] with permission.

Secondary ACS (formerly termed medical or extra-abdominal ACS) is characterized by the presence of subacute or chronic IAH that develops as a result of an extra-abdominal etiology such as sepsis, capillary leak, major burns, or other conditions requiring massive fluid resuscitation [6, 36–40]. It is most commonly encountered in the medical or burn patient [11–13, 37].

Recurrent ACS (formerly termed tertiary ACS) represents a redevelopment of ACS symptoms following resolution of an earlier episode of either primary or secondary ACS. It is most commonly associated with the development of acute IAH in a patient who is recovering from IAH/ACS and therefore represents a 'second-hit' phenomenon. Recurrent ACS may occur despite the presence of an open abdomen (known as the 'open abdomen compartment syndrome') or as a new ACS episode following definitive closure of the abdominal wall [41]. Recurrent ACS is associated with significant morbidity and mortality [42].

Occasionally, patients may demonstrate signs and symptoms consistent with both primary and secondary ACS. Examples might include a patient who develops sepsis with fluid overload after initial surgical stabilization for trauma [11, 40]. This overlap of clinical conditions and potential etiologies has added to the confusion regarding the definition of ACS. Nevertheless, the majority of IAH/ACS patients may be assigned to one of these three classes.

Pathophysiologic Implications

It is beyond the scope of this chapter to give a concise and complete review of the pathophysiologic implications of raised IAP on organ function inside and outside the abdominal cavity. Therefore, we will summarize some key-points related to each organ that may affect daily clinical practice in the ICU.

Neurologic Function

Because of the interactions between intra-abdominal, intrathoracic, and intracranial pressures, accurate monitoring of IAP in head trauma victims with associated intra-abdominal lesions is worthwhile. The presence of high IAP can be an additional extracranial cause of intracranial hypertension not only in head trauma patients but also in patients with abdominal trauma but without overt craniocerebral lesions. For these reasons, recent head injury should be considered an absolute contraindication for laparoscopic procedures. The same principles are responsible for the development of idiopathic intracranial hypertension (pseudotumor cerebri) in morbidly obese patients

Cardiovascular Function

Cardiovascular dysfunction and failure (low cardiac output, high systemic vascular resistance [SVR]) are commonly encountered in the patient with IAH or ACS. Accurate assessment and optimization of preload, contractility, and afterload is essential to restoring end-organ perfusion and function. Our understanding of traditional hemodynamic monitoring techniques and parameters, however, must be re-evaluated in the patient with IAH/ACS as pressure-based estimates of intravascular volume such as pulmonary artery occlusion pressure (PAOP) and central venous pressure (CVP) are erroneously increased. If such limitations are not recognized, misin-

terpretation of the patient's minute-to-minute cardiac status may result in the institution of inappropriate and potentially detrimental therapy. The clinician must be aware of the interactions between intrathoracic pressure, IAP, positive end-expiratory pressure (PEEP), and intracardiac filling pressures in order to correctly resuscitate these patients. Transmural filling pressures might better reflect preload and are obtained by subtracting the intrathoracic pressure from the end-expiratory pressure:

$CVP_{tm} = CVP_{ee} - ITP$
$PAOP_{tm} = PAOP_{ee} - ITP$

A quick estimate of transmural filling pressures can be obtained by subtracting half of the IAP from the end-expiratory filling pressure (since the average abdominothoracic transmission is around 50 %).

$CVP_{tm} = CVP_{ee} - IAP/2$
$PAOP_{tm} = PAOP_{ee} - IAP/2$

The surviving sepsis campaign guidelines targeting initial and ongoing resuscitation towards a CVP of 8 to 12 mmHg should be interpreted with caution in case of IAH/ACS to avoid unnecessary over- and under resuscitation. Volumetric estimates of preload status such as right ventricular (RV) end-diastolic volume index (RVEDVI), global end-diastolic volume index (GEDVI), or intrathoracic blood volume index (ITBVI) are especially useful in such patients with changing ventricular compliance and elevated ITP related to IAH. Functional hemodynamic parameters, such as stroke volume variation (SVV), pulse pressure variation (PPV) or systolic pressure variation (SPV) should be used to assess volume responsiveness. Application of an aggressive, goal-directed resuscitation strategy improves cardiac function, reverses end-organ failure, and minimizes IAH-related patient morbidity and mortality.

Respiratory Function

The presence of IAH decreases total respiratory system compliance by decreasing chest wall compliance, lung compliance being unchanged (except in cases of concomitant primary acute respiratory distress syndrome [ARDS]) finally resulting in a form of restrictive lung disease. Best PEEP can be set to counteract IAP whilst at the same time avoiding over-inflation of already well-aerated lung regions (consider applying weights on the chest at PEEP-levels above 20 cmH_2O):

Best PEEP = IAP

The ARDS consensus definitions should take into account PEEP and IAP values. During lung protective ventilation, the plateau pressures should be limited to transmural plateau pressures below 35 cmH_2O instead of the classical alveolar plateau pressures measured by the ventilator:

$Pplat_{tm} = Pplat - IAP/2$

The PAOP criterion in the ARDS consensus definitions is futile in case of IAH and should be adapted since most patients with IAH and secondary ARDS will have filling pressures above the 18 mmHg definition cut-off. The presence of IAH dramatically increases lung edema especially in cases of direct lung injury or capillary leak; within this concept monitoring of extravascular lung water index (EVLWI) seems warranted. The combination of capillary leak, positive fluid balance, and raised IAP puts the patient at an increased risk of lung edema. Body position affects IAP; put-

ting an obese patient in the upright position can cause ACS. Conversely, the abdomen should hang freely during prone positioning. The anti-Trendelenburg position may improve respiratory mechanics; however it can decrease splanchnic perfusion. The use of curarization should be balanced with, on one side, the beneficial effect on abdominal muscle tone resulting in a decrease in IAP and improvement in APP, and, on the other side, worsened lung mechanics due to the more cranial position of the diaphragm during curarization (especially in conditions of IAH or ACS). The presence of IAH will lead to pulmonary hypertension via increased intrathoracic pressures with direct compression on lung vessels and via diminished left and right ventricular compliance.

Renal Function

Decreased renal function, as evidenced by development of oliguria, is one of the first visible signs of IAH. Clinicians should be aware of elevated IAP and its effect on renal function as often the first sign of impending ACS. Renal function may be improved by paracentesis of ascitic fluid and reduction in the IAP. A prompt reduction in IAP has dramatic beneficial effects on urine output in patients with primary and secondary IAH after trauma. Within the capsule of the kidney itself, local hematoma formation may have an adverse effect on tissue perfusion causing a local renal compartment syndrome.

Liver Function

The liver appears to be particularly susceptible to injury in the presence of elevated IAP. In this condition, the plasma disappearance rate for indocyanine green (ICG-PDR) is a promising parameter as it correlates not only with liver function and perfusion but also with IAP. Since cytochrome P450 function may be altered in case of IAH/ACS, medication doses should be adapted accordingly. Within the capsule of the liver itself, local hematoma formation may have an adverse affect on tissue perfusion causing a local hepatic compartment syndrome. With increasing IAP, there is decreased hepatic arterial flow, decreased venous portal flow, and an increase in the portacollateral circulation; these features all exert physiological effects with decreased lactate clearance, altered glucose metabolism, and altered mitochondrial function.

Splanchnic Perfusion

IAH has profound effects on splanchnic organs, causing diminished perfusion, mucosal acidosis and setting the stage for multiple organ failure (MOF). IAP inversely correlates with gastric intramucoasl pH (pHi) and with ICG-PDR. IAH triggers a vicious cycle leading to intestinal edema, ischemia, bacterial translocation, and finally MOF. Maintenance of adequate perfusion pressure (APP >65 mmHg) is mandatory.

Recommendations

A summary of the recommendations developed at the recent international conference of experts on IAH and ACS is provided in Table 3.

Table 3. Consensus recommendations

Recommendation	Term	Description
Recommendation 1	Risk factors for IAH	Patients admitted to the ICU should be screened at least once (preferably on admission) for possible risk factors for the development of IAH or ACS.
Recommendation 2	Indication for IAP monitoring	If one or more risk factor is present, a baseline IAP measurement should be obtained for future reference.
Recommendation 3	Epidemiology	Studies examining the prevalence and incidence of IAH/ACS should be based on the consensus definitions and classifications.
Recommendation 4	Epidemiology	Epidemiologic data should be given for mean, median and maximal IAP values on admission and during the study stay.
Recommendation 5	Treatment	Treatment for IAH/ACS is based on three principles: – specific medical procedures to reduce IAP and the consequences of ACS – general (intensive care) support of the critically ill patient – optimization after (surgical) decompression to perhaps counteract some of the specific adverse effects associated with decompression
Recommendation 6	Medical treatment	The medical treatment options should be targeted to specific goals and can be divided into 5 groups: – Improvement of abdominal wall compliance – Evacuation of intraluminal contents – Evacuation of peri-intestinal and abdominal fluids – Correction of capillary leak and positive fluid balance – Specific treatment
Recommendation 7	Temporary abdominal closure (TAC)	It is not obligatory to use a vacuum assisted fascial closure (VAFC) as first time TAC since it is quite expensive and about one third of the patients can have their fascia closed at the second laparotomy. If used a home made VAFC is preferred initially.
Recommendation 8	IAP measurement	Future studies need to examine the ideal frequency for IAP measurement as well as the diurnal and nocturnal variations during continuous IAP monitoring.
Recommendation 9	IAP and APP tresholds	Studies looking at IAP and APP thresholds should be based on the analysis of receiver operating characteristics (ROC) and the area under the ROC-curve.
Recommendation 10	ROC curves	A good area under the ROC curve should be at least 0.75, and the best threshold needs to be identified with a sensitivity and/or specificity of at least, or close to 75%.
Recommendation 11	IAP validation	Studies examining new devices to measure IAP should always compare the new IAP measurement method with some form of gold standard.
Recommendation 12	IAP validation	The validation of the new technique should not be limited to the analysis of correlation, but it should also include a Bland and Altman analysis.
Recommendation 13	Bias	The bias or the difference between 2 IAP methods should be close to 0 mmHg (range −1 tot +1 mmHg).
Recommendation 14	Limits of agreement	The maximal allowed limits of agreement (LA) when comparing 2 IAP methods should be within a range of 4 mmHg (LA = bias ± 4 mmHg).

IAP: intra-abdominal pressure; APP: abdominal perfusion pressure; IAH: intraabdominal hypertension; ACS: abdominal compartment syndrome; LA: limits of agreement; ROC: receiver operating characteristics; TAC: temporary abdominal closure; VAFC: vacuum assisted fascial closure

Indications for IAP Monitoring

Indications for IAP monitoring should be based on the presence/absence of predisposing risk factors. Many conditions have been reported in association with IAH/ACS, and they can be classified into four categories: Factors related to diminished abdominal wall compliance; factors related to increased intra-abdominal contents; factors related to abdominal collections of fluid, air or blood; and factors related to capillary leak and fluid resuscitation (Table 4). Patients admitted to the ICU should be screened at least once (preferably on admission) for possible risk factors for the development of IAH or ACS. If one or more risk factor(s) is present at baseline, IAP measurement should be obtained for future reference.

Epidemiology

How big is the issue? The problem in answering this question is that studies have used different criteria to define IAH. Epidemiologic data on IAH and ACS depend

Table 4. Risk factors predisposing to intra-abdominal hypertension/abdominal compartment syndrome

Factors related to diminished abdominal wall compliance
• Mechanical ventilation, especially fighting against the ventilator and the use of accessory muscles
• Use of positive end-expiratory pressure (PEEP) or the presence of auto-PEEP
• Basal pleuro-pneumonia
• High body mass index
• Pneumoperitoneum
• Abdominal (vascular) surgery, especially with tight abdominal closure
• Prone and other body positioning
• Abdominal wall bleeding
• Correction of large hernias, gastroschisis, or omphalocele
• Burns with abdominal eschars
Factors related to increased intra-abdominal contents
• Gastroparesis, gastric distention, ileus, colonic pseudo-obstruction
• Abdominal tumor
• Retroperitoneal/abdominal wall hematoma
Factors related to abdominal collections of fluid, air or blood
• Liver dysfunction with ascites
• Abdominal infection (pancreatitis, peritonitis, abscess)
• Hemoperitoneum
• Pneumoperitoneum
Factors related to capillary leak and fluid resuscitation
• Acidosis (pH below 7.2)
• Hypothermia (core temperature below 33 °C)
• Polytransfusion/trauma (> 10 units of packed red cells/24 hours)
• Coagulopathy (platelet count below 50,000/mm^3 or an activated partial thromboplastin time (aPTT) more than 2 times normal or a prothrombin time (PT) below 50% or an international standardized ratio (INR) more than 1.5)
• Sepsis
• Bacteremia
• Massive fluid resuscitation (> 5 liters of colloid or crystalloid/24 hours with capillary leak and positive fluid balance)
• Major burns

on the accuracy and reproducibility of the method used to measure IAP [4]. Over the years, different threshold values have been suggested for IAH and ACS and sometimes the terms IAH and ACS have been interchanged. Others have suggested other terms, such as primary or secondary ACS, but with ever-changing definitions [6]. To date it is, therefore, very difficult to interpret the available data.

Future studies examining the prevalence and incidence of IAH/ACS should be based on the consensus definitions and classifications [10], and results should be given for mean, median and maximal IAP values on admission and during the study stay.

Treatment

General treatment for IAH or ACS should be based on three principles:

1. Specific medical procedures to reduce IAP and the consequences of ACS
2. General (intensive care) support of the critically ill patient
3. Optimization after (surgical) decompression to counteract some of the specific adverse effects associated with decompression

Medical treatment

Before surgical decompression is considered, less invasive medical treatment options should be tried as a first treatment option, especially in cases of secondary IAH or ACS. Different medical treatment procedures have been suggested to decrease IAP [22]. These include the use of paracenthesis, gastric suctioning, rectal enemas, gastroprokinetics (cisapride, metoclopramide, domperidone, erythromycin), colonoprokinetics (prostygmine), furosemide either alone or in combination with human albumin 20 %, continuous venovenous hemofiltration (CVVH) with aggressive ultrafiltration, continuous negative abdominal pressure, and finally sedation and curarization. Table 5 provides an overview of the different medical treatment options, divided according to specific goals.

Surgical treatment

Surgical abdominal decompression obviously results in an open abdomen, which can be a challenge to the ICU physicians and nurses. Fig. 2 shows a suggested surgical treatment algorithm. Several methods for temporary abdominal closure are available:

- Towel clip or moist gauze closure is often used as an initial method of temporary abdominal closure after damage control surgery, because of the speed of closure. After re-exploration, it can be replaced by one of the following techniques
- The 'Bogota bag' is a plastic sheet cut from a sterile 3 liter irrigation bag, and sewn to the skin or fascia. This system is cheap and offers the advantage that the bowel and abdominal contents can be easily inspected and accessed, but fluid losses are difficult to control which makes it a real challenge for the nursing staff.
- Removable prosthetic material was initially used in open abdomen treatment of intra-abdominal sepsis, and can be used for temporary abdominal closure in other circumstances as well. Examples are zippers and Wittmann patch (which uses a Velcro closure system).
- Vacuum assisted fascial closure systems are packing techniques that use suction or vacuum to control the fluid draining from the open abdomen (e.g., the vac-

Table 5. Medical treatment options for intra-abdominal hypertension (IAH) and abdominal compartment syndrome (ACS)

1. Improvement in abdominal wall compliance
– Sedation
– Pain relief (not fentanyl!)
– Neuromuscular blockade
– Body positioning
– Negative fluid balance
– Skin pressure decreasing interfaces
– Weight loss
2. Evacuation of intraluminal contents
– Gastric tube and suctioning
– Gastroprokinetics (erythromycin, cisapride, metoclopramide)
– Rectal tube and enemas
– Colonoprokinetics (neostigmine, prostygmine bolus or infusion)
– Endoscopic decompression of large bowel
– Colostomy or ileostomy
3. Evacuation of peri-intestinal and abdominal fluids
– Ascites evacuation
– Percutaneous drainage of collections
– CT or ultrasound guided aspiration of abscess or hematoma
4. Correction of capillary leak and positive fluid balance
– Correction of capillary leak (antibiotics, source control, ...)
– Colloids instead of crystalloids
– Dobutamine (not dopamine!)
– Albumin in combination with diuretics (furosemide)
– Dialysis with ultrafiltration
5. Specific therapeutic interventions
– Continuous negative abdominal pressure (CNAP)
– Negative external abdominal pressure (NEXAP)
– Targeted abdominal perfusion pressure (APP)
– (experimental: Octreotide and melatonin in secondary abdominal compartment syndrome)

uum pack technique and modified sandwich vacuum pack technique, or vacuum assisted closure system). These are simple solutions for the management of an open abdomen and provide easy control and quantification of fluid losses. The World Society of the Abdominal Compartment Syndrome (WSACS) advocates the use of a home-made vacuum assisted fascial closure system for first time temporary abdominal closure since this is quite inexpensive and about one third of the patients can have their fascia closed at the second laparotomy.

Implications for Future Research

Epidemiologic data

Studies examining the prevalence and incidence of IAH/ACS should be based on the above cited definitions and classifications. The results should be given for mean, median and maximal IAP values on admission and during the study stay. Future studies need to examine the ideal frequency for IAP measurement as well as the diurnal and nocturnal variations during continuous IAP monitoring.

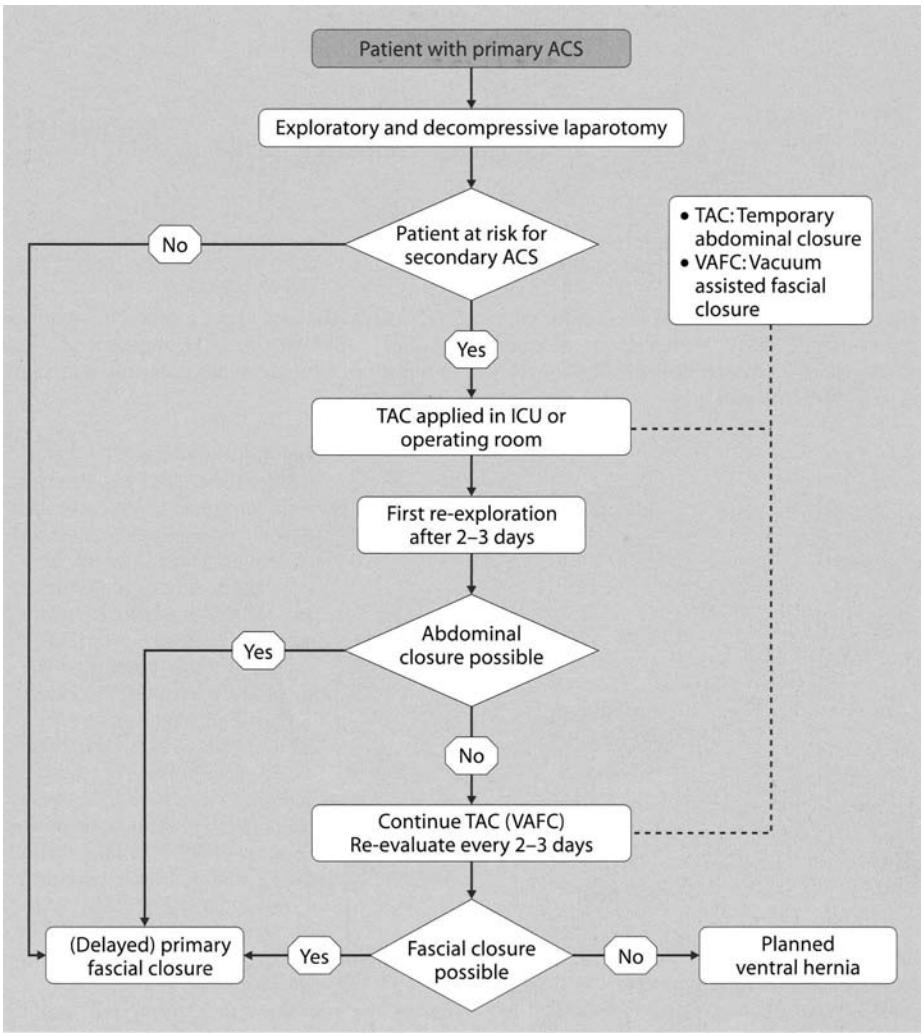

Fig. 2. Surgical treatment algorithm for ACS. Adapted from the ESICM PACT module on abdominal problems (http://www.esicm.org/PAGE_pactprogramme)

The frequency of IAP monitoring may affect the mean and maximal daily IAP-levels as well as the incidence and prevalence of IAH when different thresholds are used. Maybe we need to look at the time above a critical IAP threshold during each 24 hour period or the area under the curve above the threshold (Fig. 3).

Defining Thresholds

Studies looking at IAP and APP thresholds should be based on the analysis of receiver operating characteristics (ROC) curves and the area under the ROC-curve. As an example, in a recent prospective study, ROC-curves were generated for IAP and APP in order to identify the threshold values of each endpoint that were most

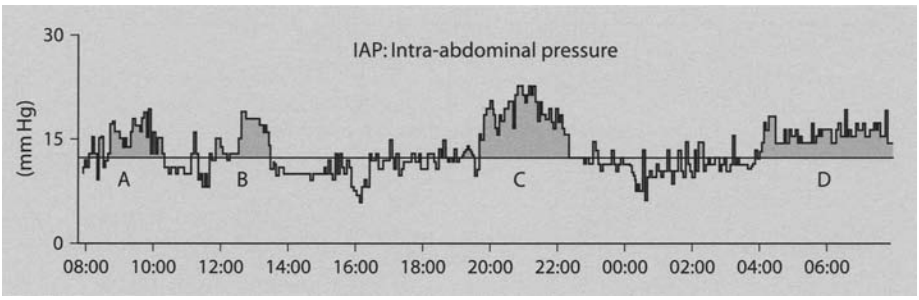

Fig. 3. Continuous IAP tracing in a patient on peritoneal dialysis. The time above a critical IAP threshold (the horizontal line at 12 mmHg) can be calculated as time A+B+C+D, i.e., or 11 hours out of 24, or 45.8%, which is probably clinically significant. The area under the curve above the same critical threshold of 12 mmHg is shaded gray.

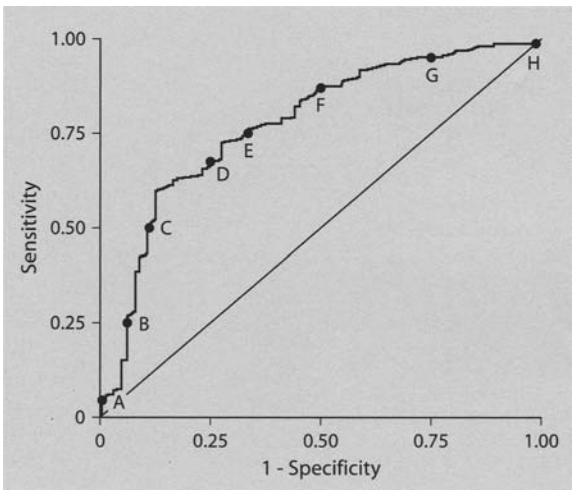

Fig. 4. Receiver operating characteristics curve (ROC) for abdominal perfusion pressure (APP) with some clinically relevant/useful thresholds or decision points (A to H). Sensitivity and specificity of APP on admission with respect to survival according to ROC curve in 235 patients. Point A: sensitivity 0%, specificity 100% (APP threshold 160 mmHg); Point B: sensitivity 25%, specificity 94% (APP threshold 80 mmHg); Point C: sensitivity 50%, specificity 90% (APP threshold 68 mmHg); Point D: sensitivity 68%, specificity 75% (APP threshold 62 mmHg); Point E: sensitivity 75%, specificity 69% (APP threshold 58 mmHg); Point F: sensitivity 87%, specificity 50% (APP threshold 52 mmHg); Point G: sensitivity 95%, specificity 25% (APP threshold 46 mmHg); Point H: sensitivity 100%, specificity 0% (APP threshold 21 mmHg). From this ROC curve analysis it becomes clear that the best APP threshold (that with the best sensitivity and specificity) is somewhere between 58 and 62 mmHg, namely 60 mmHg with a sensitivity of 72.2% and a specificity of 72.7%. The area under the curve was 0.777 (.709–.844) (adapted from [1, 12]).

predictive of patient outcome [1, 12]. ROC curves graph the sensitivity of a diagnostic test (true positive proportion) versus 1 minus specificity (false positive proportion) and provide an improved measure of the overall discriminatory power of a test as they assess all possible threshold values [43]. A test that always predicts survival has an area under the ROC curve of 1.0 and a test that predicts survival no more often than would be done by chance has an area under the ROC curve of 0.5. The point on the ROC curve closest to the upper left corner is generally considered to optimize the sensitivity and specificity of the test. In this study [1], the area under the ROC curve was 0.777 for APP (Fig. 4) and 0.685 for IAP. Although the areas under the ROC curves for APP and IAP are not statistically different, the curves demonstrate that the sensitivity and specificity of APP are both superior to those of

IAP for the clinically useful decision thresholds. Maintenance of an APP of at least 60 mmHg appears to maximize both the sensitivity (72 %) and specificity (73 %) of APP as a predictor of patient survival. While an IAP threshold of 30 mmHg achieved in another study also had a sensitivity of 70 % and specificity of 72 %, this endpoint exceeds what is now recognized as being clinically acceptable and its application would place the patient at risk for significant end-organ malperfusion [21]. Within the currently advocated ranges of 10 to 25 mmHg, IAP was specific, but not sensitive for predicting patient outcome. APP appears to be a clinically superior resuscitation endpoint and predictor of patient survival during treatment of IAH and ACS as it addresses not only the severity of IAH, but also the adequacy of end-organ perfusion. The WSACS recommends that a good area under the ROC curve is at least 0.75, the best threshold needs to be identified with a sensitivity and/or specificity of at least, or close to 75 %.

Validation of New IAP Measurement Techniques

Studies examining new devices to measure IAP should always compare the new IAP measurement method with some form of gold standard. The validation of the new technique should not be limited to the analysis of correlation, but it should also include a Bland and Altman analysis. A good and significant Pearson correlation coefficient (r^2) is not enough to compare two different methods. More detail is needed with an analysis according to Bland and Altman who proposed to test for systematic bias, precision, and agreement between two methods by plotting the mean difference against the mean of two measurements [44]. When comparing a new method of measurement with a standard method, one of the things you want to know is whether the difference between the measurements by the two methods is related to the magnitude of the measurement. A plot of the difference against the standard measurement is sometimes suggested in the literature, but this will always appear to show a relation between difference and magnitude when there is none. A plot of the difference against the average of the standard and new measurements is unlikely to mislead in this way [45]. This is illustrated in a practical example in Figure 5.

The bias or the difference between two IAP measurement methods should be close to 0 mmHg (range -1 to +1 mmHg), and the maximal allowed limits of agreement when comparing two IAP measurement methods should be within a range of 4 mmHg (limit of agreement = bias $\pm$ 4 mmHg) [10].

Interventional Studies

Future research should not only focus on epidemiology. The crucial question before widespread acceptance, practice, and clinical use of IAP monitoring still remains unanswered i.e., „Is IAP a phenomenon or an epi-phenomenon?" The impact that IAP has on therapeutic decision making and outcome when an intervention is undertaken to influence IAP still has to be studied. Before IAP monitoring is accepted as a valid tool in practice, it has to be demonstrated that interventions to treat ACS alter patient outcome (if not mortality then at least morbidity); maybe it is now time for such multicenter, multinational, interventional studies.

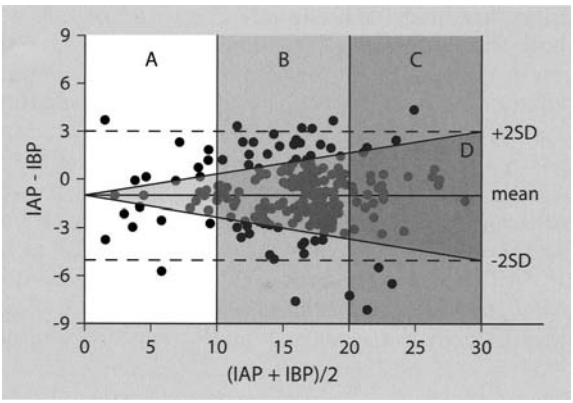

Fig. 5. Bland and Altman analysis comparing two techniques of measuring intraabdominal pressure, one via the bladder (IBP) and one via direct measurement during laparoscopy (IAP). On the X-axis the mean value of two paired measurements is plotted against the difference between the two measurements (or the bias) on the Y-axis. A good Bland and Altman analysis should obtain a bias around or close to zero (in this study the bias was -0.95 ± 1.95 mmHg). The limits of agreement, calculated as the bias plus or minus twice the standard deviation should also be within reasonable limits; in this study the limits of agreement were from −4.9 to +3 mmHg. Most studied values (>95%) should lie within these limits of agreement. These limits of agreement need also to be seen within the context of the range of studied IAP values, from normal values (area A), through slightly increased but clinically most relevant values (area B), up to highly increased (ACS) values (area C). In the analysis shown, most of the values lay within the clinically relevant range (B). Finally, the standard error around the mean bias should be within a maximum of 15% (dark shaded triangular area D). In summary, the Bland and Altman analysis presented in this figure has a good bias, and a good range of measured IAP values but the limits of agreement are too large. SD: standard deviation

Conclusion

In order to accurately compare different clinical reports, and to plan for future clinical trials, definitions are required that are comprehensive, detailed, simple, practical, and acceptable to the majority of the scientific community working in this field. In addition to reviewing the recently published consensus definitions, this chapter provides further guidelines and recommendations for all issues associated with increased IAP, which may serve as a springboard for further consensus building endeavors.

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Gut Absorption Capacity in the Critically Ill

M.M. Berger, L. Soguel Alexander, and R.L. Chioléro

Intestinal Failure: Definition and Clinical Consequences

The intestine is characterized by a large mucosal surface, a complex vascular system, a variable anatomy, a dense neuronal network, a complex mesenteric lymphatic system, and an important gut-associated lymphoid tissue (GALT). Defining intestinal failure starts with the recognition of gut function (Table 1). Intestinal failure may be chronic or acute, may be intrinsic (direct insult to the gut) or extrinsic, with hemodynamic, septic, or pharmacological causes (as in the case of the opioid bowel syndrome). Intestinal failure involves motility disorders, alteration of the barrier func-

Table 1. Functions of the gastrointestinal tract.

Function	Alteration – condition	Comment
Digestion	Impaired gastric secretion Impaired pancreatic secretion Loss of mucosal brush border enzymes	Ulcer prophylaxis in the ICU Rarely significant
Absorption nutrients	Insufficient digestion → low absorption Mucosal ischemia Mucosal edema Impaired motility Increased motility (e.g., diarrhea) causes a reduced/insufficient mucosal contact time of the nutrients	 Various shock states favor their development Opioids, sedatives, dopamine Antibiotics are the most frequent cause
Barrier – microorganisms	Bacterial and endotoxin translocation	Ischemic gut, or increased permeability states (anaphylaxis, sepsis, burns) Worsened by impaired motility, enabling proliferation of microorganisms in the gut
– water	Capillary leak of water and albumin in inflammatory patient may lead to a gut edema impairing its function. Impaired water reabsorption in the colon → diarrhea.	Explains some of the gut alterations observed in burns if enteral feeding is delayed
Immunity (GALT)	Gut ischemia/reperfusion reduced IgA production	Probably underdiagnosed
Peristalsis	Pyloric dysfunction → gastric stasis Impaired peristalsis → inadequate timing of nutrient passage	Medication, mechanical ventilation

tion (increased permeability), and decreased absorption capacity. This chapter will focus on conditions observed in the critically ill patient.

The final result of intestinal failure is a reduction in the functional intestinal mass below the minimum sufficient to allow maintenance of nutritional status. In sub-acute conditions, it can also be defined by the minimal energy and wet weight absorption required to avoid home parenteral nutrition [2]. The reduction in the functional mass may result from surgical resection of intestine causing short bowel syndrome. Mucosal injury after small bowel transplantation is also associated with a sharp reduction in absorptive area and small intestinal function. As enteral feeding is the recommended route of feeding in the critically ill, altered absorption puts the patient at risk of malnutrition [1]. Many papers have reported insufficient feed delivery by the enteral route. The causes of low intake are many, but sub-acute gastrointestinal failure is the most important.

The importance of the gut has long been underestimated in the critically ill. The systemic consequences of gut failure are many. Gut hypoperfusion is now postulated to be an important mechanism leading to post-insult multiple organ failure (MOF). Gut-derived factors present in the mesenteric lymph have been shown to contribute to distant organ injury [3]. This concept is supported by animal studies [4] indicating that division of the mesenteric lymphatic ducts prevents lung injury after hemorrhagic shock and significantly ameliorates lung injury after thermal injury. A recent study [5] clarified another mechanism of impaired host defense after gut ischemia/reperfusion, which causes GALT mass atrophy. Because GALT plays a central role in systemic mucosal immunity, its atrophy may allow pathogens and toxins to cross the mucosal barrier. Another study showed that albumin infusion after a gut ischemic insult may maintain gut immunity by preventing GALT atrophy [6].

The diagnosis of gut failure is not easy though. Unlike renal or liver failure which are monitored by functional indicators such as serum creatinine or bilirubin, the clinician lacks reliable biological markers of absorptive enterocyte mass to help in the diagnosis of small intestinal failure; plasma citrulline may have altered this issue, though [7]. The abdominal part of the Sequential Organ Failure Assessment (SOFA) score [8] is little direct help, as it is only includes hyperbilirubinemia. Nevertheless, it has been shown recently that the number of failing organs defined by the SOFA score (using a SOFA organ sub-score ≥3) helps identify patients at risk of developing abdominal compartment syndrome with its potential intestinal consequences [9].

Physiology

Digestion and Absorption

Three steps are required for normal nutrient absorption [10]: 1) luminal processing; 2) absorption into the intestinal mucosa; and 3) transport into the circulation. All three may be altered in the critically ill. Table 2 provides a short reminder of the physiological processes.

Carbohydrates: Starch (amylose, amylopectin), sucrose (= saccharose = Glu+Fru), and lactose (Glu+Gal) are the most abundant digestible carbohydrates in the human diet. In feeding solutions oligo- and polysaccharides are the predominant source. These must be broken down into their constituent monosaccharides prior to absorption. This family of substrates is probably the least likely to be altered, their absorption being rather simple.

Table 2. Digestion and absorption mechanism of the different substrates

Substrate	Location	Mechanism
Carbohydrates	Oropharynx	salivary and pancreatic amylase → digestion of amylose and amylopectin.
	Pancreas	digestion products = oligo- and disaccharides
	Small intestine	brush border disaccharidases hydrolyze oligo- and disaccharides → monosaccharides monosaccharides absorbed by either active or passive transport processes.
Lipids	Oropharynx	lingual lipase and gastric lipase → Fat hydrolysis in the stomach → free fatty acids (FA).
	Stomach	Free FA stimulate pancreatic lipase and colipase, → majority of lipid hydrolysis.
	Duodenum	Entry of protons into the duodenum → release of secretin →
	Pancreas	enhances pancreatic bicarbonate secretion, raising the intraluminal pH
	Gallbladder	Bile salts → fat solubilization → emulsion of microscopic micelles (triglycerides, cholesterol esters, and diglycerides coated by phospholipid).
	Small intestine	Pancreatic lipase degrades lipid emulsion to monoglycerides and FA. phospholipase A2 and pancreatic cholesterol esterase hydrolyze phospholipids and cholesterol Lipolytic products mixed with bile salts → micelles or liposomes bile salts remain in the intestinal lumen, reaching terminal ileum → actively resorbed (enterohepatic circulation)
Proteins	Stomach	gastric pepsins → proteolysis Amino acids released from gastric digestion → cholecystokinin (CCK)
	Duodenum	release from duodenal and jejunal endocrine epithelial cells.
	Pancreas	Duodenum, several proteases digest proteins into amino acids, or dipeptides and tripeptides.
Micronutrients	Small intestine	passive diffusion (carotenoids) carrier-mediated, non-energy requiring processes active transport systems (i.e., energy-requiring transporters working against a chemical gradient) (folate, calcium). Competitions for transporter (e.g., metallothionein: Cu, Zn)

Fat: Most dietary lipids are absorbed in the proximal two thirds of the jejunum. Absorption is influenced by several factors, including the types of dietary lipid and the presence of other ingested nutrients. Normally, more than 94% of dietary fat is absorbed. Triglycerides are the predominant lipids in diet and in feeding solutions (long chain and medium chain triglycerides). Their digestion begins in the stomach with peristaltic emulsification, and continues in the duodenum with the combined action of bile and pancreatic lipase. Absorption occurs in the proximal jejunum. The lipids (except the medium chains triglycerides) are then secreted as chylomicrons in the thoracic duct and finally reach the blood circulation.

Proteins: Protein digestion begins in the stomach by the action of gastric pepsins, and continues in the intestinal lumen by the action of the pancreatic enzymes, and by the specific peptidases of the brush border of the jejunum and ileum. The di- and tri-peptides resulting from this digestion are absorbed by enterocytes and split into amino acids by cytosolic peptidases before entering the blood circulation.

Micronutrients and minerals: these represent a wide array of compounds, possessing an equally wide array of chemical properties. Therefore, the intestine has many types of transport mechanisms to facilitate assimilation of these nutrients across the intestinal barrier. The type of transport varies in different regions of the small intestine for many micronutrients.

Malabsorption

The term, malabsorption, refers to impaired absorption of nutrients. It can result from congenital membrane transport defects (primary malabsorption) or from acquired defects in the epithelial absorptive surface (secondary malabsorption). Maldigestion, which is due to impaired digestion of nutrients within the intestinal lumen or at the terminal digestive site of the brush border membrane of mucosal epithelial cells, is another factor that can interfere with nutrient absorption. Although malabsorption and maldigestion are pathophysiologically different, they are interdependent, so that in clinical practice, the term malabsorption denotes derangements in both processes. Malabsorption may either be global or partial (isolated). Global malabsorption results from diseases associated with either diffuse mucosal involvement or a reduced absorptive surface (e.g., celiac sprue). Partial or isolated malabsorption results from diseases that interfere with the absorption of specific nutrients (e.g., vitamin B12 in pernicious anemia).

Hemodynamic Responses to Enteral Feeding

Enteral nutrition is the recommended feeding route in the majority of patients [1]. But there are a few caveats to this route in the intensive care unit (ICU) patient [11].

The normal hemodynamic response to feeding is complex, including an increase in cardiac output, vasodilation of mesenteric arteries, and a decrease in peripheral resistance. In healthy subjects, enteral nutrition induces significant increases in flow parameters in the superior mesenteric artery and portal vein [12]. A study enrolling 44 healthy subjects showed splanchnic postprandial hyperemia in response to intraduodenal feeding using Echo-Doppler technology. Postprandially, diastolic blood pressure fell, and flow in the portal vein and mean velocity in the superior mesenteric artery increased significantly [12]. These changes were paralleled by alterations in systemic hemodynamics.

In circulatory compromise, enteral nutrition may aggravate gut ischemia by a steal mechanism. Therefore, many authors recommend the use of parenteral nutrition in acute conditions and especially after surgery: these recommendations are expert opinions, with only limited and contradictory data to support that enteral nutrition contributes to this type of complication.

On the positive side, continuous enteral nutrition (800–1500 kcal/day), compared with intermittent feeding, has been shown to minimize oxygen uptake (VO_2) and myocardial oxygen consumption in patients with congestive heart failure: therefore, enteral nutrition can be provided safely from the cardiac function aspect [13]. The combination of oral food and parenteral nutrition to achieve 20 to 30 kcal/kg per day for 2–3 weeks in patients with cardiac cachexia was also associated with stable hemodynamics, unchanged whole body VO_2 and CO_2 production [14]. Enteral feeding in patients receiving inotropic support after cardiopulmonary bypass causes increases in cardiac index (CI) and splanchnic blood flow (an appropriate hemodynamic response), while the metabolic responses (endocrine profile) indicate that nutrients are utilized [15].

Our team has repeatedly shown that cautious enteral nutrition can be used during severe cardiac compromise, including cases requiring mechanical cardiovascular assist devices and high doses of vasopressor [15–17]. In a series of 23 patients with hemodynamic failure (CI between 2–2.5 l/m^2/min), jejunal absorption, determined using paracetamol absorption, was maintained compared with patients without cardiac failure [16]. Such patients can be fed under tight clinical supervision. Another recent observational study in 70 patients with circulatory compromise admitted to our ICU, showed that the feed volume was limited in the presence of severe hemodynamic compromise [17]; as a mean, a maximum of 1000 ml could be delivered by the gastric route, and 1500 ml by the postpyloric route. Among these 70 patients, 18 were dependent on intra-aortic balloon pump support: the analysis of this subset of patients with severe hemodynamic failure showed similar results, enabling the delivery of 15–20 kcal/kg/day, i.e., a total energy delivery of 50–75% of the energy target determined by indirect calorimetry. Daily enteral energy delivery should, therefore, be monitored to avoid the development of energy deficits. Combined enteral and parenteral nutrition should be used to achieve energy targets within 7 days in ICU patients staying longer than this, to avoid the malnutrition caused by a negative energy balance [18].

Multifactorial Absorption Failure: A Clinical Case

Critically ill patients with major burns are generally characterized by an intact gut, able to accommodate the large volumes of enteral feeding required by this hypermetabolic condition [19]. However, there are exceptions as shown by the following case:

A 28 year-old lady with sigmoid diverticulosis, was admitted after a 53% body surface area burn with inhalation injury. The patient was very slim (pre-injury weight 49 kg for 162 cm; BMI = 18.7 kg/m^2). Her clinical course was complicated early on, resulting in a very prolonged ICU stay (190 days – Fig. 1). By protocol, enteral nutrition was started on the day of admission, with an initial energy target set at 2000 kcal/day and adapted using indirect calorimetry determinations. Problems started with the occurrence of an early severe pneumonia causing acute respiratory failure. The patients then suffered cardiac arrest from hypoxemia requiring cardiopulmonary resuscitation (CPR) on day 9. Thereafter, she suffered three episodes of septic shock, of cutaneous, catheter, and urinary origins, before becoming colonized by *Acinetobacter baumannii* [20]. During the first septic episode, she developed transient acute renal failure. By day 25, there was no energy deficit. Early excision surgery was resumed after interruption during the hemodynamically unstable septic episodes.

On day 28, while undergoing surgical debridement, she suffered severe peri-operative hemorrhagic shock, which apparently improved with fluid resuscitation. Remarkably though, enteral nutrition was less on these days, which was attributed to the reduced feeding time between surgical sessions. In parallel, renal function had worsened. On day 30, feeding became truly difficult, with intestinal paresis, an acute abdomen, and high fever. A laparotomy showed necrosis of the right ascending and transverse colon with stercoral peritonitis, and resection of the major part of the colon and confection of a terminal ileostomy was required; the small bowel was inflammatory but not ischemic. The condition might have been a variant of non-occlusive bowel necrosis [21], aggravated by ischemia-reperfusion in the con-

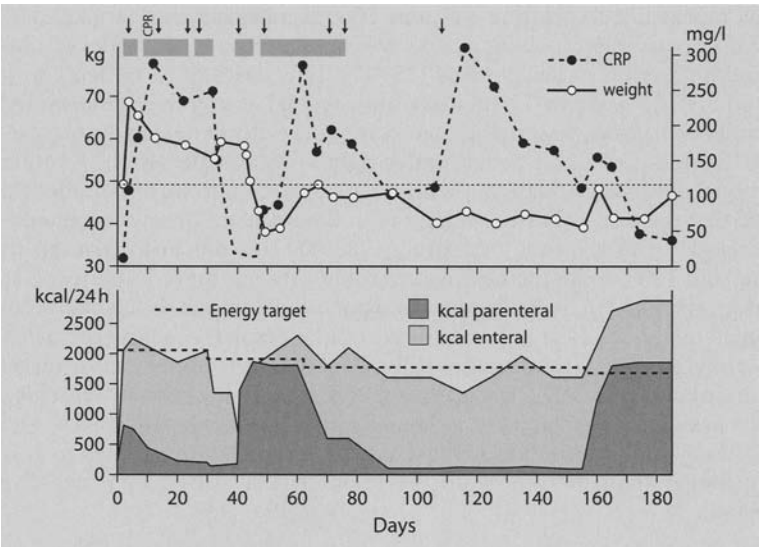

Fig. 1. Evolution of nutritional enteral and parenteral intakes, body weight, and C-reactive protein (CRP) over 190 days, in a 28 year-old lady with 53% BSA burns. The arrows represent the surgical interventions, the two hemorrhagic episodes being highlighted. Thick line on top = duration of mechanical ventilation CPR: cardiopulmonary resuscitation

text of diverticulosis. The cumulative energy balance became negative at -6300 kcal by day 35. At this time, worsening of renal failure required continuous extra-renal replacement therapy for a few days, and evolved into chronic renal failure with a creatinine clearance of 40 ml/min. On day 40, the patient developed an alithiasic cholecystitis, requiring cholecystectomy. Full parenteral nutrition was initiated, based on the diagnosis of persistent peritonitis and deficient absorption causing malnutrition. The patient's malabsorption was attributed to gastrointestinal failure caused by hemorrhagic shock, ischemia/reperfusion, and sepsis. By day 60, enteral nutrition was progressively reintroduced and apparently well tolerated, with a step-down of parenteral nutrition. The patient's weight stabilized (47 kg).

Progressive weight loss was again observed from day 100, despite provision of energy matching her expenses, resulting in a weight of 38 kg (-11 kg = -22.4% of initial weight). The paracetamol test was carried out on day 125: the absorption curve was nearly flat. Malnutrition was again diagnosed, and required reintroduction of parenteral nutrition by day 155. It was continued without stopping enteral nutrition until day 190, when the patient was again progressively weaned from parenteral nutrition, in parallel with the introduction of oral feeding.

Physiopathology of Intestinal Failure in Selected ICU Conditions

Ischemia

Splanchnic ischemia is caused by a reduction in intestinal blood flow, which most commonly arises from occlusion, vasospasm, and/or hypoperfusion of the mesenteric vessels (celiac axis, superior and inferior mesenteric arteries, or collateral circulation). Acute ischemia refers to the sudden onset of intestinal hypoperfusion.

Occlusive arterial obstruction is most commonly due to emboli or thrombosis of mesenteric arteries, while occlusive venous obstruction is most commonly due to thrombosis or segmental strangulation. Non-occlusive arterial hypoperfusion with splanchnic dysoxia is most commonly due to primary splanchnic vasoconstriction, such as is observed in a variety of critical care conditions (low cardiac output, septic shock, vasopressor therapy, abdominal compartment syndrome [ACS]).

Intestinal ischemic damage is caused both by hypoxia and by reperfusion injury after a period of ischemia, such as may be observed during acute hemorrhage or after cardiac surgery [22]. Inotropic and vasopressor drugs further interfere with splanchnic perfusion and gut function. Norepinephrine, the most frequently used vasopressor, is, however, considered to have limited effects on splanchnic perfusion [23].

The clinical consequences of intestinal ischemia can be catastrophic, including sepsis, bowel infarction and necrosis, and eventually death [11], making rapid diagnosis and treatment imperative. Early signs and symptoms of mesenteric ischemia are nonspecific, and definitive diagnosis often requires invasive testing. As a result, the diagnosis is often delayed; generally tests starts with a plain abdomen film, abdominal computed tomography scan, and eventually endoscopy.

Non-occlusive bowel necrosis is a rare, special form of ischemia associated with early enteral nutrition. In a study including 4,311 patients, non-occlusive bowel necrosis developed in 0.3%, with an onset during the second week in high-acuity patients with previous good feeding tolerance [21]. Clinical findings resemble bacterial sepsis with tachycardia, fever, and leukocytosis, and a modest decrease in gastric intramucosal pH (pHi). Gastrointestinal specific signs are not consistent or appear late.

Abdominal Compartment Syndrome

The 2006 definition of intra-abdominal hypertension (IAH) is a sustained or repeated elevation in intra-abdominal pressure (IAP) ≥ 12 mmHg, with a grading I up to IV (pressure >25 mmHg) [9]. Deleterious effects on renal, cardiac, and gastrointestinal function have been witnessed at IAP levels as low as 10–15 mmHg. ACS is defined as a sustained IAP >20 mmHg (with or without an abdominal perfusion pressure <60 mmHg) that is associated with new organ dysfunction/failure. Feeding such patients by the enteral route amplifies the risk of ischemic complications. ACS can have different causes [9], but fluid resuscitation after major trauma [24] and burns [25], and gut ischemia after major vascular surgery are predominant causes. Indeed, fluid resuscitation causes generalized edema, including edema of the abdominal viscera [26] – increasing IAP with enteral feeding will further worsen the condition; in addition, under such edema conditions, absorption is unpredictable.

Alterations of Permeability

Permeability of the gut may be altered by verious conditions, including anaphylactic reactions, major burns, severe inflammatory conditions, sepsis, and ischemia/reperfusion. Increased intestinal permeability is associated with the installation of bacteremia, sepsis, and MOF in critically ill burned and trauma patients [27]. Bacterial translocation has only been clearly demonstrated in animals, while human data are inconsistent. Nevertheless, the increased permeability may be significant *per se*, and may contribute to diffusion of endotoxins and to the propagation of inflammation.

Table 3. Tools for monitoring of intestinal absorption, permeability, and failure

Function	Tool	Method	Comment
Absorption	Citrulline [7, 34, 42]	Plasma concentration reflects active intestinal cell mass	Not tested in ICU
Absorption	Paracetamol [16, 30, 31, 41]	Plasma concentration 5 min after an enteral dose of 15 mg/kg	Sensitive marker, widely available
Absorption	Labeled ^{13}C acetate [43]	Breath test	Easy to carry out in ventilated patients but not yet available in clinical settings
	Sugar absorption tests [36–38]	Determination of urinary recovery	
Absorption	– 3–0-methyl-D-glucose	active carrier mediated absorption	
Absorption	– D-xylose	passive carrier mediated	
Absorption	– L-rhamnose	non-mediated absorption capacity	
Permeability	– Sucralose	enables assessment of the whole gut	stable in the colon Not tested in ICU
Permeability Permeability	– lactulose:rhamnose (L/R) ratio – lactulose:mannitol ratio	intestinal permeability (L/R ratio) paracellular intestinal permeability (L/M ratio)	
Permeability	PEG (polyethylene glycols)	6h urinary recovery of a mixture of different sized PEGs in liquid meal	Not tested in ICU
Ischemia	Arterial lactate, pH	Blood gas	
Ischemia	pHi, $PtCO_2$	Tonometry	
Ischemia	Intra-abdominal pressure [9, 24, 25]	Continuous/intermittent vesical pressure monitoring	

Permeability should not be confused with absorption; as shown in Table 3, the tests used to assess both are different.

Motility Disorders

Drugs, such as sedatives and especially opioids, reduce intestinal motility in the critically ill patient [28]. The alterations involve the complete gastrointestinal tract from the esophagus to the rectum. Propulsive motility of the esophageal body is significantly reduced during sedation [29]. Dopamine alters intra-gastric pressure already at low dose, and depresses motility by a direct action on gastric receptors. Pyloric dysfunction is extremely frequent, resulting in delayed absorption and high volumes of gastric residues [30, 31]. Opioids depress intestinal motility, causing pyloric dysfunction even at low doses, and reducing absorption of any substance delivered into the stomach by decreasing passage into duodenum. This opioid-mediated reduction of absorption has been shown in cardiac surgery using the paracetamol test [16]. Use of prokinetics (erythromycin more than metoclopramide) improves gastric

emptying [28, 30, 32], and increases migration of nasoenteric feeding tubes [33]. Conditions such as diabetes mellitus, severe hyperglycemia, and previous abdominal surgery, are important risk factors for pyloric dysfunction.

Intestinal Mucositis (Radiotherapy, Hematological Malignancies)

Intestinal mucositis is an important cause of cancer treatment-related morbidity and mortality [34]. Epithelial gut damage results from direct toxicity from both radiotherapy damage and myeloablative therapies. Such damage results in loss of absorption capacity, and may be a life-threatening disorder.

Gut Resection

Surgical resection is generally required in acute ischemic conditions, and will, therefore, not be discussed separately. Nevertheless, the consequences for subsequent management will be serious if less than 1 meter of small bowel is present, the ileal segment being the most sensitive, leading to the short bowel syndrome. Presence of the ileo-cecal valve is also a determinant for gut rehabilitation

Markers of Gut Failure

Clinical observation of patients remains the corner stone of gut monitoring. Therefore, the clinical follow up of patients at risk of developing splanchnic ischemia includes a careful examination of the abdomen, watching for distension or other signs of subileus.

Clinical and Paraclinical Tools

Paraclinical tools (Table 3)

1. Splanchnic ischemia may be monitored by the means of gastric tonometry, enabling the determination of gastric mucosal PCO_2 and calculation of pHi [36].
2. Monitoring IAP by means of a urinary bladder catheter is a sensitive tool. Any increase in pressure above 15 mmHg puts the gut at risk of ischemia from ACS.
3. Monitoring of arterial pH by blood gas analysis and determination of arterial blood lactate can be used to confirm intestinal ischemia; decreasing pH and increasing lactate levels usually herald the development of clinically relevant intestinal ischemia, but are late signs.

Scoring of gut failure

Recently a score was proposed for gut alterations associated with hematological malignancies and their treatment, based on six items: frequency of emesis and diarrhea, occurrence of nausea, abdominal complaints, fecal incontinence, and fecal volume [35]. Each item is allocated a score between 0 (normal) and 3 (severe). Summation of the scores yields three grades of mucositis: mild toxicity (Grade I), moderate toxicity (Grade II), and severe toxicity (Grade III). This score has not yet been applied to other critically ill patients, but could possibly be adapted to ICU settings.

Laboratory Tests

These should be used when the above observations suggest impending trouble, to refine the diagnosis.

Motility

The best bedside assessment of motility disorders is clinical examination and determination of gastric residues, which although not precise, reflect pyloric dysfunction. The paracetamol test is a good diagnostic tool. Bowel movements, gases and stools, are the output component. Bowel sounds are not specific – in particular, their absence does not mean absence of transit. Absent transit for more than 5 days, generally indicates an impending problem. Diarrhea, although feared by nurses, is not the worst complication; it is usually defined as more than 4 to 6 unformed stools per day for more than 48 hours. Most cases of acute diarrhea in the adult are due to infections – in the ICU, antibiotics are the most frequent cause.

Permeability

Currently, the sugar permeability tests are considered an objective endpoint to quantify alterations in gut barrier function [35] and treatment effects [37, 38]. Permeability is tested with substances that are not absorbed. By simultaneously using multiple sugar probes, which are chosen according to different permeation pathways and stability characteristics in the gut, information about intestinal absorptive capacity is obtained in addition to information regarding intestinal permeability. Lactulose enables the small intestine (not stable in the colon) to be investigated, while sucralose is the only disaccharide which is stable in the colon and enables assessment of the whole gut. Polyethylene glycols (PEG) using different molecular sizes are an alternative.

Absorption capacity

- Paracetamol administration is rarely contraindicated in critically ill patients, and is used to determine gastric motility and pyloric function [31, 39, 40]. We have previously shown that the typical absorption patterns after gastric or jejunal paracetamol administration are maintained even in critically ill patients with severe cardiovascular failure, during low cardiac output conditions; absorption is not suppressed, only delayed, because of decreased pyloric motility [16]. Serum paracetamol, measured 5 minutes after administration, enables the gastric or postpyloric position of the feeding tube to be determined, due to the very fast jejunal absorption (specificity 100%; sensitivity 94%) [41].
- Serum citrulline appears to be a promising marker for intestinal failure as its determination is simple and repeatable. Citrulline is an amino acid released and synthesized exclusively in small intestinal enterocytes from glutamine and then metabolized into arginine by the kidney. It has been shown to be a reliable marker for small bowel enterocyte mass [7, 34, 42]. In patients with a short bowel, citrulline concentration is correlated with bowel length [7]. This test has yet not been validated in the critically ill. In particular, the relationship between citrulline concentration and the capacity to tolerate and absorb enteral nutririon has not been established.
- The ability to determine absorption using a breath test with labeled acetate was investigated in 24 critically ill surgical patients requiring enteral nutrition [43]. ^{13}C acetate was administered by one of three routes, along with D-xylose: 1)

gastric, 2) jejunal, and 3) intravenous (control). Gastric and jejunal ^{13}C acetate were rapidly absorbed, contrasting with a depressed or delayed D-xylose absorption. $^{13}CO_2$ recovery kinetics were similar after jejunal or intravenous ^{13}C acetate and slightly depressed after gastric administration. Further studies are required to determine the value of labeled nutrients to assess gastric emptying and intestinal absorption.

Monitoring Nutritional Support

The clinical observation of critically ill edematous patients is difficult, and weight determinations are not always available. In the acute stage of critical illness, the weekly/bi-weekly measurement of albumin, transthyretin, and C-reactive protein (CRP) plasma levels seems to provide a 'window' on the metabolic condition (anabolism versus catabolism) [44].

Daily and cumulated energy balance monitoring, i.e., calculating the difference between energy target and the real energy delivery, and doing so for the total ICU stay, is important in monitoring intestinal tolerance to feeding. Deliveries of energy below 70% of target reflect a problem that must be solved if it lasts more than 3 days to avoid the development of large energy deficits. Monitoring cumulated energy delivery enables decisions to be made about the introduction of combined parenteral feeding when enteral nutrition fails/is insufficient and energy deficit reaches -8,000 - 10,000 kcal [18]. Computerized information systems facilitate this monitoring and improve nutritional support [45].

Conclusion

Bowel dysfunction is frequent in the critically ill, and is proteiform. Barrier function alterations, in particular increased permeability, are frequent and contribute to amplify the inflammatory response and probably the incidence of infectious complications. Depression of motility is a side effect of many ICU therapies. Absorption capacity is the ultimate intestinal function, and is more frequently altered than previously recognized. Absorption failure rapidly causes malnutrition during enteral feeding. We still lack a good indicator of bowel absorption capacity in the critically ill, and we still rely on the combination of various indicators for assessment. Future research should focus on this particular aspect.

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The Liver

Critical Illness and the Hepatic Microcirculation: A Review

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Introduction

One of the most important goals of therapy in critically ill patients is restoring and maintaining adequate perfusion and oxygenation of vital organs in the recovery from a variety of disruptive processes, such as circulatory failure in myocardial infarction, sepsis, and trauma. The gastrointestinal tract is generally regarded as significant in the development of shock and multiple organ failure (MOF) as a consequence of loss of its barrier function against luminal bacteria and bacterial products, such as endotoxin in hypoxic conditions. Insufficient blood flow to the splanchnic organs is believed to be the essential mechanism [1]. Translocation of bacteria and endotoxin to the lymphatic and portal system is a first step towards distant organ damage. The gut and liver macrophages (Kupffer cells) are important as a first barrier against spread of translocated bacteria and endotoxins to the bloodstream.

The autoregulation of microcirculatory blood flow distribution in the splanchnic area is compromised under septic or shock conditions. Regional blood flow distribution is marred and changes under normal circumstances are not reflected in conditions of sepsis and septic shock. The flow distribution to the mucosa is decreased in sepsis while the general splanchnic flow remains largely intact [2]. Blood flow distribution and regional perfusion has to adapt to local metabolic demand. Because the diffusion of oxygen in tissues is limited, nutritional delivery has to be through a dense, finely distributed network of capillaries. This capillary network is generally regarded as vessels smaller than 300 μm in diameter and comprises the largest endothelial surface area (>0.5 km^2).

Hiltebrand et al showed that the microcirculatory blood flow in the splanchnic organs is remarkably heterogeneous both in early, hypodynamic shock as well as later in hyperdynamic shock and cannot be predicted based upon general changes in systemic or even regional blood flow [3]. So, monitoring the microcirculation seems to be the answer to the question of how we could identify patients at risk of developing further organ system failure and how to evaluate our therapeutic interventions [4].

Unlike other organs, the microcirculation of the liver receives blood from two types of afferent vessels: The portal system and the hepatic artery. Both portal venous and hepatic arterial systems are obviously not routinely accessible for flow or other forms of direct measurement except at laparotomy. The aim of this chapter is to highlight several aspects of the hepatic microcirculation and oxygenation, and what changes occur during sepsis or other critical illness conditions.

Characteristics of Hepatic Anatomy

Together with the small bile ductuli culminating in the common bile duct, the terminal hepatic venules and the terminal hepatic arterioles form a triad, the portal tract, upon which the base of the microscopic structure of the liver rests. The hepatocytes are organized in the Kiernan's or classic lobules around central hepatic venules, which form the hepatic vein, in a hexagonal outline. The portal tracts are situated in the 'corners' of the hepatic lobules [5, 6]. Rappaport, based on intravital microscopy of the microcirculation, proposed a further refinement with the 'simple liver acinus' as the functional and structural basic liver unit, consisting of the smallest portal tract including the terminal portal venules and hepatic arterioles as the afferent vessels together with the bile ducts, lymphatic vessels and nerves at the center, and the terminal hepatic venule as the efferent vessel in the periphery. The zone closest to the afferent vessel is referred to as 'zone 1', the area surrounding the peripheral efferent hepatic venule as 'zone 3'. 'Zone 2' is located between these two areas (Fig. 1). This morphologic and functional structure provides a rational way to study the heterogeneous hepatic metabolism in each zone [7]. For instance, there is an oxygen gradient from zone 1 to zone 3, making zone 3 cells the most vulnerable to oxygen deprivation.

The portal venous system, terminating in the portal venules, and the hepatic arterial system derived from the celiac trunk, forming terminal hepatic arterioles, confluence into the liver capillary bed, called the sinusoids. These have characteristic structures, different from normal capillaries, e.g., they have sinusoidal endothelial fenestrae and the basement membrane beneath the sinusoidal endothelial cells is absent (Fig. 2) [5, 8]. The hepatic arterial blood flows indirectly into the sinusoids via an anastomosis between the terminal hepatic arteriole and the portal venule, but also directly into the sinusoids [8, 9]. Regulation of the blood flow into the sinusoids is maintained by the relaxation and contraction of a precapillary sphincter at the end of the terminal hepatic arteriole and also by the coordinated contraction and dilatation of the sinusoidal endothelial fenestrae around the portal tract zone and by regulators in the portal venous system [9]. Furthermore, the stellate or Ito cells have an important regulatory role to be discussed hereafter.

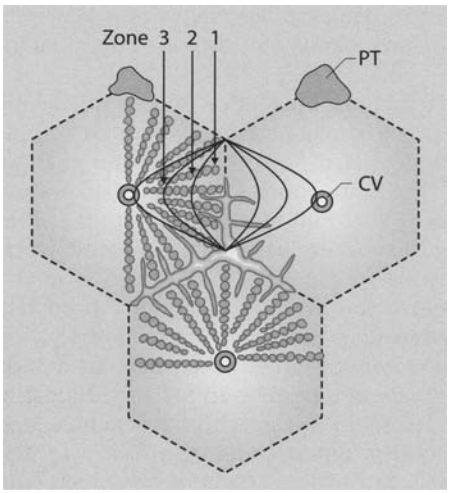

Fig. 1. A schematic drawing of 'simple liver acinus" grouped around the portal tract (PT) with the zonal arrangements of the hepatocytes. The arterial and portal blood flow congregates at the central venule (CV).

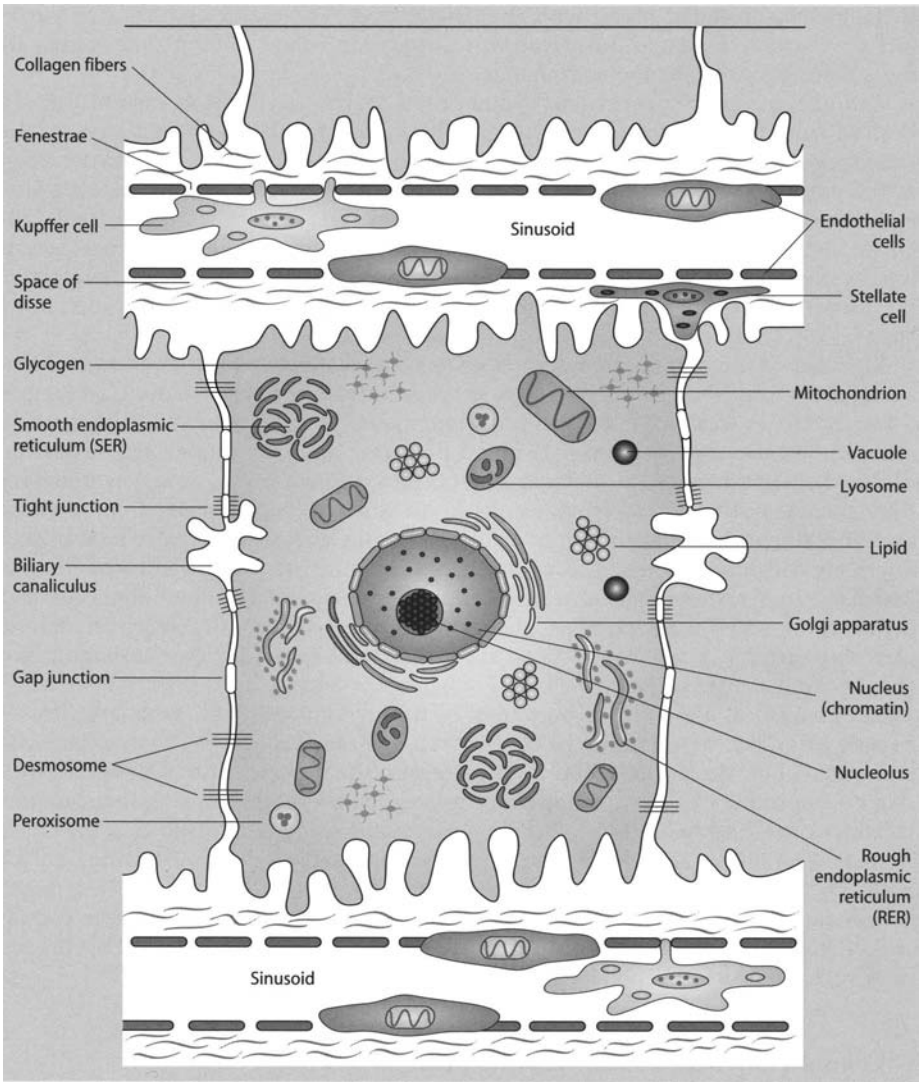

Fig. 2. Relationship between sinusoid, sinusoid lining cells, and hepatocyte. The sketch illustrates the various hepatocyte organelles. Note Kupffer cells of which cytoplasmic processes are anchored in endothelial fenestrae. From [8] with permission.

Current Physiology of the Hepatic Microcirculation

The terminal hepatic arteriole supplies oxygen enriched blood to the bile ducts, the walls of the portal venules as 'vasa vasorum', the nerves, and the connective tissue [5, 9]. The portal system carries nutrients from the digestive tract to the sinusoids and hepatocytes. Blood flow in the sinusoids, examined by intravital video enhanced contrast microscopy, was found to be significantly slower (400–450 μm/sec) than in normal or 'true' capillaries (500–1000 μm/sec), thus facilitating the metabolic

exchange of sinusoidal blood with the hepatocytes. The diameter of the sinusoids was smaller and the blood flow velocity was slower in zone 1 than in zone 3, indicating a distinction in metabolic exchange.

Endothelins as vasoconstrictors and nitric oxide (NO) as a vasodilator are derived from the endothelial cells and are important for the local regulation of the microcirculation in the liver [5, 6, 10]. Endothelin infusion results in a constriction of the portal venules and terminal portal venules, comparable to the effects of norepinephrine: the sinusoidal blood flow slows down, leading to stasis and final collapse of the sinusoids. The sieving plates or fenestrae of the sinusoids contract, as well as the terminal hepatic arterioles directly connected to the sinusoids, contributing to an overall elevation of portal pressure [9]. The role of NO will be discussed later.

The role of the autonomic nervous system has not been clarified entirely. Parasympathetic branches from the vagus nerve innervate the liver, as well as sympathetic branches derived from the celiac ganglion. Although the sympathetic and parasympathetic influences on liver blood flow have not been established, excitation of the sympathetic nerves leads to a general flow reduction [6]. Complete denervation, such as in liver transplants, results in an almost complete loss of epinephrine and norepinephrine concentrations in the liver, but effects on hepatic macro- and microcirculation are contradictory. Although in humans, total liver blood flow is increased after orthotopic liver transplantation, experimental animal models of liver denervation showed a variable picture of decreased hepatic microcirculation, increased hepatic artery blood flow, stable hepatic venous flow, or no significant changes whatsoever [11].

About 25% of the cardiac output is distributed to the liver, with a portal to hepatic arterial ratio of about 3:1 to 4:1, with a compensatory mechanism between both, i.e., when the portal blood flow decreases, the hepatic arterial flow increases and *vice versa* [12–14]. One of the proposed regulatory mechanisms in reduced portal flow is the 'adenosine washout hypothesis': Adenosine is released into the space of Mall (surrounding the hepatic arterioles and portal venules) and washed out by both systems, maintaining a constant concentration. When the 'wash-out' is reduced by a reduction in flow of one of the systems, the adenosine concentration increases and dilates the hepatic artery resulting in increased arterial blood flow. This theory, however, is speculative and subject to debate [15].

Cellular function in the Hepatic Microcirculation

Endothelial cells that enclose Disse's space underneath the fenestrated cell processes, line hepatic sinusoids with an average diameter of about 10 μm. The hepatocyte surface delineates the other border of this space, in which the stellate or Ito cells are situated, their long cytoplasmic processes surrounding the sinusoids (Fig. 2). The endothelial basement membrane is typically absent in the sinusoids. In liver cirrhosis, the hepatic sinusoidal endothelial fenestrae are decreased in diameter and number and formation of a basal membrane is found beneath the sinusoidal endothelium, influencing normal vascular tone and exchange of nutrients. This change is induced by endothelin-1 and contributes to the development of portal hypertension [16].

Hepatic stellate cells represent 5–8% of all liver cells in humans. On the luminal side of the endothelium lie the phagocytic Kupfer cells and liver-associated lym-

phatic cells [8]. Smooth muscle cells in portal venules and hepatic venules regulate the pre- and post sinusoidal vascular resistance. In the sinusoids the endothelial and stellate cells are the resistance regulators.

Several substances influence the stellate cell tonus *in vitro*, e.g., endothelins, angiotensin II, prostaglandin F2α, and vasopressin are constrictive; dilatation was shown with NO, carbon monoxide and prostaglandin E_2 [6, 17]. In addition to their influence on vascular tone, in the normal liver the predominant function of the stellate cells is storage of vitamin A, but when stimulated, such as in sepsis, they go through myofibroblastic transformation with loss of vitamin A and release of pro-inflammatory and pro-fibrinogenic mediators [17].

The role of arginine metabolism and nitric oxide

The arginine-NO pathway plays an important role in infection, inflammation and organ failure. NO is derived from arginine by normally active endogenous, and inducible NO synthases (NOS). These are located in different cell types, but mainly in endothelium and hepatocytes from which the inducible form was first found in humans [18]. NO acts as a strong vasodilator; it precludes leukocyte adhesion to endothelium and inhibits thrombocyte aggregation. It regulates immunologic cytotoxicity and lymphocyte function.

The physiological role of NO in the regulation of the hepatic microcirculation remains complex. Endogenous NOS (eNOS) produces NO 'on demand'.

eNOS in the liver is expressed by sinusoidal endothelial cells only and is upregulated, e.g., in endotoxemia, in an attempt to maintain adequate sinusoid perfusion. In several conditions, such as ischemia/reperfusion (discussed below in detail), hemorrhagic shock, and endotoxemia, eNOS-produced NO overall exerted a beneficial and cell-protective effect [18]. Li et al. disclosed in an *in vivo* animal model that augmentation of endogenous NO production by L-arginine and 3-morpholinosydnonimine, an NO donor, increased portal blood flow. Inhibition of endogenous NO production had no effect on portal hemodynamics [19].

All liver cells have been reported to express inducible NOS (iNOS) [18, 20]. iNOS once expressed continually produces NO and, in contrast to endogenous NO-synthase (eNOS), is independent of intracellular Ca^{2+} levels. The role of iNOS was more difficult to establish than for eNOS. For instance, in cold ischemia/reperfusion injury, iNOS had a cytoprotective, anti-apoptotic effect, but selective NO blocking in hemorrhagic shock experiments showed a general deleterious effect of iNOS [18]. Recently, in a rabbit model of hemorrhagic shock, Lhuillier et al showed a rapid and continuous increase in hepatic NO, that could only partly be blocked by an NOS inhibitor, suggesting enzyme-independent NO sources, e.g., tissue-stored S-nitroso-compounds, nitrosyl-hemoglobin, and nitrite ions [21].

Arginine, an NO donor, and arginine metabolism and its role in the regulation of microcirculatory flow have been investigated with great interest in recent years. Asymmetric dimethylarginine (ADMA), a derivative of arginine metabolism, inhibits the actions of NO and acts as a natural compensatory feedback mechanism for NO production. Symmetric dimethylarginine (SDMA), another arginine metabolite, has no NOS inhibiting properties, but competitively inhibits arginine uptake by the cell through an action on the so-called y^+-pump.

In a rat model of endotoxic shock, a significant role of the liver in extracting ADMA from the circulation through dimethylarginine dimethylaminohydrolase

(DDAH) was disclosed [22]. Nijveldt et al. confirmed the role of the kidney and the liver in the elimination of ADMA by DDAH. In careful organ balance studies in a rat model, they could establish a predominant elimination of ADMA in the liver, thereby regulating NO synthesis. Blocking of DDAH, which has been found in oxidative stress and inflammation, could lead to elevated ADMA levels and consequently blocking of hepatic NO production, leading to decreased sinusoid blood flow and further damage to hepatocytes and ultimately liver failure [23].

In critically ill patients, plasma ADMA concentration was found to be independently related to the presence of hepatic failure, to lactic acid and bilirubin concentration as markers of hepatic function and was a better predictor of outcome than traditional scoring systems [24, 25]. Significantly higher ADMA levels were found in non-survivors.

Recently, a relationship between arginine-NO metabolism and insulin was revealed. The infusion of L-arginine together with insulin caused a dose-dependent vasodilator effect of L-arginine. A vasodilatory effect of L-arginine infusion in kidney and ocular vasculature was enhanced by insulin, possibly by facilitated L-arginine membrane transport, but enhanced intracellular NO production or increased NO bioavailability were also proposed [26]. Evidence for a correlation between ADMA and intensive insulin therapy in the critically ill and the liver was established with a significant increase in plasma ADMA levels in traditionally treated patients as opposed to stable levels in the intensive insulin group. Modification of ADMA concentrations by insulin, with improved NO mediated microcirculation and perfusion, may in part explain the beneficial role of one of the most discussed therapeutic strategies in critically ill patients developed in recent years [27].

Ischemia/reperfusion Injury of the Liver

Ischemia-reperfusion injury of the liver occurs in several clinically relevant conditions, such as hemorrhagic shock, liver transplantation, liver resection, and sepsis. Interruption of blood flow followed by reperfusion in liver transplants leads to significant cellular damage. Experimental evidence suggests that activation of Kupffer cells and T cells mediates the activation of polymorphonuclear cells (PMN), sinusoidal endothelial cells and the activation of reactive oxygen species (ROS) [18, 28]. In response to ischemia/reperfusion, sinusoidal endothelial cells become activated and on reperfusion express an array of adhesion molecules and MHC antigens. This results in further PMN interactions and disruption of sinusoidal blood flow. PMN-induced hepatocyte injury results from adhesion of the two cell types, release by the PMNs of toxic enzymes like elastase, and induction of oxygen free radical species [28, 29].

Mitogen activated protein kinases (MAPKs) play a pivotal role in regulating cytokine production in ischemia/reperfusion injury [30]. Activation of p38 MAPK by tumor necrosis factor (TNF)-α exposure stimulates further production of TNF-α, creating a vicious circle. Inhibition of TNF-α by pentoxifylline resulted in improved sinusoidal blood flow [30].

Carbon monoxide acts as a regulatory molecule similar to NO and is released from heme in ischemia/reperfusion. It activates soluble guanylate cyclase (sGC) leading to smooth muscle relaxation and endothelial vasodilation, positively influencing the sinusoidal blood flow. It also inhibits platelet aggregation and suppresses iNOS in the hepatocytes, which has been shown to be detrimental in warm ische-

mia/reperfusion injury in the liver [18, 28]. The role of NOS in ischemia/reperfusion remains controversial though: Experiments in eNOS knockout mice demonstrated an increase in hepatotoxicity in ischemia/reperfusion liver damage, while iNOS was shown to contribute to warm ischemia/reperfusion injury, but to beneficially influence apoptosis in cold ischemia/reperfusion injury as seen in organ transplantation [18]. Thus the mechanisms by which NO exerts an effect depends on the type of ischemia/reperfusion and the source of the NO produced.

Ischemia/reperfusion also causes extensive DNA damage, with overactivation of poly(ADP-ribose)-polymerase (PARP). PARP consumes large quantities of oxidized NAD and adenosine triphosphate (ATP), depleting cellular energy and leading to cell death. A reduction of hepatic microvascular damage was demonstrated by PARP inhibition after warm ischemia of the liver and after hemorrhagic shock [31, 32]. This is a promising novel approach in reducing hepatic microcirculatory damage in ischemia/reperfusion injury.

Conclusion

Impairment of the microcirculation with altered tissue oxygenation is central in critical illness and therapeutically correcting and improving this correlates with outcome. Knowledge of the microcirculatory alterations in sepsis and other clinically relevant situations is expanding and will be of crucial importance in the development of new treatment strategies. Evaluating therapeutic interventions with monitoring of the hepatosplanchnic microcirculation seems feasible, but is, unfortunately, not routinely available in everyday ICU practice.

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The Hepatic Response to Severe Injury

M.G. Jeschke and D.N. Herndon

Introduction

After severe injury, such as thermal injury, a variable degree of liver injury is present and it is usually related to the severity of the thermal injury. Fatty changes, a very common finding, are *per se* reversible and their significance depends on the cause and severity of accumulation [1]. However, autopsies of burned children who died have shown that fatty liver infiltration was associated with increased bacterial translocation, liver failure, and endotoxemia, thus delineating the crucial role of the liver during the post-burn response [2–4]. In a recent study in 102 children, 41 females and 61 males with a total body burn size of 58 ± 2% and third degree burns in 45 ± 2%, we found that liver size and weight significantly increased during the first week post-burn (+85 ± 5%), peaked at 2 weeks post-burn (+126 ± 19%), and was increased by +89 ± 10% at discharge. At 6, 9, and 12 months the liver weight was increased by 40–50% compared to predicted liver weight. In addition, liver protein synthesis was impaired for a 6-month period with a shift from constitutive hepatic proteins to acute phase proteins [5]. Liver enzymes were significantly elevated over the first 3 weeks post-burn, normalizing over time. These findings indicate that the hepatic acute phase response perseveres for a longer time period than previously thought [5, 6].

Immediately after the burn injury, liver damage may be associated with an increased hepatic edema formation. In an animal model, we have shown that the liver weight and the liver to body weight ratio increased significantly 2 to 7 days after burn injury when compared to controls [7]. As hepatic protein concentration was significantly decreased in the burned rats, we suggest that the liver weight gain is due to increased edema formation rather than increases in the number of hepatocytes or protein levels. An increase in edema formation may lead to cell damage, with the release of hepatic enzymes [7]. The three enzymes that achieve abnormal serum levels in hepatic diseases and during the aftermath of a severe injury are alkaline phosphatase, serum glutamic oxalacetic transaminase (SGOT), and serum glutamic pyruvic transaminase (SGPT). Serum aspartate transaminase (AST), alanine transaminase (ALT) and alkaline phosphatase (ALP) are elevated between 50 to 200% when compared with normal levels. We observed that serum AST, ALT, and ALP peaked during the first week post-burn and approached the normal range 3–5 weeks post-burn. If liver damage persists or sepsis occurs, enzymes stay elevated or increase again [5–7].

Hepatocyte Proliferation and Death

Liver damage has been associated with increased hepatocyte cell death [7]. In general, cell death occurs by two distinctly different mechanisms: Programmed cell death (apoptosis) or necrosis [8]. Apoptosis is characterized by cell shrinkage, DNA fragmentation, membrane blebbing, and phagocytosis of the apoptotic cell fragments by neighboring cells or extrusion into the lumen of the bowel without inflammation. This is in contrast to necrosis, which involves cellular swelling, random DNA fragmentation, lysosomal activation, membrane breakdown, and extrusion of cellular contents into the interstitium. Membrane breakdown and cellular content release induce inflammation with the migration of inflammatory cells and release of pro-inflammatory cytokines and free radicals, which leads to further tissue breakdown [8]. Pathological studies found that about 10% to 15% of thermally injured patients have liver necrosis at autopsy [1, 9]. The necrosis is generally focal or zonal, central or paracentral, sometimes microfocal, and related to burn shock and sepsis. The morphological differences between apoptosis and necrosis are used to differentiate the two processes.

A cutaneous thermal injury induces liver cell apoptosis (Fig. 1) [7]. This increase in hepatic programmed cell death is compensated by an increase in hepatic cell proliferation, suggesting that the liver attempts to maintain homeostasis (Fig. 1).

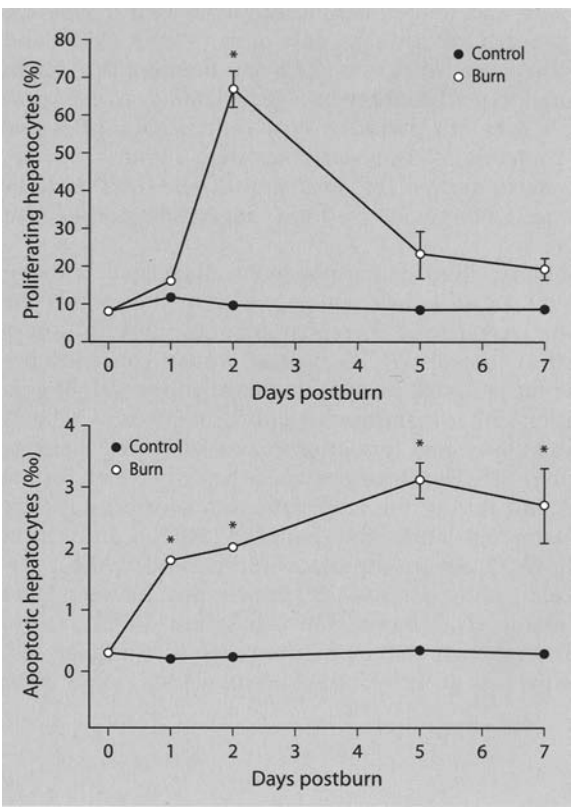

Fig. 1. Top panel: Percent of proliferating cells measured by PCNA. Lower panel: Apoptotic cells measured by TUNEL assay 1, 2, 5, and 7 days after burn, expressed as positive apoptotic hepatocytes per one thousand hepatocytes. Burned rats had significantly higher rates of hepatocyte apoptosis when compared to controls. Data presented as means ± SEM. (Burned animals n=7 and controls n=2 per time point). * $p < .05$ burn vs. control. Modified from [7] with permission

Despite the attempt to compensate for increased apoptosis by increased hepatocyte proliferation, the liver cannot regain hepatic mass and protein concentration, as we found a significant decrease in hepatic protein concentration in burned rats. It has been shown that a cutaneous burn induces small bowel epithelial cell apoptosis [10]. In the same study, the authors showed that small bowel epithelial cell proliferation was not increased, leading to a loss of mucosal cells and hence mucosal mass. Similar findings were demonstrated in the heart [11–14]. Burn induced cardiocyte apoptosis, however, cardiocyte proliferation remained unchanged causing cardiac impairment and dysfunction [11–14].

The mechanisms whereby a cutaneous burn induces programmed cell death in hepatocytes are not defined. Studies suggested that, in general, hypoperfusion and ischemia-reperfusion are associated to promote apoptosis [15–17]. After a thermal injury it has been shown that the blood flow to the bowel decreases by nearly 60% of baseline and stays decreased for approximately 4 hours [18]. It can be surmised that the hepatic blood flow also decreases, thus causing programmed cell death. In addition, pro-inflammatory cytokines such as interleukin (IL)-1 and tumor necrosis factor (TNF) have been described as apoptotic signals [19, 20]. We have shown in our burn model that after a thermal injury serum and hepatic concentrations of pro-inflammatory cytokines, such as IL-1β, IL-6, and TNF, are increased [21–23]. We, therefore, suggest that two possible mechanisms, decreased splanchnic blood-flow and elevation of pro-inflammatory cytokines, are involved in increased hepatocyte apoptosis by initiating intracellular signaling mechanisms. Signals that may be involved encompass many signals that play an important role during the acute phase response.

A pathophysiologic association with apoptosis is mitochondrial impairment and dysfunction. After severe stress, mitochondrial function and structure is impaired as shown in patients with systemic inflammatory response syndrome (SIRS), sepsis, or after burn injury [24–27]. Our group recently showed that post-burn mitochondrial state-3 respiration and the respiratory control index were significantly attenuated in muscle and liver. In addition, we found that calcium dependent onset of mitochondrial permeability transition (MPT) was markedly accelerated indicating severe mitochondrial structure damage post-burn (Fig. 2). Hepatocyte apoptosis was significantly increased along with increased caspases-3 and -9 and decreased Bcl-2 concentration post-burn [28]. We concluded that a burn induces hepatocyte apoptosis

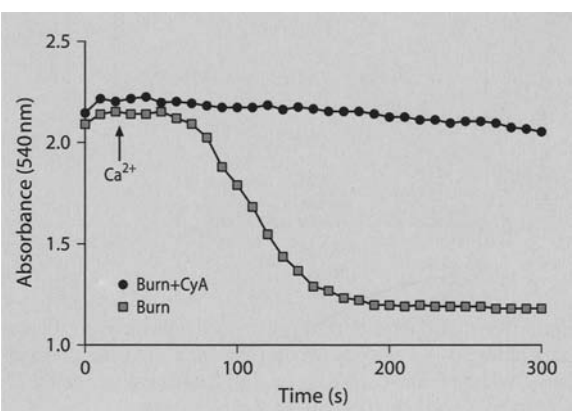

Fig. 2. Calcium dependent mitochondrial permeability transition (MPT). Burn injury induces damage to the mitochondrial membrane, which leads to a significant accelerated onset of MPT.

via caspases-3 and -9 and causes a significant impairment to mitochondrial function and structure. We suggest that mitochondrial dysfunction and increased hepatocyte apoptosis could be the underlying mechanism for the marked hepatomegaly observed after a severe burn.

Bile Formation

Bile secretion is an active process, relatively independent of total liver blood flow, except in conditions of shock. Bile is formed at two sites: a) the canalicular membrane of the hepatocyte, and b) the bile ductules or ducts. Total unstimulated bile flow in a 70 kg man has been estimated to be 0.41 to 0.43 ml/min. Eighty percent of the total daily production of bile (approximately 1500 ml) is secreted by hepatocytes and 20% is secreted by the bile duct epithelial cells. In trauma and sepsis, intrahepatic cholestasis occurs frequently and appears to be an important pathophysiologic factor, occurring without demonstrable extrahepatic obstruction. This phenomenon has been described in association with a number of processes, such as hypoxia, drug toxicity, or total parenteral nutrition [29]. The mechanisms of intrahepatic cholestasis seem to be associated with an impairment of basolateral and canicular hepatocyte transport of bile acids and organic anions [30, 31]. This is most likely due to decreased transporter protein and RNA expression thus leading to increased bile. Intrahepatic cholestasis, which is one of the prime manifestations of hepatocellular injury, was present in 26% of patients in a clinical study [9]. All of these cases were concurrent with sepsis. The cellular damage observed in sepsis is more likely the result of decreased hepatic blood flow than of direct cellular damage [32]. We have recently shown that a burn causes a significant decrease in bile acid output (Fig. 3) [33]. As bile acids are contributors to hepatic regeneration it seems likely that decreased bile acid output represents another factor for hepatocyte damage post-burn [34].

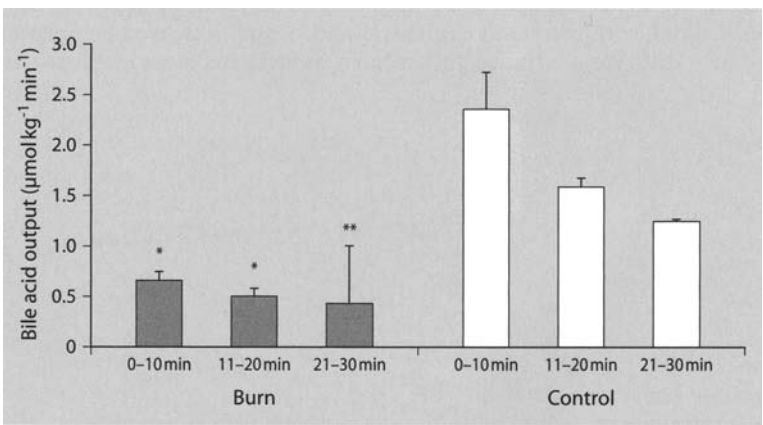

Fig. 3. Bile acid output of thermally injured animals in comparison to control rats. Bile was collected over consecutive 10 min periods. When comparing the corresponding periods, bile acid secretion was significantly reduced in the burned group. *$p < 0.001$ burn vs. control; **$p < 0.01$ burn vs. control. From [33] with permission. Copyright 2006 The Endocrine Society

Hepatic Acute Phase Response

The acute phase response is a cascade of events initiated to prevent tissue damage and to activate repair processes (Fig. 4) [35]. The acute phase response is initiated by activated phagocytic cells, fibroblasts, and endothelial cells which release pro-inflammatory cytokines leading to the systemic phase of the acute phase response. The systemic reaction affects the hypothalamus which leads to fever, the pituitary-adrenal axis so as to release steroid hormones, the liver which causes the synthesis and secretion of acute phase proteins, the bone marrow which promulgates further hemopoietic responses, and the immune system which allows activation of the reticuloendothelial system and the stimulation of lymphocytes. However, a crucial step in this cascade of reactions involves the interaction between the site of injury and the liver, which is the principle organ responsible for producing acute phase proteins and modulating the systemic inflammatory response [35].

After major trauma, such as a severe burn, hepatic protein synthesis shifts from hepatic constitutive proteins, such as albumin, pre-albumin, transferrin, and retinol-binding protein to acute phase proteins [35–37]. Acute phase proteins are divided into type I acute phase proteins, such as haptoglobin and α_1-acid glycoprotein, mediated by IL-1-like cytokines (IL-1α/β, TNF) and type II acute phase proteins, such as α_2-macroglobulin and fibrinogen, which are mediated by IL-6-like cytokines (IL-6, IL-11) [35–37]. We recently evaluated the inflammatory-cytokine expression profile after a severe burn and found that pro-inflammatory mediators, namely IL-6, IL-8, monocyte chemoattractant protein (MCP)-1 and TNF, increase 2–100 fold immediately post-burn. The inflammatory cascade decreases over time and by 5–6 weeks most of the cytokines approach normal levels [38].

The signal cascade of cytokines is the following: The cytokines bind to their receptors and activate intracellular signals by tyrosine phosphorylation, for the type I acute phase response c-Jun/c-fos, hepatic nuclear factor-kappa B (NF-κB) or the CCAAT/enhancer-binding-proteins (C/EBPs) [39–42]. The intracellular signal cascade for type II has been shown to be a tyrosine phosphorylation and activation of intracellular tyrosine kinases (JAKs), latent cytoplasmic transcription factors, STAT1, STAT3, and STAT5 (signal transducer and activator of transcription),

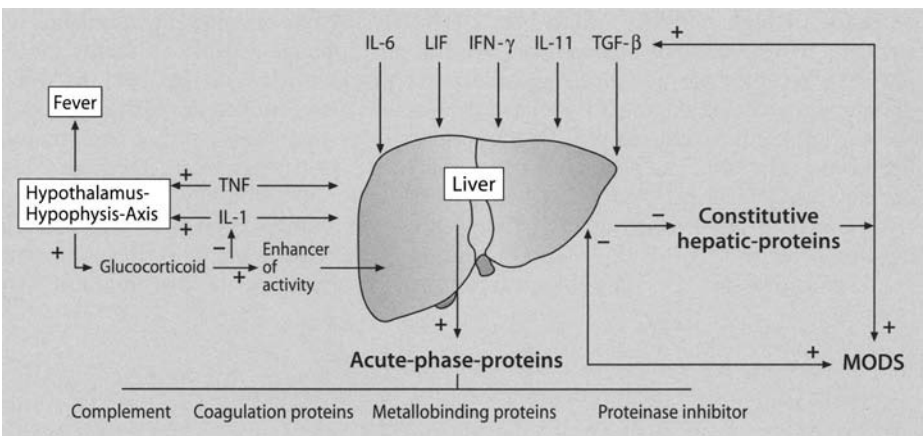

Fig. 4. Scheme of the hepatic acute phase response.

or mitogen-activated protein [40, 43, 44]. These signals activate transcription, translation, and expression of acute phase proteins. IL-6, in particular, has been speculated to be the main mediating cytokine. IL-6 activates glycoprotein 130 and the JAK-kinases (JAK-1) leading to activation of STAT1 and 3 translocating to the nucleus. The intranuclear genes for acute phase proteins are turned on.

In contrast to acute phase proteins, constitutive hepatic proteins are downregulated [35, 37, 45, 46]. After a thermal injury, albumin and transferrin decrease by 50–70% below normal levels [5, 6]. Studies have shown that two mechanisms are responsible for the decrease in constitutive hepatic proteins. First, the liver re-prioritizes its protein synthesis from constitutive hepatic proteins to acute phase proteins [35]. This has been shown in many studies in which the mRNA synthesis for constitutive hepatic proteins is decreased. The other mechanism for decreased constitutive hepatic protein concentration is the capillary leakage and the loss of these proteins into the massive extravascular space and burn wound. Albumin and transferrin, however, have important physiologic functions as they serve as transporter proteins and contribute to osmotic pressure and plasma pH [45, 46]. The downregulation of albumin and transferrin after trauma has been described as potentially harmful and the synthesis of these proteins has been used as a predictor of mortality, nutritional status, and severity of stress, and as an indicator of improved recovery [39, 46–48]. The question whether albumin substitution in patients with hypoalbuminemia is beneficial or detrimental represents a current study focus.

The aim of the acute phase response is to protect the body from further damage, and this aim will be achieved when all elements of the acute phase response coalesce in a balanced fashion. However, a prolonged increase in pro-inflammatory cytokines and acute phase proteins has been shown to be associated with a hypercatabolic state, increased risk of sepsis, multiple organ failure (MOF), morbidity, and mortality [39, 47, 48]. Therefore, an important therapeutic approach to improve survival after trauma may be the modulation of the acute phase response by decreasing acute phase proteins and pro-inflammatory cytokines and increasing constitutive hepatic proteins [49]. The use of antibodies against pro-inflammatory cytokines such as TNF, IL-1β, or their receptors showed promising results *in-vitro* and in animal models by increasing survival rates in septicemia [50–53]. However, when these approaches entered clinical trials it became evident that these promising animal data could not be repeated in humans. New approaches encompass the attenuation or blockade of high mobility group box 1 (HMGB1) a late mediator responsible for lethality in the state of septicemia [54–57], macrophage inhibitory factor (MIF) [58–62], receptor for advanced glycation end products (RAGE) [63–66], or other danger signals (e.g., damage-associated molecular pattern molecules [DAMPs]) [67]. We and others have chosen a different approach. We hypothesized that endogenous hormones can alter inflammation and the acute phase response. Over the last decade, we determined the effects of growth hormone, hepatocyte growth factor (HGF), insulin-like growth factor-I (IGF-I), ICF-I in combination with its principle binding protein-3 (IGF-I/BP-3), and insulin on the hepatic acute phase response and cytokine expression as a physiological approach to attenuate the pro-inflammatory cascade.

Possible Treatments to Alter the Acute Phase and Inflammatory Response

Recombinant Human Growth Hormone

Recombinant human growth hormone (rhGH) modulates the acute phase response by affecting pro-inflammatory, IL-1-like cytokine expression followed by decreased type I acute phase proteins and increasing constitutive hepatic proteins [23, 68]. No effect on IL-6-like cytokines and type II acute phase proteins could be demonstrated. RhGH administration increased endogenous albumin levels, reducing the amount of required exogenous substitution to maintain normal serum albumin levels. Similar to acute phase proteins, the mechanisms by which rhGH increases endogenous albumin concentrations are unknown; however, rhGH may exert this effect through activation of C/EBPβ [69]. Another side effect of rhGH that has been recently delineated is an increase in hepatic triglyceride concentration and development of a fatty liver [23, 70, 71]. RhGH administration over 10 days increased hepatic triglyceride concentration by nearly 50% in burned rats [23]. The mechanisms have been discussed in clinical studies, where the authors speculated that rhGH increased peripheral lipolysis and due to a lack of transporter proteins (low density lipoprotein [LDL], high density lipoprotein [HDL]) triglycerides accumulate in the liver [70, 71]. We demonstrated in pediatric burn patients that rhGH increased free fatty acid concentration when compared to placebo, indicating that rhGH stimulates peripheral lipolysis and subsequently free fatty acid concentration [68]. Given the fact that the acute phase response is a contributor to mortality after trauma, rhGH administration appears not to cause an increase in mortality in severely burned children as described by Takala et al. in trauma and septic patients [72], as rhGH does not cause an increased and prolonged acute phase response.

Hepatocyte Growth Factor

Administration of hepatocyte growth factor (HGF) stimulates constitutive hepatic proteins after burn injury *in-vivo* [73]. In fact, serum transferrin reached normal levels 7 days after injury with HGF treatment, whereas in saline-treated animals, serum transferrin remained low. Serum albumin levels decreased; however, beginning at day 2 after burn, HGF attenuated this drop in serum albumin. The exact mechanisms by which HGF stimulates constitutive hepatic proteins are unknown; however, HGF is capable of stimulating the synthesis of C/EBPβ, which regulates constitutive hepatic proteins [74]. In contrast to recent *in vitro* studies, where the authors demonstrated that HGF decreased acute phase proteins, we showed *in vivo* that HGF increased serum α_2-macroglobulin (type II acute phase protein), with no effect on α_1-acid glycoprotein and haptoglobin (type I acute phase proteins) [75, 76]. Type II acute phase proteins are mediated through IL-6 like cytokines, including cytokines such as IL-6 and IL-11 [35]. IL-6, secreted by Kupffer cells in the liver, is capable of regulating the synthesis of transcription factors that have response elements in the 5'-flanking region of the HGF gene that may be potentially utilized in inducing HGF gene expression at the transcriptional level [77, 78]. Therefore, IL-6 appears likely to substantially and quickly upregulate HGF mRNA and HGF mRNA receptor expression [79]. However, the interaction between HGF and IL-6 *in vitro* has been shown to be complex and controversial [75, 80]. In our study, we demonstrated that administration of rhHGF stimulated serum IL-6, along with an increase

in its dependent type II acute phase proteins, serum α_2-macroglobulin and TNF [73]. HGF has been shown to have some beneficial effects and to be a potential therapeutic agent; however, more studies need to be done before this growth factor can be applied in patients.

Insulin-Like Growth Factor-I in Combination with its Principle Binding Protein-3

Insulin-like growth factor-I is a 7.7 kDa single chain polypeptide of 70 amino acids with sequence homology to pro-insulin [81]. In the body, 95–99% of IGF-I is bound and transported with one of its six binding proteins (IGFBPs) 1–6 [81]. The majority of IGF-I is bound to IGFBP-3. Administration of the IGF-I/BP-3 complex as a therapeutic agent provides several advantages over the administration of IGF-I alone, because when IGF-I is already bound to IGFBP-3, it rapidly transforms into a ternary complex, which confers decreased serum clearance and allows the delivery of significantly larger amounts of IGF-I without inducing hypoglycemia and electrolyte imbalances. In general, IGF-I has been shown to improve cell recovery, wound healing, peripheral muscle protein synthesis, gut and immune function after thermal injury [82, 83]. Recent evidence suggests that IGF-I is instrumental in the early phases of liver regeneration after trauma and modulates the hepatic acute phase response in burned rats [22, 84]. In thermally injured children, rhIGF-I in combination with its principle binding protein modulates the hepatic acute phase response by decreasing the pro-inflammatory cytokines, IL-1β and TNF, followed by a decrease in type I acute phase proteins. IGF-I/BP-3 had no effect on IL-6 and type II acute phase proteins. Decreases in acute phase protein and pro-inflammatory cytokine synthesis were associated with increases in constitutive hepatic protein synthesis [85, 86]. Attenuating the hepatic acute phase response with IGF-I/BP-3 modulated the hypermetabolic response, which may prevent MOF and improve clinical outcome after a thermal injury without any detectable adverse side effects. The data shown would make IGF-I/BP-3 an ideal therapeutic agent; however, recently our group found that IGF-I/BP-3 increased the risk of peripheral neuropathies, thus limiting the use of this agent [unpublished observations].

Insulin

In severely burned rodents, insulin significantly improved hepatic protein synthesis by increasing albumin and decreasing c-reactive protein and fat, while insulin decreased the hepatic inflammatory response signal cascade by decreasing hepatic pro-inflammatory cytokine mRNA and proteins, IL-1β and TNF, at pre-translational levels [21, 28]. Insulin increased hepatic cytokine mRNA and protein expression of IL-2 and IL-10 at a pre-translational level when compared with controls. In addition, insulin affected hepatic signal transcription factors and attenuated inflammation at a molecular level. Insulin increased hepatocyte proliferation along with Bcl-2 concentration, while decreasing hepatocyte apoptosis along with decreased concentrations of caspase-3 and -9, thus improving liver morphology (Fig. 5) [21, 28]. From this animal study, we concluded that insulin attenuates the inflammatory response by decreasing the pro-inflammatory and increasing the anti-inflammatory cascade, thus, restoring hepatic homeostasis. This effect was not only limited to the post-burn response but also to other post-stress models [87, 88]. We showed that insulin administration during endotoxemia (lipopolysaccharide [LPS] administration) improved hepatic protein synthesis, inflammation, acute phase protein synthesis,

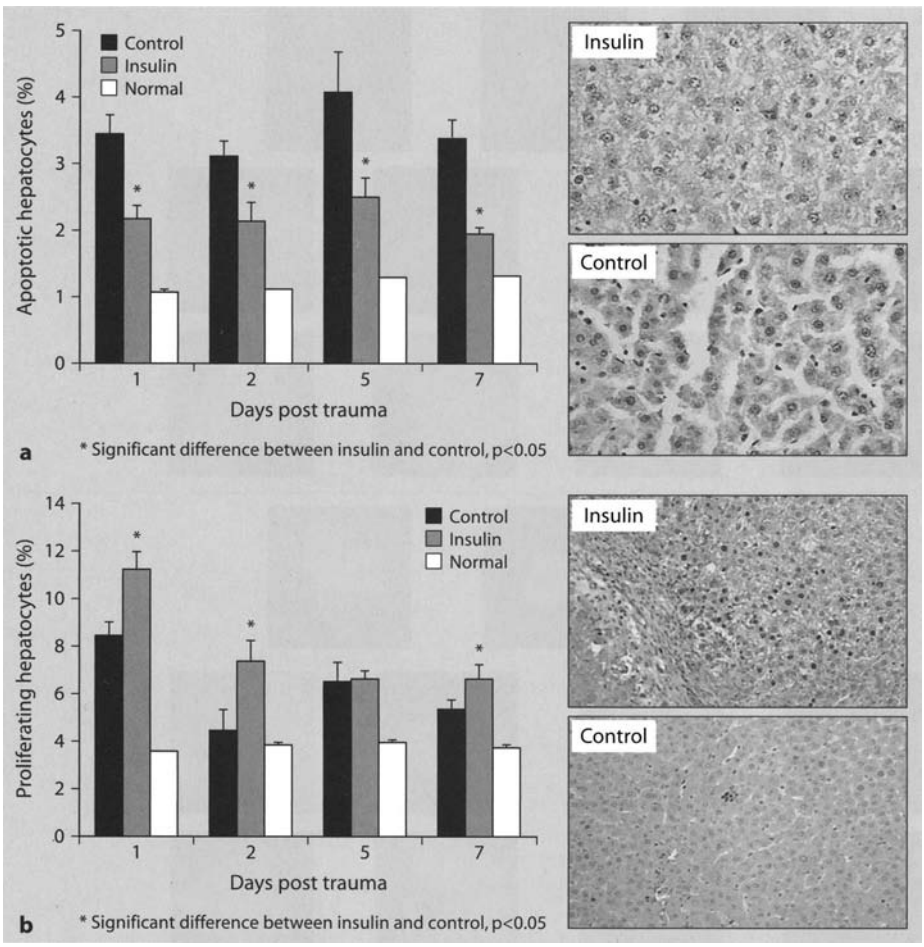

Fig. 5. Burn caused a significant increase in hepatocyte apoptosis compared to normal. Insulin administration decreased hepatocyte apoptosis at all time points. *$p < 0.05$ insulin vs control. Data presented as mean ± SEM with $n = 7$ for each group and each time point. Insulin significantly increased hepatocyte proliferation on days 1, 5, and 7 compared to controls. Modified from [28] with permission

and homeostasis. Bioluminescence showed that insulin improved hepatic glucose metabolism and glycolysis (Fig. 6). Gene chip analysis revealed a strong anti-inflammatory effect of insulin on inflammatory mediators. In order to confirm our animal data, we conducted a human study [89]. Insulin administration decreased pro-inflammatory cytokines and proteins, while increasing constitutive-hepatic proteins. Burned children receiving insulin required significantly less albumin substitution to maintain normal levels compared to controls. Insulin decreased free fatty acids and serum triglycerides when compared to controls.

In conclusion, several studies have shown that insulin attenuates the inflammatory response by decreasing the pro-inflammatory, and increasing the anti-inflammatory cascade, thus, restoring systemic homeostasis, which has been shown to be critical for organ function and survival in critically ill patients. Insulin appears to be

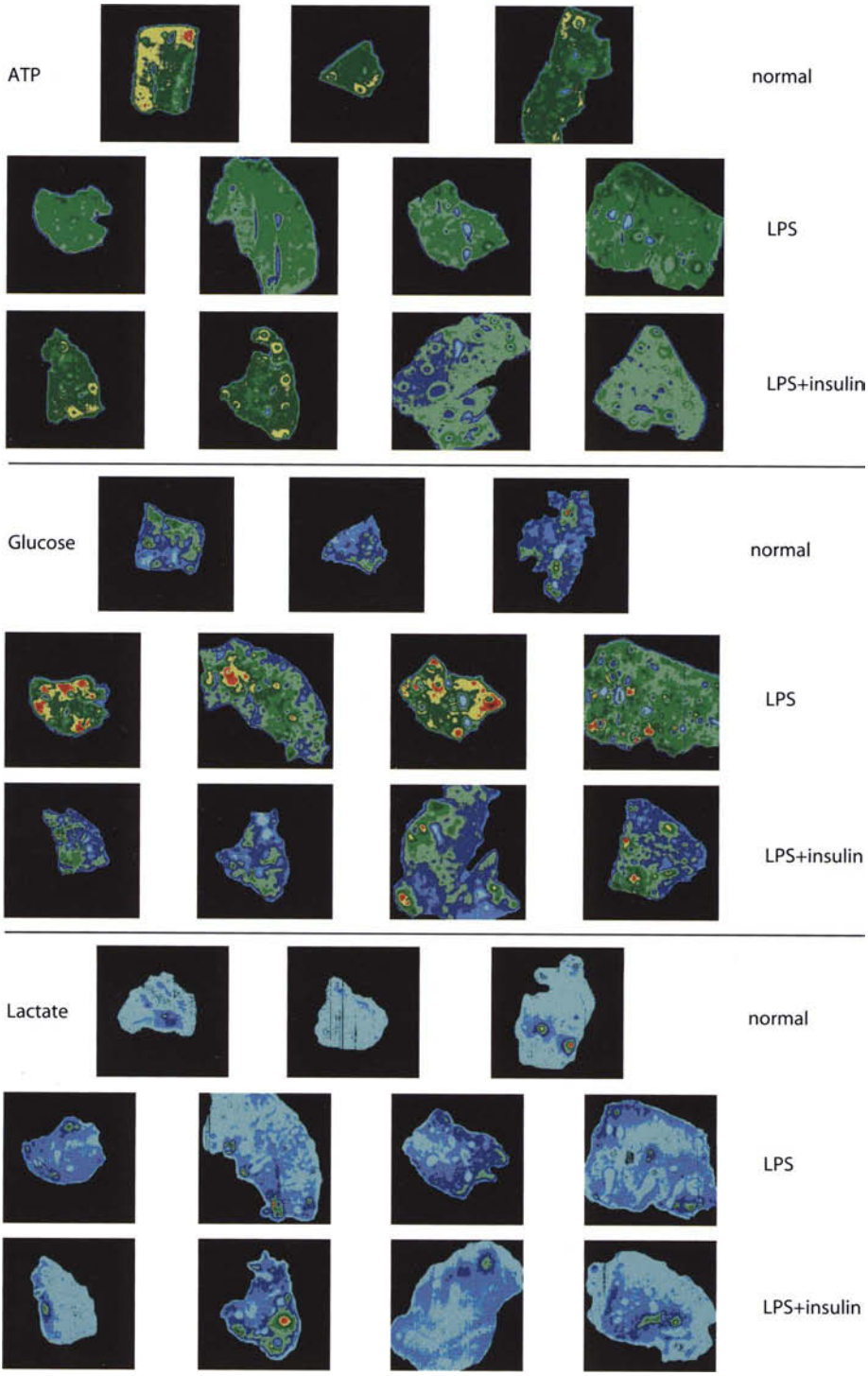

Fig. 6 *(Legend see p. 661)*

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Fig. 6. Bioluminescence of the liver. ATP was present in normal animals. Endotoxemia caused decreased levels of intracellular hepatic ATP. There were no significant differences between LPS and LPS+insulin. Intrahepatic glucose levels were low. LPS caused an increase in intrahepatic glucose levels, which were decreased to normal concentrations with insulin administration. Rats receiving LPS+insulin had normal hepatic glucose levels, $p<0.05$. Endotoxemia caused a significant increase in lactate, whereas animals receiving LPS+insulin had normal lactate levels, $p<0.05$. Color interpretation from highest to lowest concentration: red>orange>yellow>green>light blue>dark blue. From [88] with permission from the European Association for the Study of the Liver

a safe and effective drug to affect hepatic dysfunction. Moreover, as tight euglycemic control has been shown to be advantageous, studies in this area are warranted.

Propranolol

Finally, we would like to mention propranolol, a non-selective β_1/β_2 blocker. Beta blockade results in a decrease in urinary nitrogen loss, with decreased peripheral lipolysis and whole body urea production [90], decreased resting energy expenditure, and improved skeletal muscle protein kinetics [91]. Furthermore, propranolol preserved fat-free mass when compared to controls [91]. Propranolol also decreases hepatic fat storage by limiting fatty acid delivery in severely burned pediatric patients [92]. In addition, we showed that propranolol decreased peripheral lipolysis and improved insulin responsiveness [93]. Recently, we further showed that propranolol has a profound effect on fat infiltration of the liver by reversing hepatomegaly [92]. We propose that propranolol reduces hepatomegaly by inhibiting lipolysis and reducing liver blood flow, and, in turn, delivery of fatty acids to the liver. The effect of propranolol on the hepatic acute phase response, systemic inflammatory reaction, and immune system is being examined in ongoing clinical studies at our institute.

Conclusion

The liver plays a crucial role in the aftermath of a thermal injury. The synthesis of constitutive hepatic proteins, acute phase proteins, cytokines, and other mediators makes it a determining factor for survival. For a long time, hepatic dysfunction was tolerated without any treatment option. In the field of burns, it appears that new treatment options will be available to successfully attenuate the hypermetabolic hepatic acute phase response. A new approach for improving hepatic function may be the use of anabolic, anti-inflammatory agents; however, there is currently no effective treatment for hepatic dysfunction.

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The Management of Liver Trauma

A.B. Cresswell, M.J. Bowles, and J.A. Wendon

Introduction

The adult liver weighs around 1500 g and lies mainly in the right upper quadrant of the abdomen, immediately beneath the diaphragm [1]. The liver is the most commonly injured intra-abdominal organ and is found to be damaged in 30% of patients undergoing laparotomy for penetrating injuries and in 15–20% of laparotomies for blunt injuries [2].

An appreciation of basic liver anatomy is essential in understanding the mechanisms and consequences of hepatic trauma. The liver is divided into left and right lobes by an imaginary plane (the principal plane) which runs between the inferior vena cava (IVC) and the port hepatis and gall bladder. There is further subdivision into eight segments based on portal venous, hepatic arterial and bile duct anatomy, first described by Couinaud [3]. The majority of the liver is covered by visceral peritoneum which condenses to form the diaphragmatic attachments of the coronary, left and right triangular, and falciform ligaments. The vascular inflow to the liver is provided by the hepatic artery and portal vein, which lie with the common bile duct at the porta hepatis, and drainage is into the IVC via the three hepatic veins and also by small direct tributaries between the caudate lobe (segment 1) and the anterior surface of the IVC.

Mechanisms of Injury

Liver injuries can be broadly categorized as being due to blunt or penetrating mechanisms of trauma, with violent behavior and road traffic accidents accounting for the majority of cases in the UK [4]. There is a clear pattern to the distribution of injury with blunt mechanisms prevailing in European reports [5, 6], usually as the occupant of a motor vehicle [7] compared to a preponderance of penetrating injury in North America [8] and South Africa [9].

Blunt injury causes either deceleration or crush pattern injuries, with deceleration resulting in movement of the liver against its attachments and crush causing direct compression of the hepatic parenchyma. The distinction is important as deceleration injuries tend to create lacerations, primarily within the right lobe, which can include vascular disruption, whereas direct crush tends to involve the central parts of the liver [10]. Massive unsurvivable liver injuries are usually the result of high energy deceleration resulting in major vascular disruption or avulsion of the liver from the IVC [11].

In patients who are hemodynamically stable, a careful history from the patient, witnesses, and emergency services can provide useful information in predicting the likely pattern of potential hepatic injuries.

Table 1. The Hepatic Injury Scale is the most commonly used grading system and was produced by the Organ Injury Scaling Committee of the American Association for the Surgery of Trauma

Grade	Injury	Description
I	Hematoma	Subcapsular <10% of surface area
	Laceration	Capsular tear <1 cm of parenchymal depth
II	Hematoma	Subcapsular 10–50% of surface area Intra-parenchymal <10 cm in diameter
	Laceration	1–3 cm parenchymal depth and <10 cm length
III	Hematoma	Subcapsular >50% of area or expanding. Ruptured subcapsular or parenchymal hematoma. Intraparenchymal hematoma >10 cm or expanding
	Laceration	>3 cm parenchymal depth
IV	Laceration	Parenchymal disruption involving 25–75% of hepatic lobe or 1–3 segments within a single lobe
V	Laceration	Parenchymal disruption involving >75% of hepatic lobe or >3 segments within a single lobe
	Vascular	Juxta-hepatic venous and caval injuries
VI	Vascular	Hepatic avulsion

Grading of Liver Injury

The severity of liver injury ranges from relatively minor subcapsular lesions to unsurvivable hepatic avulsion. The Hepatic Injury Scale (Table 1) is the most commonly used grading system and was produced by the Organ Injury Scaling Committee of the American Association for the Surgery of Trauma in 1989 [12] and subsequently revised in 1994 [11]. This scale grades injuries from I to VI with grade I or 2 injuries considered minor and usually amenable to non-operative treatment; such lesions account for 80 to 90% of liver trauma [2, 13]. Grade VI injuries, in which there is avulsion of the liver from its vascular attachments, are much less common and tend to be incompatible with life.

Initial Management and Investigation

Early management should be in accordance with a set trauma protocol, such as the Advanced Trauma Life Support guidelines of the American College of Surgeons Committee on Trauma [14], with attention first directed to airway maintenance and ventilation. The correct approach to fluid resuscitation with potentially uncontrolled hemorrhage remains contentious, with growing advocacy of permissive hypotension [15] and a move away from 'aggressive', high-volume fluid replacement [16].

The key underlying determinant of subsequent management is, of course, hemodynamic stability. Unstable patients, in the context of abdominal injury with signs of peritonism, should undergo immediate resuscitative laparotomy without further investigation [14]. However, emergency room laparotomy for those too unstable to survive transfer to the operating room is probably futile [8, 17]. Irrespective of hemodynamic stability, all cases of gunshot wound to the abdomen require exploratory laparotomy, given the radiological difficulties in following a bullet track [18].

In cases where hemodynamic stability is maintained with or without volume resuscitation, and abdominal injury is suspected, prompt radiological imaging

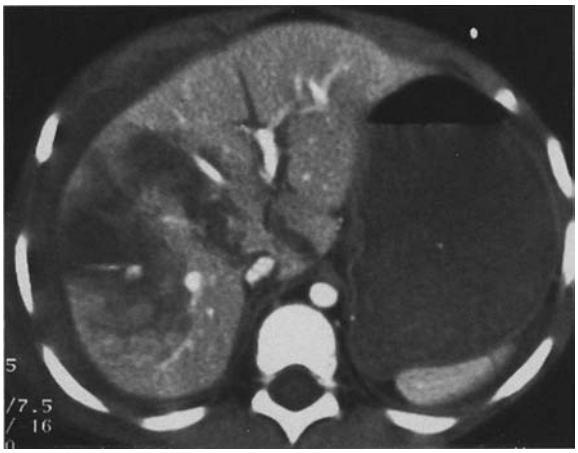

Fig. 1. A CT scan displaying a grade 5 hepatic injury with an extensive laceration involving disruption of >75% of the right lobe and extending into the porta hepatis. This patient was managed non-operatively on liver intensive care and did not develop any late complications.

should be performed. Features of the plain trauma series radiographs, which are suggestive of hepatic injury include fractures of the lower ribs, elevation of the right hemi-diaphragm and loss of the psoas shadow [10].

Ultrasound, even in the most expert hands, has a reported sensitivity of around 88% for detection of intra-abdominal injuries [19] and computed tomography (CT) is the investigation of choice to accurately detect and categorize liver injuries [20, 21], with the caveat that CT is known to slightly overgrade the category of liver injury when compared to operative findings [22] (Fig. 1).

The timing of scan is an important consideration and there is some suggestion that increasing the duration between injury and scanning aids differentiation between hematoma and lacerations [20]. Intraperitoneal pooling of contrast correlates with ongoing hemorrhage [23], tracking of fluid around the portal structures is associated with injuries to the portal triad [24], and recognition of the 'sentinel clot' and active extravasation are features associated with high likelihood of requirement for active intervention [25].

Magnetic resonance imaging (MRI) is yet to be shown to have a role in the acute assessment of liver trauma, a fact which may simply reflect the increased time required for scanning and a general lack of availability in the acute setting. Other, more targeted investigations such as endoscopic retrograde cholangiopancreatography (ERCP) or angiography, may have a complementary role with CT in the management of acute liver injuries but cannot provide a comparative global assessment tool of the liver and the rest of the abdomen.

Definitive Management of Liver Trauma

Following the acute assessment and initial resuscitation, patients must be selected for either an operative or non-operative approach to management with a conservative approach appropriate in around 80% of cases [26]. Selection is, of course, a dynamic process and all patients require close observation as failure of conservative management, due to continued or recurrent bleeding can be expected in around 5–11% of cases [26, 27]. For both groups, possible complications include early hemorrhage and later bile leak or intra-abdominal collections [26].

Table 2. The outcome of liver injury by grade in a series reported by Schweizer et al from Switzerland in 1993 [6]

Grade	Mortality (%)
I	11
II	10
III	13
IV	
V	33

The major causes of death in liver injury are uncontrolled hemorrhage early in the clinical course, and sepsis with multiple-organ failure (MOF) in the longer term [8].

Overall, a non-operative approach is associated with a lower transfusion requirement and a lower incidence of abdominal complications [26]. The association of solid organ injury with damage to a hollow viscous is a concern in both groups and, for the non-operative cases especially, a careful review of the radiological imaging is required to exclude concomitant damage in the knowledge that incidence increases with the number of solid organs injured [28].

The outcome after liver trauma has been shown to be dependent on the severity of the liver injury, the mechanism, and also the presence of any other associated injuries [8] with a commonly quoted overall mortality of around 12%. Only one study primarily addressed mortality based on grade of liver injury and a summary of these 175 patients is displayed in Table 2 [6].

Liver trauma itself and especially operative intervention, with or without packing, carries a high incidence of intra-abdominal hypertension and the abdominal compartment syndrome (ACS) [29]. Whether or not the patient undergoes surgery the intra-abdominal pressure should be routinely measured and if high, in the presence of organ dysfunction, decompression should be performed. Following decompression, or if the abdominal wall is found to be tight at the time of closure following operative management, a temporary abdominal dressing will be required. Our own preference is for insertion of a silastic mesh, which is usually removed around day 5–10 with partial or staged fascial closure, or occasionally skin only closure. Several other techniques are described for temporary abdominal closure and at present none has been shown to be superior in the context of a randomized trial.

Non-operative Management of Liver Trauma

The conservative approach to liver trauma has evolved from pediatric practice [30] and is now well established in the adult setting [31]. The success of a non-operative strategy is impossible to predict, and is not related to the grade of injury noted either by CT or subsequently at operation [26, 32].

The patient must be placed appropriately, usually within the critical care unit, and certainly within an area allowing close monitoring of hemodynamic and hematological parameters. In addition, repeated clinical examination, ideally by the same clinician, is essential. Requirements for invasive monitoring have not been absolutely established and will, to some degree, be determined by the patient's clinical condition. As an absolute minimum, the entirely stable patient requires frequent checks of heart rate and non-invasive blood pressure, with wide bore venous access in place, although some units would advocate a more aggressive approach and there are no evidence based guidelines.

In cases managed non-operatively, hemodynamic instability is an absolute indica-

tion for intervention and is the commonest early complication, occurring in 5–11% of cases [26]. The liver does, however, have a remarkable capacity to bleed substantially and then spontaneously stop, so the presence of a large amount of intra-peritoneal blood is not an indication for laparotomy *per se*, unless it is accompanied by hemodynamic instability.

Despite recommendations that repeated scans only be performed in the event of clinical deterioration [33], it has been found that 49% of follow-up scans after operative management of severe liver injuries demonstrate new liver related complications [34], most of which require intervention. With this in mind, the majority of units would perform a second scan in all cases of significant liver trauma prior to discharge, and usually around 7–10 days after the original imaging.

Late complications of liver injury can occur following non-operative and operative management and include secondary bleeding (usually from pseudoaneurysms Fig. 2), infected collections and bile leaks (Fig. 3), the majority of these complications can be dealt with by radiological intervention, with percutaneous drainage of collections, localization and temporary stenting of bile leaks by ERCP and angiographic embolization of secondary hemorrhage [35, 36] (Fig. 4).

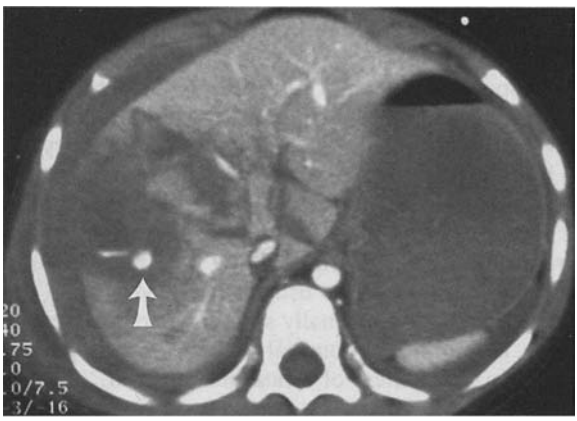

Fig. 2. A CT scan following a significant liver injury showing a traumatic pseudoaneurysm in the right lobe, which required angio-embolization.

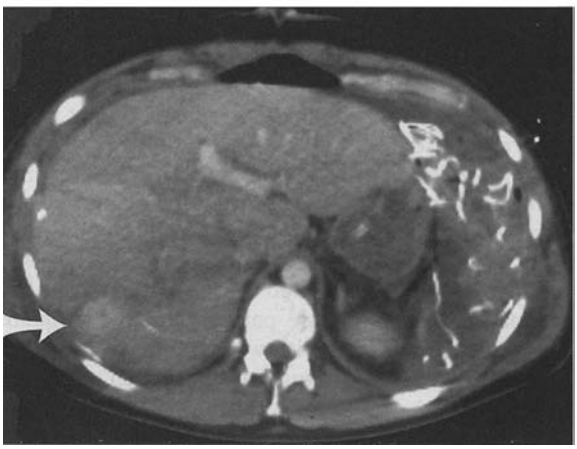

Fig. 3. An early follow-up CT scan performed after a liver injury showing a bile leak at the posterior aspect of the right lobe.

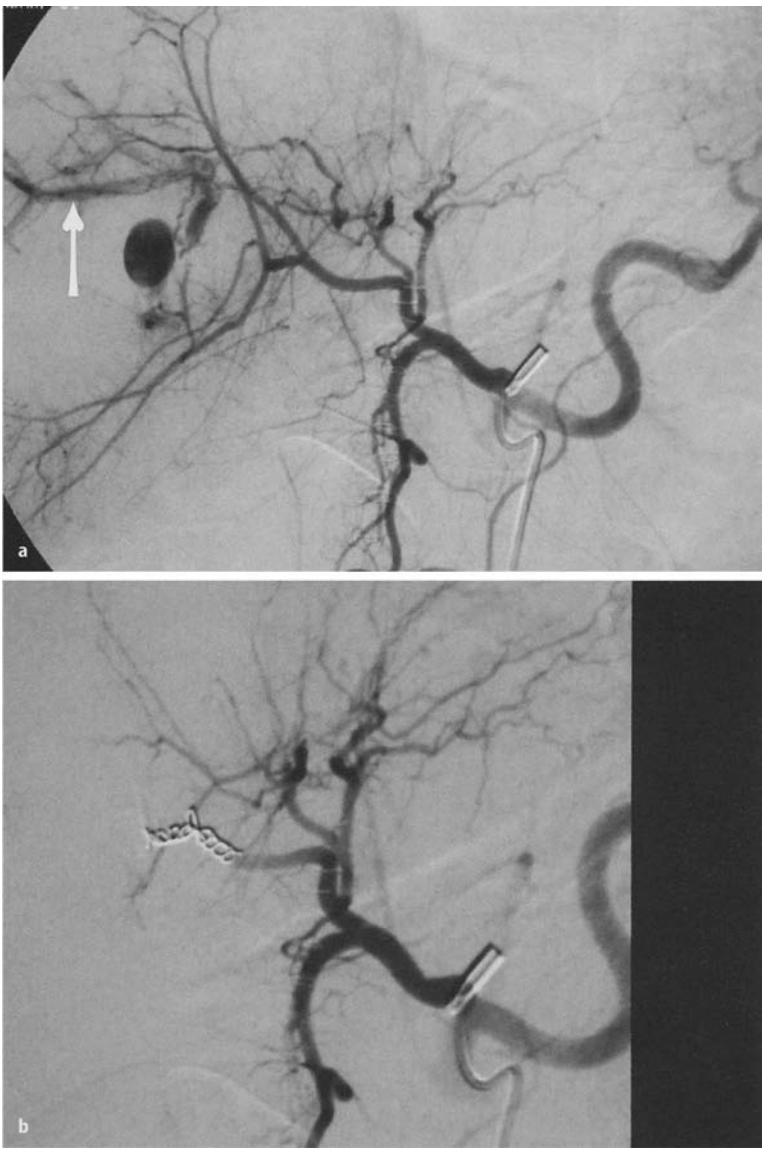

Fig. 4. Angiographic images of the pseudoaneurysm shown in Fig. 2. The first image clearly shows passage of contrast into the venous system (arrow). The second image is obtained following control of the aneurysm by selective coil embolization of the right hepatic artery.

Operative Management of Liver Trauma

Patients undergoing primary operative management of liver trauma are, by definition, hemodynamically unstable, and can also be expected to exhibit other features of critical illness such as coagulopathy, renal dysfunction, and acute lung injury. The chosen surgical intervention should reflect this fact and, by and large, be limited to

damage control procedures [37], with the key underlying principle being to limit intervention as far as possible.

In an ideal world, such patients should be managed by specialist hepato-biliary surgeons; however the majority of unstable liver injuries will be treated initially by general or trauma surgeons at the admitting hospital. In this setting, the only appropriate intervention is packing of the liver via a midline laparotomy [38], with subsequent transfer as indicated.

Specialist surgical interventions include liver resection, selective hepatic artery embolization, repair of hepatic vein or caval injuries, and *ex-vivo* liver resection or liver transplantation. The various surgical strategies will be briefly described, along with their relative merits, however, this chapter is by no means a definitive surgical text.

Isolated injury to the extra-hepatic biliary tree is encountered only rarely in the trauma population and the mainstay of treatment is control of hemorrhage with adequate drainage pending definitive reconstruction [39]. Isolated gall bladder injuries may be treated by primary cholecystectomy; however, traumatized bile ducts are best treated by biliary-enteric anastamosis with a roux-en-Y type reconstruction in a manner identical to the management of iatrogenic injuries [40].

Strategies for Hemostasis and Hepatic Packing

Following opening of the peritoneal cavity and initial evacuation of blood and clots, four quadrant packing should be employed to control hemorrhage and no further attempts made to evaluate the extent of liver injury until adequate volume resuscitation [10] and relevant blood products (red cells, platelets, fresh frozen plasma cryoprecipitate, and occasionally recombinant factor VII) have been administred. Temporary packing has been shown to be effective in the majority of patients, the exception being in the presence of major vascular injury [41]. When stable, the packs can be gently removed from each quadrant in turn and the injuries carefully evaluated.

When the right upper quadrant packs are removed it may be possible to control light bleeding with diathermy (ideally via argon beam coagulater, which prevents the tip of the diathermy probe becoming adherent to the tissues [42]), direct pressure applied for several minutes or the topical application of synthetic absorbable hemostatic materials. Visible vessels can be suture ligated or clipped. If bleeding cannot be easily controlled it may be necessary to re-apply packs and to occlude the inflow to the liver by compression of the portal structures (hepatic artery and portal vein) in the free edge of the lesser omentum (Pringle's maneuver). Inflow control may arrest the bleeding and allow sufficient time for clot formation (especially if any coagulopathy is reversed during this time by the intravenous administration of clotting factors), and can be maintained for up to one hour [43]. Bleeding that is temporarily controlled by inflow occlusion is likely to be arterial in origin and may require ligation of the relevant right or left branch. This is, however, a relatively demanding procedure in the face of active hemorrhage.

Continued bleeding with the inflow clamped indicates hepatic vein or caval injury, both of which lie behind the liver and are relatively inaccessible. In such circumstances, manipulation and mobilization of the liver should be kept to a minimum as it may lead to extension of venous lacerations or air embolism and the combination of the weight of the liver and packing can be utilized to tamponade the bleeding, pending specialist input.

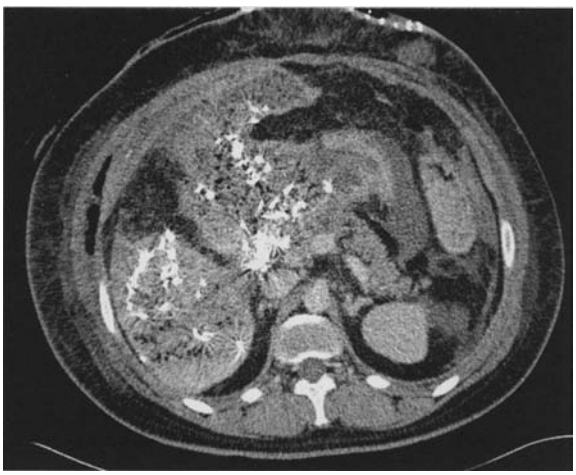

Fig. 5. A CT scan following the incorrect packing of a patient transferred from a peripheral hospital following significant liver injury. Packs have been forced into the cavity formed by the initial laceration causing further parenchymal disruption and the radio-opaque tapes of the packs are clearly seen within the liver substance. At laparotomy, amongst other injuries, the right hepatic artery was found to have been completely transected in the packed cavity.

The technique for packing is important, and ideally, the liver should be partially mobilized from its diaphragmatic attachments for packs to be inserted over the superior border. Under no circumstances should packs be inserted into cavities within the liver parenchyma as this will lead to further distraction and disruption of blood vessels (Fig. 5). Such cavities should be manually closed and held in position by direct pressure and peri-hepatic packing. The former practice of hepatorrhaphy, whereby sutures are used to compress the hepatic parenchyma, is best avoided due to the risk of inducing hepatic necrosis with subsequent infection [2, 13]. It is generally accepted that packs should not remain in position for more than three days due to the risk of infection [41] and that intravenous antibiotic and anti-fungal prophylaxis should be administrated [44].

Following immediate stabilization, transfer of a patient with significant liver trauma to a specialist hepato-biliary surgical unit should be considered as early as possible, even if the initial management has been non-operative.

Specialist Surgical Interventions

Following transfer, reassessment of hemodynamic stability will take place. If stable, a triple phase CT scan is usually performed to reassess the injury and specifically look for extravasation of contrast in the arterial or venous phases. The indications for surgery are the same (hemodynamic instability or concurrent injury to other viscera requiring surgical intervention) and packing remains the mainstay of hemorrhage control. Occasionally, further surgery is necessary but this is best performed after the patient has been fully stabilized in terms of hemodynamics, coagulation, and temperature and should only be undertaken by an experienced specialist surgeon.

Liver resection

Resectional debridement involves the non-anatomical excision of devitalized liver tissue along the lines of the previous injury [9]. The technique is rarely required but

may be indicated if part of the liver becomes necrotic and the source of uncontrollable sepsis. Particular attention must be made to identifying and ligating or repairing segmental bile ducts to avoid subsequent bile leak.

Formal anatomical liver resection in the context of acute trauma with active bleeding is a technically difficult exercise and is best avoided. There are only a few reports from highly specialized centers where this technique has been employed successfully in preference to resectional debridement for injuries involving major vessels or bile ducts [45, 46].

Selective Hepatic Artery Ligation and Embolization

Although surgical ligation of the hepatic artery or its branches has been largely displaced by modern surgical techniques it does remain a viable option where perihepatic packing fails to control hemorrhage and temporary hepatic artery occlusion is effective [13].

Radiological embolization by placement of coils and/or gel within the arterial tree is an emerging interventional option and data, although limited, are encouraging [47]. The technique has been described for acute and life-threatening hemorrhage from a hepatocellular adenoma [48] and also in the treatment of post-traumatic pseudoaneurysm [35]. Angiography with a view to embolization should now be the investigation of choice for suspected arterial bleeding in a stable patient.

As with surgical ligation, gangrenous cholecystitis remains a possible complication where the right hepatic artery has been occluded [49] (and sometimes following packing). In these circumstances, cholecystectomy is required.

Repair of Hepatic Vein and Caval Injuries

Failure of the Pringle maneuver to arrest the hemorrhage raises the possibility of either aberrant vascular anatomy or else hepatic vein or caval injury. In this situation, the most common abnormality is an anomalous right hepatic artery arising from the superior mesenteric artery which is present in around 15% of cases and is usually located posteriorly at the porta hepatis [3].

In order to achieve total vascular exclusion of the liver, in addition to inflow control as previously described, it is also necessary to occlude the supra-hepatic and infra-hepatic vena cava and, in the context of trauma, this maneuver is usually combined with a shunting procedure to maintain venous return. Such injuries carry a reported mortality of between 50 and 80% [50] with very few centers having any great experience. In one of the largest series, from China, isolated left hepatic vein injury was associated with a higher survival (five out of five survivors) than isolated right hepatic vein (8% mortality) and combined right and left hepatic vein (100% mortality) [51].

Liver Transplantation

Data on acute liver transplantation due to uncontrollable hemorrhage are extremely limited. The only true series considered eight patients who underwent total hepatectomy with temporary portocaval shunting as a life-saving manuever following failure of other surgical procedures. Six of the eight patients died from MOF or sepsis [52]. Beyond this small series, occasional case reports exist, however the outcome of transplantation for primary hemorrhage control is universally poor [51].

Some success has been seen with transplantation for the treatment of sub-acute liver failure due to reperfusion injury on removal of tight packs. In these circumstances, profound hemodynamic and respiratory compromise occur in the operating room, which is irreversible and may only be controlled by total hepatectomy. Such patients must be super-urgently listed for transplantation and will only survive if a suitable donor liver becomes available within two to three days of the patient being anhepatic.

Conclusion

Despite its relatively protected location, the liver is the most commonly injured intra-abdominal organ and liver injury is often associated with injuries to other structures. The majority of liver injuries can be managed non-operatively and grade of injury is not useful in selecting patients for, or predicting outcome of, non-operative management. The initial management of liver injuries is effective trauma resuscitation and prompt transfer of unstable patients to the operating room, where correctly performed peri-hepatic packing can be a life-saving, temporizing measure. The effective surgical management of complex hepatic injuries is best provided in a dedicated specialist center with access to the appropriate surgical, radiological and critical care expertise. High energy deceleration injuries resulting in significant juxta-hepatic vascular and caval injuries are accompanied by an extremely high mortality rate despite aggressive surgical intervention but there is no clear role for acute liver transplantation in these cases at present.

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The Pathology and Management of Intracranial Hypertension in Acute Liver Failure

N. Murphy

Definition

Acute liver failure is a syndrome manifest by the rapid cessation of hepatic function in previously normal individuals. The rate of decline in function dictates the manner in which the syndrome manifests and influences the outcome. The etiology of the insult to the liver is the main influence on the rate of progression and the likelihood of spontaneous recovery [1].

The different patterns of progression from initial symptoms to the onset of hepatic encephalopathy, dictate the manner in which the syndrome presents and the associated complications. In the most recent definition, the terms hyperacute, acute, and subacute liver failure are recognized [1].

In most of Western Europe and the USA, the commonest presentation is hyperacute liver failure characterized by the onset of encephalopathy within seven days from initial symptoms. Hyperacute liver failure has a high rate of spontaneous recovery with medical management but also has the highest incidence of cerebral edema and intracranial hypertension. The cause of the vast majority of hyperacute liver failure is acetaminophen toxicity [2, 3]. Patients who present with jaundice to encephalopathy from 8 to 28 days have an acute presentation and have a high incidence of cerebral edema but a lower rate of spontaneous recovery, and those presenting from 5 to 28 weeks have a subacute presentation, a low rate of spontaneous recovery but a reduced incidence of cerebral edema [1].

Etiology

Cerebral edema was noticed and commented on in the seminal clinico-pathological review of servicemen presenting with fulminant epidemic hepatitis during the East Asian campaign of the Second World War [4]. However, the recognition that cerebral edema was a distinct clinical entity and cause of death associated with acute liver failure did not become clear until much later [5, 6].

The Munroe-Kelly hypothesis states that the cranial vault is a closed space that contains brain, cerebrospinal fluid (CSF), and blood. Each of these components is incompressible. If the volume of one increases there is displacement of the others to a point where there is failure of compensation. After this point, a small change in volume will result in a large change in pressure within the skull. CSF displacement compensates during the early stages of cerebral edema in acute liver failure. Following on from this, reduction in blood volume can result in cerebral ischemia and displacement of the brain substance results in caudal herniation.

The recognition that brain swelling is an important component of acute liver failure is now well established. Management strategies place the risk of intracranial hypertension at the forefront of care, and prophylactic therapy, monitoring, and treatment are all widely debated in the literature [7, 8]. The cause of cerebral edema and intracranial hypertension in acute liver failure are not completely understood but in recent years progress has been made into the processes involved [9]. Ammonia has long been implicated as important in hepatic encephalopathy and, as the evidence mounts, is increasingly recognized as an important factor in the etiology of cerebral edema in acute liver failure [10]. Recent work has shown that whole blood ammonia predicts outcome and the chances of cerebral herniation [11, 12]. Electron-microscopic analysis of post-mortem brain biopsies together with gravemetric analysis of brain water content have, in animal models, shown that swelling occurs in the gray matter and that astrocytes are the main target [13, 14]. How ammonia induces astrocytes to swell is incompletely understood but there are a number of possible mechanisms including an osmotic effect and cellular toxicity.

Ammonia is detoxified in astrocytes by combining with glutamate to produce glutamine. Astrocytes are the only cell type in the brain that contain glutamine synthetase and this normal pathway maintains the ratio of glutamate to glutamine in astrocytes and neurons. In the event of acute liver failure, marked increases in serum ammonia induce a build up of glutamine within astrocytes. This increase in intracellular glutamine results in an increase in cellular osmotic potential. This is offset by exclusion of intracellular ions but these processes are overwhelmed because of the rapid rise in glutamine. This results in an increase in cellular volume. Evidence of this effect can be inferred by the fact that inhibition of glutamine synthetase, which catalyzes the reaction, prevents brain swelling in experimental models [15]. An osmotic effect may not be the only mechanism by which glutamine induces cellular swelling, however, but there is circumstantial evidence that it occurs to some extent. In subacute and chronic liver disease there is time for adaptation to the increase in astrocyte osmotic potential by the excretion of intracellular osmolytes such as myo-inositol [16]. In acute liver failure there is not time for adaptation because of the rapid increase in intracellular glutamine. Recently, however, the direct correlation between astrocyte glutamine levels and cell volume has been questioned. Experimental data have not been able to show a correlation between the two, with peak glutamine levels occurring before cellular volume reaches its maximum [17, 18]. A clinical study using cerebral microdialysis showed abnormal glutamate/glutamine trafficking within the brain extracellular space with initial high levels of glutamate but then a reduction to very low levels without any correlation to intracranial pressure (ICP) [19]. Extracellular lactate concentration was shown to rise prior to surges in ICP, however [19].

A breakdown in cellular energy metabolism induced by an increase in intracellular glutamine and ammonia is another possible mechanism for the cellular swelling. Impairment of alpha-ketogluterate dehydrogenase and pyruvate dehydrogenase induced by oxidative stress and ammonia results in a breakdown in astrocyte energy production, and an increase in glycolysis. This suggestion is supported by an increase in extracellular lactate and brain lactate flux seen in clinical studies [19, 20]. Mitochondrial dysfunction induced by ammonia may also play a role [10].

In addition to the cytotoxic edema seen early in acute liver failure, changes in cerebral blood volume appear to play an important role in the development of intracranial hypertension, in some patients at least. Cerebral blood flow (CBF) shows wide variation in patients with acute liver failure and the normal close relationship

between cerebral metabolism and flow appears to be lost [21]. In animal models of liver failure there is a gradual increase in cerebral blood flow but this situation is less clear in human studies [9, 22]. Cerebral metabolism is generally reduced in high-grade encephalopathy; however, because of the breakdown in autoregulation there is evidence that relative or absolute cerebral hyperemia can contribute to intracranial hypertension [23, 24]. An increase in CBF may induce intracranial hypertension by a number of mechanisms. It may induce further cerebral swelling. This could occur due to an increase flux of potentially toxic metabolites to the brain such as ammonia or by an increase in cerebral water content because of an increase in cerebrovascular hydrostatic pressure. Neither of these hypotheses has been proven and the evidence suggests that the blood brain barrier is relatively intact in acute liver failure suggesting that hydrostatic or vasogenic edema is uncommon [14]. Autoregulation is lost in acute liver failure [22, 25]. The cause is unknown but a gradual cerebral vasoparesis concurs with clinical observation. Autoregulation can be restored by hyperventilation and mild hypothermia but not by the use of indomethacin [26]. It has been suggested that the increase in astrocyte glutamine plays a role in this gradual vasoparalysis and loss of autoregulation by the induction of local nitric oxide (NO) or carbon monoxide [9]. It is relatively common to find a patient with a high ICP and jugular venous oxygen saturation above 80%. This suggests a state of luxury perfusion in which an increase in cerebral blood volume in an already swollen brain accounts for the associated increase in ICP [27].

Management

In the management of intracranial hypertension in acute liver failure it is important to target those at risk. Intracranial hypertension remains a leading cause of death despite advances in the understanding of etiology and management of patients [28, 29]. Monitoring the brain in these patients is difficult and invasive. Despite investigation, a reliable non-invasive method for the evaluation of CBF, cerebral oxygenation, and ICP remains elusive [30]. There are important points that need to be considered when managing a patient with acute liver failure and possible increased ICP:

- Prediction of patients at risk of intracranial hypertension
- How to monitor the brain
- Prophylactic management
- Treatment of established intracranial hypertension

Predicting Intracranial Hypertension

In acute liver failure, the development of cerebral edema is seen in patients with the shortest time between the development of jaundice and the onset of encephalopathy [1]. The fulminating presentation, lack of time for cerebral adaptation, and systemic burden of a necrotic liver appear to be the likely reasons. Patients with acetaminophen (paracetamol) toxicity make up the largest number of this group, athough other etiologies can fall into the hyperacute group including patients with acute liver failure due to hepatotrophic viruses, particularly hepatitis B. In contrast, patients with subacute liver failure due to seronegative hepatitis or non-ABCDE hepatitis have a smaller risk.

It has been suggested that the incidence of intracranial hypertension following acetaminophen toxicity has fallen since the mid 1980s [31]; however, it still repre-

sents a significant complication in this setting. Recent work from King's College Hospital in London suggests an incidence of 20–30% in all patients with acute liver failure [28]. Data from our own unit suggests that intracranial hypertension is implicated in the death of 25% of all patients with acute liver failure and 35% of those following acetaminophen-induced toxicity [29].

Young age has consistently been found to be a risk factor [5, 28]. Arterial ammonia concentration has been shown to correlate with death and cerebral herniation [11, 12, 32]. Recent commentary suggests that arterial ammonia should be measured serially in all patients with acute liver failure and ICP monitoring be instituted if the concentration is greater or equal to 150 μmol/l [27].

Monitoring the Brain

There are a number of monitoring devices and methods that can be undertaken to screen for raised ICP or cerebral ischemia or both. Some of these techniques are more invasive than others and the possible risks and benefits need to be understood.

Computed tomography (CT) is a standard investigation in any patient with suspected intracranial pathology. Cerebral edema can be recognized on CT scans of patients with acute liver failure and the severity correlates crudely to encephalopathy grade but the correlation between imaging and severity of ICP measurement is poor [33, 34]. As little additional information is gained, very careful consideration should be undertaken before transporting this very sick group of patients to the CT scanner. Occasionally there are diagnostic difficulties or a suspected complication of ICP bolt insertion and CT scanning might be considered.

Functional brain imaging, using single positron emission tomography (SPECT), has been used to investigate the distribution of CBF in acute liver failure and magnetic resonance imaging (MRI) has been used to investigate the distribution of intracerebral water but neither has found a place in clinical practice [35, 36].

In patients with suspected intracranial hypertension, direct monitoring of cerebral oxygenation and blood flow are appealing but current methods have technical and clinical limitations. Tissue PO_2 and interstitial metabolites, using intra-parenchymal probes, have been investigated in traumatic brain injury (TBI) and to a limited extent in acute liver failure [19, 37]. These techniques have the advantage in traumatic injury of providing localized information around the area of injury. The use of cerebral microdialysis remains a research tool in acute liver failure at the present time.

Methods used for the estimation of global cerebral oxygenation include the sampling of jugular venous blood for oxygen saturation, and products of metabolism such as lactate. A jugular venous saturation of less than 55% suggests an ischemic brain. This can be due to a reduction in blood flow in excess of demand because of brain swelling or cerebral vasoconstriction due to hypocarbia, if the patient is being hyperventilated. An increase in demand due to seizure activity can also manifest as a reduction in jugular venous saturation [38]. High jugular venous oxygen saturation (>80%) may represent a hyperemic brain and steps can be made to reduce cerebral blood volume if ICP is raised. Very high jugular venous oxygen saturation is often seen as a terminal event and may represent a complete loss of oxygen extraction by the brain.

Near infrared spectroscopy (NIRS) is a non-invasive technique used to assess the oxygen content of various organs. NIRS can be used to determine cerebral oxygena-

tion and changes in cerebral perfusion in acute liver failure and warrants further investigation [39].

Non-invasive measurement of CBF using transcranial Doppler has been investigated in acute liver failure and found to be predictive of changes in CBF induced by hyperventilation; however, the technique does not provide data on cerebral oxygenation and so could not be recommended without the addition of a jugular venous catheter [40].

The recognition that ICP is raised in a significant proportion of patients with acute liver failure and that this is implicated in significant morbidity and mortality led to the use of direct measurement of ICP with various forms of monitor [5, 41]. These techniques, while fully supported internationally in TBI, are controversial in the field of management of acute liver failure and there remains a dichotomy of opinion in most countries with some units using them and others not [7, 8, 42, 43].

Controversy revolves around the lack of evidence of improved outcome associated with the monitoring of ICP and the risk of intracranial bleeding associated with insertion. The reported risk of bleeding, from survey data, is between 10 and 20% overall, the majority of which is not clinically significant. Mortality has been reported at between 1 and 3% (Lee, unpublished data from Edinburgh, UK) [41, 43]. The risk of bleeding is higher than that seen following TBI but there is uncontrolled evidence that activated factor VII can reduce this risk [44].

Clinical signs of raised ICP are inconsistent and often occur late when intervention is usually futile. In essence, the argument for the use of pressure monitoring is that with more and earlier information, interventions will not be futile and may either prevent cerebral swelling and or hyperemia or prevent their complications.

It has not been possible to prove that ICP monitoring improves survival in acute liver failure in itself, as a randomized, controlled clinical trial has not been performed to evaluate this question. However, it is undoubted that medical intervention can reduce ICP and prevent cerebral ischemia and brain herniation in patients with acute liver failure [27]. Data also suggest that having an ICP monitor increases the intervention rate compared to patients without, and increases the length of survival in the critical care unit, but not overall survival [45]. Many studies have shown that simple interventions such as osmotherapy, sedation, hyperventilation, indomethacin, and hypothermia can reduce ICP, but it is also true that the majority of patients with acute liver failure die of multiple organ failure (MOF) due to sepsis. It may be that interventions to reduce ICP just delay death.

The corollary is that without monitoring ICP one is unable to intervene until clinical signs, such as pupilary dilatation, are present. While the risks of monitoring are documented, the risks of not monitoring are less clear and not monitoring will deprive the patient of interventions proven to reduce ICP, even if not proven to increase survival. This is surely a nihilistic view.

The use of ICP monitoring should be seen in context. Without monitoring ICP there is a tendency toward therapeutic paralysis because of uncertainty and to manage all patients as if they had raised ICP. The reassurance of a normal ICP enables a reduction in sedation and paralysis. It enables tracheal suctioning and other nursing care without the uncertainty of worsening an unknown ICP. With ICP monitoring, modest increases in ICP can be treated early before clinical signs suggest impending brain herniation. Monitoring ICP enables the calculation of cerebral perfusion pressure and, together with the monitoring of jugular oxygen saturation, allows a more complete picture of cerebral perfusion and oxygenation. The use of ICP monitoring has been advocated in the setting of liver transplantation for acute

liver failure and, of course, enables continued research into the management of cerebral edema in the clinical setting.

Prophylactic Measures

In patients at high risk of cerebral edema, a number of prophylactic interventions have been shown to reduce the incidence of intracranial hypertension. Serum sodium is often low in patients with acute liver failure. In a consecutive group of patients with acute liver failure from acetaminophen overdose admitted to King's liver ICU 65% of them were hyponatremic on arrival (Will Bernal, personal communication). Hyponatremia is associated with a poor outcome in acute liver failure [46]. Based on retrospective data showing an inverse relationship between ICP and serum sodium in patients with acute liver failure, moderate hypernatremia was investigated as a possible prophylactic intervention [47, 48]. Using hypertonic saline, serum sodium was maintained between 145 to 155 mmol/l. This simple prophylactic measure was found to reduce ICP from baseline and reduce the incidence of surges in ICP [47] (Figs. 1 and 2). Hypothermia improves outcome following out-of-hospital cardiac arrest and has been investigated in patients with TBI. Early reports suggest that hypothermia can reduce ICP in patients with acute liver failure. Prophylactic hypothermia is currently being investigated in this setting. Simple measures such as raising the head of the bed to a 30° angle and the avoidance of excessive stimulation are prudent.

Cerebral Perfusion Pressure

In TBI there is a consensus of opinion supporting the use of cerebral perfusion pressure (CPP) as a treatment goal [49]. In acute liver failure, the concept of CPP-directed therapy is less useful. To assume a correlation with CBF there has to be a consistent cerebrovascular resistance and this is not the case in acute liver failure

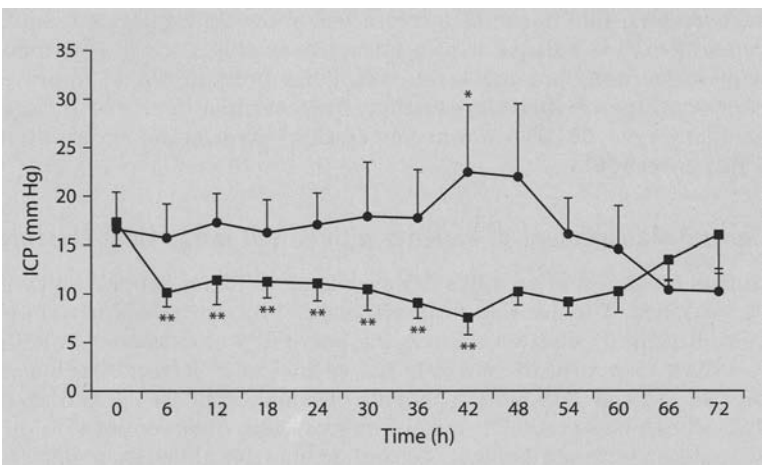

Fig. 1. Intracranial pressure in patients treated with normal saline to maintain serum sodium between 145 to 155 mmol/l, and control patients [47] (● = control, ■ = treatment). Mean and standard error; *: p=0.04 Mann-Whitney U test; **: p=0.001, Quade ANOVA)

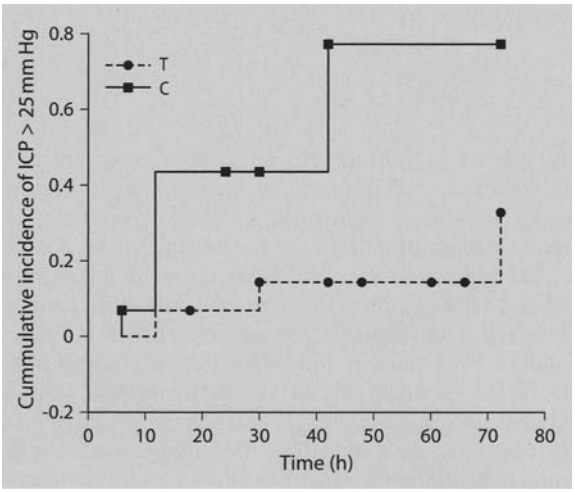

Fig. 2. Kaplan-Meyer analysis of patients in Figure 1. Cumulative hazard for ICP > 25 mmHg, (p=0.04, Breslow test) T = treatment group, C = control group.

[50]. This is due to the loss of cerebrovascular autoregulation and attempts to increase cerebral perfusion are often unsuccessful as the use of vasopressors often results in an increase in ICP as brain blood volume increases [24, 51]. However, this is not to say that CPP should be ignored entirely but the safe lower limit of CPP has yet to be defined, as there are many reports of patients surviving with normal cerebral function despite a low CCP [52]. The normal lower limit of cerebral autoregulation is reached at a mean arterial blood pressure (MAP) of about 50 mmHg, below which flow becomes pressure dependent. In patients with absent autoregulation, such as in acute liver failure CPP should probably be maintained above 40 mmHg (the normal lower limit of autoregulation with an ICP of 10 mmHg or less) but there are no data to back this statement up. Maintenance of CPP in acute liver failure is best achieved by decreasing ICP and aiming for a MAP, using fluid and vasopressors, that does not increase ICP above 25 mmHg. Attempting to improve cerebral oxygen balance is also attractive in this setting. This may be attempted with intravenous indomethacin, which has been shown to improve CPP without compromising cerebral oxygenation, hypothermia, increased sedation, and hyperventilation [26, 53–55]. Monitoring cerebral oxygenation is very useful during such a maneuver [50].

General Management of Patients with Raised Intracranial Pressure

In patients at risk of or with suspected cerebral edema prophylactic measures should be instituted. The decision to insert an ICP bolt or not will have to be made by the clinical team. If inserted there is the possibility of managing ICP. ICP is normally less than 15 mmHg in an adult. The definition of intracranial hypertension is not precise and will vary among patients. Available data are derived from patients with TBI where observational studies suggest that interventions to reduce pressure should be instituted between 20 and 25 mmHg, although pupillary abnormalities and brain herniation can occur at lower pressures [56]. There have not been any studies investigating treatment thresholds in patients with acute liver failure and so similar thresholds to those in TBI are used.

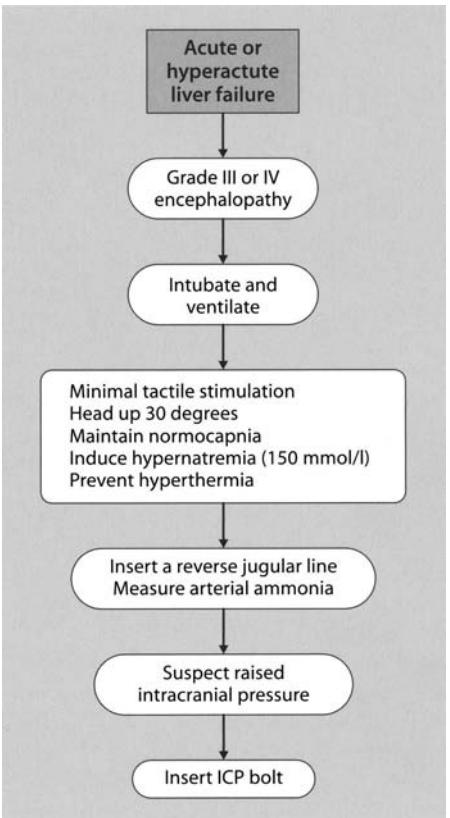

Fig. 3. Initial management of a patient at risk of intracranial hypertension.

The management of intracranial hypertension is usually escalated along standard algorithms (Fig 3–5). Sit the patient at an angle of 30° and avoid tight straps around the neck to encourage venous drainage. ICP tends to increase during nursing interventions. If this takes more than a couple of minutes to recover it can suggest poor intracranial compliance. Treatment is usually instituted for a sustained rise in ICP (>5 to 10 minutes) or clinical signs suggesting cerebral ischemia or impending herniation. Sedation should be increased. Propofol is probably the agent of choice [30]. Osmotherapy is the mainstay of treatment following these simple measures. Mannitol as a rapid infusion (0.5–1.0 g/kg) has been shown to reduce ICP reliably in acute liver failure [57]. The dose can be repeated but care must be used in renal failure due to accumulation and multiple administrations can result in a hyperosmolar syndrome. Plasma osmolality should be monitored if multiple doses are used. Current practice is to remove 500 ml of ultrafiltrate via continuous veno-venous hemofiltration (CVVH) following each bolus dose of mannitol. Bolus doses of 20 ml hypertonic saline (30%) have a similar effect to mannitol in this setting (personal observation). Hypertonic saline has a higher reflectance coefficient at the blood brain barrier compared to mannitol and there appears to be less tachyphylaxis to multiple administrations [47].

In patients with a raised ICP and cerebral hyperemia, suggested by a jugular venous oxygen saturation of 80% or greater, (luxury perfusion) short-term hyper-

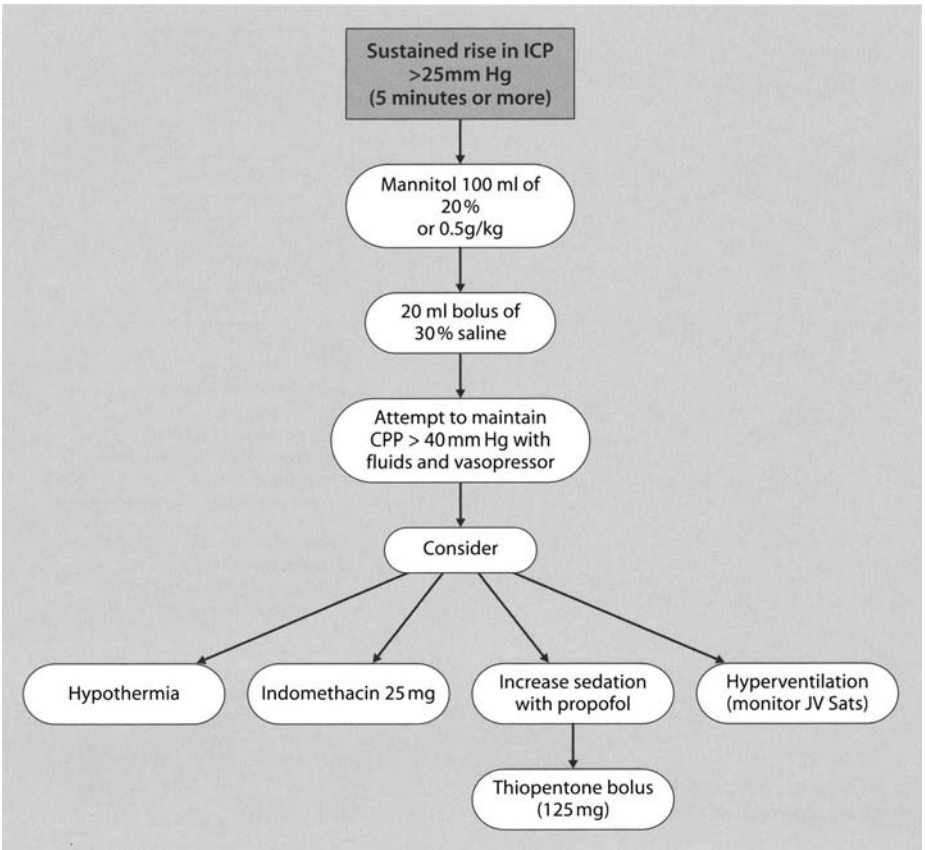

Fig. 4. Management of a sustained rise in intracranial pressure.

ventilation will induce cerebral vasoconstriction and reduce blood volume. This maneuver has been shown not to impair cerebral oxygenation but close monitoring of cerebral oxygenation should be employed if it is attempted [55]. Short-term hyperventilation has not been shown to improve overall survival in acute liver failure but has been shown to delay ICU death [58]. Hyperventilation may be life saving and buy time for definitive treatment (transplantation). Indomethacin induces cerebral vasoconstriction and reduces ICP in patients with both TBI and acute liver failure without impairing cerebral oxygenation although confirmatory studies are needed [26].

Seizures

Ammonia toxicity and cerebral edema are associated with seizure activity and it has been recognized that sub-clinical seizures occur more commonly than thought previously [38]. The use of mechanical ventilation facilitated by sedatives and muscular paralysis can mask the clinical signs. Seizures adversely affect cerebral oxygen consumption and may contribute to cerebral edema and are a cause of low jugular oxygen saturation. It has been suggested that prophylactic phenytoin be used in all

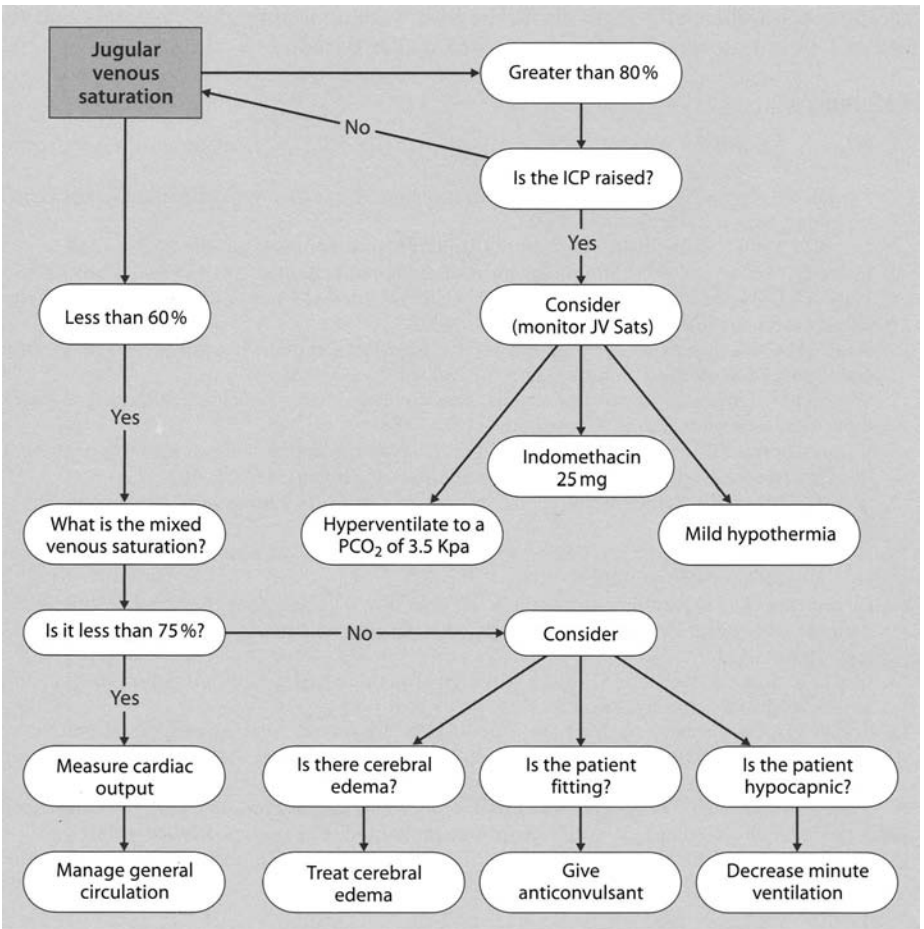

Fig. 5. Interpretation of abnormal jugular venous oxygen saturation.

patients with acute liver failure and high-grade encephalopathy. Others have questioned this approach because of the significant side effects and apparent lack of effect on outcome [59]. If confirmed, seizure activity should be managed along standard management guidelines.

Conclusion

Acute liver failure is a rare but often fatal syndrome in which liver transplantation is the only definitive treatment option. Cerebral edema is a severe complication in a significant proportion of patients with a hyperacute and acute mode of presentation. Improved understanding of the pathology and clinical observation has enable a more focused approach to its management. Management options now include prophylactic therapy such as hypertonic saline and possibly mild hypothermia, and treatment options for established intracranial hypertension have broadened over

recent years. Debate still rages about the best way to monitor these patients and the use of ICP monitoring remains very much center based.

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Neurological Issues

Continuous EEG Monitoring in the ICU

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Introduction

Since the first human electroencephalogram (EEG) was recorded in 1929 by Hans Berger, enormous advances have been made in EEG recording technology and data analysis [1, 2]. The recording period was extended and long-term EEG monitoring became technically feasible when computer applications were introduced in the 1970s and digital EEG recording systems established. However, the use of long-term EEG monitoring was mostly limited to epilepsy monitoring units and only subsequently found its way to the intensive care unit (ICU).

Yet, an old comparison to cardiology still holds true: While it is an unchallenged standard to continuously monitor heart function in all ICU patients with abnormal cardiac function, brain activity is often not monitored in patients with acute brain damage, mainly because brain function has been more difficult to monitor. A single or even repeated EEG represents only a small sample of data. For example, seizures in the ICU setting are usually brief and paroxysmal and can easily be missed when EEG recording is performed intermittently. Fortunately, over the past 15 years, rapid advances in computer technology and digital data transmission and analysis have made it possible to introduce continuous EEG monitoring in the ICU.

The goal of continuous EEG monitoring is to enable intensive care physicians to predict impending central nervous system (CNS) injury at a time when intervention is still possible. The challenge for the future will be to demonstrate that intensified monitoring of brain function by continuous EEG will result in better outcomes.

Indications for Continuous EEG in the ICU

Detection of Seizures

The underlying rationale for continuous EEG monitoring in the ICU is to detect secondary insults that may lead to further injury of the already damaged and vulnerable brain, thus enabling intensive care physicians to promptly initiate treatment [3]. Here, seizures are of utmost importance. In the intensive care setting, there are two main types of seizures: 1. classical convulsive seizures, which may result in generalized convulsive status epilepticus, a life-threatening entity; and 2. subtle, clinically invisible, non-convulsive seizures, with non-convulsive status epilepticus as a maximal form.

The annual incidence of generalized convulsive status epilepticus ranges between 3.6 and 6.6 per 100,000 and of non-convulsive status epilepticus between 2.6 and 7.8 per 100,000 [4]. Mortality and morbidity of both types of seizures are influenced by the underlying etiology; this will be discussed in more detail later.

However, there is a general consent that seizures and status epilepticus, both convulsive and non-convulsive, are potentially life-threatening complications in patients with acute brain injuries. Today, there is little doubt that unrecognized and untreated non-convulsive seizures and non-convulsive status epilepticus exacerbate neuronal injury [5]. For example, DeGiorgio et al [6] have shown that neuron-specific enolase is elevated in patients with convulsive or non-convulsive seizures. Importantly, enzyme levels were highest in those with non-convulsive seizures. This observation clearly suggests that seizure activity without clinical convulsions can produce neuronal injury [6].

Non-convulsive seizures and non-convulsive status epilepticus in patients with reduced consciousness

Although non-convulsive seizures and non-convulsive status epilepticus were first described more than 100 years ago, this diagnosis is often missed in ICU patients. Continuous EEG monitoring is the only reliable means to rule out non-convulsive seizures or non-convulsive status epilepticus in stuporous or comatose patients, and without continuous EEG monitoring early diagnosis and appropriate treatment are often delayed. Thus, it has become clear that the majority of seizures in ICU patients are clinically not visible and will be missed without continuous EEG recording [7].

In a study of 124 critically ill neurological patients, 35% presented with non-convulsive seizures and 76% of those were even in non-convulsive status epilepticus [8]. Another prospective study of 198 patients with altered levels of consciousness showed that 37% of patients had non-convulsive seizures [9]. Finally, in a study of 236 comatose patients who were admitted to a medical ICU, excluding all patients with any clinical suspicion of seizure, continuous EEG still detected non-convulsive status epilepticus in 8% [10].

A retrospective study of 570 patients monitored by continuous EEG identified 110 patients with seizures (19%), and 101 of the 110 (92%) had exclusively non-convulsive seizures. In the same study, the authors found that in 95% of non-comatose patients, the first non-convulsive seizure was detected within the first 24 hours of continuous EEG monitoring; however, in comatose patients, the first seizure was detected after 24 hours in 20%, and after 48 hours of continuous EEG monitoring in 13%. The authors concluded that a period of 24 hours was usually sufficient to detect non-convulsive seizures in patients who were not comatose and one of 48 hours or more in comatose patients. Without EEG monitoring, the diagnosis of non-convulsive seizures would have been missed [11].

Early diagnosis is crucial

As discussed above, it is well known that early recognition of status epilepticus is crucial for effective treatment and results in better outcomes. A study conduced by Lowenstein and Alldredge showed that the efficacy of first-line therapy in status epilepticus decreased from 80% when therapy was initiated within 30 minutes after onset to 40% when treatment started after 2 hours [12].

Focusing on non-convulsive status epilepticus, Young et al. showed that, in patients with non-convulsive status epilepticus lasting less than 10 hours, 60% returned home and 10% died. However, if the non-convulsive status epilepticus lasted longer than 20 hours, none of the patients returned home and 85% died [13]. The same study found that the mortality of patients with non-convulsive status epilepticus depended on the time needed for a proper diagnosis to be made: only 36%

of patients in whom status epilepticus was diagnosed within 30 minutes died compared to 75% of those in whom diagnosis was delayed for more than 24 hours [13].

Detection of non-convulsive seizures/non-convulsive status epilepticus after status epilepticus

Continuous EEG monitoring is crucial for detecting non-convulsive seizures and non-convulsive status epilepticus in patients with status epilepticus who do not quickly regain consciousness following cessation of convulsions [14]. A study including 164 patients with generalized convulsive status epilepticus found that 48% of patients had non-convulsive seizures and 14% were in non-convulsive status epilepticus after clinical convulsions stopped. In this study, the mortality was significantly higher in those with non-convulsive seizures or non-convulsive status epilepticus than in those without [15]. Another study, comprising 33 patients with refractory status epilepticus who were treated with intravenous midazolam, showed that 18% had seizures within the first 6 hours after treatment, 56% had seizures more than 6 hours after initiation of therapy, and 68% had seizures after treatment was completed. Of these seizures, 89% were clinically subtle or purely non-convulsive seizures [16].

Detection of seizures after acute brain damage

Although various studies have been conducted, the exact incidence of non-convulsive seizures and non-convulsive status epilepticus following specific brain injuries such as traumatic brain injury (TBI), hemorrhagic or ischemic stroke, and subarachnoid hemorrhage (SAH) still remains unclear. There are two main reasons for this: first, until recently, none of the studies employed continuous EEG monitoring in these specific entities; second, electrographic manifestations of non-convulsive seizures or non-convulsive status epilepticus in critically ill patients are quite different from those in patients with epilepsy (see EEG patterns in the ICU).

The clinical incidence of early posttraumatic seizures ranges between 2 and 12% [17]. One prospective study, including 94 moderately-severe TBI patients monitored by continuous EEG, detected seizures in 22%, 52% of which were non-convulsive seizures and one-third non-convulsive status epilepticus [18].

In patients with intracerebral hemorrhage (ICH), the clinical incidence of early seizures has been reported to be between 4.9% and 17% [19]. In a recent, prospective ICU study monitoring 63 patients with ICH by continuous EEG, seizures were detected in as many as 28% of the patients. In 18 patients presenting with seizures, these were not clinically visible in 16, and were non-convulsive status epilepticus in 8 [20]. In this study, seizures were observed in 34% of patients with lobar hemorrhage and, surprisingly, in 21% with deep subcortical hemorrhage. The authors observed that non-convulsive seizures were associated with an increased amount of midline shift and the outcome tended to be worse [20]. However, in another prospective study of 9 patients with ICH who were admitted to a stroke unit and monitored by continuous EEG, only one developed electrical epileptic activity [21].

The reported incidence of early clinical seizures in patients with acute ischemic stroke varies between 4.4% and 13.8% [19]. One prospective study using continuous EEG monitoring in 46 patients with ischemic stroke detected non-convulsive seizures or convulsive seizures in 6% [20]. In another prospective study, 91 consecutive patients with ischemic stroke admitted to a stroke unit were monitored with continuous EEG, 16 of whom (17%) developed electrical epileptic activity [21].

Early clinical seizures after SAH have been reported in 1.1 to 16% of patients [19]. A study including 26 patients with SAH who were stuporous or comatose and were monitored by continuous EEG showed that eight of them (30%) had non-convulsive status epilepticus. All eight patients were receiving prophylactic anticonvulsive therapy [22].

Monitoring treatment

Once ongoing seizures are detected, continuous EEG is essential to monitor the effects of therapeutic interventions, particularly when the therapeutic goal is to titrate medications to a burst-suppression pattern, a frequent endpoint in the management of refractory, generalized status epilepticus. Recently, recommendations of the European Federation of Neurological Societies on the management of status epilepticus were published [4]. These guidelines recommend titrating the treatment towards a burst suppression pattern for at least 24 hours in patients with refractory status epilepticus [4].

Continuous EEG monitoring of non-convulsive seizures and non-convulsive status epilepticus and outcome

Young and al. found in a multivariate analysis that the two variables associated with mortality in 49 patients with non-convulsive status epilepticus were seizure duration and delay to diagnosis [13]. Another retrospective study involving 105 patients who underwent continuous EEG monitoring in an ICU found that continuous EEG was essential for the diagnosis and treatment of non-convulsive seizures and refractory status epilepticus. However, only a minority of patients had a favorable neurological outcome [23]. We already know that the prognosis of non-convulsive status epilepticus in the ICU setting is poor: in different patient series the overall mortality was 30% to 50% [13, 22], and refractory non-convulsive status epilepticus after severe TBI and SAH has been associated with 100% mortality [4, 22]. Therefore, the challenge of demonstrating that the aggressive, early termination of non-convulsive status epilepticus in these patients can result in better outcomes remains.

Moreover, further studies are needed to determine which EEG patterns typically summarized as non-convulsive seizures in critically ill patients indicate impending brain damage and whether changing the therapeutic strategy when such EEG patterns are recorded will improve clinical outcome.

Detection of Ischemia

Continuous EEG monitoring has been widely used in the last 30 years to detect acute cerebral ischemia during carotid artery surgery or intracranial endovascular treatment. However, continuous EEG was only recently introduced to monitor the course of spontaneous acute ischemic stroke [24].

EEG is a highly sensitive tool to detect brain ischemia. Cortical layers 3 and 5, which are particularly sensitive to oxygen deficits, contribute most to the generation of electrical dipoles detected by EEG. Cerebral ischemia results in EEG changes as has been demonstrated by positron emission tomography (PET) and Xenon-computed tomography (CT) study of cerebral blood flow (CBF) [25, 26].

EEG patterns usually begin to change when reversible neuronal dysfunction occurs. At this time CBF decreases to 25 to 30 ml/100 g/minute, a level at which therapeutic interventions could be instituted to prevent permanent brain damage [27]. Cell death occurs when CBF drops to 10 ml/100 g/minute. EEG signs of ischemia

include loss of beta activity, followed by activity in theta and delta ranges and finally flattening of the EEG with burst suppression or continuous suppression [18]. These EEG signs can be detected far sooner and are more sensitive than clinical examinations, which are often of limited use in ICU patients [28].

EEG monitoring also helps to demonstrate the recovery of brain function following reperfusion. A study comparing EEG and CBF with Xenon CT CBF measurements showed a hemispheric slowing of EEG activity that correlated with moderate-to-severe reduction of CBF. Upon application of hypervolemic therapy, EEG patterns improved and ischemia was resolved [29]. Another study in ischemic stroke patients showed that a pattern of regional attenuation without delta activity predicted massive infarction with malignant edema [28].

Continuous EEG may also be used to detect reversible ischemia in SAH. A study in 32 patients with low-grade SAH using quantitative, continuous EEG showed that the loss of relative alpha variability preceded angiographic vasospasm or changes in transcranial Doppler findings by at least 2 days; sensitivity was 100% [30]. In another study, 42 grade IV-V SAH patients were monitored by quantitative, continuous EEG for 7 days. It was shown that a reduction of more than 50% of the alpha/delta ratio from the baseline could predict cerebral ischemia with an 89% sensitivity and 84% specificity [31].

Despite promising data, the utility of EEG parameters to detect ischemia in the ICU setting has yet to be demonstrated. Further research studies of ischemic stroke should combine continuous EEG and advanced imaging techniques such as PET and perfusion CT and magnetic resonance imaging (MRI). Improvements in real-time ischemia detection systems and software with automatic alarms are needed to establish wider application of continuous EEG monitoring in ischemic stroke [32].

Other Indications

Prognosis

Continuous EEG monitoring provides prognostic information. For example, Nei et al. showed that, in patients in status epilepticus, the occurrence of periodic epileptic discharges was associated with a poor outcome [33]. In another continuous EEG study including 89 patients after moderate-to-severe TBI, a Glasgow Coma Scale score lower than 9 and a persistently impaired variability of relative alpha predicted poor outcome or death (positive predictive value of 86%) [34].

Continuous EEG influences therapeutic management

Few studies have been carried out on the effect of continuous EEG monitoring in the ICU on therapeutic management. In a study of 124 critically ill neurological patients, continuous EEG monitoring had an impact on clinical decisions in 51% of cases and made a significant contribution in 31% [8]. In another study including 15 neuro-critical care patients, continuous EEG influenced therapeutic management on almost 50% of monitoring days [35]. Further research is required to determine whether continuous EEG monitoring results in better outcomes.

EEG Patterns in the ICU

Generalized Status Epilepticus

Treiman identified five EEG patterns that occur in a predictable sequence during the course of secondarily generalized status epilepticus (Table 1) [36]. However, other investigators have not found this sequential EEG pattern [33, 37]. It is well known that in later stages of generalized status epilepticus, electroclinical dissociation, termed 'subtle' status epilepticus, may develop [36]. During this period, the patient may demonstrate slight twitching of the limbs, facial muscles or jerking eye movements. This last sign is correlated with phase 4 and 5 of the EEG of Treiman.

Table 1. Sequence of EEG patterns in secondary generalized status epilepticus. Modified from [36]

1. EEG changes of discrete seizures with interictal slowing
2. Merging seizures with waxing and waning ictal discharges.
3. Continuous ictal discharges.
4. Continuous ictal discharges with 'flat' periods.
5. Periodic epileptiform discharges on a 'flat' background

Non-convulsive Seizures and Non-convulsive Status Epilepticus

Identifying EEG seizures in patients with severe brain lesions is challenging even for experienced EEG experts. The classic ictal patterns of seizure may not be evident in patients with diffuse brain injury and profound EEG background suppression [38]. Moreover, no widely accepted criteria to diagnose non-convulsive seizures or non-convulsive status epilepticus have been defined yet. For example, it is controversial whether certain EEG patterns, such as periodic lateralized epileptiform discharges (PLEDs), bilateral independent PLEDs (BIPLEDs), periodic epileptiform discharges (PEDs), focal or generalized, and generalized triphasic waves (TWs), are ictal or interictal [37, 39–41]. Moreover, the dichotomy of EEG patterns into ictal or nonictal clearly represents an oversimplification [7].

Furthermore, EEG patterns seen in metabolic encephalopathies cannot always be distinguished from non-convulsive status epilepticus and EEG patterns can be abolished by benzodiazepines [42]. Until last year, no generally accepted nomenclature existed to describe the EEG patterns encountered in ICU patients monitored by continuous EEG, and there is no consensus regarding which patterns are associated with ongoing neuronal injury and require intervention [43].

As a first step towards standardizing terminology, a group of experts has proposed a new classification for rhythmic and periodic EEG patterns encountered in critically ill patients [43]. This approach will facilitate multicenter research projects that, for instance, should aim at determining which EEG patterns are associated with ongoing neuronal damage.

Stimulus-induced Rhythmic, Periodic, or Ictal Discharges

Recently, a new EEG phenomenon in critically ill patients was recognized, 'stimulus-induced rhythmic, periodic, or ictal discharges' (SIRPIDs) [44]. SIRPIDs were observed in 33 of 150 (22%) patients monitored by continuous EEG. All of these periodic, rhythmic or ictal-appearing discharges were consistently induced by alerting stimuli such as examination, chest percussion, or loud noise. Furthermore, 18 of

these patients fulfilled the criteria for ictal discharges with rhythmic patterns and temporal evolution. There was no significant difference in the incidence of clinical seizures in patients with or without SIRPIDs; however, clinical status epilepticus was more common in patients with focal or ictal-appearing SIRPIDs than in those without. Recording video or documenting patient stimulation was necessary to distinguish SIRPIDs from spontaneous seizures. Indeed, further research is necessary to determine the pathophysiologic, prognostic, and therapeutic significance of SIRPIDs [44].

Staffing and Technical Requirements for Implementing Continuous EEG Monitoring in the ICU

Staffing Requirements

A well-trained continuous EEG monitoring team is fundamental for the successful implementation of any continuous EEG monitoring program on the ICU. EEG experts should be available 24 hours per day to guarantee proper interpretation of the generated data. Furthermore, specialized training of the ICU nursing and physician staff is indispensable, as are frequent visits by the EEG technician.

Data Analysis

The ongoing analysis of continuous EEG data is a major task due to the sheer volume of information generated and the need for near real-time interpretation of EEG patterns. Visual unprocessed EEG analysis performed by experienced readers at relatively low review rates represents the classic and standard method of assessing EEGs and is an excellent means of recognizing seizures and detecting changes in a standard EEG. However, standard visual analysis of a continuously recorded EEG in the ICU setting is impractical: it is far too time-consuming since an experienced electroencephalographer would have to be constantly available for continuous real-time interpretation of EEG patterns.

Nowadays, technological advances in digital EEG data acquisition, storage, computer processing, transmission, and display of large amounts of data have made continuous EEG monitoring in the ICU technically feasible [45, 46].

Usually, the classic 16-channel EEG plus an electrocardiogram channel are recorded. Electrodes are placed using the international 10–20 system, sometimes with modifications. More electrodes provide greater spatial coverage but are technically more difficult to maintain and may provide redundant data. In digital EEG, data from a wide variety of placements can be recorded and retrospective montaging is also possible.

Continuous EEG data collected over long periods of time can be transformed into a more 'user-friendly' summary format that can be interpreted by non-electroencephalographers in real-time. The computer analysis technique transforms the continuous EEG data into power spectra by fast Fourier transformation (FFT), creating quantitative EEG (qEEG) parameters [47]. To date, most qEEG tools use amplitude-integrated EEG (AEEG) or frequency-domain methods to simplify the EEG. The qEEG can be displayed graphically as compressed spectral arrays (CSAs), a kind of picture that is easy to interpret, and that could be useful as a means for non-expert caregivers to screen cerebral function at the bedside [45]. CSA has not been formally tested in the ICU setting [32]. Furthermore, it is not known which of the qEEG tools are best for recognizing seizures or ischemia. Comparison of currently available and

developing methodologies will facilitate the creation of more sensitive and specific quantitative assessment tools.

Numerous specialized EEG signal processing software packages are available for screening large continuous EEG datasets to detect possible electrographic seizure activity for ambulatory patients with epilepsy; however, as already discussed, EEG patterns of seizure activity in critically ill patients are different from those in ambulatory patients.

Surprisingly, little systematic research has been conducted to evaluate the sensitivity and specificity of the currently available qEEG tools to detect seizures and ischemia in the ICU. Good clinical practice requires that the underlying EEG signal must be available for review by an experienced electroencephalographer to confirm the significance of any changes suggested by methods that have simplified the EEG data stream. One group recommends reviewing the electrographic data at least twice a day and even more frequently if clinically warranted [32].

The ongoing development of new methods to display and analyze continuous EEG data will result in the widespread adoption of continuous EEG monitoring technology in the ICU. For an overview of the currently available techniques to display and analyze continuous EEG data see the excellent reviews published by Scheuer and Wilson [45] and Kull and Emerson [46].

Recording Electrodes

One of the main limiting factors of continuous EEG monitoring in the ICU setting is the recording electrode [48]. From the vast array of electrodes used, the only electrode that, once placed, never requires further adjustment for weeks is the chronic silver-silver/chloride (Ag-Ag/Cl) sphenoidal (Sp) electrode. This electrode has been recently modified to permit subdermal placement. Importantly, this new electrode is also MRI and CT compatible [48].

Concomitant Video Monitoring

The use of digital video/continuous EEG has been shown to be particularly helpful in identifying subtle ictal phenomena (facial twitching or rhythmic eye movements) and artifacts that mimic seizures. One study found that all artifacts were easily and quickly recognized on video recordings but were quite difficult to identify without this technique [49]. Valid interpretation of continuous EEG and artifact recognition in the absence of video monitoring is only possible if the ICU staff accurately notes any external manipulation of the patient.

Artifacts

EEG artifacts are recorded electrical phenomena that do not arise from the brain. An EEG recorded in the ICU setting is often contaminated by artifacts arising from monitoring equipment, life support systems, and personnel. Traditionally, artifacts have been divided into exogenous and biologic/physiologic artifacts:

There are a large number of sources of exogenous artifacts in the ICU, some of them mimicking seizures or other important EEG patterns, including electronic devices that generate alternating current fields and structures that generate static charges such as dripping intravenous fluids and water commonly found moving in ventilator tubing. Dislodged EEG electrodes and electrodes that have dried out, with

serious mismatches in impedance between electrodes, are also responsible for artifacts, as are vibrating beds, pumps, monitors, ventilators, and pacemakers. Even the movement of the patient by doctors or nurses may be a source of electric artifacts. Completely disconnected electrodes occasionally may record activity that looks similar to that of a comatose patient with moderate to severe diffuse slowing and attenuation. Therefore, electrodes should be secured with collodion and checked at least twice per day and after any manipulation or transport of the patient [50]. Drug-induced artifacts on the EEG are also well-recognized, the most frequent being beta activity due to benzodiazepines. Electromagnetic fields from cellular phones, radios or beepers produce artifacts in general digital monitoring of patients, and future investigations must study such effects on continuous EEG monitoring.

Biologic/physiologic artifacts include EEG changes induced mainly by the patient's heart beats or respiration. Finally, it must be considered that sedating medications, which severely alter brain activity and hence EEG patterns, are routinely used in the ICU.

The Future: Multimodal Neuromonitoring in the ICU

The monitoring standards of patients with catastrophic intracranial diseases are changing and an active search for secondary insults such as ischemia, hypoxia, or seizures is an important target in order to improve the outcome of these critically ill patients.

Today, in addition to the traditional monitoring of intracranial pressure (ICP) and cerebral perfusion pressure (CPP), it is possible to measure global oxygen delivery via jugular bulb oximetry or to continuously record focal brain tissue oxygen tension and brain temperature using intracranial probes. Intracerebral microdialysis provides further information about glucose metabolism in the brain. In most centers CT and MRI scans of the brain are available 24 hours a day. Finally, we can continuously monitor regional CBF with an intracranial probe using laser Doppler or thermal diffusion techniques.

Software with which these data can be fully integrated is being developed. The integrative use of all or a specific selection of some of these techniques, in combination with general monitoring of a patient, has been termed multimodal neuromonitoring. However, only continuous EEG can directly show seizures. And, as discussed above, every patient with a catastrophic intracranial disease is at risk of developing seizures and should be monitored by continuous EEG.

The issue of monitoring techniques that may be of special use in which intracranial disease, and more importantly, in which specific patient, must be addressed in future multicenter trials.

Conclusion

Continuous EEG recording is strongly recommended for patients with acute intracranial diseases who are at risk of developing seizures. As a result of recent technological improvements, continuous EEG and video monitoring for over 48 hours or longer is now feasible.

Although previously thought to be uncommon, non-convulsive seizures and non-convulsive status epilepticus are being identified more frequently. In fact, anyone

who works with critically ill neurologic patients and does not see this entity on a regular basis is missing the diagnosis [7]. Prompt diagnosis and treatment of non-convulsive status epilepticus is crucial, and delayed treatment has been associated with poor outcome.

We currently face four major challenges in further establishing continuous EEG monitoring in the ICU setting:

1. Standardized terminology must be used.
2. Continuous EEG monitoring must be transformed into a more user-friendly but also highly sensitive and specific method.
3. The incidence of non-convulsive seizures and non-convulsive status epilepticus following catastrophic intracranial disease must be determined.
4. Most importantly, we must determine whether continuous EEG monitoring leads to therapeutic decisions that significantly influence the outcome of our patients.

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The Acute and Chronic Management of Large Cerebral Infarcts

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Introduction

Stroke remains a major source of morbidity and mortality throughout the world representing the third leading cause of death in North America and the second leading cause of death in Asia [1]. Large hemispheric strokes account for a majority of these deaths and represent a significant proportion of stroke patients treated in an intensive care unit (ICU). Our understanding of the secondary processes that occur after the initial stroke has changed our approach to the management of this population. We will review and discuss the new management strategies that have been developed to decrease the morbidity and mortality of patients with large hemispheric infarctions.

Definitions, Acute Presentation, and Clinical Course

Strokes that involve greater than 50% of a cerebral hemisphere are generally considered large cerebral infarcts. These occur secondary to acute occlusions of the internal carotid (ICA) or middle cerebral artery (MCA). The most common pathological processes include acute carotid artery dissections, *in situ* thrombosis of a near occluded carotid artery, and cardiac or artery-to-artery emboli to the stem of the MCA. The extent and size of the resultant cerebral infarct depends upon a number of factors including the exact location of the occlusion, the extent of collateral circulation, core body temperature, blood pressure, and if and when recanalization occurs [2].

Patients with large hemispheric cerebral infarcts present with acute contralateral hemiparesis or hemiplegia, hemianesthesia, and hemianopsia. Involvement of the frontal eye fields leads to the development of a gaze deviation ipsilateral to the side of the stroke and contralateral to the hemiparesis. Patients with dominant hemispheric stroke will present with a global aphasia. Non-dominant hemispheric stroke will present with spatial neglect. Apraxia of eye opening leading to an inability to open the eyes is common and often misleads the examiner into believing the patient is unresponsive. A Horner's syndrome suggests dissection or acute occlusion of the ipsilateral internal carotid artery.

Clinically, patients may be awake and agitated or alternatively may appear drowsy or stuporus. Hypertension is common and is worsened by agitation, and confusion. Seizures can occur but are unusual.

Most patients will stabilize both neurologically and hemodynamically a few hours post ictus. Secondary neurological deterioration occurs due to the development of

cerebral edema. The pathological time course of the development of cerebral edema was quantified by Ng and Nimmannitya [3] Typically maximal swelling peaks 3–5 days post infarct; however, it can occur up to 7 days post infarct. Neurological deterioration parallels the development and worsening of cerebral edema and is heralded by decreasing levels of consciousness, pupillary abnormalities, flexor or extensor posturing, and Cheynes-Stokes or ataxic respirations. Neurological deterioration usually mandates endotracheal intubation, mechanical ventilation, and treatments designed to decrease cerebral edema and lower intracranial hypertension.

Despite maximal medical treatment, many patients will worsen and progress to brain death. The mortality rates for large hemispheric strokes are approximated to be between 40–60% in many clinical studies. European studies have listed the mortality as high as 80% after the development of 'malignant' cerebral edema. Survivors are severely disabled and usually unable to reintegrate into normal activities of daily life [4, 5].

Mechanisms of Neurological Deterioration

Early clinical studies attributed neurological deterioration to downward displacement of the brain caused by increased intracranial hypertension. A clinical syndrome of central herniation was proposed and codified by Plum [6]. Thus, the main management strategy was to use agents to reduce increased intracranial pressure (ICP) [6].

Ropper, however challenged this dogma [7]. In a series of patients with supratentorial mass lesions Ropper was unable to correlate between downward displacement of brain structures and the patients' level of consciousness. Level of consciousness, however, correlated strongly with horizontal displacement of the pineal imaged on sequential computed tomography (CT) scanning. Ropper thus demonstrated that expanding mass lesions inducing horizontal and not vertical displacement of the rostral diencephalon (using the pineal as a surrogate marker) were responsible for neurological deterioration [7]. Ropper postulated that ICP differentials and not total increases in ICP were responsible for this. Frank later verified this hypothesis by placing ICP monitors in 19 patients with large cerebral infarctions and subsequent neurological deterioration. A large majority of patients showed neurological deterioration without an increase in ICP. Patients with increases in ICP tended to be younger [8].

The above finding had significant implications for the treatment of patients with expanding hemispheric infarctions. In some patients intracranial hypertension may be problematic. However, lowering ICP may not be effective if global increases in ICP are not the source of neurological deterioration. In addition there was growing concern that traditional measures to decrease ICP (i.e., placement of a ventriculostomy, mannitol, hyperventilation) may actually worsen tissue shifts and cause neurological deterioration by shrinking of normal tissue disproportionately to damaged tissue. Kaufmann and Cardoso had similarly shown in a cat stroke model that repeated dosing of mannitol accumulated in damaged brain tissue and, thus, could theoretically worsen midline shift [9].

Manno and Videen directly addressed these issues in a series of studies [10, 11]. Eight patients with midline shift and neurological deterioration after a large hemispheric stroke were given a 2 g/kg dose of mannitol. Sequential magnetic resonance imaging (MRI) was obtained during and for up to 30 minutes after the infusion. A

volumetric analysis revealed that the non-infarcted tissue decreased in size by 8% compared to no change in the infarcted tissue. However, this was not a large enough change to affect either vertical or horizontal shifts of midline structures [10, 11].

Initial Management of Large Hemispheric Cerebral Infarcts

The acute management of large ischemic strokes includes the basic assessments of assessing the airway breathing and circulation. Most patients with large strokes will not require endotracheal intubation unless they have aspirated. However, patients with a depressed level of consciousness may lose pharyngeal tone and develop airway obstruction. Under these circumstances a nasal or oral airway can be used to maintain airway patency. Adequate intravenous fluid replacement to maintain euvolemia is necessary to avoid periods of hypotension. Normal saline solutions are preferred to avoid decreases in serum sodium levels, and dextrose solutions are generally not used to prevent hyperglycemia. Emergent head CT scanning is required [2].

In North America, intravenous tissue plasminogen activator (t-Pa) can be used within 3 hours of stroke onset if patients meet a set of radiologic, hemodynamic, and laboratory criteria [12]. Patients outside of the 3-hour window may be eligible for intra-arterial t-Pa or mechanical clot retrieval through endovascular methods [13].

Hypertension is common after large hemispheric strokes and may represent a normal physiological response to maintain adequate cerebral perfusion or could be secondary to an acute stress response, agitation, pain, or noncompliance with antihypertensive medication. Control of hypertension in an acute stroke should be judicious and used only if there are concerns of cardiopulmonary deterioration. Overzealous control or attempts to normalize blood pressure can theoretically extend the infarct by decreasing cerebral perfusion [14].

Anticoagulation is controversial. Larger strokes are at risk for hemorrhagic conversion and enthusiasm for the use of heparin in acute strokes has waned in recent years. Indications for anticoagulation include atrial fibrillation, suspected myocardial infarction, or a visualized thrombus or large akinetic segment of the myocardium detected on echocardiography. Re-embolization has been reported to be as high as 21% within the first 3 weeks under these circumstances. Goal anticoagulation should be between 1.5–2.0 times above baseline control activated partial thromboplastin time (aPTT) [2].

Management of Neurological Deterioration

Neurological deterioration after large strokes may be multifactorial but most commonly accompanies the development of cerebral edema and its resultant tissue shifts. Neurological deterioration usually occurs within 96 hours of ictus but there is wide variation ranging from a few hours to a week post ictus [13]. A unilateral headache or vomiting may precede drowsiness. Cheynes-Stokes respiration progresses to hyperventilation and irregular respiratory patterns. Endotracheal intubation is usually required if a patient progresses to stupor. Unilateral anisocoria and bilateral ptosis are evidence of parahippocampal gyrus compression of the ipsilateral third nerve and warrant immediate intervention [2]. Mechanical ventilation for ischemic stroke is in itself a poor prognostic sign. Prospective series have reported 66–76% mortality. Mortality, however, can be improved with acute interventions [15–17].

ICP monitoring can be used but, as noted, brain tissue shifts may occur due to variations in pressure gradients that do not manifest as elevated ICP [7]. Hyperventilation is commonly employed to decrease ICP. Arteriolar vasoconstriction in response to a decrease in cerebrospinal hydrogen ion concentration subsequently decreases cerebral blood flow (CBF) and volume. Hyperventilation can be used acutely to lower ICP and should be utilized if neurological deterioration develops rapidly. The effect of hyperventilation on ICP, however, is short lived and ICP will return to baseline in a few hours [18]. Hyperventilation, therefore, does not represent a long-term treatment.

The mainstay of treatment for neurological deterioration is osmotic therapy. Mannitol is the most commonly used agent. In Europe, intravenous or enteric glycerol are also used to lower ICP [19]. There are several potential mechanisms which may explain how mannitol can lower ICP. As an osmotic diuretic, mannitol can create an osmotic gradient favoring movement of water into the intravascular space, which can subsequently be excreted. Paczynski et al. [20] were able to demonstrate dehydration of an ischemic cerebral hemisphere in a rat stroke model using progressively increasing doses of mannitol. This process, however, took several hours to develop [20]. More commonly, the rheological effect of mannitol is advocated to account for the effect on ICP. According to this theory, mannitol lowers ICP by decreasing cerebral venous engorgement. A bolus of mannitol will lead to an influx of fluid into the intravascular space. The subsequent hemodilution leads to a decrease in serum viscosity. If flow remains constant, passive vasoconstriction should occur leading to a decrease in CBF and cerebral blood volume. This effect takes approximately 30 minutes to occur and closely matches the clinical effect of mannitol on ICP [21].

More recently, hypertonic saline has been advocated as a potential substitute for mannitol. Theoretically, by inducing acute changes in serum osmolality, hypertonic saline should operate under the same mechanisms as mannitol. The advantages of hypertonic saline would include fewer electrolyte abnormalities, nephrotoxicity, and volume depletion [22]. Suarez et al. have also used hypertonic saline successfully in head trauma patients as salvage treatment for patients with recalcitrant intracranial hypertension [23]. Implementation in acute stroke, however, has been limited due to a lack of standardization of both timing and dosage.

Osmotic therapy is typically initiated at first evidence of neurological worsening (i.e., worsening level of consciousness, cerebral ptosis, or anisocoria). Mannitol is given in large doses, up to 1 g/kg, which can be repeated in approximately 30 minutes if needed. Scheduled dosing may be given. Neurological and critical care textbooks suggest that serum osmolality should not be increased beyond 320 mOsm/l due to concerns about nephrotoxicity [2]. A recent review, however, suggested that nephrotoxicity with mannitol use is probably overstated [24]. All the proposed mechanisms of action of mannitol require the development of an osmotic gradient for mannitol to be effective. Thus, at higher osmolality mannitol may be less effective. The osmolal gap (i.e., the difference between measured and calculated osmolality) appears more useful then serum concentrations of mannitol [25].

Previously stated concerns about mannitol becoming trapped inside the tissue and worsening cerebral edema may be overstated [8]. Maiorielloet al. [26] described a patient with end stage renal disease and a large stroke who was given radiolabeled mannitol for treatment of cerebral edema before and after dialysis. The labeled mannitol seen inside the infarcted tissue was excreted uniformly after dialysis suggesting that trapping of mannitol inside these tissues does not occur [26].

Hypothermia has been advocated as a potential treatment for large cerebral infarctions. Animal models strongly support that acute and delayed elevations in brain temperature worsen focal and global ischemia after cerebral infarction [27]. Both retrospective and prospective studies have revealed a strong relationship between hyperthermia and poor outcome after ischemic stroke [27]. Schwab et al. were able to demonstrate that brain temperature exceeded core body temperature by approximately 1–2 °C after stroke [28]. Hypothermia has been shown to be effective in improving neurological outcome after cardiac arrest in two large studies [29, 30].

Hypothermia has been studied in large strokes. Moderate hypothermia to 33 °C was induced initially in 25 patients after large cerebral infarcts. The overall mortality was 47 % compared to 80 % of historical controls. Follow up work in 50 patients suggested that hypothermia is feasible but is associated with several medical complications including coagulopathies, pneumonia, and bradycardia. Rebound increases in intracranial hypertension also proved to be problematic [31].

The optimal method for providing temperature control in acute neurological conditions is under study. Mayer et al. demonstrated that 'traditional' methods to cool neurological patients (i.e., acetaminophen, air cooling blankets) were largely ineffective [32]. Newer non-invasive surface cooling devices applied directly to the skin, accompanied by pharmacologic and non-pharmacologic measures to control shivering, have proved superior for controlling fever and inducing hypothermia. Temperature regulated invasive catheters attached to central line catheters have similarly been shown to be effective in temperature modulation [33]. A large company-sponsored safety and feasibility trial studying external cooling devices in ischemic stroke is currently enrolling patients.

Barbiturates have been found to be ineffective in the treatment of large cerebral infarcts. Schwab et al. induced barbiturate coma in 60 patients with large hemispheric strokes and recalcitrant intracranial hypertension. The effect of barbiturate coma on ICP, however, proved to be transient, with 50 patients returning to baseline ICP elevation within 3 hours. The remaining non-responders did not survive [34].

Decompressive hemicraniectomy has recently been used for treatment of large cerebral infarcts. This strategy has largely taken introduced because of our new understanding of the horizontal tissues shifts involved with cerebral herniation syndromes and the failure of medical therapies to significantly impact morbidity and mortality. In this procedure, a large bone flap (almost half of the skull) over the involved hemisphere is removed. An incision is made in the underlying dura and a dural sac is added to allow edematous brain to swell outside the cranial cavity. The bone and dura are usually replaced several weeks later [2] (Fig. 1).

Several successful hemicraniotomy procedures were reported in the 1980s [35]. Decompressive hemicraniectomy began to gain favor as a series of uncontrolled trials reported improvements in morbidity and mortality [35]. Rieke et al. [36] in an open non-randomized trial compared the results of 32 patients with large hemispheric infarctions treated with hemicraniectomy to 21 patients managed medically. Approximately two-thirds of the surgery group had either a good outcome or were moderately disabled compared to only 24 % of the medical group who were moderately disabled. Mortality was 11 % for the surgical group compared to 76 % for the medical group [36].

The Hemicraniectomy and Duratomy upon Deterioration From Infarction Related Swelling Trial (HeADDFIRST) was a 3-year multicenter, randomized, prospective pilot study. Patients with large hemispheric infarcts who displayed evidence of neurological deterioration were randomized to standard medical therapy or stan-

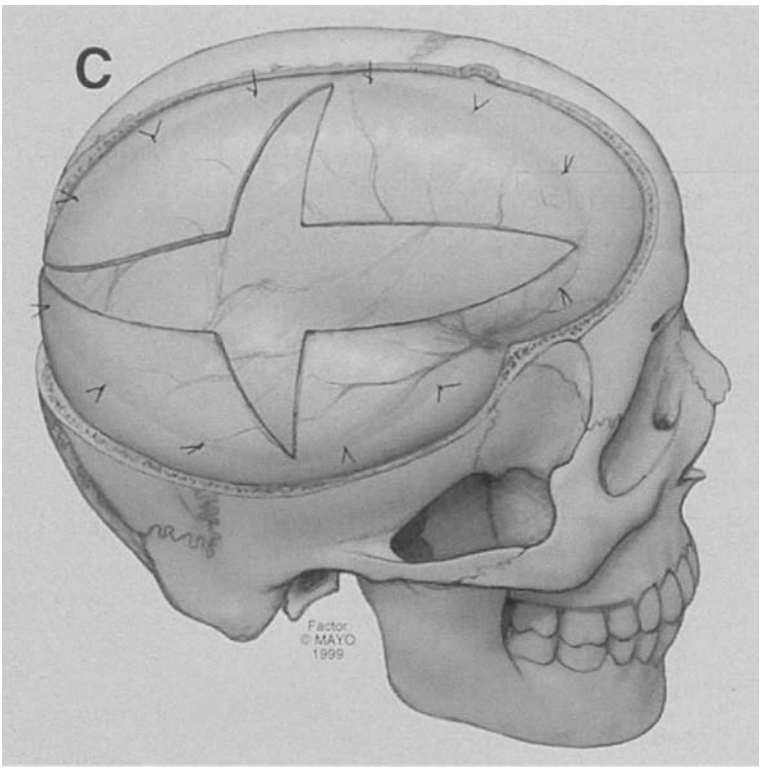

Fig. 1. Schematic representation of a hemicraniectomy with the bone flap removed, incision of the dura, and placement of a dural sac to accommodate for the expanding brain tissue outside of the cranial cavity (with permission of the Mayo foundation for Medical Education and Research)

dard medical therapy plus hemicraniectomy at the first sign of neurological deterioration. Sixty-six patients screened from almost 5000 met enrollment criteria; 41 developed neurological deterioration and were randomized. The results showed a trend ($p < 0.10$) for improvement in the surgical group (Frank JI, unpublished data, presented at the American Academy of Neurology, Honolulu, April 2003). The Hemicraniectomy After MCA Infarction with Life-Threatening Edema Trial (HAMLET) is an ongoing European open multicenter trial comparing standard medical therapy to standard medical therapy plus hemicraniectomy. One year measures of outcome and disability will be evaluated.

Standardization of clinical trials has been difficult due to considerations about the timing, method, and location of surgery. Some advocate the use of early surgery prior to neurological deterioration. The theoretical basis for this was provided by Doerfler et al. [37], who performed a series of hemicraniectomies at various time periods post-infarction in a MCA occlusion rat model. The infarcted brain volume was markedly smaller with early surgery. Infarct size was unchanged compared to control rats if surgery was delayed for 36 hours. Improved surface collateral blood flow and perhaps local hypothermia were postulated to account for the difference [37]. Schwab et al. [38] reported a trend for improved mortality in patients who had decompressive surgery within the first day of ictus compared to delayed surgery.

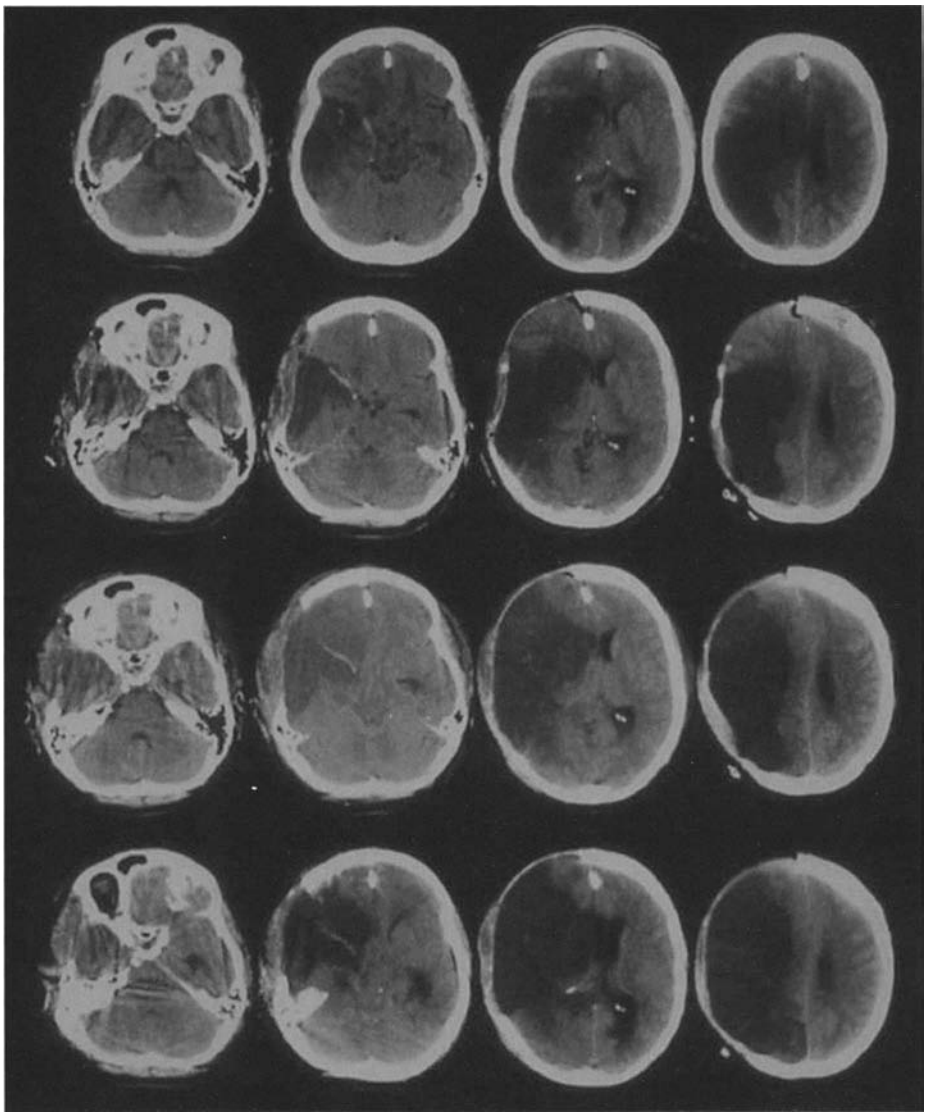

Fig. 2. Computed tomography images of brain herniation outside a craniotomy used for decompression. Note the hyperdense right MCA sign.

Evidence of uncal herniation prior to surgery was postulated to represent a poor prognostic sign for recovery [38]. Most authors, however, recommend delaying surgery. The rationale is that in some cases surgery can be avoided and, in the case of progressive neurological deterioration, medical therapy can at least provide some temporizing measures until surgery can be arranged.

Some surgeons have recommended removal of the uncus or a temporal lobectomy in addition to hemicraniectomy [38]. Kalia and Yonas reported good outcomes in 4 patients who had necrotic cerebral tissue, as identified by Xenon CT, removed [39].

There has also been considerable debate about long-term outcomes after surgery. A systematic review of 138 patients followed for at least 4 months after hemicraniectomy revealed that 7% of patients were independent, 35% moderately disabled, and 58% dead or severely disabled [40]. Older patients (>50 years) fared much worse (80% dead or severely disabled). The timing of surgery, hemisphere infarcted, or signs of herniation did not affect outcome [40].

The growing consensus that hemicraniectomy improves mortality has lead to attempts to identify early markers of neurological deterioration. Radiological markers extracted from the large National Institute of Neurological Disorders and Stroke (NINDS) t-PA trial suggested that a hyperdense MCA sign, signifying clot in the MCA, and greater than 50% involvement of the MCA territory were predictors of neurological deterioration [41]. Manno et al., however, reported that only the hyperdense MCA sign had significant predictive power [42]. Large areas of infarction were only predictive of neurological deterioration if they were found on the initial CT scan [42] (Fig. 2). Newer studies using CT perfusion suggest that infarcted tissue may be able to be identified early in the clinical assessment of patients [43]. A recent paper has identified cellular-fibronectin, a component of the cerebral endothelium, as a potential marker for the amount of cerebral tissue at risk of developing cerebral edema [44]. Elevation of >16.6 μg/ml was a powerful predictive index for subsequent neurological deterioration.

Conclusion

Large hemispheric cerebral infarcts are common strokes admitted to both neuro and general ICUs. The morbidity and mortality associated with these infarcts is significant and is related to the development of cerebral edema. Medical management designed to decrease cerebral edema has been disappointing. Our understanding of the mechanics of the subsequent tissue shifts involved has led to the development of surgical procedures to allow swelling to occur outside of the cranial cavity. A large series of studies has uniformly reported improvements in mortality with these procedures. Morbidity may also be improved in younger patients. Significant questions remain as to the timing and location of surgery and the predictive power of early clinical, radiographic markers of neurological deterioration. The results of large randomized trials are awaited.

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Cooling Therapies after Neuronal Injury: Direct Brain Cooling and Systemic Hypothermia

P.J.D. Andrews, E.L. Anderson, and M. Saxena

Introduction

Acute brain injury is a frequent cause of disability and death worldwide. Common forms of acute brain injury include perinatal birth asphyxia, traumatic brain injury (TBI), stroke, and out-of-hospital cardiac arrest. These conditions affect patients with a wide age range from the young to the elderly. Interruption of cerebral oxygen and nutrient delivery by cardio-respiratory insufficiency or by a vascular lesion may precipitate cerebral ischemia. The initial pathology may not induce immediate cell death, but can precipitate a complex biochemical cascade leading to delayed neuronal loss, the end result being death or disability (Fig. 1). This chapter reviews the current evidence on temperature reduction after neuronal injury.

Preclinical studies have demonstrated that temperature reduction preceding, during, and after experimental neurological injury can beneficially modulate outcomes. A recent systematic review of the efficacy of hypothermia in animal models of acute ischemic stroke has suggested that a 44% reduction (95% CI, 40% to 47%) in infarct volume was achieved with temperature reductions to 31°C. Interestingly, temperature reduction to 35°C also appeared to substantially reduce infarct volume (30%, 95% CI, 21 to 39%), suggesting that smaller temperature reductions may still have clinically worthwhile effects (personal communication, Malcolm McLeod) offering the possibility of a reduction in the burden of systemic complications with this less challenging intervention.

We believe that there are two distinct clinical hypotheses that require *separate* evaluation:

First, can temperature reduction to 32–34°C (mild systemic hypothermia) improve clinical outcomes if applied early, in the first minutes to hours, after neuro-

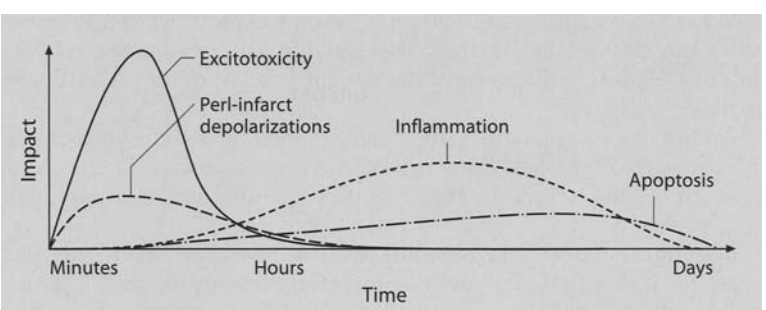

Fig. 1. Temporal window following acute brain disease

nal injury? There is evidence to support this hypothesis in adults following cardiac arrest and in neonates following perinatal hypoxia.

Second, in the immediate days after neuronal injury, can preventing pyrexia reduce neuronal injury and improve clinical outcomes. At present there are no completed interventional trials that explore this latter hypothesis.

Within the discussion of these two hypotheses, we also discuss the concept of direct brain cooling and contrast its potential applications with current techniques of inducing systemic hypothermia. We believe that the second hypothesis in particular warrants further investigation. The interventions that may prevent pyrexia in the first days after neuronal injury may, arguably, have wider applicability than systemic hypothermia, which may be considered a more complex and intensive therapy to administer. Hence, reliable demonstration of even a small absolute benefit by preventing pyrexia would have the potential to avoid thousands of deaths and disabilities worldwide. Similarly, because some of these strategies are already widely used in an ad hoc manner after neuronal injuries, reliable refutation of any benefit would protect thousands of patients from unnecessary intervention with associated side effects, and reduce costs.

Hypothesis 1: Can Temperature Reduction to 32–34 °C (Mild Systemic Hypothermia) Improve Clinical Outcomes if Applied Early in the First Minutes to Hours After Neuronal Injury?

There are many techniques of administering systemic hypothermia and these include air or water-circulating surface cooling blankets, endovascular cooling systems with catheter placement in the inferior vena cava [1], and the administration of ice-cold intravenous fluids [2, 3]. The primary advantage of systemic hypothermia is that target core body temperatures can be achieved rapidly. However, systemic hypothermia has implications in terms of not only safety and potential side-effects, but also resource, including equipment and staff expertise, and hence represents an intervention that is relatively expensive and complex.

The effects of systemic hypothermia on platelet function and coagulation cause concern in the early phase after hemorrhagic brain injuries, such as after TBI, intracerebral hemorrhage, or subarachnoid hemorrhage. Further practical concerns center on the challenge of initiating and maintaining systemic hypothermia while transfers, further investigations, or procedures are carried out. To facilitate achieving systemic temperatures between 32–34 °C, it may be necessary to administer a general anesthetic and this mandates the involvement of intensive care services. This is associated with an increase in the level of resources required and increases the complexity of the intervention further. This has implications for the risk/benefit profile of the intervention and may limit the patient population to which this strategy may be applied.

Additional complications of systemic cooling include immunosuppression and infection, cold-induced diuresis, electrolyte imbalance [4], and shivering. A hyperadrenergic state is present after TBI and hypothermia may paradoxically aggravate this condition.

Systemic hypothermia warrants further investigation, in the early phase after neuronal injury, but the results from trials in cardiac arrest [5–7] and perinatal birth asphyxia [8, 9] may be challenging to replicate in other patient populations such as patients with TBI, stroke, or subarachnoid hemorrhage. In the former condi-

Table 1. Methods of direct brain cooling

- **Non-invasive methods**
  - heat loss from the upper airways – nasal gas flow and lavage
  - heat loss through the skull – external forced convection (fanning, cooling hoods) and conduction (i.e., cooling caps)
- **Invasive methods**
  - antegrade cerebral perfusion
  - intracarotid flush
  - open and semi-closed irrigation
  - contact cooling of specific areas of the brain.

tions, the patient populations reach health care services rapidly and without posing a diagnostic or therapeutic dilemma. Therefore, intervention with mild hypothermia may be implemented within a short time window and early after neuronal injury. It must also be emphasized that the therapeutic benefit of systemic hypothermia may reduce with increasing time from neuronal injury. Hence the management of the latter conditions and the complexity of systemic hypothermia as an intervention may adversely affect the risk-benefit ratio by prolonging the time from neuronal insult to achieving the therapeutic target temperature.

In TBI there have been several systematic reviews in the last few years [10–13]. The major issues for the final analysis in this area include the heterogeneity of inclusion criteria, the time to target temperature, the degree and duration of hypothermia, rewarming methodology, and the management of the control groups. With the exception of McIntyre's review in 2003 [13], these reviews have suggested that there is little evidence for the use of therapeutic hypothermia at present. The study by Liu et al. [14] with both general hypothermia and direct brain cooling, however, suggests that direct brain cooling (Table 1) may offer some advantage.

A recent Cochrane review of cooling therapies after acute stroke (ischemic and intracerebral hemorrhage, but not including subarachnoid hemorrhage) did not identify any completed randomized controlled clinical studies [15]. We have recently carried out a systematic review of cooling therapies after subarachnoid hemorrhage (unpublished data) and this also failed to identify any randomized controlled clinical studies.

In summary, there are no randomized, controlled clinical trials that support the use of mild systemic hypothermia after stroke, subarachnoid hemorrhage or TBI. It may be a significant challenge to replicate the promising data demonstrated with the intervention of systemic hypothermia following cardiac arrest and perinatal birth asphyxia in other patient populations. Further clinical studies are warranted in this area.

Hypothesis 2: In the Immediate Days after Neuronal Injury Can Preventing Pyrexia Reduce Neuronal Injury and Improve Clinical Outcomes

Targeting cooling at the brain parenchyma is logical since brain rather than trunk temperature is important in cerebral protection [16, 17]. In a fetal model of brain asphyxia, direct brain cooling showed a reduction in neuronal loss throughout deep brain structures. The intervention was a cooling cap placed on the cranium of a pig-

let [16] and this achieved a significant temperature reduction in both superficial and deep structures of the brain [18, 19]. Therefore it was concluded that direct brain cooling may prove to be effective for cerebral resuscitation in pediatric practice. Mustafa et al., have designed a neck collar perfused with cooled glycol (-1 to -40°C) to induce cooling and vasodilatation of the carotid artery [20-22]. Calculations based on a theoretical model have shown that neck cooling of arterial blood can achieve 1.1°C reduction in brain temperature [23]. In human thermoregulatory physiology research, there are also some experimental data to support direct brain cooling mechanisms [23-25].

In Edinburgh, two randomized controlled direct brain cooling trials have been conducted in brain injured, orally intubated patients. In the first trial, air was continually flowed through both nostrils at rates equivalent to normal minute ventilation. This was not associated with direct brain cooling assessed using a Camino pressure/temperature device placed in the frontal cortex [26]. However, in a subsequent trial [27] where nasal air flow and head fanning were performed in combination and alone, there was evidence of direct brain cooling in the combined cooling group with a mean brain temperature reduction of 0.41°C within 30 minutes.

Therefore, direct brain cooling may be achieved by conductive cooling of the neck and/or convective cooling using simple fans and other devices that optimize airflow and heat loss from the scalp [28, 29]. These non-invasive techniques require further evaluation in terms of both feasibility and efficacy. They represent techniques that could be used to test the hypothesis that preventing pyrexia in the first few days after neuronal injury may beneficially modulate patient outcome and subsequently have potentially wide applicability [30-32] (in conjunction with supportive care and medication, see below).

In an observational study of patients with acute stroke, increased body temperature was associated with large lesion volumes, high case fatality, and poor functional outcome. Reith and colleagues demonstrated in a prospective observational study that a 10°C increase in body temperature after stroke, increased the odds of a poor outcome by a factor of 2.2 [33]. These data suggested that interventions that induce temperature reductions of this magnitude after stroke warrant further evaluation.

Adjunctive Pharmacology

Drug therapy could include the administration of acetaminophen (paracetamol), non-steroidal anti-inflammatory drugs, and selective cyclooxygenase inhibitors, although concerns about the anti-platelet effect of the latter two classes of drugs may limit their use in brain injury. Dippel et al. have argued that an acetaminophen-induced tympanic temperature decrease of 0.27°C may reduce the relative risk of poor outcome after acute ischemic stroke by 10-20% [34, 35]. The risk of a poor outcome has also been found to rise by a factor of 2.2 for each degree centigrade increase in body temperature (95% CI 1.4 to 3.5) after acute ischemic stroke [33, 36]. Two randomized double blind clinical trials in patients with acute ischemic stroke have recently shown that treatment with a daily dose of 6g acetaminophen resulted in a small but rapid and potentially worthwhile reduction of 0.3°C (95% CI: 0.1-0.5) in body temperature [34, 35]. There is a large multi-center randomized controlled clinical trial underway exploring the question of whether 1g of acetaminophen given every 4 hours over 3 days to patients with acute ischemic

stroke can improve patient outcomes (www.strokecenter.org/trials and www.pais-study.org/).

Drug therapy and the techniques of direct brain cooling described above have independent mechanisms of action and hence may have additive effects, providing a temperature reduction of an order of magnitude that may be clinically important and therefore requires formal evaluation.

Conclusion

Today several different methods of temperature reduction are available for the treatment of brain injured patients. Therapeutic hypothermia in the immediate hours after neuronal injury has been found to be neuroprotective in animal models, as well as in clinical studies after cardiac arrest and neonatal encephalolpathy. Direct brain cooling and drug therapy may be better suited to answering the research question of whether preventing pyrexia in the first days after neuronal injury can improve patient outcomes. Both approaches warrant further investigation.

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Non-traumatic Subarachnoid Hemorrhage

J.I. Suarez

"When the patient appears to all his friends to be in perfect health, and to bid fair for a long life, he is sometimes struck dead instantaneously, without a sign or a groan. ... A very unfavorable prognostic is also drawn from the patient's putting his hand to his head"

John Cheyne (1812) Cases of Apoplexy and Lethargy: With Observations upon the Comatose Diseases. T. Underwood, London, pp 3–14.

Introduction: Demographics of Subarachnoid Hemorrhage

Non-traumatic subarachnoid hemorrhage (SAH) has distinct risk factors, demographics, and treatment from other forms of stroke. Spontaneous SAH, mostly aneurysmal, accounts for about 2–5% of all strokes, afflicting 37,500 cases of stroke per year in the United States [1]. A cerebral aneurysm is an outpouching of the brain arteries that eventually ruptures. The incidence of non-traumatic aneurysmal SAH has remained stable over the past 30 years [2]. Although the incidence of non-traumatic SAH varies from region to region, the aggregate worldwide incidence is about 10.5 per 100,000 person years [3, 4]. Women have a 1.6 times (95% confidence interval [CI] 1.5–2.3) higher risk than men [5] and people of African descent a 2.1 times (95% CI 1.3–3.6) higher risk than whites [6]. The major risk factors for non-traumatic SAH include cigarette smoking, hypertension, cocaine use, and habitual heavy alcohol intake [7]. Other factors, such as a family history of first-degree relatives with the disease and heritable connective-tissue disorders, also play a role [7].

The average case fatality for SAH is 51% with approximately one-third of survivors remaining dependent [7]. SAH is also associated with significant costs most of which are direct because of inpatient days during the first year [8]. Despite advances in diagnosis, treatment, and prevention of complications of SAH, there has been modest improvement in outcome [7]. Thus, there are still many challenges facing clinicians with regards to non-traumatic SAH. The development of treatments to prevent neurological complications with subsequent improvement in outcome and reduction of hospital stay will certainly have a tremendous impact for patients with non-traumatic SAH.

Clinical Presentation and Diagnosis

Patients experiencing a non-traumatic SAH typically present with sudden onset of severe, exploding headache ("the worst headache of my life"), usually accompanied

Table 1. Various clinical gradings of non-traumatic subarachnoid hemorrhage [11–13]

Botterell-Lougheed	
Grade I:	(minimal bleed) alert, no neurological deficit.
Grade II:	(mild bleed) alert, minimal neurological deficit such as third nerve palsy, stiff neck.
Grade III:	(moderate bleed) drowsy or confused, stiff neck, with or without neurological deficit.
Grade IV:	(moderate or severe bleed) semi-coma, with or without neurological deficit.
Grade V:	(severe bleed) coma and decerebrate movements.
Hunt-Hess	
Grade I:	Asymptomatic, or minimal headache and slight nuchal rigidity
Grade II:	Moderate to severe headache, nuchal rigidity, no neurological deficit other than cranial nerve palsy
Grade III:	Drowsiness, confusion, or mild focal deficit
Grade IV:	Stupor, moderate to severe hemiparesis, possibly early decerebrate rigidity and vegetative disturbances
Grade V:	Deep coma, decerebrate rigidity, moribund appearance
The World Federation of Neurological Surgeons	
Grade I:	Glasgow Coma Scale (GCS) score of 15, no motor deficit
Grade II:	GCS score 13–14, no motor deficit
Grade III:	GCS score 13–14, with motor deficit
Grade IV:	GCS score 7–12, with or without motor deficit
Grade V:	GCS score 3– 6, with or without motor deficit

by nausea, vomiting, and meningeal irritation with or without loss of consciousness [4, 9]. Headache may be the only presenting complaint in about 40% of patients and may completely resolve by the time patients seek medical attention. Such headaches have been called "warning leaks" and they are usually followed by the full-blown picture a few days later [10]. Several grading systems have been used to determine predictors of mortality and disability based on the initial clinical presentation (Table 1) [11–13]. The World Federation of Neurological Surgeons grading scale is the most reliable and should be used. Higher scores mean worse level of consciousness and prognosis.

Diagnosis of SAH is confirmed by the presence of hyperdense areas in the subarachnoid space by head computed tomography (CT) and/or blood in the cerebrospinal fluid (CSF) [4]. Head CT scanning should be the first diagnostic study in patients experiencing symptoms suggestive of non-traumatic SAH (Fig. 1). The amount of blood shown by the initial head CT scan is associated with outcome and with the frequency of neurological complications. Several radiologic scales have been proposed to evaluate this issue (Table 2) [14, 15]. The scale proposed by Classen et al is more reliable and reproducible [15]. Higher gradings are associated with worse outcome and a greater number of neurological complications.

In up to 50% of patients presenting with non-traumatic SAH, an incorrect diagnosis may be given [4, 9]. Misdiagnosis is usually associated with worse clinical outcome and increase morbidity and mortality. A suggested indication for the various diagnostic studies is presented in Table 3.

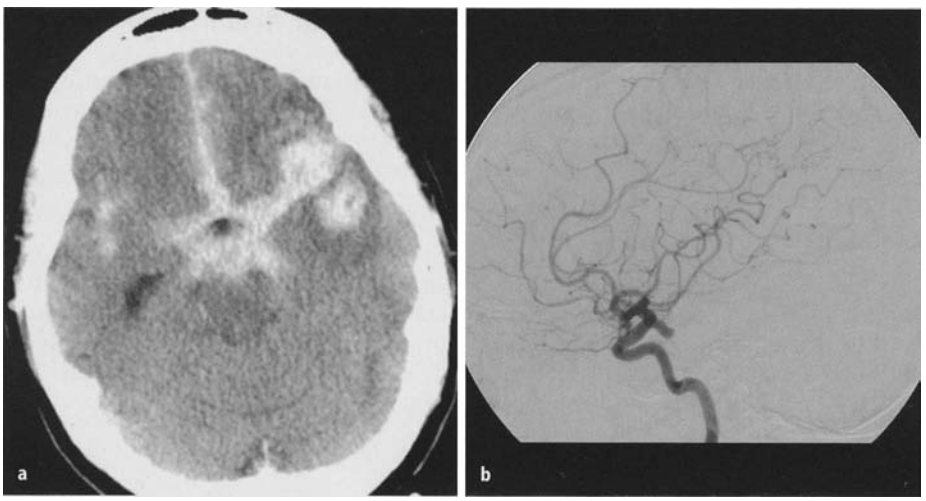

Fig. 1. A 36 year-old woman with a history of heavy smoking, presents with the worst headache of her life followed by blurred vision. Physical examination discloses nuchal rigidity and left third nerve palsy. A head CT scan reveals significant subarachnoid hemorrhage with thick clot in the left Sylvian fissure (Panel **a**). A cerebral angiogram shows a left posterior communicating aneurysm which explains her clinical presentation.

Table 2. Radiologic grading scales for non-traumatic subarachnoid hemorrhage (SAH) [14, 15]

Fisher Scale	
Grade I	No blood detected
Grade II	Diffuse, thin layers of SAH less than 1 mm thick
Grade III	Localized clot and vertical layers of blood more than 1 mm thick
Grade IV	Intracerebral or intraventricular hemorrhage, with little or no SAH
Classen et al.	
Grade 0	No SAH or intraventricular hemorrhage (IVH)
Grade I	Minimal/thin SAH, no IVH in both lateral ventricles
Grade II	Minimal/thin SAH, with IVH in both lateral ventricles
Grade III	Thick SAH*, no IVH in both lateral ventricles
Grade IV	Thick SAH*, with IVH in both lateral ventricles

* Completely filling 1 cistern or fissure. The 10 cisterns or fissures evaluated include: the frontal interhemispheric fissure, the quadrigeminal cistern, both suprasellar cisterns, both ambient cisterns, both basal sylvian fissures, and both lateral sylvian fissures

Table 3. Diagnostic investigations for subarachnoid hemorrhage (SAH)

1. Head CT scan without contrast material: **ALL** patients with suspected non-traumatic SAH
2. Lumbar puncture: **ALL** patients with suspected non-traumatic SAH **AND** negative head CT scan
3. Cerebral angiography: **ALL** patients with positive head CT scan **OR** ALL patients with negative head CT scan and positive lumbar puncture (xanthochromia or elevated red blood cell count unchanged from tube 1 to tube 4)
4. Head CT angiogram: can be performed instead of cerebral angiography in those places where it is available
5. Repeat cerebral angiography 1–3 weeks after initial one: **ALL** patients with positive head CT scan or lumbar puncture **AND** negative initial cerebral angiography
6. MRI of the brain/brainstem and spinal cord: **ALL** patients with two negative cerebral angiographies

Neurological Complications after Subarachnoid Hemorrhage

The most common neurological complications associated with non-traumatic SAH are rebleeding, hydrocephalus, and delayed cerebral ischemia [4]. Delayed cerebral ischemia is the leading cause of morbidity and mortality in patients with SAH. Because of its importance we will discuss this topic in more detail below.

Rebleeding

The risk of rebleeding, presumably from aneurysm re-rupture, can be 35–40% within the first month after non-traumatic SAH [16]. Rebleeding carries a high morbidity and mortality [17]. The risk of rebleeding can be prevented by giving patients antifibrinolytic therapy [18] or by performing early aneurysm surgery [19–22]. The current trend is toward performing early aneurysm surgery.

Hydrocephalus

Hydrocephalus, caused by ventricular dilatation, can be seen in 20–28% of patients with non-traumatic SAH [23, 24]. The occurrence of hydrocephalus is related to the presence of intraventricular blood and its presence increases mortality, particularly if left untreated. The increased mortality may be related to the presence of cerebral infarcts and decreased intravascular volume [24]. Current treatment of hydrocephalus consists of insertion of an external ventricular catheter.

Delayed Cerebral Ischemia

Diagnosis and management of delayed cerebral ischemia

Delayed cerebral ischemia is a complex phenomenon occurring in patients with SAH that is accompanied by decreased cerebral blood flow (CBF) and subsequent infarction. The most common cause of delayed cerebral ischemia is cerebral vasospasm, which is a mutivascular or diffuse phenomenon in most patients [25]. Cerebral vasospasm has been defined as the delayed narrowing of large arteries at the base of the brain following SAH [26]. Depending on the criteria used to define it, cerebral vasospasm can be detected on 30–70% of cerebral angiographies done after SAH [27–29]. Angiographic vasospasm usually starts between 3–5 days after SAH with maximal narrowing between 5–14 days, and gradual resolution over 2–4 weeks [27]. However, only about 20–30% of patients with angiographic vasospasm suffer from neurological deterioration, a condition that has been termed symptomatic vasospasm. We will use the term symptomatic vasospasm in this manuscript to denote onset of symptoms between 4–12 days with angiographic or ultrasonographic confirmation in the absence of any other condition that may explain the symptoms [30].

Symptomatic vasospasm is the most important cause of mortality in patients with SAH, representing about 25% of the total number of deaths [23]. Therefore, prevention or effective treatment of symptomatic vasospasm is important if we are to improve clinical outcome after SAH.

The risk of symptomatic vasospasm is associated with the amount of subarachnoid blood [18] and its onset can be predicted by transcranial Doppler ultrasonography [31]. Daily monitoring with transcranial Doppler ultrasonography could provide early identification of patients at risk for symptomatic vasospasm particularly

those with rapidly increasing mean CBF velocities (i.e., an increase >50 cm/sec within 24 hours). Once symptomatic vasospasm is suspected or confirmed by transcranial Doppler ultrasonography or angiography, patients are initially managed with increased amounts of intravenous fluids and elevation of systemic blood pressure the so-called hypertensive, hypervolemic, and hyperdynamic therapy (triple H). Current evidence supporting the use of triple H therapy comes from uncontrolled studies [32–34]. However, it has been reported that triple H therapy can reverse symptoms of symptomatic vasospasm in up to 75% of patients [33]. The risks associated with this therapy include rebleeding of an untreated aneurysm, cerebral edema, hemorrhagic transformation of infarcted areas, congestive heart failure, and myocardial infarction [2, 35]. The subsequent or concomitant use of endovascular treatments, such as cerebral angioplasty and infusion of intra-arterial papaverin has also become common practice in tertiary care centers. The available reports, also from uncontrolled case series, report sustained improvement in over 50% of treated patients [36–39]. The major risks associated with endovascular therapies are rebleeding, hyperperfusion injury, and vessel rupture.

Mechanisms responsible for subarachnoid hemorrhage-induced cerebral vasospasm

Understanding the mechanisms underlying the development of cerebral vasospasm has remained a challenging task. Despite the fact that our knowledge of molecular events leading to vasopasm after SAH remains incomplete, over the past few years several mechanisms have been investigated. It is now widely accepted that blood products, particularly oxyhemoglobin, contribute to cerebral vasospasm mediated by an insofar-unknown mediator [40, 41]. A number of mechanisms are thought to play a role in cerebral vasospasm including the following:

1. Endothelium-derived mediators that will cause impaired endothelium-dependent relaxation and increased production of endothelium derived constricting factors [40]. The end result is vessel constriction. Mediators implicated in endothelial alterations include nitric oxide (NO), oxygen free radicals, endothelin, lipooxygenases, and cyclooxygenases and their metabolites [42–47].
2. Vascular smooth-muscle-derived mediators which may lead to dysfunction of ion pumps, inhibition of potassium channels, activation of calcium channels (mostly potassium and calcium), reduction in second messengers (cAMP and cGMP), and protein kinase C activation [48–50].
3. Pro-inflammatory mediators that induce blood-brain barrier disruption, cytokines and adhesion molecules that promote leukocyte migration [51–54].
4. Platelet activating factors which lead to release of potent growth factors for cells in the vascular wall, such as platelet-derived growth factors, transforming growth factors, and vascular endothelial growth factor [55, 56]. The release of these growth factors induces cellular proliferation with subsequent vessel wall thickening.
5. Stress-induced gene activation, such as heat shock proteins and heme oxygenase, which may alter arterial contraction [57].

Prevention of cerebral vasospasm

The rationale for the use of preventive measures for SAH-induced cerebral vasospasm is based on the assumption that they will modify one or more of the molecular events discussed above. Several methods have been used, including blocking calcium entry into the vascular smooth muscle cell, inhibition of lipid peroxidation, scavenging of free radicals, inhibition of platelet activation, prophylactic volume

expansion, subarachnoid clot lysis, and prophylactic vessel angioplasty. Clinical trials have been undertaken with three calcium antagonists: nimodipine, nicardipine, and AT877 [58–62]. When analyzed separately, treatment with nimodipine was associated with a significant reduction in the proportion of patients with a poor outcome, without affecting the incidence of angiographic vasospasm. On the other hand, treatment with nicardipine and AT877 reduced the incidence of angiographic vasospasm but not the proportion of patients with a bad outcome. When analyzed in combination, calcium antagonists improve outcome (absolute risk reduction 5.1%) but mostly due to the nimodipine data [63]. It is not clear whether nimodipine works by exerting neuroprotection, improving vasospasm or both. Oral nimodipine has become the standard of care for patients with SAH. Other agents used without proven benefit include tirilazad [64, 65], nicaraven [66], ebselen [67], aspirin [68], dipyridamole [69], nizofenone [70], cataclot [71], and OKY-46 [72]. A small-randomized study using magnesium sulfate against placebo found no difference in outcome [73]. Another strategy that has been tested is the prophylactic use of hypervolemic therapy [74–76], under the assumption that circulating blood volume may frequently be contracted and this may be a risk factor for vasospasm [77]. However, studies have shown no difference in outcome or in physiological surrogate measures. Finally, prophylactic cerebral vessel angioplasty has also been advocated but no controlled studies are available [78]. In conclusion, short of a modest effect of nimodipine on outcome there is no other effective preventive therapy for cerebral vasospasm. The reason for this failure may be that most of the treatments tested may have limited mechanisms of action. Therefore, the use of an agent with multifunctional aspects and various actions would be the next logical step. A promising treatment that is currently undergoing further evaluation in a clinical trial is 25% human albumin [79, 80].

Treatment of Ruptured Cerebral Aneurysms

The treatment of ruptured cerebral aneurysms focuses on two modalities: surgical clipping and endovascular coiling [4, 81]. The former technique has been available for over 40 years whereas the latter was developed in the 1990s. The main advantage of surgical clipping is the long-term follow up of aneurysm obliteration. However, surgical clipping involves performing a craniotomy and brain tissue manipulation, which may be associated with complications, such as rebleeding, cerebral ischemia, and edema. Endovascular coiling involves the deployment of titanium coils into the aneurysm to exclude it from the circulation. The main advantage of endovascular coiling is its less invasive nature. However, like surgical clipping, endovascular coiling is also associated with complications including rebleeding (during the procedure and after due to incomplete treatment of aneurysm), cerebral infarction (due to branch occlusion or distal emboli), and mass effect.

The landmark International Subarachnoid Aneurysm Trial (ISAT) prospectively evaluated patients with ruptured aneurysms who were considered equally suitable for either endovascular coiling or surgical clipping [82, 83]. The ISAT investigators reported that for the study patients, the outcome, defined as survival free of disability at 1 year, was significantly better in patients treated with endovascular coiling. They also found that the risk of epilepsy was substantially lower in patients allocated to endovascular coiling, but the risk of rebleeding was higher. Furthermore, in those patients who underwent follow-up cerebral angiography, the rate of complete aneu-

rysm occlusion was greater with surgical clipping. After ISAT was published a great deal of controversy was created regarding the study results. What ISAT really did was to validate the endovascular coil technique. It is also important to point out that the ISAT results only apply to those aneurysms that could be treated by either surgical clipping or endovascular coiling. Obviously, many ruptured cerebral aneurysms are not equally suitable for both treatments. Several factors have been reported as being crucial when deciding on the best treatment approach for an individual patient including patient age, underlying medical problems, aneurysm location and morphology, and relationship of the aneurysm to adjacent cerebral vessels [84–86].

Patients in whom surgical clipping is preferable including the following:

- Wide neck aneurysms (ratio of neck diameter to largest dome >0.5)
- Aneurysms associated with large parenchymal hematomas
- Aneurysms causing local mass effect
- Aneurysms with normal branches arising from the base or dome
- Middle cerebral artery aneurysms

Patients in whom endovascular coiling is preferable include the following:

- Elderly patients
- Patients in poor medical condition
- Aneurysms of the vertebrobasilar system
- Aneurysms deep in the skull base (e.g., paraophthalmic aneurysms)

Due to the complexity of patients presenting with ruptured cerebral aneurysms, it is recommended that a group of individuals with a detailed knowledge of neurovascular surgery, endovascular coiling, and neurocritical care perform thorough evaluations of these patients. A team approach may be in the best interest of the patients.

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Promising Concepts in Subarachnoid Hemorrhage

A.M. Naidech

Introduction

Management strategies for subarachnoid hemorrhage (SAH) include early aneurysm obliteration, pharmacologic prevention of vasospasm, therapeutic (but not prophylactic) hyperdynamic therapy, targeted critical care, and rehabilitation. Despite recent advances, major gaps in our knowledge remain, and many potentially salvageable patients have devastating outcomes. Neuroprotective, prognostic, and pharmacologic developments are especially promising. This chapter highlights areas of intensive study in SAH management, with hopes that the efforts of dedicated clinician-scientists will come to fruition in the near future. I mean to highlight promising areas of active study with preliminary data or research funding, not today's state-of-the-art. There are no express or implied guarantees about actual progress.

Background

SAH care has vastly improved. In the past, prophylactic aneurysm obliteration was performed on a case-by-case basis with unknown probability of rupture and risk of treatment. Management strategies for SAH used to involve watchful waiting during the vasospasm period, microvascular clipping (then a still-developing technique) after the risk of vasospasm was passed, avoidance of volume overload, and seizure prophylaxis.

Table 1. Trends in management of SAH

	Past	Present	Future
Risk assessment of unruptured aneurysms	Size	Size, location	Shape and blood flow
Neuroprotection	N/A	Limited	Multiple specific targets
Aneurysm obliteration	Clipping	Clipping or coiling	Vessel reconstruction, endothelial repair
Anticonvulsants	Common prophylaxis	Institution dependent	Targeted prophylaxis, EEG screening
Vasospasm	Watchful waiting, prediction	Volume repletion, some Rx prevention	Genomic risk stratification and modification

EEG: Electroencephalogram; N/A: not available

SAH and aneurysm management will continue to evolve (Table 1). Research progress is expected in:

- predicting which unruptured aneurysms will bleed
- vascular repair as opposed to aneurysm obliteration
- vasospasm prevention and treatment
- transfusion and anemia management
- neuroprotection
- rehabilitation

Prediction of Aneurysm Rupture

Since 1% of the population has an intracranial aneurysm, it is essential to be able to predict which unruptured aneurysms will bleed. While aneurysm size, uncontrolled hypertension, tobacco use, and connective tissue disorders predispose to rupture, our ability to predict rupture, while improved, remains imperfect.

The best data available on unruptured aneurysms point to aneurysm diameter, aneurysm location, and history of SAH as important prognostic factors [1] in patients whose aneurysms are not obliterated. First-degree relatives of patients with SAH have a higher risk of intracranial aneurysms, but pre-emptive repair may not be superior to watchful waiting when the complications of repair are taken into account [2]. Patients with SAH have a large lifetime risk of rupture of another aneurysm, with aneurysm size and continued tobacco use prominent risk factors [3]. While the available data have not shown repairing small aneurysms is worthwhile, many patients are terrified of a 'ticking time bomb' in their head, and the average aneurysm size in SAH registries is < 10 mm. Since many small or medium size anterior-circulation aneurysms rupture, we need better tools to predict which of these 'low risk' (by diameter) aneurysms are more dangerous than they might appear on angiography.

Aneurysm rupture is likely to be predicted by change in size over time. The hypothesis that a growing aneurysm is likely to keep on growing and rupture is attractive, and several centers are pursuing this line of prospective study. Complicating factors include the population (randomly selected, referred, relatives of SAH patients, etc.), imaging modality (computed tomography [CT] angiography, magnetic resonance angiography, catheter angiography), and follow up time (3 months, 12 months, 5 years, etc.).

Aneurysm morphology relates to mathematical risk of rupture, too. A daughter aneurysm may be protective initially and decrease wall stress, while subsequent growth may lead to increased wall stress and imminent rupture [4]. Spherical aneurysms may have more wall stress than other shapes, and the dome may not be the site of highest wall tension [5], where the calculated probability of rupture is highest.

Inflammation may play a role in aneurysm formation and rupture. Ruptured aneurysms have increased levels of tumor necrosis factor (TNF)-α, but not T-helper cell produced interleukins [6]. Further work will be necessary to determine if TNF levels produce aneurysms or wall stress, or are caused by rupture, and if modification of TNF expression is safe.

Matrix metalloproteinase 9 (MMP9) is involved in degradation of proteins in the vessel wall [7] and may lead to vessel wall instability and rupture. MMP9 levels are not elevated by SAH alone but are associated with subsequent vasospasm [8]. Further work may elaborate which of these proteins are most important for the develop-

ment of vasospasm and the process by which important genes are activated, transcribed, and have downstream effects. These insights will hopefully lead to pharmacologic targets.

Rebleeding is recognized as an often preventable catastrophe. Predicting rebleeding is straightforward: The larger the aneurysm and the worse the neurologic grade, the higher the risk [9]. The most certain way is to obliterate the aneurysm with clips or coils at admission. For patients who must be transported to a referral center or encounter an unavoidable delay, pro-thrombotic agents can be used until definitive care [10]. Pro-thrombotic agents given throughout the vasospasm period, however, cause as many complications as they prevent.

Vasospasm Prevention and Treatment

Oxidative stress is a part of the inflammation associated with vasospasm. Superoxide anion, a highly reactive oxygen species that causes cellular damage, is implicated in animal models of vasospasm [11]. There are some data to suggest that high levels of superoxide dismutase (SOD), a protective protein, reduce vasospasm [12] and improve vasomotor reactivity [13] in rats but not in dogs [14]. Further work on antioxidants and methods to induce and deliver them is underway.

SAH is associated with altered nitric oxide (NO) metabolism. Vasospasm may be partially a NO deficiency state, and strategies to increase NO may prevent or lessen the impact of vasospasm. Oxyhemoglobin from degenerating clot scavenges NO [15]. Inhibitors of NO are strongly associated with vasospasm [16]. Endothelial NO synthase (NOS) increases NO to pharmacologic levels, but not enough to produce systemic effects [17]. In a primate model of SAH, infusions of low dose nitrite led to increased cerebrospinal fluid (CSF) nitrite concentrations and decreased cerebral vasospasm [18]. It is possible that inducing NOS, or repleting NO stores, will reduce vasospasm and stroke after SAH.

It may also be possible to increase NO through pharmacologic mechanisms. Sildenafil and related compounds antagonize phosphodiesterase, which leads to increased cyclic GMP and subsequent vasodilatation. A dose consistent with sildenafil's package labeling leads to improved cerebrovascular reactivity as measured by transcranial Doppler [19].

Endothelium Repair

Aneurysm obliteration should prevent rebleeding, but is unlikely to restore a normal endothelium. When aneurysms are coiled, normal endothelium is not in place between the obliterated aneurysm sac and the parent vessel. This is a goal of surgical clipping, but the success is difficult to determine, and the endothelium is unlikely to be normal immediately after surgical manipulation. Given the risk of vasospasm and cerebral infarction in the repaired vessel's distribution, we will need to learn to protect and assist endothelial growth after aneurysm obliteration.

Endothelial manipulation is possible. In an animal model of vascular injury, genetic delivery of endostatin altered endothelial function [20]. This proves the concept that we can alter endothelial function through gene therapy. Many more key questions need to be answered, such as the important genes involved, the optimal method of transfer, the timing of therapy, and the risks of distant side effects.

It may also be advantageous to use genetic manipulation in coiled aneurysms. Possible strategies include targeting adenovirus to the coils themselves with tags, altering expression of MMP or endothelial growth factors, or activating genes with external radiation [21]. These strategies might help to promote complete thrombosis, endothelial repair, and aneurysm reformation.

Neuroprotection

Neuroprotection has been oft-quoted as a way to minimize brain damage from aneurysmal rupture, endovascular repair, temporary surgical occlusion, or vasospasm. The promise of neuroprotection has generally gone unfulfilled, but is progressing fitfully. Although albumin does not improve outcomes in general intensive care unit (ICU) patients [22], in phase II studies it seems to improve outcome in acute ischemic stroke [23]. The presumed mechanism is that albumin functions as a sink for toxic metabolites and scavenges free radicals. A phase III study of albumin after ischemic stroke is under way. The available data also associate albumin use with improved outcomes after SAH [24]. A phase II study of albumin for SAH patients has been funded [25]. NXY-059, which may be beneficial in acute ischemic stroke [26], may also have a role in SAH. Another free-radical scavenger reduced the incidence of cerebral infarction after SAH [27].

Transfusion and Anemia Management

The role of hemoglobin and cerebral oxygen delivery after SAH is an area of active research. The traditional practice is to hemodilute (or at least not transfuse) until the hematocrit is 30% to ensure optimal viscosity, although actual data for this strategy are lacking. More than half of SAH patients have at least one measured hematocrit $<30\%$ (hemoglobin about 10 g/dl) [28]. Anemia and transfusion are associated with poor outcome in multivariate analysis [29]. Intra-operative and postoperative transfusion of SAH patients has been linked to poor outcome in post-hoc univariate analysis [30]. Patients with death or poor outcome after SAH have lower hemoglobin throughout the acute hospital course after correcting for neurologic grade, age, and vasospasm [31]. It is unclear if this relationship is causal, and a prospective, randomized, pilot study is under way to determine if higher goal hemoglobin is safe and feasible [32].

Erythropoietin (EPO) may be beneficial after SAH. EPO raises hemoglobin and decreases the need for transfusion in ICU patients [33]. In animal models of SAH, EPO is neuroprotective [34]. A pilot clinical trial of EPO after SAH recently completed enrollment [35]. EPO might both increase hemoglobin and provide neuroprotection after SAH.

Like hemoglobin, alterations in platelet number and function have been associated with outcomes after SAH. Within 24 hours of SAH there is widespread microvascular platelet aggregation that resolves by 48 hours after hemorrhage [36]. A decrease in the platelet count is associated with symptomatic vasospasm [37]. This implies that vasospasm is related to platelet consumption or reduced production (less likely). Microvascular platelet activation might trigger symptoms of vasospasm through cerebral ischemia. The processes that activate platelets, where they might be activated, and how this process could be manipulated are unclear.

Platelet inhibition is probably related to the amount of initial hemorrhage and the risk of rebleeding. Aspirin use is very common in outpatients, and other antiplatelet drugs, such as clopidogrel, are increasingly prevalent. There are few data relating SAH to methods of pharmacologic platelet inhibition, measurements of platelet function, and outcome for patients with antiplatelet treatment. Aspirin use after SAH is feasible and probably safe [38].

Seizures

Seizures are a feared complication of SAH. Especially in an unruptured aneurysm, seizures can increase blood pressure, raise intracranial pressure, and lead to rebleeding. In SAH patients with an abnormal mental status, convulsive and non-convulsive seizures are strongly associated with poor outcomes and should be aggressively treated [39]. While phenytoin has a long history of use, its side effects are also potentially serious. The available data associate its use with functional and cognitive disability in a dose-dependent manner [40]. Further refinement of anticonvulsant protocols and screening for seizures are needed.

After hospitalization, SAH patients often suffer from neurocognitive deficits. Recovery of memory and personality may be as important as motor strength: The ability to walk to the corner grocery is important, but so is remembering why you walked there.

Apolipoprotein (Apo)E4 has been shown to correlate with dementia and memory disorders, and is also linked to recovery after SAH. Among conscious patients, ApoE4 was associated with worse cognitive function and more ischemic deficits [41]. Insulin-like growth factor and TNF have also been associated with outcome [42]. Genetic testing will make prognosis more accurate, and the possibility of gene transfer may open therapeutic windows for recovery of neurocognitive function.

Conclusion

SAH is morbid and deadly, but not as morbid and deadly as it used to be. As our understanding of the disease process increases we are moving from a policy of reacting to events to predicting which will occur, mitigating against them, and designing specific therapies. Past performance is no guarantee of future success, but prospective research should shed new light on the basic physiology of aneurysm growth and rupture, vessel and endothelial repair, neuroprotection and recovery. In the meantime, be sure to have your high blood pressure treated and avoid tobacco exposure.

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Nitric Oxide Metabolism after Traumatic Brain Injury

L. Rangel-Castilla and C.S. Robertson

Introduction

Nitric oxide (NO) is a cell membrane-permeable free radical gas and is the smallest known biologically active molecule. NO can be produced by nearly all tissues of the body. NO is synthesized from the semi-essential amino acid, L-arginine, by the enzyme, nitric oxide synthase (NOS). Three isoforms of NOS exist: Endothelial (eNOS), neuronal (nNOS), and inducible (iNOS). Agents that selectively inhibit the individual isoforms of NOS, as well as transgenic mice that are deficient in each of the isoforms of NOS, have played important roles in understanding the normal functions of NO and the changes in NO metabolism that occur with neurological disorders like traumatic brain injury (TBI) [1].

NO plays a role in numerous general physiological processes of the brain, including maintenance of basal vasomotor tone, inhibition of platelet and leukocyte aggregation, macrophage-mediated cytotoxicity, neurotransmission, selective neuroprotection, apoptosis, synaptogenesis, intercellular signaling, synaptic plasticity, and memory formation. Under pathologic conditions, both excesses and deficiencies of NO may have deleterious effects. Depletion of NO produced by eNOS could potentially result in inadequate cerebral perfusion and excesses of NO produced by nNOS and iNOS could result in neurotoxicity and cellular injury. Such changes in NO metabolism have been implicated in the pathophysiological changes that occur after TBI.

Chemistry, Synthesis, and Metabolism of Nitric Oxide

NO is a signaling molecule with a number of physiological functions, including the regulation of blood pressure, neurotransmission, and tissue metabolism. Although it is considered a free radical, NO is not highly reactive *per se*. NO mediates biologic function through both direct and indirect effects. The most well-known direct effect of NO is its activation of soluble guanylate cyclase, which results in an increase in intracellular cyclic guanosine monophosphate (cGMP), an important regulator of smooth muscle contractility, platelet aggregation, and leukocyte adhesion.

NO is produced from a five-electron oxidation of the guanidine nitrogen of L-arginine. The oxidation of L-arginine to L-citrulline occurs via two successive monooxygenation reactions that produces N^{w}hydroxy-L-arginine as an intermediate. This reaction is catalyzed by the enzyme NOS, which has a complex structure and function.

NOS requires the following five bound cofactors for normal function: Flavin adenine dinucleotide (FAD), flavin mononucleotide (FMN), heme, tetrahydrobiopterin, and Ca^{2+}-calmodulin. NOS protein is constructed as a dimer formed of two sub-

units, each containing three distinct domains: A reductase domain, a calmodulin-binding domain, and an oxygenase domain. The reductase domain contains the FAD and FMN, and acts to transfer electrons from nicotinamide adenine dinucleotide phosphate (NADPH) to the oxygenase domain of the opposite sub-unit of the dimer. The calmodulin binding domain contains the binding site for Ca^{+2} calmodulin. The oxygenase domain contains the binding sites for tetrahydrobiopterin, heme, and arginine, and catalyzes the conversion of arginine to citrulline and NO. In addition to the five cofactors, NOS also requires the following three co-substrates: L-arginine, oxygen, and NADPH.

Three isoforms of NOS have been identified. Two of the isoforms are expressed constitutively, nNOS (type 1) and eNOS (type 3), and one isoform is induced under pathological conditions, iNOS (type 2). All three isoforms are found in the brain. Under normal conditions, only the constitutive isoforms of NOS can be detected in brain using immunohistochemical methods. nNOS is localized in neurons and perivascular nerves. Very low levels of nNOS are detected in astrocytes. eNOS is found in cerebrovascular endothelium. iNOS exists in astrocytes and microglia, vascular smooth muscle, and endothelial cells.

NO produced by nNOS normally mediates synaptic plasticity and neuronal signaling but may also have cytotoxic activity under pathological conditions, like trauma. NO generated by nNOS causes cell death by excitotoxicity and oxygen free radical mechanisms. NO reacts with superoxide ($O_2^{\cdot-}$) to form peroxynitrite ($ONOO^-$), which is a highly toxic free radical capable of oxidizing proteins, lipids, and DNA and homolytically decomposing to yield even more potent neurotoxins, like the hydroxyl radical.

NO is a vasodilator in cerebral vessels. NO produced by eNOS plays a role in maintaining resting cerebral blood flow (CBF). NO produced by nNOS participates in the cerebrovascular responses to metabolic activity. Some studies have also suggested a role for NO in pressure autoregulation in the brain.

Activation of iNOS mediates inflammatory and cytotoxic actions. Calmodulin is tightly bound to iNOS, and is not influenced by fluctuations in cytoplasmic levels of calcium. Once expressed, iNOS becomes activated and leads to generation of NO for several days. Regulation of iNOS activity is complex. It has been proposed that agents elevating cAMP and NO itself (by promoting nuclear translocation of nuclear factor-kappa B [NF-κB]) may induce iNOS. L-arginine and availability of cofactors such as tetrahydrobiotperin are additional factors that may also affect iNOS activity. The inducible form of NOS is likely to be important in pathophysiological mechanisms of trauma because this isofrom is activated by cytokines and produces an excess of NO which is toxic to neurons.

Methods for Measuring NO in the Brain

NO is difficult to measure *in vivo* because of its short half-life. In laboratory studies, NO has been measured directly with a NO electrode or by using a substrate, such as hemoglobin, to trap NO in a stable form that can be assayed [2–4]. NO has also been measured in brain tissue using electron paramagnetic resonance spectroscopy [5, 6]. In humans, direct measurement is not very practical, but the end products of NO metabolism, nitrate and nitrite (NOx), can be studied as an index of NO production. These end-products of NO can then be measured in cerebrospinal fluid (CSF) or in microdialysate samples [7–10].

Nitric Oxide after Traumatic Brain Injury

Observations about changes in NO metabolism that occur with brain trauma as well as the consequences of these changes are complex because NO has multiple functions within the central nervous system (CNS). A triphasic (high-low-high) change in the concentrations of NO in the brain after trauma has been observed in experimental studies.

Immediate Increase in NO Concentrations after Traumatic Brain Injury

The immediate hemodynamic response to TBI, consists of a transient hypertensive surge followed by hypotension [11, 12]. A transient rise in intracranial pressure (ICP) accompanies the increase in blood pressure; and these cardiovascular changes are thought to be caused by massive sympathetic discharge.

During this very early time period post-injury, NO has only been studied in the brain in animal models. NO measured directly with an electrode and indirectly from microdialysate concentrations of NOx show a transient increase in NO concentrations in injured brain tissue during the first 5 to 10 minutes post-injury [13]. Constitutive NOS activity in the injured brain is also increased at 5 minutes post-injury and returns to normal by 30 minutes [14]. iNOS activity is not detectable in the brain at 5 minutes or 6 hrs post-injury [15].

The consequence of NO production for the injured brain depends on the origin. Both of the constitutive isoforms of NOS contribute to this early increase in NO, although NO produced by nNOS probably predominates [5]. NO produced by nNOS during this early time period is likely to have adverse effects on outcome. NO produced by eNOS during this early time period is likely to be beneficial and act to preserve CBF in the injured brain. These differential effects of NO have been studied by using selective NOS inhibitors in experimental models of TBI (Table 1).

Administration of the non-selective NOS inhibitor, nitro-L-arginine-methyl ester (L-NAME), prior to injury inhibits the initial transient accumulation of NO in the brain after cortical impact injury [13]. However, the effect of inhibitors on outcome after trauma has been quite variable. In one study, mortality rate was increased to 70% with pre-injury administration of L-NAME. Death was due to prolonged hypertension and pulmonary edema [16]. In the fluid percussion injury model and cold injury model, pre-injury administration of non-selective NOS inhibitors does not alter lesion volume [14, 17, 18]. In contrast to the non-selective NOS inhibitors, pre-injury administration of the nNOS inhibitor, 3-bromo-7-nitroindazole (7-NI), has more consistently been found to have protective effects [14, 17].

Other significant metabolic and biochemical changes occur in the brain during this early time period. At the time of traumatic impact of the brain, widespread depolarization occurs. Potassium concentrations in the extracellular space rapidly increase and calcium accumulates intracellularly [19]. Brain glucose metabolic rates are dramatically increased as the injured tissues reestablish normal ionic gradients following the trauma-induced depolarization. Glutamate release mediates some of these findings [20]. One possible mechanism for the increased NO in the early post-injury period is that glutamate stimulation of NMDA receptors causes postsynaptic calcium influx and, therefore, activation of the constitutive isoforms of NOS.

Table 1. Summary of studies using NOS inhibitors or L-arginine in traumatic brain injury (TBI).

Agent	Time of administration	First author, year [reference]	Result
Non-specific NOS inhibitors			
L-NAME or L-NNA	Pre-injury	Wada, 1998, 1999 [14,17]	No change in contusion volume or behavioral deficits
	Pre-injury	Stoffel, 2001 [18]	No change in cold lesion volume
	Pre-injury	Lu, 1997 [16]	Increased mortality
	Post-injury	Mesenge, 1996 [34]	Decreased behavioral deficits
	Post-injury	Lu, 1997 [16]	No change in mortality or behavioral deficits
	Post-injury	Wada, 1998 [14]	No change in contusion volume
	Post-injury	Gahm, 2005 [35]	Promoting neuronal survival
Selective nNOS inhibitors			
7-NI	Pre-injury	Wada, 1998, 1999 [14,17]	Decreased contusion volume and behavioral deficits
	Post-injury	Wada, 1998 [14]	No change in contusion volume
	Post-injury	Mesenge, 1996 [34]	Decreased behavioral deficits
Selective iNOS inhibitors			
L-NIL	Post-injury	Gahm, 2006 [35]	Increased neuronal survival
	Post-injury	Louin, 2006 [46]	Decreased behavioral deficits
1400W	Post-injury	Jafarian, 2005 [47]	Improves histopathological outcome
	Post-injury	Louin, 2006 [46]	Decreased behavioral deficits
Aminoguanidine	Pre-injury	Stoffel, 2001 [18]	Decreased cold lesion volume
	Post-injury	Sinz, 1999 [50]	Worse behavioral deficits, increased neuronal loss
	Post-injury	Gorlach, 2000 [48]	Decreased cold lesion volume
	Post-injury	Lu, 2003, [49]	Decreased behavioral deficits and improved neuronal survival
	Post-injury	Louin, 2006 [46]	Decreased edema and decreased behavioral deficits
NOS substrate			
L-arginine	Pre-injury	Stoffel, 2001 [18]	No change in cold lesion volume
	Post-injury	Mesenge, 1996 [34]	No change in behavioral deficits
	Post-injury	Wada, 1999 [17]	Decreased contusion volume
	Post-injury	Cherian, 1999 [38]	Decreased contusion volume, improved cerebral blood flow
	Post-injury	Hlatky, 2003 [7]	Improved cerebral blood flow

Early (30 min–6 hour) Decrease in NO Concentrations after Traumatic Brain Injury

The early response to severe brain injury after the immediate hypertensive surge is characterized by intracranial hypertension, systemic hypotension, and a reduction in CBF. At least part of the low CBF observed during this period is a consequence of a decreased cerebral perfusion pressure (CPP). However, CPP is not sufficiently low to fully explain the 50% reduction of CBF that occurs in the cortical impact injury model.

A similar pattern of hypoperfusion has been observed during the first few hours after a head injury in humans. Bouma et al. [21] have observed a regional CBF less than 18 ml/100gm/min in 37% of patients during the first 6 hours after TBI. Martin

et al. [22] described an evolving pattern for CBF following traumatic injury with hypoperfusion on the day of injury and hyperemia on days 1–3 after injury.

During this period of early low CBF, most studies in experimental models of trauma have demonstrated relative deficiencies of NO. The concentration of NO is consistently reduced in injured brain between 5 minutes and 3 hours after cortical impact injury [13], impact acceleration injury [23], and fluid percussion injury [24]. At 5 minutes after fluid percussion injury, cNOS activity is increased, by 30 minutes cNOS activity is returned back to normal, and from 1 to 7 days after injury cNOS activity is reduced [14]. In transgenic mice deficient in the eNOS isoform, CBF is lower and does not respond to administration of L-arginine following cortical impact injury [25].

Many pathological processes induced by trauma could result in either a reduction in NO production or rapid inactivation of NO. Free radical production is a prominent part of early TBI pathology. Superoxide radical rapidly reacts with NO to form peroxynitrite, and this reaction may quickly remove any NO that is produced, preventing NO from being available for physiological functions. Studies using free radical scavengers suggest that superoxide radicals do play some role in the reduction in tissue NO concentrations and also the reduction in CBF during the early post-injury period. Administration of superoxide dismutase (SOD) and catalase prior to injury and during the early post-injury period minimizes both the reduction in tissue NO levels and in CBF in the cortical impact injury model [26]. Administration of SOD in the fluid percussion injury model prevents early cerebral hypoperfusion [27].

Reactive oxygen species (ROS) also damage genes from which mRNA is transcribed as part of the endogenous neuroprotective response. The NOS gene itself could be injured by oxidative damage following trauma. Depending on the severity of damage, this oxidative damage of the NOS gene might result in production of non-functioning mRNA transcripts or might inhibit production of mRNA transcripts [28]. Oxidative DNA damage can often be repaired with time, and typically results in only a delay in production of functional mRNA. This is consistent with the time course of vascular injury after TBI with the greatest incidence of low CBF during the first few hours after injury and subsequent spontaneous recovery by 6–12 hrs after injury in most patients.

Other pathways could also play a role in reducing availability of L-arginine, which is the substrate for the enzyme NOS, and, thereby, reduce NO production. As oxygen supplies become limited, less citrulline is converted to arginine since that is an energy-dependent reaction. This mechanism could partially explain decreased NO concentrations after TBI, since hypoperfusion and depletion of tissue oxygen occurs at the contusion site [29]. Another enzyme, arginase, hydrolyzes the conversion of L-arginine to ornithine and urea as part of the urea cycle and has the potential to modulate NOS activity by altering the availability of L-arginine [30]. Arginase is induced in the brain after ischemia and could compete with NOS for available L-arginine [31].

Inhibition of NOS activity may also occur after trauma. Cortical impact injury induced a 10- to 20-fold increase in ornithine decarboxylase activity and a 4- to 5-fold increase in putrescine in the ipsilateral cortex [32]. The adverse effects of polyamines in stroke and trauma are usually thought to be cytotoxic. However, polyamines may also have vascular effects, by inhibiting NOS [33].

Effects of treatment with non-selective and neuronal NOS inhibitors during this early post-injury time has been inconsistent (Table 1). Treatment with L-NAME or

7-NI between 5 and 30 minutes post-injury has not improved contusion volume in the fluid percussion model, even in models where pre-treatment with these inhibitors had been neuroprotective [14]. One study in closed head injury in mice has shown neuroprotective effects with both L-NAME and 7-NI administration between 5 minutes and 1 hr after injury [34]. In a weight drop model of TBI, administration of 7-NI improved neuronal survival [35].

Administration of L-arginine during this early time post-injury has had more consistent beneficial effects on CBF and on neurological outcome in multiple studies and TBI models [36–39]. The improvement in CBF with L-arginine administration has been accompanied by restoration of NO levels in the brain. Studies in transgenic mice deficient in eNOS suggest that the endothelial isoform is necessary for these neuroprotective effects of L-arginine [25].

Late (>6 hour) Increase in NO after Traumatic Brain Injury

The late hemodynamic response to brain injury has been described primarily in human head injury and is characterized by return of CBF to normal or even elevated levels. Cerebral metabolic rate tends to remain reduced unless the patients rapidly recover consciousness, and the arteriovenous oxygen difference is low. Under the circumstances, even a normal level of CBF can be considered to be excessive for the reduced cerebral metabolic rate.

Most of the measurements of NO in the brain have been in human studies at this later time period after injury. CSF NOx concentrations have been reported to peak at 20 hrs and 30–42 hrs after injury at a concentration of 25 ± 6.2 μmol/l and 26.4 ± 3.3 μmol/l, respectively [9, 10]. Patients who died of their brain injury had a significantly higher CSF level of NOx than patients who survived.

Expression of NOS in the brain has been studied in both experimental models and human tissue. In weight drop and fluid percussion models of TBI, nNOS expression is observed initially at 3 hr post-injury, while iNOS appears around 12 hr after injury [40–42]. Expression of iNOS has been observed as long as 7 days after injury [15]. Expression of iNOS protein in cerebrovascular smooth muscle cells and infiltrated neutrophils after TBI has suggested that iNOS may play a role in the cerebral hyperemia that occurs 2–3 days after TBI [40]. In the weight drop model, the peak in CBF at 3 hrs after injury paralleled expression of iNOS in cerebral vessels [43].

Expression of iNOS after brain trauma has also been examined in human tissue in a similar time course. In contused brain tissue removed at surgery, increases in iNOS-positive cells are detectable within 6 hours after trauma and peak at 8 to 23 hours with expression of iNOS detectable in neurons, macrophages, neutrophils, astrocytes, and oligodendrocytes [44]. In autopsy specimens, iNOS immunoreactivity in the brain was observed between 2 and 8 days after injury [45].

The effect of the selective iNOS inhibitors on outcome has been variable in TBI (Table 1). In several studies, aminoguanidine, L-N iminoethyl-lysin (L-NIL), and 1400W have been shown to improve neurobehavioral outcome, and neuronal survival after TBI [18, 35, 46–49]. In the cortical impact injury model in rats, aminoguanidine has been found to worsen cognitive performance and to increase loss of neurons in the hippocampus [50]. Transgenic mice deficient in iNOS had worse cognitive performance following cortical impact injury [50]. Further studies in these iNOS knock-out mice suggest that NO produced by iNOS has antioxidant properties, which may be neuroprotective following TBI [6].

Conclusion

NO has multiple physiological roles in the normal brain. The role of NO in the evolution of brain injury is also complex, but the availability of selective NOS inhibitors and of knock-out mice has begun to help elucidate some of the mechanisms for NO in the pathology that occurs after TBI. The initial immediate peak in NO in the brain after injury is probably due to the activity of eNOS and nNOS. Pre-injury treatment with 7-NI, which inhibits the immediate increase in NO produced by nNOS is effective in improving neurological outcome in some models. After the initial peak in NO, there is often a period of relative deficiency in NO. This period of low NO levels is associated with a low CBF. Administration of L-arginine at this early time improves CBF, and outcome in many models. The late peak in NO after traumatic injury is due primarily to the activity of iNOS. Inhibition of iNOS is neuroprotective in most models of TBI.

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Modulation of Blood Pressure in Traumatic Brain Injury

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Introduction

The modulation of arterial pressure is an important stage in the care of a patient with a cerebral lesion. International guidelines recommend a level of cerebral perfusion pressure (CPP = mean arterial pressure [MAP] – intracranial pressure [ICP]) that is superior to 60 mmHg. On the other hand, a level that exceeds 70 mmHg in the absence of cerebral ischemia must be avoided given the risk of acute respiratory distress syndrome (ARDS) [1]. Moreover, a single episode of hypotension defined as systolic arterial pressure <90 mmHg in a patient with severe head trauma is associated with an increase in mortality and morbidity [2].

In fact, the variations induced by pharmacologic treatment of arterial or perfusion pressure have different effects depending on the preservation of cerebral vasomotor reactivity (auto-regulation). Arterial pressure is not correlated to the velocity of cerebral blood flow (CBF) when auto-regulation is intact; in this case, its increase triggers vasoconstriction, a reduction in cerebral volume and, therefore, a decrease in ICP. On the other hand, the correlation between arterial pressure and the velocity of CBF is significant in patients who have lost self-regulation [3]. Treatment must, therefore, take the presence or absence of auto-regulation into account.

An analysis of a data bank of 392 patients with Glasgow Coma Scale (GCS) scores between 3 and 8 revealed that a MAP inferior to 70 mmHg was associated with a poor prognosis in patients with severe head trauma. It should be noted that the results were not the same when the level of MAP was fixed at 80 mmHg [4]. In such patients, it is, therefore, advisable to maintain a MAP that is superior to 70 mmHg with CPP between 60 and 70 mmHg.

The aim of this chapter is to define the role of catecholamines in the maintenance of these therapeutic objectives. For that, an analysis of the literature using the Pubmed database was performed using the following key words and their combinations: catecholamines, norepinephrine, epinephrine, dopamine, dobutamine, dopexamine, isoprenoterol, head injury, cerebral perfusion pressure, intracranial pressure, cerebral blood flow, trauma. Randomized clinical trials were specifically targeted.

The Effects of Hypertension on a Cerebral Lesion

Increasing CPP beyond 70 mmHg is inadvisable given the resulting extracranial complications. In one study, increasing CPP by 20% using norepinephrine in head trauma patients reduced the volume of the ischemic zone, improved flow metabolism coupling and increased the ICP by 2 mmHg [5]. In another study, an increase

in MAP of 14 ± 5 mmHg triggered an increase in ICP from 16 ± 9 to 19 ± 9 mmHg. In this population, a decrease in ICP of more than 20% was observed in 77% and 49% of the patients treated by hyperventilation or metabolic suppression (propofol), respectively, whereas this occurred in only 5.5% of the patients with induced hypertension [6].

The effects of arterial pressure variations were studied in 13 head trauma patients during the first three days of hospitalization. The initial MAP was 94 mmHg. It then decreased to 68 mmHg and subsequently went up again to 126 mmHg. In the six patients with an ICP >24 mmHg, the reduction in CPP increased ICP and reduced cerebral tissue oxygen partial pressure ($PtiO_2$). The patients without intracranial hypertension did not present significant variations during the various tests [7].

A study of CBF around the contusion zone provided interesting data. The flow and the volume of the cerebral blood compartment increased in the region of the contusion. The increase in CPP from 70 to 90 mmHg had effects on the perilesional zone that were not significant. Overall, the ischemic regions remained ischemic [8]. Thus, the effects of hypertension on the hemodynamics of head trauma patients depend in part on self-regulation and the ICP level. Locally, a reduction in ischemic zones or a modification in ($PtiO_2$) is sometimes observed. In any case, these modifications have no clearly defined clinical consequences.

The Effects of Catecholamines and other Vasopressors

The Systemic Effects of Catecholamines

The systemic effects of catecholamines depend on their affinity for α- and β-receptors. Briefly, seven subtypes of α-receptors have been described but only α1 and α2 are of practical interest. These receptors respond, in order of strength, to norepinephrine > epinephrine > isoprenoterol. Norepinephrine has a sensitivity that is higher for α1 than for α2 receptors. α1 receptors activate the Gq protein which increases intracellular calcium via phospholipase C. This results in muscle contraction whereas stimulation of α2 receptors induces vasodilatation via inhibition of adenyl cyclase. In addition, α2 receptors have central effects such as sedation, anxiolysis, and analgesia.

β-receptors are different from a pharmacologic point of view. The order of sensitivity for β-receptors is isoprenaline > epinephrine > norepinephrine. These receptors activate a Gs protein and increase the concentration of cyclic AMP via the activation of adenyl cyclase which in turn triggers muscular relaxation.

The Cerebral Effects of Catecholamines

Norepinephrine

Norepinephrine is a central nervous system (CNS) endogenous mediator. The administration of exogenous norepinephrine induces dose-dependent effects. At a low dose, the β1 effect predominates, inducing an increase in cardiac output, whereas strong doses stimulate α1 receptors, inducing intense vasoconstriction of the arterial and venous territories. In head trauma patients, increasing arterial pressure with norepinephrine has no effect on renal function [9].

Old data suggest that norepinephrine reacts through a local mechanism during a cerebral lesion. The application of topical norepinephrine on large cerebral arteries induces vasoconstriction. This is not the case for small arteries [10]. The absence of

a response is probably due to a modest sympathetic innervation of the small arteries and to the liberation of nitrogen monoxide at their level [11, 12]. Intraventricular injection of a 40 μg/kg bolus of norepinephrine significantly increases CBF, oxygen and cerebral glucose consumption. After chemical rupture of the hemato-encephalic barrier, intra-carotid injection of 50 ng/kg/min of norepinephrine increased CBF, cerebral oxygen and glucose consumption. The response to norepinephrine is, therefore, dependent on the integrity of the hemato-encephalic barrier and the increase in CBF is secondary to that of cerebral metabolism [13]. These results are in agreement with those of a study performed on a culture of astrocytes. The formation of CO_2 due to glucose oxidation increases in the presence of norepinephrine. Oxidative metabolism is linked to the stimulation of α1 and 2 receptors [14]. In an experiment on conscious rats, an elevation in arterial pressure induced by the administration of 10 μg/kg/min of norepinephrine did not modify the CBF/glucose utilization ratio or the blood brain barrier [15]. Another study performed in conscious rats reported a reduction in glucose metabolism with unchanged CBF during perfusion of norepinephrine which produced moderate hypertension [16]. However, the clinical relevance of these studies is debatable since experimental conditions are far from clinical practice. The doses of norepinephrine were 20 times more than those used in clinical practice, the injection sites were inappropriate, and the clinical condition of the animals did not mimic that of a cerebral lesion.

Two arguments suggest that the local effect of norepinephrine on cerebral hemodynamics is not significant. First of all, in healthy tissue, norepinephrine boutons are directly apposed to the capillary wall at sites of glial end-feet discontinuities, leading one to suppose that the latter are not very accessible (Fig. 1) [17]. In a head trauma animal model, self-regulation was stopped in the area of the lesion. During rupture of the blood brain barrier, the local effect of norepinephrine should induce vasoconstriction. However, elevated MAP increases local CBF with arteriolar vasodilatation, confirming the abolition of auto-regulation [18]. Second, a study performed in healthy volunteers revealed that an increase in cerebral vascular resistance during perfusion of norepinephrine is the result of self-regulation with constant CBF despite the increase in MAP. In order to decouple the effects of MAP and norepinephrine, an antihypertensive agent (phentolamine) was used to normalize MAP, keeping the norepinephrine perfusion constant and, thereby, preserving the local effect. CBF remained constant and vascular resistances regained their initial values [19]. This study confirmed the specific local effect of norepinephrine when the blood brain barrier is intact. Although data remain uncertain in head trauma patients, it would appear that the local effect is not predominant.

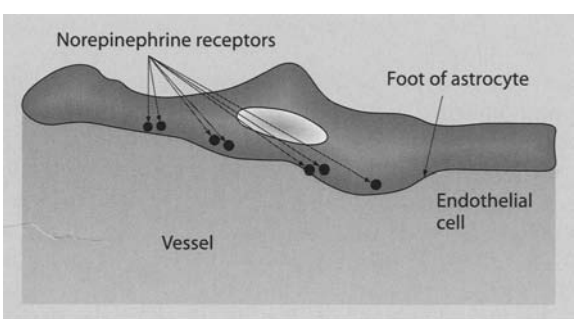

Fig. 1. Relation between norepinephrine receptors and cerebral vessels.

Dopamine

Dopamine is the immediate precursor of norepinephrine. It has dose-dependent actions as a neurotransmitter via activation of the D_1 receptor and as an indirect agent by inhibiting the liberation of norepinephrine via the D_2 receptor. Doses that range from 2 to 10 μg/kg/min have positive chronotropic and inotropic effects by activating the β1-receptor. Beyond 10 μg/kg/min, the α1-receptors trigger vasoconstriction.

In the large cerebral arteries, dopamine-induced relaxation appears to be linked to dopaminergic receptors which predominate over the α vasoconstrictor effect at a low concentration [20]. In conscious rats treated with dopamine (200 μg/kg/min) in order to reach a MAP of 150 mmHg, the CBF/glucose utilization ratio and the blood brain barrier permeability increased with the administration of dopamine [15]. On the other hand, two animal studies revealed that dopamine perfusion triggered an increase in MAP without modifying CBF [21, 22].

Another study has clarified this contradiction by stressing the effect of the dose. Rats were subjected to head trauma, then randomized to either a treatment group (dopamine at 40–50 μg/kg/min) or a placebo group (saline solution). CBF decreased by 46% at the level of the cerebral contusion in all of the rats. The administration of dopamine at 10–12 μg/kg/min did not modify MAP or cortical cerebral blood pressure compared with the placebo. On the other hand, a dose of 40–50 μg/kg/min increased MAP from 89 to 120 mmHg and CBF in the contusion zone by 35%. However, flow was not modified in the contralateral hemisphere. Cerebral swelling, water content, and the concentration of glutamate and hypoxanthine in the cerebrospinal fluid (CSF) were not affected by the perfusion of dopamine. In fact, the administration of dopamine revealed a local change in self-regulation [23].

In a rodent model, the effect of dopamine was determined using models of a rapid increase in ICP due to a secondary lesion and a cortical contusion. Dopamine restored cerebral perfusion in the first model with partial restoration of CBF. Magnetic resonance imaging (MRI) showed an elevation in cerebral water content four hours after the lesion. Dopamine perfusion increased the quantity of water. In the contusion model, the administration of dopamine aggravated the edema in the homo- and controlateral zones [24].

In conclusion, dopamine is effective in restoring CPP but increases CBF depending on the area of the lesion. Its effect on the volume of the lesion has not been defined but requires caution.

Other catecholamines

Epinephrine is an endogenous catecholamine which, at a low dose (0.015 μg/kg/min), activates the β1 (increase in cardiac output) and β2 (bronchodilatation and vasodilatation) receptors. At high doses, the α receptors are stimulated, leading to vasoconstriction. The cerebral effect of epinephrine was tested on a sheep model without cerebral lesion. In this model, the hypertension induced by a clinically significant dose of epinephrine did not alter CBF, ICP, or cerebral consumption of oxygen [25].

Among the agents that preferentially act on β-receptors, dobutamine is a synthetic catecholamine derived from dopamine that acts predominantly on β1 receptors. The cerebral effects of dobutamine have been analyzed in a sepsis model excluding meningitis (loss of auto-regulation). Dobutamine perfusion increased CBF and MAP [26]. MAP modification was the only effect reported in healthy volunteers [27]. These results alone do not make it possible to make conclusions on the possi-

ble local effects of dobutamine although it would appear that this agent does not have an effect on healthy brain. Auto-regulation is probably stopped in the case of sepsis. The increase in CBF, parallel to that of MAP, suggests passive vasodilatation linked to the increase in flow. However, one cannot exclude the possibility that dobutamine, through its β effect, triggers vasodilatation due to the change in the blood brain barrier [26, 27]. In conclusion, since these agents have a predominant β receptor effect, they must be excluded from the therapeutic arsenal used to maintain adequate CPP.

A non-catecholamine vasopressor: Vasopressin

Vasopressin is a natural hormone secreted from the posterior pituitary gland. It is active via the V_1, V_2, and V_3 receptors. V_1 receptors activate a Gq protein which produces an elevation in intracellular calcium concentration and consequently a contraction of smooth muscle. This system is an alternative to the catecholamine system for strong vasoconstriction. V_2 receptors make up the antidiuretic system at the level of the kidneys whereas V_3 receptors are stimulated by adenocorticotropic hormone (ACTH).

Vasopressin has a marked local effect with a probable role in vasospasm via activation of the V_1 receptors. In fact, in a goat model, incremental doses of vasopressin (0.03–1 μg) administered in the internal mammary artery significantly increased cerebral vascular resistance. This effect was not observed with desmopressin, which elicits V_2 receptor action [28]. In a murine model, vasopressin was implicated in the vasospasm secondary to subarachnoid hemorrhage (SAH) [29]. In a head trauma model followed by hemorrhagic shock, the effects of volemic expansion, with or without administration of a vasopressor (phenylephrine or vasopressin), were compared [30]. The addition of vasopressors prevented an increase in ICP and a reduction in CPP. Lactate plasma levels remained elevated in the group treated with vasopressin whereas it decreased in the other groups [30]. The effects of vasopressin on cerebral hemodynamics remain to be clarified but its vasoconstrictive properties with its probable implication in vasospasm make its use advisable.

Comparison of Norepinephrine and Dopamine

Animal model

The effects of norepinephrine and dopamine were studied in a murine model with cerebral lesion (by hammer) followed by hypoxic and hypotensive shock with the aim of maintaining CPP greater than 70 mmHg. In the control group, CPP decreased with an increase in ICP and a reduction in CBF. Surprisingly, the CPP objective was not achieved with either catecholamine. Moreover, ICP was higher in the catecholamine groups than in the control animals. Local CBF fell in a similar manner in the three groups. According to the authors, hypovolemia, as an explanation for the resistance to catecholamines, was improbable given the volemic expansion performed [31].

Human studies

Four studies have compared norepinephrine and dopamine in head trauma patients (Table 1) [32–35]. The first study was prospective and not randomized with the choice left to the doctor who received the patient. Nineteen patients were included with an initial ICP of 29 ± 10 mmHg. Catecholamines were crossed over after the first data collection. The important result of this study was the significant increase

Table 1. Human studies comparing norepinephrine (NE) and dopamine (DA).

Reference	Design Patients (*n*)	Objectives	Dopamine	Norepinephrine	Conclusion
[32]	Prospective (19)	CPP > 60 mmHg $SjvO_2$ > 55%	↗ ICP		NE > DA (effect ICP)
[33]	Prospective, randomized (11)	↗ CPP 65 mmHg 85 mmHg	Less predictable	↗ O_2 local ↘ ΔO_2 art-vein	NE > DA (prediction)
[34]	Prospective, randomized (10)	↗ CPP 65 mmHg 85 mmHg	Less predictable		NE > DA (prediction)
[35]	Prospective, randomized (15)	↗ CPP 65 mmHg	↗ HR, CI	↗ creatinine clearance ↘ pHi	No difference

CI: cardiac index; HR: heart rate; ICP: Intracranial pressure; CPP: cerebral perfusion pressure; $SjvO_2$: oxygen jugular venous saturation

in ICP with a reduction in CPP in the dopamine group. Transcranial Doppler and oxygen saturation data, measured in the jugular vein, were similar in both groups. Given the results, it was not possible to know how many patients lost cerebral autoregulation. Norepinephrine, therefore, had a better performance than dopamine for the selected criteria [32].

In order to increase CPP from 65 to 85 mmHg, the effects of norepinephrine and dopamine were compared in a randomized study that included 11 patients with severe head trauma (GCS < 10) [33]. A microdialysis system analyzed local oxygen exchange. An increase in CPP induced by norepinephrine significantly reduced the arterio-venous difference in oxygen and increased local oxygenation. This result was not obtained with dopamine. There was no difference between dopamine and norepinephrine in terms of ICP, although the response to dopamine was less predictable [33].

A cross-over randomized study was performed by the same team in 10 patients who had initially been treated with norepinephrine or dopamine [34]. CPP was set at 65 mmHg, was increased to 75 mmHg and finally to 85 mmHg, with a cross-over of catecholamines. On admission, GCS scores were between 3 and 8 (the study was performed three days after admission). Norepinephrine predictably increased the velocity of mean cerebral artery flow estimated by transcranial Doppler at each stage of the experiment whereas dopamine produced unpredictable results. However, no significant difference was observed in terms of the absolute value of ICP or CBF. The high levels of CPP required in this study are not currently recommended which limits the scope of these results [34].

In a study using the same protocol and performed in 15 patients with GCS scores from 3 to 8, the aim was to maintain CPP at 65 mmHg. Norepinephrine and dopamine doses were 0.27 ± 0.2 μg/kg/min and 11.3 ± 3 μg/kg/min, respectively. Heart rate and cardiac index were higher in the dopamine group and a reduction in intramucosal pH was observed in the norepinephrine group without hyperlactatemia. Creatinine clearance was significantly increased in this same group. CPP and ICP on inclusion were 15 and 55 mmHg, respectively. The CPP target (>65 mmHg) was

reached with no significant difference between the groups. The velocities measured by transcranial Doppler increased uniformly after treatment and ICP increased non-significantly (18 ± 9 mmHg) [35].

Of these studies, three concluded in favor of the use of norepinephrine in head trauma patients. The first study showed an elevation in ICP after the administration of dopamine but the absence of randomization obviously limits the interpretation of the results [32]. The second and third studies were published by the same group, the same year in two different journals. Both studies indicated better norepinephrine predictability but the CPP objectives are no longer recommended in daily clinical practice [33, 34]. The fourth study was published in abstract form [35]. The CPP target was 65 mmHg. No difference in cerebral hemodynamics was observed. None of these studies had a large enough cohort to make definitive conclusions. Moreover, there are no data on the effect of catecholamines on the recovery and mortality of patients with severe head trauma, which illustrates the need for future clinical studies. The results of the above studies encourage the use of norepinephrine given its better predictability, despite the fact that this result only appears indirectly in these studies.

Conclusion

The data on the effects of catecholamines on cerebral hemodynamics are complex and variable depending on the type of artery (large or small), the condition of the hemato-meningeal barrier, changes in self-regulation, and the objectives of the study. Agents with a predominantly β-adrenergic action (dobutamine, isoprenaline) should not be used in head trauma patients to increase CPP. Norepinephrine and dopamine are the two best studied catecholamines. Overall, a local effect probably exists when there is rupture of the hemato-meningeal barrier but does not appear to be prominant in clinical practice. Administration of a catecholamine for a cerebral lesion restores CBF in the injured zones but the repercussions in terms of oxygenation are less obvious. There are few human studies and their methodology limits their scope. However, norepinephrine appears to be more appropriate than dopamine for the maintenance of CPP in patients with a cerebral lesion.

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The Delirious Patient in the ICU

K.H. Polderman and A.J.C. Slooter

Introduction

Until a few years ago, the occurrence of delirium or 'intensive care unit (ICU) psychosis' was regarded as a routine feature of life in the ICU. Delirium occurred with such a high frequency that it was often considered to be a routine consequence of prolonged stay – a combined effect of the patient's underlying illness and perhaps the administration of large amounts of sedatives.

Attending physicians tended not to worry about delirium too much; it was assumed that the problem would resolve once the patient's somatic condition improved, and delirium was regarded more as a nuisance and a patient management problem than as a life-threatening event. Delirium was usually not treated; treatment was mostly given only if the patient was completely unmanageable due to his/her restlessness, or became violent towards the medical and nursing staff. In many of these cases delirious patients were treated with sedatives rather than with antipsychotics.

In general, until the early 1990s, ICU policies regarding use of sedatives, morphine-like analgesics and neuromuscular blocking agents in mechanically ventilated patients were much more liberal, and very large doses were routinely used in most ICU patients. The reason for this was the widely held assumption that patients could otherwise not tolerate mechanical ventilation and other invasive ICU treatments, and that sedation and paralysis would also allow the patient to 'rest' and recover. More mundane factors also played a role; often the practices and treatments used for general anesthesia during major surgery were simply continued for longer-term management of patients in the ICU.

This attitude began to change in the mid-1990s, when it was realized that long-term use of very high doses of sedatives, opiates, and neuromuscular blocking agents was linked to adverse outcome and increased length of stay in the ICU. Kress and co-workers showed that length of stay in the ICU and hospital could be reduced by early tapering and daily interruption of sedative-drug infusions in mechanically ventilated patients [1]. These observations have led to a significant decrease in the use of sedatives, opiates and neuromuscular blocking agents in recent years, and thus to a rise in the number of more or less conscious patients in the ICU, especially in the recovery phase after acute illness. This in turn led to the realization that even more patients than presumed develop alterations in their mental status in the acute phase of critical illness and in later phases.

The true incidence of delirium remains a matter of debate. Various studies, using different assessment methods, have reported that between 16% and 83% of ICU patients become delirious during their stay in the ICU [2–13]. Part of this wide

range is probably explained by use of different assessment methods (see below) and differences in case mix. In addition, there are three distinct subtypes of delirium (hyperactive, hypoactive, and mixed), which may complicate the diagnosis as they, especially the hypoactive form, may be difficult to recognize particularly in ICU patients. Therefore, we will briefly discuss the difficulties and diagnostic aspects of delirium in critically ill patients.

Terminology and Definition

The terminology and definitions of ICU delirium can sometimes be somewhat confusing. Delirium is described in the Diagnostic and Statistical Manual of Mental Disorders (DSM IV) as "a disturbance of consciousness with inattention, accompanied by a change in cognition or perceptual disturbance, that develops over a short period of time (hours to days) and fluctuates over time" [14]. However, many different terms have been used to describe the syndrome of cognitive impairment in critically ill patients. Sometimes these terms are linked to specific disease states or conditions, such as the term septic encephalopathy. Sometimes they refer to a general state of confusion, e.g., terms such as ICU psychosis, acute confusional state, acute brain failure, ICU syndrome, etc. Sometimes the term encephalopathy is used to describe hypoactive delirium, whereas the other terms listed above refer to hyperactive delirium. As explained above the true incidence of delirium (especially in the ICU) remains a matter for debate, but it is clear that hypoactive delirium is far more common than the more easily recognizable pure hyperactive delirium. For example, Peterson et al. reported a frequency of delirium of 61% in a population of 614 critically ill patients, of whom only 2% had pure hyperactive delirium, while 43% had hypoactive delirium, and 55% had mixed-type delirium [10]. The pure hyperactive form was observed more frequently in younger patients, whereas the purely hypoactive form was more common in older patients [10].

Various assessment methods have been developed specifically to establish a diagnosis of delirium in critically ill patients. In the early 1990s, Inouye et al. developed the Confusion Assessment Method (CAM) for use as a quick delirium screening tool for patients in the general ward [15]. This method was subsequently adapted for use in the intensive care setting by Ely and co-workers, as the Confusion Assessment Method for the ICU (CAM-ICU); the first paper describing this screening method was published in 2001 [2]. Another screening system specifically designed for use in the ICU setting, the Intensive Care Delirium Screening Checklist (ICDSC) developed by Bergeron et al., was published in the same year [3].

Studies using the CAM-ICU scale to screen for delirium have mostly reported higher incidences of delirium than those using the ICDSC; this may be partly due to the assessment of patients with decreased consciousness, who are excluded in the ICDSC scale but who may be diagnosed as delirious with the CAM-ICU system.

Incidence and assessment systems

The reported incidence of delirium in ICU patients has varied significantly. The following numbers have been reported in the medical literature in recent years: 11% [4], 16% [3, 5], 19% [6], 22% [7], 32% [13], 48% [8], 70% [9], 71.8% [10], 81.3% [11], 81.7% [12], and 83.3% [2]. These differences may be due to variations in case

mix and severity of illness in the populations studied, as well as differences in age and other factors. In addition, the screening method used (CAM-ICU, ICDSC, or other systems not specific for ICU patients) may play a role, with studies using the CAM-ICU systems mostly reporting a higher incidence of delirium (48%-83%) than those using the ICDSC system (11%-32%).

Detecting delirium in the ICU can be difficult because many patients are mechanically ventilated and are, therefore, not able to communicate verbally. In addition, the patient's level of consciousness and awareness may be affected by treatments with sedatives, opiate analgesics and other drugs. Peterson et al. identified several independent risk factors for under-recognition of delirium by nurses in the ICU setting. These included hypoactive delirium (odds ratio [OR] 7.4, 95% confidence interval [CI] 4.2–12.9), age ≥80 years (OR 2.8, 95% CI 1.7–4.7), vision impairment (OR 2.2, 95% CI 1.2–4.0), and pre-existing cognitive impairment (OR 2.1, 95% CI 1.2–3.7). The risk for under-recognition increased with the number of risk factors present, from 2% when no risk factors were present to 44% when 3 or more risk factors were present [10].

Impact on Clinical Outcome

In spite of the relatively wide range in the reported incidence of delirium in the ICU, most of the studies listed above have reported an association between delirium and adverse outcome, increased length of stay in the ICU, and hospital and higher mortality [5, 8, 11–13]. One study reported a threefold increase in risk of death associated with the development of delirium [12]. The association between delirium and unfavorable outcome persists when adjustments are made for age, co-morbidity, and severity of illness, which suggests that delirium may be causally related to these adverse events. This has been demonstrated most clearly in severely ill patients with prolonged length of stay in the ICU; however, similar observations have been made in less severely ill patients. For example, Thomason and associates found that delirium was a predictor of longer stay in the ICU (average: 1 day) and hospital (average: 2 days) in non-mechanically ventilated patients with an average APACHE score at admission of 15 [8].

An association between delirium and adverse outcome, including mortality, has also been reported outside the ICU setting in general hospital wards and other settings [20–21]. Not all studies have found a higher mortality associated with development of delirium, but almost all have linked delirium to increased length of stay in the ICU and hospital, regardless of the initial severity of illness [1–13].

Most of the studies listed above [5–8, 11–13,] as well as various others have also reported that developing delirium leads to significant additional costs. One study in ICU patients reported an average increase in costs of 40%, after adjustment for variables such as severity of disease and co-morbidities [22]. Ely has estimated that the annual additional health care expenditure linked to ICU delirium in the USA alone lies between $1.5 billion and $20 billion (€1.2–16 billion) [23]. Even if the true figure lies at the low end of this estimate or even lower, the potential for reducing expenditure by prevention or early treatment of delirium seems huge.

However, it should be realized that although many studies have documented a relationship between delirium and adverse outcome, no prospective studies have so far been performed showing that early and/or aggressive treatment of delirium can actually improve patient outcome [25]. However, this does seem highly likely (par-

ticularly in hyperactive delirium, which increases the risk of complications such as auto-extubation and accidental removal of central lines), and the viewpoint that recognition and early treatment of delirium is an important issue in critically ill patients has become more or less generally accepted.

Risk Factors and Therapeutic Options

Numerous risk factors for developing ICU delirium have been identified in various clinical trials. These are listed in Table 1. As explained above, no prospective studies assessing the impact of preventive and therapeutic measures against delirium on ICU mortality have so far been performed. However, it is generally accepted that prevention of delirium is likely to improve outcome, if only through prevention of complications such as self-extubation and other ICU complications [23–26].

It has been shown that simple preventive measures can be highly effective in preventing and/or mitigating delirium. These are also listed in Table 1. Inouye et al. were able to reduce the incidence of delirium by 40% in elderly patients who had been admitted to a general ward through a combined strategy of avoiding sleep deprivation, dehydration, electrolyte disorders and hypoxemia, and by applying cognitive therapies, correcting visual and auditory impairments, repeated re-orientation, and early mobilization [27]. Preventive measures should also include regular screening of ICU patients for delirium, and increasing awareness in the medical and nursing staff.

Another potential method to reduce the incidence of delirium is the administration of prophylactic drugs. Kalisvaart and co-workers [28] recently published the results of a randomized placebo-controlled trial in which low-dose prophylactic treatment with haloperidol was used to prevent delirium in the post-operative setting. Although the overall incidence of delirium was not reduced significantly in this study, the authors did report a positive effect on the severity and duration of delirium. In addition, patients receiving haloperidol prophylaxis had a decreased length of stay in the hospital. Trials are ongoing to assess whether haloperidol prophylaxis could have a similar beneficial effect in ICU patients.

Development of delirium may signify the presence of an acute somatic illness, such as a nosocomial infection or a severe metabolic disorder. This should be kept in mind, and the presence of an underlying somatic disorder should be ruled out if a patient develops delirium. Care should also be taken to determine that a patient's acute confusional state is not caused by pain.

The next step is to make sure whether the 'simple' preventive measures listed above and in Table1 have been implemented, and if not to rectify this situation. Normalization of sleep-wake cycles may be particularly important, and this is something often overlooked in the daily routine in the ICU. Aizawa et al. performed a randomized study to assess the effect of diazepam and pethidine administration during the first three nights following a surgical intervention, and reported a reduction in the occurrence of delirium from 35% to 5% [29]. This would suggest that the prophylactic use of sedatives to simulate a normal sleep-wake rhythm could be an effective strategy to prevent delirium. However, others have reported that the use of benzodiazepine prophylaxis can actually *increase* the risk for delirium [30]. Thus further studies will be needed to clarify this issue.

Pharmacologic options include discontinuation of drugs that can cause or aggravate delirium (including benzodiazepines and narcotics, which are often inappropri-

Table 1. Risk factors and therapeutic options in delirium.

Risk Factors for Delirium

Prior medical history
High age
Dementia
Other neurological diseases with cognitive impairment
Vascular disease
Hypertension
Diabetes
Smoking
Alcohol abuse
Visual impairment
Hypothyroidism

Status at ICU admission
Greater severity of illness (as indicated by high APACHE, SAPS or SOFA scores)

Course of illness during ICU stay
Development of sepsis/nosocomial infections
Development of organ dysfunction
Hypoxemia
Electrolyte disorders

ICU treatments
(Excessive) use of sedatives
(Excessive) use of opiate analgesics
Sleep deprivation
Dehydration
Restraints, immobilization
Number of different drugs used to treat the patient

Therapeutic Options

Preventive strategies
Awareness in medical and nursing staff
Early and regular screening for delirium (especially hypoactive form)
Avoiding sleep deprivation
Cognitive stimulation
Early mobilization
Avoiding or promptly treating hypoxia, dehydration, uremia and electrolyte disorders
Early use of visual aids (glasses) and hearing aids
ICU noise-reduction strategies
Music listening, television, etc.
If possible: early removal of central lines, catheters etc.
Consider family involvement
Avoid excessive use of sedatives and opiates

Pharmacological options
Conventional antipsychotic agents
- Haloperidol
- Droperidol
- Chlorpromazine

'Second generation' antipsychotic agents
- Olanzepine
- Quetiapine
- Risperidone
- Ziprasidone

ately used in the ICU to treat 'confusion'). This is especially important if the patient is in pain; this of course requires appropriate analgesia, and sedating patients who are in pain will significantly increase the risk of developing delirium (pain + sedation/decreased consciousness = delirium). Some preliminary evidence suggests that perhaps the use of short-acting sedatives such as dexmedetomidine could help decrease the risk of developing delirium, particularly for short but painful and/or unpleasant procedures [23, 31]. However, the evidence for this is still limited.

Ouimet and co-workers recently reported that even extremely brief periods of sedation, for example to facilitate the performance of procedures such as a bronchoscopy, were associated with increased risk of delirium [13]. For this and other reasons the unnecessary and/or excessive use of sedatives (and opioids) should be avoided in critically ill patients. In addition, doses of sedation (and opiate analgesia) should be tapered whenever possible (while, of course, avoiding under-treatment and providing sufficient levels of comfort to the patient with the lowest possible doses).

If and when delirium develops in ICU patients, treatment with antipsychotics such as haloperidol should be considered. Unfortunately, no prospective studies assessing the use and effectiveness of haloperidol in the ICU setting have so far been performed. However, based on anecdotal reports and non-randomized studies, haloperidol appears to be effective, and its use to treat delirium in the ICU is recommended in the guidelines of the Society of Critical Care Medicine (SCCM) [32]. Haloperidol is a dopamine D_2 receptor antagonist which may work in part by enhancing the release of acetylcholine [33]. Other drugs with similar mechanisms include droperidol (which has just been re-introduced to the market) and chloropromazine.

Other antipsychotics and neuroleptic agents with broader receptor affinities (such as risperidol, olanzapine, ziprasidone) may be (more) effective, especially in non-agitated hypoactive delirium. Apart from the dopamine D_2 receptors these drugs also target other neurotransmitters such as serotonin, acetylcholine, and norepinephrine [34]. However, these drugs have not been well studied in the ICU setting. Therefore, in most situations haloperidol remains the drug of choice to treat delirium in the ICU.

All patients receiving first or second generation antipsychotics should be carefully monitored for side effects such as QT prolongation and polymorphous ventricular tachycardia ('torsade de pointes') [26]. This is especially important because many other drugs commonly used in the ICU (macrolides, fluoroquinolones, azole antifungals, amiodarone, some calcium channel blockers) can also influence QT time, putting the ICU patients receiving several of these drugs simultaneously at a cumulative risk of developing QT prolongation. Other side effects include extrapyramidal effects, malignant hyperthermia, hypotension, and anticholinergic effects as well as glucose and lipid dysregulation. Although antipsychotics are generally well tolerated and the likelihood for developing these side effects is relatively low, patients should nevertheless be carefully monitored. In recent years a number of authors have described successful treatment of persistent delirium with cholinesterase inhibitors [35–37]. This deserves further study in ICU patients.

Conclusion

ICU patients appear to be at high risk of developing delirium. Various studies have shown that developing delirium significantly increases the risk of death, as well as the length of stay in ICU and hospital, and health care costs. Therefore, a concerted

effort to prevent and aggressively treat delirium seems warranted, although no prospective studies have so far been performed demonstrating benefits in survival resulting from these strategies.

A number of preventive measures can help decrease the likelihood of developing delirium, and these should be applied (adapted to local circumstances) to improve outcome and prevent delirium in the ICU. A potential problem is that inattention and disorganized thinking, which are considered to be the key features of delirium, can be difficult to assess in patients who are mildly or moderately sedated. Hypoactive ('silent') delirium may, in particular, be easily overlooked.

Two assessment systems, CAM-ICU and ICDSC, are available, which have been designed specifically to screen for delirium in patients who are unable to communicate verbally. A number of other assessment systems have been tested only outside the ICU. At this moment insufficient evidence exists to recommend one over the other. Based on the available evidence all forms of delirium should be treated promptly, and a pro-active approach with early and regular screening for delirium seems warranted. If preventive measures are insufficient, treatment with haloperidol or another antipsychotic should be initiated, and patients should be carefully monitored for side effects, especially QT prolongation.

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Management of Burns

Emergency Room and Acute Care of the Critically Ill Burned Patient

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Introduction

The natural history of serious burns is characterized by burn shock, which can be fatal within the first few hours to days, particularly in those with untreated large burns. Burn wound sepsis is the major cause of mortality among those who survive the burn shock. Survival and outcome after major burn injury have improved over the last 20 years due to improved understanding of the pathophysiologic nature of burn injury, better resuscitation, and advances in control of post-burn sepsis including early, aggressive surgical treatment [1].

Burn Injury Pathophysiology

Severe burn injury results in significant hypovolemic shock and tissue trauma. The volume loss is related to the release of local and systemic inflammatory mediators at local and distant sites. Increases in pulmonary and systemic vascular resistance in association with myocardial depression occur despite adequate fluid resuscitation [1]. Mediators implicated in the pathogenesis of burn injury include histamine, serotonin, kinins, oxygen free radicals, and products of the arachidonic acid cascade [2–4]. These mediators alter vascular permeability directly and/or indirectly by increasing microvascular hydrostatic pressure or surface area via arteriolar vasodilation. Because of the multiplicity of mediators, therapy to antagonize one single mediator (e.g., histamine) has not proved successful. Sympathetic stimulation and hypovolemia related to the injury result in release of catecholamines, vasopressin, angiotensin II, and neuropeptide Y leading to vasoconstriction and increased systemic vascular resistance (SVR) [3]. Increased SVR immediately after burn injury is also partly the result of increased blood viscosity secondary to hemoconcentration from fluid loss, which contrasts with other forms of trauma where red cells are also lost. Organs particularly susceptible to ischemia due to inadequate resuscitation and vasoconstriction are the kidneys and gastrointestinal (GI) tract. Myoglobinemia due to muscle damage can also contribute to the renal insult [5]. Sustained vasoconstriction of the GI tract can occur even with adequate resuscitation, leading to ischemia and bacterial translocation [6].

The fluid loss of burns occurs not only at the area of burn wound but also at distant non-burned tissues and can occur up to at least 24–48 hours after the injury [7]. The generalized capillary leak leads to decreased plasma volume, cardiac output, and urine output. Thus, the initial therapeutic goal is restoration of intravascular volume in order to preserve tissue perfusion and minimize the ischemia/reperfusion

injury. Pulmonary edema is not uncommon, especially after the fluid resuscitation phase and restoration of capillary integrity (48–72 hours after burn injury), when the edema fluid is reabsorbed leading to hypervolemia. Initially, the pulmonary edema is the result mainly of increased capillary pressure secondary to increased pulmonary vascular resistance. It is likely that some left heart failure also contributes to the pulmonary edema [8]. Developing hypoproteinemia may be an important contributing factor for post-burn pulmonary edema [9]. Pulmonary dysfunction associated with inhalation injury is discussed later.

The early phase of burn injury (first 1–2 days), characterized by decreased cardiac output, and metabolic rate, is followed by a phase of increased cardiac output and metabolic rate, which plateaus around post-burn day five. This hypermetabolic and hyperdynamic response is more severe and sustained than any other form of trauma [3, 10] and lasts even as long as 9 to 12 months post-injury in patients with major burns [11]. As a result, lean muscle mass continues to decrease with a negative nitrogen balance despite aggressive nutritional support even with insulin. Loss of a quarter of total body nitrogen balance can be fatal and this limit can easily be reached within 3–4 weeks in burn patients not receiving maximal nutritional support [12].

Initial Evaluation

Between 5–7% of patients admitted to burn centers suffer from non-thermal traumatic injuries. Therefore, all burn patients should be approached initially as multiple trauma patients [13]. Securing the airway is the first priority during the initial evaluation; safe airway management begins with its assessment. The presence of airway injury, signs of airway obstruction, and presence of preexisting airway abnormality should be obtained as soon as the patient arrives at the hospital. Airway injuries may not be evident initially but, with massive fluid resuscitation, airway edema may result. As a general rule it is safer to intubate the patient early than risk a difficult intubation later when airway swelling has occurred. With severe injuries of the face or neck, direct laryngoscopy may be difficult or impossible. When laryngoscopy and endotracheal intubation are anticipated to be difficult, options include a cricothyroidotomy or tracheostomy.

Inhalation Injury

Inhalation injury increases the resuscitation fluid requirements by up to 50% and is a major source of mortality in burn patients [14]. A history of exposure to fire in a closed space, loss of consciousness, and presence of chemical irritants, in combination with the physical examination revealing carbonaceous sputum, singed nasal or facial hair are all suggestive of inhalational injury. Chest X-rays are usually normal until secondary complications, such as atelectasis or pneumonia develop. Fiberoptic bronchoscopy may be used to support the diagnosis, which may reveal carbonaceous debris, erythema, or ulceration. The mechanism of inhalation injury consists of a combination of: 1) direct thermal injury to the upper airway from inhalation of hot gases; 2) damage to the cellular and oxygen transport processes by inhalation of carbon monoxide and cyanide; and 3) chemical injury to the lower airways caused by inhalation of the toxic products from the fire.

Direct heat injury to the upper airway can lead to marked swelling of the tongue, epiglottis, and glottic opening resulting in airway obstruction [15]. Airway swelling may not occur immediately but may develop over a period of hours (especially with concurrent fluid resuscitation). Therefore, a high index of suspicion and frequent re-evaluations are essential. Upper airway edema will have more immediate consequences in smaller children. Signs of impending upper airway obstruction include hoarseness, chest retraction, and stridor. If the history and physical examination are suggestive of inhalational injury one should have a low threshold for early intubation, particularly in children. If intubation is delayed and significant swelling occurs, intubation can become difficult or impossible. Upper airway edema usually resolves in 7–14 days and is facilitated by elevation of the head of the bed and avoidance of excessive fluid administration.

Carbon Monoxide Poisoning

Because carbon monoxide binds to hemoglobin, 200 times more readily than oxygen, it can significantly reduce the oxygen carrying capacity of blood [16]. Binding of carbon monoxide to hemoglobin also shifts the oxyhemoglobin dissociation curve to the left. In addition, carbon monoxide interferes with peripheral oxygen utilization by binding to molecules such as myoglobin, NADPH reductase, and the cytochrome oxidase system resulting in impaired oxidative phosphorylation at the mitochondria [16]. The mitochondrial dysfunction due to carbon monoxide has best been documented in the heart, where it produces myocardial stunning [17]. The decreased oxygen delivery to the tissues, impaired release of the available oxygen at the capillaries, and weakened ability to utilize the delivered oxygen results in tissue hypoxia and metabolic acidosis.

The clinical findings of carbon monoxide poisoning are variable and largely nonspecific, and include headache, nausea, shortness of breath, tachypnea, angina, and changes in mental status [17]. The half-life of carboxyhemoglobin is 4 hours when breathing room air. This is reduced to 40–60 minutes when breathing 100% oxygen. Hyperbaric oxygen will further reduce the half-life of carboxyhemoglobin to 23 minutes [17]. In those patients with more severe exposures (carboxyhemoglobin level greater than 30% or neurologic changes), hyperbaric oxygen has been suggested to diminish the incidence of long term neurological sequelae [18]. The hyperbaric chamber is a difficult environment to monitor, administer fluid resuscitation, and provide acute burn care.

The absorbance spectrum of carboxyhemoglobin and oxyhemoglobin are similar. Therefore, standard pulse oximeters cannot distinguish between the two forms of hemoglobin. Oximeter readings will therefore be normal even when lethal amounts of carboxyhemoglobin are present in the blood. The PaO_2 measured from arterial blood gas sample reflects the amount of oxygen dissolved in blood and does not indicate oxygen bound to hemoglobin (saturation). Thus, the PaO_2 can be normal even with high levels of carboxyhemoglobin. The diagnosis of carbon monoxide poisoning is made by measuring the carboxyhemoglobin level in arterial blood, expressed as a percent saturation of hemoglobin. Because of the inevitable time delay between exposure and testing, prophylactic high concentrations of oxygen therapy are indicated even before results of carbon monoxide levels. The levels of carboxyhemoglobin measured may not reflect the true extent of poisoning especially if the patient has been breathing a high concentration of oxygen.

Cyanide Poisoning

Hydrogen cyanide (HCN) is a toxic gas produced in fires by the burning of nitrogenous materials, including natural fibers (wool and silk) and synthetic polymers (polyurethane, polyacrylonitrile and acrocyanate). HCN binds to mitochondrial cytochrome oxidase, which catalyzes the last step in the oxidative phosphorylation (ATP formation) pathway, preventing utilization of oxygen by mitochondria. HCN also arrests the tricarboxylic acid cycle. The pathophysiological sequel of cyanide poisoning is that cells can only generate ATP via anaerobic metabolism, which results in a metabolic acidosis from lactic acid production.

As with carbon monoxide poisoning, HCN toxicity can be difficult to diagnose, but should be suspected in any patient with a history of inhalation injury. Concentrations greater than 20 parts per million (ppm) are considered dangerous. Early symptoms include headache, dizziness, tachypnea, and tachycardia. HCN toxicity may manifest as S-T segment elevation on the electrocardiogram (EKG), mimicking acute myocardial infarction. Cyanide increases minute ventilation through carotid body and peripheral chemoreceptor stimulation. Concentrations of 100 ppm can lead to seizures, coma, respiratory failure, and death. Laboratory findings include an anion gap metabolic acidosis that does not respond to oxygen administration. The mixed venous oxygen saturation (SvO_2) in cyanide poisoning is often elevated suggesting an inability to utilize oxygen [19]. Direct detection of cyanide poisoning in the blood is difficult. Cyanide has a short half-life in blood and measurement is not universally available.

The deleterious effects of HCN are normally neutralized by the conversion of cyanide to thiocyante, which is excreted in the urine. This can be enhanced by the administration of exogenous thiosulfate [20]. Cyanide can also combine with hydroxycobalamin (vitamin B12), which forms cyanocobalamin. Nitrate administration results in the oxidation of hemoglobin to methemoglobin, which can combine with cyanide to form cyano-methemoglobin. Methemoglobin, however, does not transport oxygen and may thus be harmful in a patient whose oxygen carrying capacity is already compromised because of carboxyhemoglobin.

Chemical Injury to the Lower Airways

The burning of many materials in a house fire can release combustion products that are toxic and damaging to the lower airways, including respiratory epithelium and capillary endothelium of the airway and alveoli. The damage to epithelium results in destruction of mucociliary transport, which impairs clearance of bacteria. Alveolar collapse and atelectasis can occur because of loss of surfactant production or from plugging due to mucus debris [21]. Chemical damage to alveoli and its capillaries will lead to extravasation of plasma proteins. Activation of injury-induced alveolar macrophages will lead to further inflammatory response and damage. Bronchial swelling and bronchospasm can lead to obstruction of both large and small airways. The end result is respiratory failure from increased V/Q mismatch, decreased lung compliance, and increased dead-space ventilation generally occurring 12–48 hours after the inhalation event [15]. The respiratory failure may further worsen several days later from continued airway mucosal sloughing, barotrauma, bacterial invasion, and pneumonia [22].

Injury to the lung can occur in patients with severe cutaneous burns in the absence of inhalational injury [23]. Mechanisms include inflammatory mediators from the burn-injured area, effects of fluid resuscitation, and infection. Pulmonary

edema often occurs after a large burn injury because of decreased oncotic pressure, and pulmonary artery hypertension. After restoration of the capillary integrity, the edema fluid from throughout the body is resorbed and can lead to hypervolemic pulmonary edema.

Fluid Resuscitation

Multiple fluid resuscitation formulae exist for estimating fluid needs. As a general rule, burns of < 15% total body surface area (TBSA) are not associated with extensive capillary leak and can be managed with fluid of 1.5 times maintenance rate and careful attention to the hydration status. The commonly used resuscitation formulae differ somewhat in their recommendations of the amount of crystalloid and colloid (Table 1). Most formulae recommend isotonic crystalloid initially and later use of colloids [24]. The time at which colloid administration is initiated varies from institution to institution, and depending on the size of the burn, patient age and other cardiorespiratory parameters. Lactated Ringer's solution, or similar composition-solutions, is often the crystalloid chosen as it contains physiologic concentrations of major electrolytes and lactate replaces some of the chloride in the solution resulting in less hyperchloremic metabolic acidosis compared to normal saline. In younger children and patients where hypoglycemia is a potential concern, 5% dextrose can be added to the lactated Ringer's solution.

Once capillary integrity returns, generally by 24–48 hours, most resuscitation formulae recommend administration of colloid. Most authorities advocate 5% albumin in isotonic crystalloid, which is ideally administered by continuous infusion at a dose adjusted by burn size. Side effects of large volume crystalloid resuscitation include pleural and pericardial effusions, and intestinal ileus with abdominal compartment syndrome. Thus, more burn units are advocating early use of colloids. Resuscitation with hypertonic saline is not part of practice in most burn units since there was a significant increase in renal failure and deaths in patients treated with hypertonic saline compared to lactated Ringer's solution [25].

The Parkland formula remains the most widely used resuscitation formula for burn injury in the United States. The Parkland formula, 4 ml per %TBSA burn per kg body weight, is administered over the first 24 hours with one half of the calculated volume administered during the first eight post-injury hours [26]. The remain-

Table 1. Formulae for estimating burn resuscitation fluid needs

Crystalloid formulae		
Parkland	Lactated Ringer's	4 ml/kg/% TBSA burn
Modified Brooke	Lactated Ringer's	2 ml/kg/% TBSA burn
Colloid formulas		
Evans	Normal saline	4 ml/kg/% TBSA burn
	Colloid	1 ml/kg/% TBSA burn
	5% dextrose	2000 ml/24 hours
Brooke	Lactated Ringer's	1.5 ml/kg/% TBSA burn
	Colloid	0.5 ml/kg/% TBSA burn
	5% Dextrose	2000 ml/24 hours

TBSA: total body surface area

Table 2. Burn resuscitation end-points

Arousable and comfortable
Warm extremities
Systolic blood pressure: For infants, 60 mmHg; for older children, 70–90 mmHg + 2 x age (in years); for adults, mean arterial pressure >65 mmHg or within 20% of baseline
Heart rate: 80–150 bpm (age dependent)
Urine output 0.5–1 ml/kg/hr (glucose negative)
Base deficit <2 mEq/l

ing half is administered over the next 16 hours. If resuscitation is delayed, this volume is administered so that infusion is completed by the 8th post-injury hour. No matter which formula is used, it should serve only as a guideline and fluid resuscitation be titrated to physiologic endpoints (Table 2). Actual fluid requirements can vary depending on size of the burn, patient's weight, interval from injury to start of resuscitation, presence of associated injuries and presence of inhalational injury.

Base deficit is another indicator of global tissue perfusion and is calculated from an arterial blood gas using normograms. In a retrospective study in burn patients, Kaups et al. showed the base-deficit was predictive of fluid requirements and survival [27]. In burn injury, when tissue perfusion is not uniform throughout the body, an indirect measure of less well-perfused tissues may prove useful. One such measure that has been described is the intramucosal gastric pH (pHi), as measured by gastric tonometry. After burn injury, blood flow to the heart, brain, and kidneys is maintained at the expense of splanchnic blood flow. Several studies have shown that a lower pHi is predictive of organ failure and increased mortality and have suggested the use of pHi as a guide to resuscitation [28, 29]. This technique, however, has not become routine in clinical practice.

A small percentage of patients fail to respond to conventional fluid resuscitation. These patients frequently have large, deep burns, are at extremes of age, or have inhalational injury or coexisting medical conditions [30]. If the total fluid requirement exceeds 6 ml/kg/%TBSA/24 hours, it is advisable to obtain more information regarding intravascular volume. This information can be obtained by physical exam or by measurement of central venous pressure (CVP) and/or pulmonary artery pressure. Based on the information, inotropic support may be required. Echocardiographic evaluation of ventricular volume and function has been used in burns [31]. After 24–48 hours, capillary integrity returns to normal in non-burned areas especially with repletion of circulating volume. At this stage, fluid requirements dramatically decrease; it is important to decrease fluid administration promptly as overzealous administration of fluid can be associated with substantial morbidity.

Estimation of Size/Depth of burn

The magnitude of burns is classified according to the TBSA involved, depth of the burn, and the presence or absence of inhalational injury. The burned TBSA can be estimated in adults using the 'Rule of Nines'. Each of the upper arms and head in the adult contribute to 9% of TBSA, while the front and back of the trunk, and each of the lower limbs contribute to 18% TBSA. Alternatively, estimation by palmar surface of the hand (without the fingers, 0.5% TBSA) is age invariant and can also provide a quick estimate [32]. The depth of skin destruction is characterized as first-, sec-

ond- or third-degree, based on whether there is superficial, partial, or full thickness destruction of the skin. Fourth degree is used to describe burns that have injured deeper structures such as muscle, fascia and bone. Deep second and third degree burns require surgical debridement and grafting, whereas more superficial burns do not. Revisions of burn-depth estimations are often necessary in the first 24 to 72 hours. This is especially true in patients with thin skin, who often sustain deeper burn injuries than evident on initial examination. Skin can be presumed to be thin in young children and the elderly. Mortality from burn injury is related to the TBSA of deep second or third degree burns. A large analysis revealed three risk factors as predictive for death after burns: Age more than 60 years, burn size more than 40% TBSA, and inhalation injury. Mortality rates were 0.3, 3, 33, or 90% depending on whether 0, 1, 2, or 3 risk factors, respectively, were present [33].

Burn Center Referral and Organ Specific Care

Data exist linking improved outcomes from major burns with early referral to a burn center [34]. It is recognized that burn care requires specialized expertise, personnel, and equipment which are not cost-effectively maintained in low volume centers. Following establishment of the airway and correction of immediate life-threatening problems, the next section focuses on aspects of the neurologic, otolaryngologic, ophthalmic, chest, cardiac, abdomen, genitourinary, and extremity issues that are related to acute burn injury.

Neurologic

Central nervous system (CNS) function can be altered by inhalation of neurotoxic chemicals, effects of hypoxia and hypotension, and from the effects of anxiety and pain or their treatment. It is essential to rule out coexisting intracranial and cervical spine injury by history, clinical examination and radiologic imaging. Patients with serious injuries commonly become obtunded because of hemodynamic instability as well as from the administration of drugs for sedation and analgesia. Therefore, it is important to know that this change does not represent a missed intracrainal injury. In rare instances, patients with deep neck burns may need escharotomies at that site to facilitate venous drainage.

Otolaryngologic and Ophthalmic

The primary otolaryngologic and ophthalmic evaluation includes assessment and initial treatment of burns to the airway, corneal epithelium, and the external ear. Signs of airway involvement include perioral and oropharyngeal burns, presence of carbonaceous sputum, and signs of hoarseness. Hot liquid can be aspirated in conjunction with a scald injury to the face and can result in rapid airway compromise. One should have a low threshold for intubation when potential airway involvement exists. The globes of the eye should be examined early since adnexal swelling can make the examination difficult. Severe corneal burns are usually obvious by the cloudy appearance they impart, but damage is more often subtle requiring florescein staining. Topical antibiotics are the initial treatment if an injury is present. Burns to the external ear can be complicated by suppurative chondritis. Treatment with topical mafenide acetate cream may decrease its development.

Chest

The focus of the initial evaluation of the chest is to ensure chest wall compliance of both hemithoraces. Impaired chest wall compliance can result from deep circumferential eschar impairing chest wall excursion and/or bronchospasm resulting from inhalation of airway irritants. The inhalation of toxic fumes may precipitate bronchospasm in a patient with a previous history of asthma. A patient with decreased compliance because of a circumferential eschar will exhibit rapid shallow respirations. A patient on a ventilator will show an increase in peak airway pressures. Escharotomy is the treatment of the latter condition while bronchodilators, pulmonary toilet, and ventilation strategies to minimize breath stacking are used to treat bronchospasm. Severe inhalational injury may result in thick secretions and the sloughing of airway mucosa, which can occlude the endotracheal tube or distant bronchi resulting in atelectasis and collapse. In these instances, suctioning and bronchoscopy may be required.

Abdomen

Primary objectives in the evaluation of the abdomen are to exclude associated injuries, ensure that eschar does not impair adequate abdominal wall compliance to permit ventilation, decrease the risk of gastric dilation, and prevent gastrointestinal ulceration. Coincident abdominal trauma should be evaluated with imaging studies or diagnostic peritoneal lavage if indicated. Occult abdominal trauma can explain excessive fluid resuscitation requirements or a paradoxical fall in hematocrit in the early phase of burn injury. In some cases, torso escharotomies may be necessary to facilitate spontaneous respiration or mechanical ventilation in patients with deep circumferential eschars. Circumferential abdominal eschar, accumulation of intraperitoneal fluid, or bowel edema can lead to abdominal compartment syndrome, leading to diminished urine output, decreased pulmonary compliance, and hemodynamic instability [35]. Obtaining bladder pressure measurements can be useful in the diagnosis. In some cases abdominal decompression may be necessary. Patients with severe burns often develop a paralytic ileus and require nasogastric decompression for varying lengths of time. Gastroduodenal ulceration is a risk in severe burn injury and ulcer prophylaxis with H_2 receptor antagonists or proton pump inhibitors should be initiated as early as possible.

Genitourinary

Catheterization of the bladder is important in patients with moderate to severe burns, who, therefore, require intravenous fluid resuscitation, since it facilitates the use of urine volume and quality as an indicator of adequacy of resuscitation. Soft tissue swelling in the genital area can be significant with severe burn injury whether or not the burn involves the genital region. This can make urinary catheterization more difficult as time passes in the acute resuscitation phase. For this reason an appropriate size Foley catheter should be inserted as early as possible. In males it is important to ensure that the foreskin is reduced over the urinary catheter after its insertion to prevent the development of paraphimosis as soft tissue edema develops.

Extremities

Exclusion of associated (non-burn) injuries and monitoring of peripheral perfusion are the initial priorities in evaluation of the extremities. Extremity perfusion can be compromised by soft tissue swelling in the noncompliant fascial compartments or by circumferential eschar. Extremities that are at risk for ischemia, especially in those with circumferential burns or with electrical injury, should be monitored closely for tense fascial compartments and signs of impaired perfusion. Frequent checks of pulses, capillary filling, venous congestion, and Doppler blood flow are important. Dressings should be loosely applied to facilitate frequent examination. Tense extremities should be decompressed by escharotomy and/or fasciotomy when clinical examination reveals signs of impaired perfusion. Escharotomies can be performed at the bedside with use of electrocautery to minimize blood loss. The need for escharotomy usually becomes apparent in the early hours of acute resuscitation. Fasciotomies are generally performed in the operating room to minimize damage to the underlying structures that can be obscured by the tissue edema.

Antibiotics

Prophylactic antibiotics have no proven role in burn care and are not routinely given [36]. All burn injuries are potentially contaminated soft tissue wounds, and, therefore, tetanus toxoid should be given to all burned patients [37]. If the patient has not been previously immunized, tetanus immunoglobulin as well as tetanus toxoid should be administered.

Electrical Injuries

Electrical burns can have acute and chronic effects not seen with other types of burn injury, and with morbidity much higher than expected based on burn size alone [38]. Approximately 15% of patients sustaining electrical injury suffer other traumatic injury in addition to their burn. These injuries often involve falls, being thrown against an object, or result from tetanic muscle contractions.

Both arrhythmias and direct myocardial injury can result from electrical injury. Creatine kinase and MB-creatine kinase enzymes are poor indicators of myocardial injury in the absence of EKG findings, particularly if muscle injury is present [39]. The diagnostic value of cardiac troponin levels has not been evaluated in this setting. The myocardial injury behaves more like a cardiac contusion than a myocardial infarction, with minimal hemodynamic consequences. This may be related to the fact that the heart, unlike the skeletal muscle cannot sustain tetanic contractions. Virtually any cardiac arrhythmia can result from electrical injury. Ventricular fibrillation is the most common cause of death at the scene of the injury. Arrhythmias from electrical injury are managed using the same medical therapies as those resulting from any other cause. Patients with electrical injury should have EKG monitoring during transport to the hospital, in the emergency room, and afterwards. Indications for more prolonged cardiac monitoring include: 1) documented cardiac arrest; 2) cardiac arrhythmia on transport or in the emergency room; and 3) abnormal EKG [40].

The hidden (deeper) injury associated with electrical burn makes the standard fluid resuscitation formula inaccurate. Adequate fluid resuscitation is obtained by

achieving the standard resuscitation endpoints described previously. Myoglobinuria due to muscle damage will manifest as pigmented urine and usually indicates more severe muscular damage. Myoglobin and hemoglobin pigments pose risk for acute renal failure and require prompt treatment with crystalloid loading to a target urine output of 2 ml/kg/hr. Addition of sodium bicarbonate to intravenous fluid may facilitate pigment clearance and minimize renal injury. Mannitol and furosemide are also effective in promoting a prompt diuresis, but compromise the value of urine output as an indicator of adequacy of resuscitation.

Conclusion

Care of the severely burned patient requires the team effort of emergency room physicians, anesthesiologists/intensivists, psychiatrists, surgeons, nursing staff, and the paramedical personnel. Intensive care management of the patient necessitates an understanding of the early and late pathophysiology of the injury and careful attention to detail. An organized team approach will lead to safe management and avoidance of complications, and thus decrease morbidity and mortality.

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Early Manipulation of Metabolic Changes due to Severe Burns in Children

W.B. Norbury, M.G. Jeschke, and D.N. Herndon

Introduction

Burns account for around 700,000 emergency department visits every year resulting in around 50,000 admissions to hospital in the United States [1]. Around 50% of these admissions have burns of less than 10% total body surface area (TBSA) and, as such, have near normal metabolic rates. For the remainder, the rise in metabolic rate is linked to burn size and for those with severe thermal injuries (>40% TBSA) the change in patient metabolism is, if left unchecked, set to last for more than 12 months. The change contributes, at least in part, to long term deleterious effects on the individual. It has been previously shown that the ensuing period of hypermetabolism and catabolism following a severe burn leads to impaired immune function, decreased wound healing, erosion of lean body mass, and hinders rehabilitative efforts delaying reintegration into normal society. However, the magnitude and longevity of these changes has yet to be fully elucidated. Strategies for attenuating these maladaptive responses may be divided into pharmacological and non-pharmacological. Non-pharmacological approaches include prompt, early excision and closure of wounds, pertinacious surveillance for and treatment of sepsis, early commencement of high protein high carbohydrate enteral feeding, elevation of the immediate environmental temperature to 31.5 °C (± 0.7 °C), and early institution of an aerobic resistive exercise program. Several pharmacotherapeutic options are also available to further reduce metabolic rate and as such attenuate the erosion of lean body mass; these include anabolic agents such as recombinant human growth hormone, insulin, and oxandrolone and also beta blockade using propranolol. This chapter will mention what has been shown in the past concerning these metabolic changes during the acute admission, but will concentrate on the long term sequelae of these changes and how they can be attenuated by early institution of different pharmacological interventions.

Acute Metabolic Alterations Following Burn Injury

The Hypermetabolic Response

Severe burns lead to a hypermetabolic response far in excess of that seen in any other disease state [2] and although patients admitted with multiple traumatic wounds have an increase in metabolic rate that rises further when placed on a ventilator to between 30 and 75% that of normal, those admitted with burns involving more than 40% of the TBSA have increases in metabolic rate of between 80 to 200% of normal. The subsequent wound and metabolic response result in a nitrogen defi-

cit of up to 30 g/day. This hypermetabolic response is characterized by a hyperdynamic circulation, hyperthermia, increased oxygen and glucose consumption, carbon dioxide production, glycogenolysis, lipolysis, proteolysis, and futile substrate cycling [3]. The magnitude of the response is dependent on body weight at admission, time from burn to removal of eschar and the percentage of TBSA that is burned [4] (Fig. 1, 2). Recently gender has been shown, in pediatric patients, to have a direct effect on resting energy expenditure with female children having a reduced resting energy expenditure (REE) at all time points during acute hospitalization up to 9 months post-burn [5]. The rise in metabolic rate has a large net catabolic effect on the individual, the magnitude of which is dependent both on the severity of hypermetabolism and the development of sepsis during admission [4]. REE rises in a curvilinear manner from around normal levels for small burns of less than 10% TBSA to double the predicted level for individuals with burns in excess of 40% TBSA. For those patients maintained at thermal neutrality (33°C) the REE increase is attenuated at 1.8 times predicted during acute admission, this then reduces to 150% when fully healed, 140% at 6 months post burn, 130% at 9 months and 110% at 12 months [6] (Fig. 3).

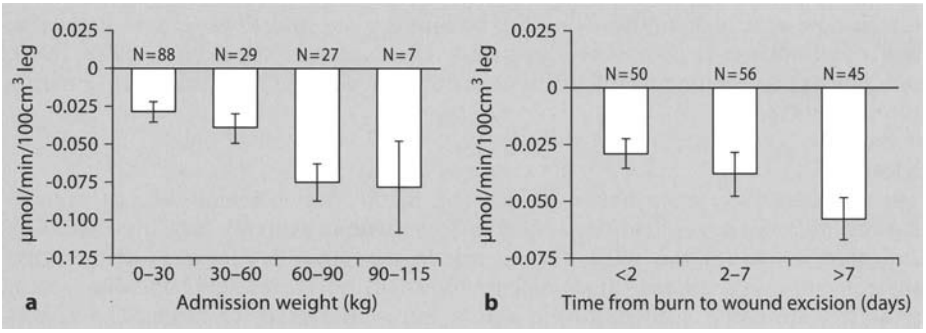

Fig. 1. a Association between admission weight and negative protein balance; **b** Association between time to primary wound excision and negative protein net balance. Data presented as mean ± SEM. From [4] with permission

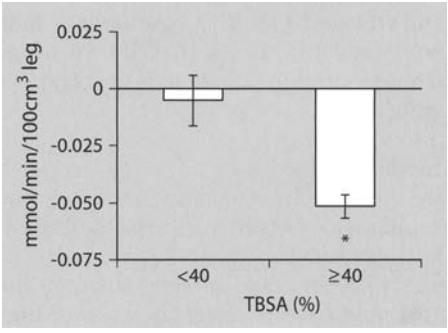

Fig. 2. Influence of burn size area (< or ≥40% total body surface area [TBSA]) on catabolism. Data presented as mean ± SEM. *$p < 0.0001$ by Student t test. From [4] with permission

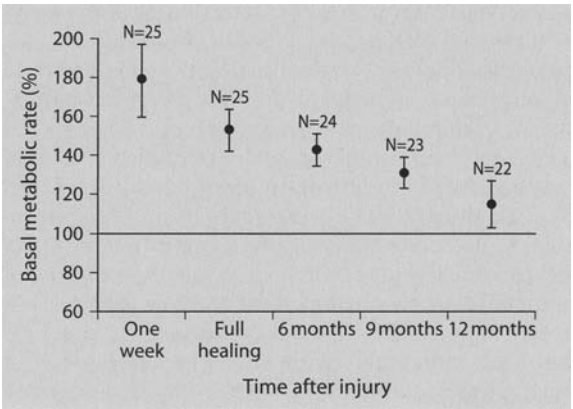

Fig.3. Resting energy expenditure. Indirect calorimetry was used to measure energy expenditure in a resting state at admission, full healing, and 6, 9, and 12 months after burn. At all time points, the energy expenditure was higher than the basal metabolic rate predicted for age-, sex-, weight-, and height-matched individuals by the Harris-Benedict equation. Error bars represent 95% confidence intervals. From [6] with permission

Mediators of Hypermetabolism

The cause of the hypermetabolic response is unclear; however, endotoxin, platelet-activating factor (PAF), tumor necrosis factor (TNF), interleukin (IL)-1 and IL-6, arachidonic acid metabolites using the cyclooxygenase and lipoxygenase pathways, neutrophil-adherence complexes, reactive oxygen species (ROS), nitric oxide (NO), and the coagulation and complement cascades have all been implicated in regulating this response [7].

Cytokines

IL-1 and TNF have been linked in the past to the rise in metabolic rate seen in chronic inflammatory conditions such as rheumatoid arthritis [8]. Together with IL-6 these cytokines play an important role in the initial development of the acute phase metabolic response [9]. A recent study [10] has highlighted the changes in cytokine expression seen following severe burn injuries in children: The results showed significant increases in both pro- and anti-inflammatory cytokine expression immediately following the initiating event. During the first week following injury, significant increases were seen in IL-1β, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12 p70, and IL-13 as well as interferon γ (IFNγ), monocyte chemoattractant protein 1 (MCP-1), macrophage inflammatory protein 1β, and granulocyte colony-stimulating factor (G-CSF). However within 5 weeks the serum concentrations of most cytokines had reduced again but remained above those for non-burned patients.

Catecholamines

The increase in circulating levels of catecholamines has long been attributed to a compensatory mechanism countering the internal cooling of the patient caused by fluid evaporation from the wound. This increase in catecholamine consequently leads to an increase in metabolic rate. However, the entire increase in REE cannot be attributed to this effect as when α and β-adrenoceptor blockade is initiated the resulting reduction in REE amounts to only about 15% [4].

Cortisol

Cortisol has been implicated in both the initiation and maintenance of the hypermetabolic response albeit via different receptors.

It is well established that a combination of these factors is responsible for the initial response to severe burn injury. However, little is known about the factors that maintain the hypermetabolic response once these have reduced to near normal levels. Cytokine levels are close to normal 5 weeks from initial injury, cortisol levels are almost normal at around 100 days, and catecholamine levels are back to near normal at around 100 days following initial injury. So how can we explain the sustained increase in REE that is seen following severe injury? Typically the acute response to stressful stimuli commences in the hypothalamus with the release of corticotropin releasing factor (CRF) which in turn binds to CRF-1 receptors in the anterior pituitary. Although almost certainly linked to the initiation of hypermetabolism, recently the CRF type-2 receptor ligand has been proposed as integral to maintenance of the hypermetabolism associated with burns [11]. The receptor ligand most likely to be involved is one of the urocortin (UCN) peptides. Localization studies have proposed UCN2 or UCN3 to be the peptides affecting the CRF-2 receptors in the hypothalamus [12]. Once levels of CRF have fallen to near normal levels it is the UCNs that have been implicated in maintaining this deleterious effect [11].

Consequences of Acute Metabolic Changes

Alterations in Metabolism of Carbohydrate, Protein, and Fat

The increase in energy expenditure is mirrored by substrate oxidation resulting from increases in ATP consumption. Increases in catecholamine, glucagon and glucocorticoid production lead to enhanced glycogenolysis and protein breakdown in both the liver and skeletal muscle. This in turn leads to increases in triglyceride, urea, and glucose production (gluconeogenesis), which consequently leads to hyperglycemia. The process of substrate cycling leads to increased thermogenesis, which raises core and skin temperature to 2°C above that of normal, unburned patients. Raised catecholamine levels also increase peripheral lipolysis and subsequent triglyceride-fatty acid cycling lead to fatty infiltration of the liver such that the liver weight increases by 120% [13] (Table 1); this, has been associated with an increased incidence of sepsis; however, no causative effect has been found.

A large proportion of the glucose produced by the liver is directed towards the burn wound where it is consumed by the anaerobic metabolism of inflammatory cells, fibroblasts, and endothelial cells; this in turn produces lactate which is recycled back to the liver and into gluconeogenic pathways. The catabolism of protein in skeletal muscle produces three carbon amino acids, such as alanine, that are also recycled to the liver to contribute to gluconeogenic pathways. The release of catecholamines increases glucagon secretion, which in turn promotes gluconeogenesis. The relative insulin resistance seen following a major burn combined with increased hepatic gluconeogenesis lead to hyperglycemia; patients in this situation have been

Table 1. Liver weight per body weight (BW) for normal vs burned patients (2 months to 15 years of age) [13]

	Full-Thickness Burn (%)	Liver wgt/BW (g/kg)	Weight Increase (%)
Normal (n = 14)	0	34.3 ± 1.1	–
Burn (n = 14)	76 ± 5	75.6 ± 6.0*	120

burn size and liver weight ratios are means ± se, *$p < 0.001$

shown to have an increased rate of muscle protein breakdown [14]. A study measuring whole body protein flux in normal individuals showed a three fold increase in the rate of protein catabolism with no accompanying alteration in protein synthesis during a period of hyperglycemia [15]. Endogenous anabolic hormone levels change, with both insulin-like growth factor (IGF)-I and IGF binding protein (IGFBP)-3 significantly lower immediately after burn, and neither reaching normal levels after 40 days post-burn. Serum insulin levels are significantly increased during the same time period with female patients producing up to 3 times normal levels; however, in the presence of insulin resistance hyperglycemia remains a problem. Endogenous growth hormone levels also fall 4 to 5 fold initially and remain below half the normal level during the first 40 days. The result of these levels combined with relative insulin resistance in the burns patient leads to a marked reduction in protein synthetic ability which can only be reversed by restoration of more normal levels from an exogenous source.

Erosion of Lean Body Mass

The change in regulation of skeletal muscle during the stress response following major trauma is due to the activation of pathways of protein breakdown. Recent studies have shown one of the chief protagonists to be the ubiquitin-proteasome pathway [16]. Ubiquitin is a common 8 kDa peptide found throughout all eukaryotic cells (hence the name). During skeletal muscle degradation it is activated in a stepwise process to covalently attach to other proteins, reducing their ability to disassociate from proteosomes and subsequently leading to degradation of the protein it has attached to. Ubiquitin has seven lysine residues, the use of which confers different functions. Chains of ubiquitin peptides linked via lysine 48 lead to degradation of the target protein by the proteasome. However, those linked by lysine 63 appear to confer signaling functions in the nuclear factor-kappa B (NF-κB) pathway, and act as mediators in DNA repair and the stress response. The role of ubiquitin peptides linked by other lysine residues is still unclear. The ubiquitin pathway is stimulated by TNF and the rise in glucocorticoids seen following severe thermal injury. The other main reason for the net loss of skeletal muscle is due to an imbalance in the rate of amino acid production secondary to protein breakdown and the ability of the cell to retain and re-use these amino acids. A study comparing the protein turnover in patients suffering from massive burns with that in normal individuals reported an increase in both muscle protein degradation and muscle protein synthesis in the burns group [17]. However, there was an 83% increase in muscle protein degradation compared with a 50% increase in muscle protein synthesis. In the same study absolute values of inward transport of phenylalanine, leucine, and lysine were not significantly different in the two groups. However, the ability of transport systems to take up amino acids from the bloodstream, as assessed by dividing inward transport by amino acid delivery to leg muscle, was 50–63% lower in the patients. In contrast, outward phenylalanine and lysine transport were 40% and 67% greater in the patients than in the controls, respectively [17]. These results suggest that the increased protein synthesis seen is secondary to the rise in amino acid concentration; however this synthetic rate is unable to keep up with the acceleration in protein breakdown. The increased net efflux of amino acids from the cell is facilitated by accelerated outward transmembrane transport and impaired influx due to the hyperdynamic circulation caused by the rise in catecholamine release [17].

Wound Healing Delays

This rise in metabolic rate and resulting loss of total body protein results in decreased immune defenses, decreased wound healing, and exhaustion which hinders rehabilitation [6].

Effects of Cortisol

The 8-fold increase in urinary cortisol that is seen in the acute stages following burn injury has been linked to a marked decrease in bone formation and mineral apposition, a lack of detectable surface osteoblasts, a reduction in type-I collagen expression in bone, and a reduction in biochemical markers of osteoblast differentiation, all consistent with an effect of excessive glucocorticoids [18]. Increases in glucocorticoids, together with Fas ligand have been shown to induce lymphocyte apoptosis following burn injury. Hypercortisolemia has been shown to increase REE and glutamine flux in a dose-dependent manner through an increase in *de novo* synthesis. The increase in REE was not accompanied by any significant change in respiratory quotient, therefore, it has been surmised that this increase is due to raised oxidation of fat. Typically, following a severe thermal injury a protracted amount of time is spent in intensive care undergoing multiple operations in order to speed the recovery of the patient. Much of this time is spent in bed with much less time spent exercising than for normal individuals. The combination of hypercortisolemia and prolonged inactivity substantially increases muscle protein catabolism via a reduction in muscle protein synthesis [19].

Effects of Catecholamines on Infection and Inflammation

The increase in catecholamines following burn injury has been implicated in several deleterious outcomes. Stimulation of the α1-adrenergic receptor population acts as an upstream activator of p38 mitogen activated protein kinase (MAPK), JNK, and NF-κB in burn trauma [20]. Since these molecules are important in the signal transduction pathway that induces inflammatory cytokine biosynthesis, the alpha-receptor may be an important mediator of burn-induced inflammatory cytokine secretion. The increase in norepinephrine has been linked to increased CCL2 production and the generation of the type 2 T cell phenotype [21]. Norepinephrine has also been linked to reduced production of burn associated CCL3 [22], an important β-chemokine that regulates migration of monocytes, T cells, neutrophils, eosinophils, basophils, and natural killer (NK) cells.

CXC Chemokines and Burns

The levels of CXCL8 (IL-8), an important factor in chemoattraction and activation of polymorphonuclear neutrophils (PMN) are raised far in excess of normal range in burns patients in the first 5 days after injury. When burns patients become septic there is another large rise in CXCL8 that correlates closely with patient mortality; this may be in part due to the reduced surface expression of CXCR2 (one of the receptors for CXCL8) on PMN.

CC Chemokines and Burns

As stated earlier there is an increase in CCL2 (MCP-1) production that is 80 fold higher than normal in those patients with large burns; this in turn stimulates type 2 T cell generation and subsequent IL-4 production by the activated T cells. IL-4 and IL-3 produced by activated Th2 cells stimulate production of ineffective macrophages via increases in CCL17 (thymus and activation regulated chemokine, TARC). Therefore, both CCL2 and CCL17 are seen as deleterious to the outcome of the burns patient, leading to increased incidence of infection. CCL3 and CCL5 have all been shown to be decreased in burns patients. CCL3 is an important modulator of host defense against bacterial infection.

Delayed Metabolic Changes

As has been explained earlier, the expression of cortisol in the acute and delayed stages of burn recovery appears to be governed by two different pathways. Whatever the mechanism leading to the protracted course of hypercortisolemia, the effects are the same. Acutely, osteoblasts increase the production of receptor activator of NF-kB ligand (RANKL) in response to rises in glucocorticoid, which in turn increases osteoclastogenesis and bone resorption. By 2 weeks following burn, the derangement has shifted from boney resorption to reduction of bone formation, with reduction of osteoblasts on the bone surface and reduced marrow stromal cell differentiation into osteoblasts [18]. Therefore, bone mass is lost due acutely to the pro-resorptive combination of cortisol together with cytokines (IL-1β and Il-6) and their subsequent cessation of bone formation. This, together with reduced bone loading secondary to reduced patient function, leads to inexorable bone loss. The bone loss found in those children that have suffered a severe thermal injury is associated with an increase in extrapolated fracture incidence of two-fold in male and one third in female children [23]. Also, as previously alluded to, insulin resistance remains a problem long after the burn wound has been closed. The mechanisms for this are as yet unclear, however, a picture of hyperinsulinemia together with hyperglycemia is typical following severe thermal injury.

Consequences of Delayed Metabolic Changes

Up to 12 months, there is severe retardation in weight, height, lean body mass, and bone mineral content; however a recent study has shown that at around 18 months following injury the repair mechanisms of the injured individual are restored such that there are significant increases in all four parameters (Fig. 4) [24].

These effects may have been elicited by increases in both IGFBP-3 and parathyroid hormone levels that were seen to be significantly increased when compared to serum levels at discharge. Improvements in muscle strength, power, the muscle's capacity for work, and aerobic capacity can all be increased with resistive training in a supervised program [25].

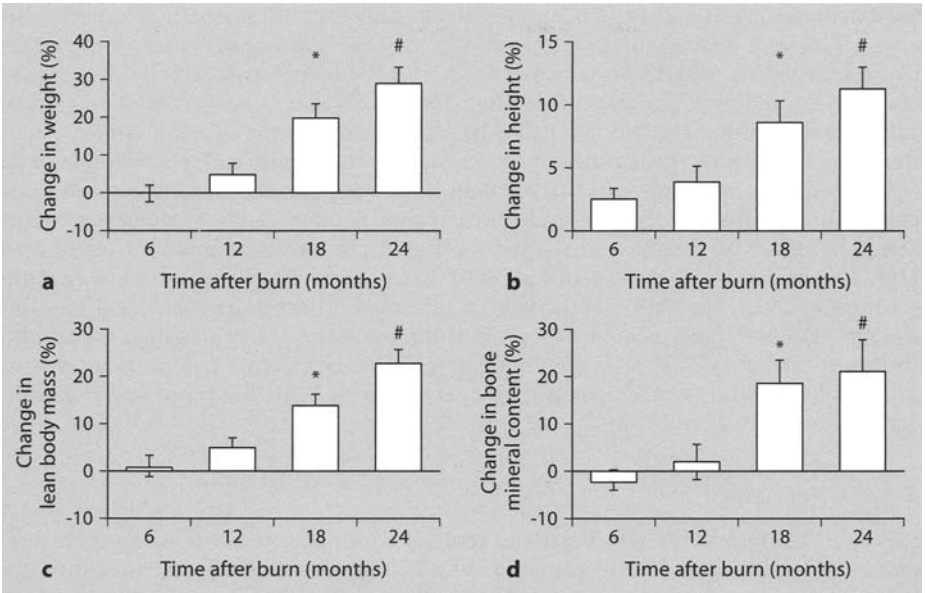

Fig. 4. Percentage change in weight (**a**), height (**b**), lean body mass (**c**), and bone mineral content (**d**) at 6, 12, 18, and 24 months after discharge in 25 pediatric patients who had suffered >40%TBSA burns, compared with discharge values. Values are means ± SEM. There was a significant difference between the first year and second year after injury for all values, * 18 months, # 24 months. From [24] with permission

Treatment Options for Attenuation of Deleterious Effects of Hypermetabolism

Propranolol

Propranolol has been used successfully to block the effects of endogenous catecholamines that have been implicated as primary mediators of the hypermetabolic response. In the initial stages after burn, levels of catecholamines show a 10-fold increase. The resulting hyperdynamic circulation, increased basal energy expenditure, and catabolism of skeletal muscle proteins are all deleterious for the patient. As described at the beginning of the chapter, catecholamines stimulate lipolysis via the β2-adrenoceptor. The effects of propranolol in the burn patient include reduced thermogenesis, tachycardia, cardiac work, and REE. The dose used is different for each patient; however, a reduction in heart rate by 20% is seen to produce reduced cardiac work load and fatty infiltration (secondary to reducing peripheral lipolysis and hepatic blood flow) [26]. Propranolol has been shown to enhance intracellular recycling of free amino acids leading to reduced skeletal muscle wasting and increased lean body mass [27]. The exact mechanisms for the beneficial changes seen in the burns patient following administration of this mixed β1/ β2 adrenoceptor antagonist remain to be identified.

Oxandrolone

Oxandrolone is a synthetic testosterone analog; it can be taken orally, is inexpensive, and has only 5% of the virilizing action seen in testosterone. Use of oxandrolone in

the burns setting at a dose of 0.1 mg/kg twice daily increases protein synthetic efficiency [28] and anabolic gene expression in muscle, and improves lean body mass by increasing net muscle protein synthesis, thereby attenuating muscle wasting. In severely burned children treated during acute hospitalization, oxandrolone significantly improved net protein synthesis, lean body mass, bone mineral content, synthesis of the hepatic constitutive proteins such as albumin and pre-albumin, and attenuated the acute phase reactive protein levels [29]. Oxandrolone improved body composition and strength in severely burned children during the 12 months of treatment. Its effect on height and weight continued after treatment was discontinued [29]. The ability of this treatment option to increase lean body mass in an outpatient setting together with the enteral route of administration makes it an ideal medication in the post-burn rehabilitation of children. Bone mineral content was also shown to be improved following long term treatment with oxandrolone versus unburned controls. A recent multicenter trial also showed that there was a significant decrease in acute hospital stay [30].

Growth Hormone

Recombinant human growth hormone (rhGH) administered via injection at a dose of 0.2 mg/kg during the acute admission resulted in reduced donor site healing time by 25%, reduced length of stay in hospital from 0.80 days/%TBSA to 0.54days/%TBSA [31] and improved quality of wound healing with no increase in scarring [32]. The growth retardation also typically seen following severe burns in pediatric patients was prevented during administration of rhGH during hospital admission [33]. A favorable attenuation of the hepatic acute phase response was also seen, with increased concentrations of IGF-I (the secondary mediator of rhGH) and increased albumin production. When given at a dose of 0.05 mg/kg/day for the first year following burn injury, improvements in height, lean body mass, and bone mineral content were seen. These improvements remained after the treatment had been stopped. Additionally, rhGH has a positive effect on immune function by reducing Th2 and enhancing Th1 cytokine production [34]. The benefits of rhGH are not without some side effects, most notably hyperglycemia during the acute admission. An increased mortality rate seen in non-burned critical care patients [35] is not present in burned pediatric patients [36]. Improved wound healing, reduced tissue wastage and length of stay in hospital are all major benefits that will improve both the physiological and psychological rehabilitation of the patient. Currently the drawbacks for rhGH are the side effects and mode of delivery; ongoing investigations are addressing these points along with trials incorporating beta-blocking agents.

Ketoconazole

Ketoconazole is an imidazole antifungal agent. As with other imidazoles, it has a five-membered ring structure containing two nitrogen atoms. Ketoconazole is available as oral tablets, a cream, and a dandruff shampoo formulations. The oral formulation has been available in the USA since 1981. Like all azole antifungal agents, ketoconazole works principally by inhibition of cytochrome P450 14a-demethylase (P45014DM) an enzyme in the sterol biosynthesis pathway that leads from lanosterol to ergosterol [37]. Ketoconazole inhibits the 11β-hydroxylation and 18-hydroxylation reactions in the final steps during the synthesis of adrenocorticosteroids [38] and may even function as a glucocorticoid receptor antagonist [39].

Insulin

Recently it has been restated that severe hyperglycemia in patients suffering from massive burns is associated with an increase in muscle protein catabolism [14], reduced graft take, and an increase in mortality [40]. Euglycemia maintained using insulin for non-burned, surgical critical care patients significantly reduced the incidence of infection and mortality [41]. The use of insulin has been shown to significantly reduce donor site healing time from 6.51 (±0.95) days to 4.71 (±2.3) days [42]. A continuous infusion used in burn patients prevented muscle catabolism and conserved lean body mass in the absence of increased hepatic triglyceride production [43]. Submaximal doses (3 mU/kg/min) of insulin administered via infusion to burns patients resulted in net protein muscle anabolism without the need for large doses of carbohydrate [44]. Insulin has been shown to attenuate the inflammatory response by decreasing the pro-inflammatory and increasing the anti-inflammatory cascade, thus restoring systemic homeostasis and reducing the drive of the hypermetabolic response. Continuous intravenous insulin infusions at doses that will maintain euglycemia (glucose between 100 and 140 mg/ml) after severe burns downregulates acute phase protein levels and attenuates muscle catabolism, preserving lean muscle mass [45]. Recently, insulin administered to burned children was shown to blunt the increase in C-reactive protein (CRP), IL-1β, and TNF levels after injury, in the absence of normoglycemia. In another recent study involving pediatric patients in whom the glucose levels were maintained at between 90 and 120 mg/ml, intensive insulin therapy was shown to be safe and effective, reducing infection rates and improving survival [46]. The mechanism are unclear for this response; however, it is likely to be caused by inhibition of NF-kB with stimulation of IkB in monocytes [47]. This would result in reduced length and severity of infections and attenuate multiorgan dysfunction associated with burn shock. Although pharmacological doses of insulin have been shown to increase glucose uptake into tissue and this uptake is accompanied by increased amino acid uptake and increase lactate release, the exact mechanisms are still unclear. Proposed pathways include activation of sodium-dependent transport systems, initiation of protein translation and direct regulation of proteolytic activities. Metformin may also be used to attenuate hyperglycemia in patients with severe burns, thereby increasing muscle protein synthesis. Other anti-hyperglycemic agents such as dichloroacetate may also have beneficial results in reduction of post-burn hyperglycemia [48].

Insulin-like Growth factor-1

The beneficial effects of rhGH are derived through IGF-I and IGFBP-3 the levels of which are raised by 100% during treatment, relative to healthy individuals. Therefore, an infusion of equimolar doses of IGF-I and IGFBP-3 has been shown to improve protein metabolism in both adult and pediatric burn patients with significantly less hyperglycemia than rhGH alone [49]. Interestingly, there was no additional benefit seen with higher doses of the infusion; using 1 mg/kg/day was sufficient to achieve the desired effect. Attenuation of the type I and II acute phase response was seen following infusion leading to reduced acute phase protein production and increased constitutive protein production by the liver. Another potentially beneficial effect of an infusion of IGF-I/IGFBP-3 has been shown in a human model where there was a partial reversal of the detrimental change in the Th1/Th2 cytokine profile [50]. Typically, following massive thermal injury, there is a shift to

a predominant Th2 cytokine response resulting in increases in lymphocyte production of IL-4 and IL-10, together with decreased production of IL-2 and IFNγ. However, this combination drug has yet to become commercially available and further studies are required.

Conclusion

As well as the exercise programs outlined above, several pharmacological options for attenuation of the hypermetabolic response are available. Propranolol has now been shown to have a prolonged effect on heart rate and REE, and to improve weight gain, lean body mass, and bone mineral content. Recent developments have shown a link between propranolol and attenuation of infection rates. The mechanisms underlying the changes seen are most likely due to the interaction of catecholamines and chemokine expression, particularly the reduction in the normally protective CCL3 (MIP-1α) and the increase in both CCL2 (MCP-1) and CCL17 (TARC), which act together for a combined systemic inflammatory response. The testosterone analog, oxandrolone, at a dose of 0.1 mg/kg given twice daily for 12 months following injury raised serum levels of IGF-1, T3 uptake, and free thyroxin index, leading to improved lean body mass and bone mineral content [29]. When looking at the effects of recombinant rhGH given over a 12 month period we find that there are significant improvements in height, weight, lean body mass, bone mineral content, cardiac function, and muscle strength. The long term effects of extended treatment with insulin are currently being examined.

The hypermetabolic response that follows a severe burn cannot be halted or reversed; however, its effects can be limited by prompt surgical removal of the burn eschar, aggressive treatment of developing sepsis, early enteral feeding with a high carbohydrate high protein diet, and a program of resistance exercises. The addition of anabolic and anticatabolic agents is increasing the improvements seen during burn recovery. The aims of ongoing research in this field are to further delineate the mechanisms involved in these processes allowing us to prevent the hypermetabolic response from gaining momentum.

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Antithrombin in Burn Trauma

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Introduction

In the United States, more than 1 million burn injuries occur every year. Although the survival from burn injury has increased in recent years with the development of effective fluid resuscitation management and early surgical excision of burned tissue, the mortality of burn injury is still high. In these fire victims, progressive pulmonary failure and cardiovascular dysfunction are important determinants of morbidity and mortality. The morbidity and mortality increases when burn injury is associated with smoke inhalation. Smoke inhalation and pneumonia increase mortality of burn patients by 20 and 40%, respectively [1], suggesting that the pulmonary involvement is a very important risk factor in burn trauma. Our group and others have reported various factors that are involved in pathophysiology of acute lung injury (ALI) in burn, including coagulopathy [2, 3]. It was earlier described that burn injury is associated with a hypercoagulable state [4]. The coagulopathy seen in these burn patients is associated with marked depletion of a major endogenous regulator of blood coagulation, antithrombin [3–5]. In the present chapter, we will discuss a possible role of antithrombin in the pathophysiology of ALI induced by combined burn and smoke inhalation and review the therapeutic approaches.

Antithrombin

Antithrombin is a plasma-derived, single-chain glycoprotein with a molecular weight of 58 kDa. It inactivates a number of proteinases of the blood cascade, especially thrombin and activated factor X (FXa) [6]. However, it has been shown that antithrombin inhibition of FXa and thrombin is a slow process. This slow rate dramatically increases in the presence of heparin. For instance, thrombin inhibition is accelerated 2000-fold by heparin and FXa inhibition 600-fold [7, 8]. Binding of heparin to antithrombin is believed to be a pre-requisite for this rate enhancement effect, and it is mediated by a unique heparin pentasaccharide sequence. Interaction between this pentasaccharide sequence and antithrombin induces a conformational change in the latter molecule that enhances the ability of antithrombin to inhibit FXa and related serine proteases, but not thrombin. Heparin species with longer polysaccharide chains appear to be required in order to enhance the inhibition of thrombin by antithrombin [9–11]. Thus, antithrombin exerts a potent anticoagulant effect as an antithrombin/heparin complex.

Of particular interest, antithrombin has been shown to exert an anti-inflammatory effect. In fact, antithrombin can modulate inflammation through inhibiting

coagulation factors, FXa and thrombin, or independently from its anticoagulant properties. Activation of the blood coagulation factors results in thrombin formation and fibrin deposition at the vascular wall, as well as in the formation of platelet-leukocyte co-aggregates, leading to severe disturbance of the microcirculation, capillary leakage, tissue damage, organ failure, and death [12]. Antithrombin has been shown to inhibit many of these pro-thrombotic and pro-inflammatory changes. Antithrombin may inhibit a number of inflammatory mediators such as interleukin (IL)-6, IL-8, monocyte chemoattractant protein (MCP)-1, E-selectin, intercellular adhesion molecule (ICAM)-1, and vascular cell adhesion molecule (VCAM)-1, through inhibiting FXa [13].

As mentioned, antithrombin is a natural inhibitor of thrombin, a central coagulant factor with potent pro-inflammatory activities. Thrombin itself interacts with cells by binding to protease-activated receptors (PARs) leading to transmembrane signaling of the G-protein and the subsequent cellular reactions [14, 15]. Thrombin has also been shown to induce expression of P-selectin and platelet-activating factor (PAF) by endothelial cells, stimulate leukocyte/monocyte adhesion, and increase expression of IL-8 [16, 17]. Thus, antithrombin can modulate both coagulation and inflammation through inhibiting the major blood clotting factors, FXa and thrombin. In Figure 1 we summarize possible pathways of involvement of procoagulants in the pathophysiology of organ dysfunction.

There are many reports on antithrombin's coagulation-independent anti-inflammatory effects. It has been suggested that antithrombin promotes prostacyclin production from endothelial cells [18]. Souter et al. documented that antithrombin inhibited lipopolysaccharide (LPS)-induced IL-6 in multiple cell types [19]. Recently, Kaneider et al. described that antithrombin affects neutrophil migration via its heparin binding site and action on cell surface syndecan-4 [20]. Thus, antithrombin displays a dual mode of action: Namely control of coagulation and inflammation. However, this important molecule, which modulates coagulation and inflammation to a physiologically meaningful level, is depleted in burn trauma.

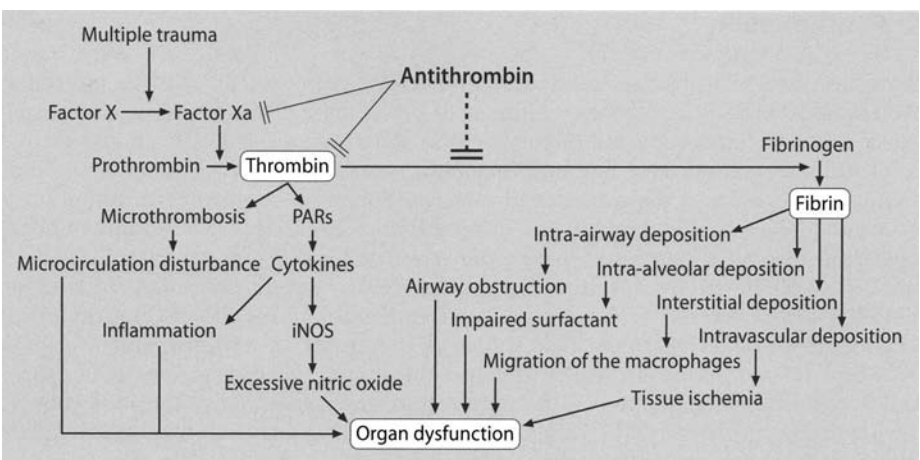

Fig. 1: The possible pathways by which procoagulant factors contribute to organ dysfunction in pathological conditions. PARs: protease-activated receptors; iNOS: inducible nitric oxide synthase.

Antithrombin and Acute Lung Injury

We have previously described the pathophysiology of ALI in sheep with combined burn and smoke inhalation injury [2, 21, 22]. An increase in airway blood flow, increased pulmonary vascular permeability with subsequent edema formation, airway obstruction, and pulmonary shunting are major factors that result in pulmonary dysfunction. We have also suggested various treatment approaches targeting the above-described pathogenic factors. We found that the pathophysiological alterations seen following burn and smoke inhalation injury are associated with marked depletion of endogenous antithrombin, which has potential physiological importance in regulating the inflammatory and coagulatory responses. It was earlier suggested that increases in plasminogen activator inhibitor type-1 (PAI-1) and antithrombin depletion in the initial (24 h) post-burn period might cause hypercoagulability in burn patients [23]. As shown in Figure 2, plasma levels of antithrombin were markedly depleted over time in sheep exposed to combined burn and smoke inhalation injury. There was an almost 80% depletion of plasma antithrombin 12 h post-injury, which recovered slightly over time and was ~ 50% at 24 and 48 h post-injury. In support of our findings, Kowal-Vern et al. reported that plasma concentration of antithrombin was reduced by 50% in burn patients [3, 4]. Aoki et al. documented that plasma antithrombin decreased to 41 ± 4 in burn patients at 24 h post-injury followed by a gradual increase to reach normal values 120 h post-burn. It is worth noting that these patients were transfused with ~1.6 L of fresh-frozen plasma during the first 24 h [23]. Since fresh frozen plasma is a good source of antithrombin, the true value of the plasma antithrombin measured could be even lower. Similar results were documented by Garcia-Avello et al. who reported that the hypercoagulable state in burn patients during the initial 24 h was associated with high levels of activated factor VII (FVIIa), thrombin-antithrombin complex (TAT), PAI-1, and low levels of antithrombin and protein C [5]. Taken together the results of the above studies could suggest that a high rate of fibrin or thrombin formation and their diffuse deposition in the microvasculature during the first days following injury, accompanied by a relatively ineffective fibrinolysis due to excess PAI-1 could play a pathophysiological role in the development of organ dysfunction after burn trauma.

We have recently described an important role of fibrin clots in the formation of airway obstructive casts in sheep after combined burn and smoke inhalation injury

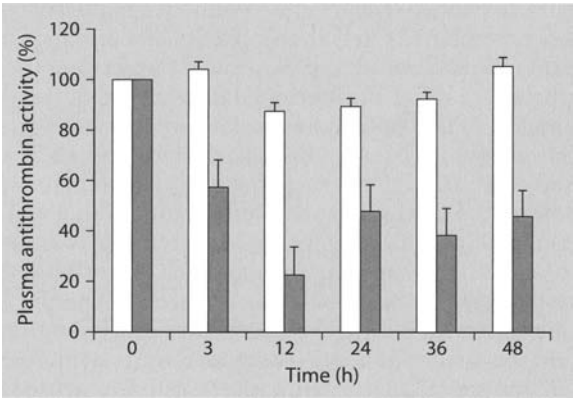

Fig. 2: Plasma antithrombin activity in sheep subjected to combined burn (40% total body surface area, 3rd degree, flame) and smoke inhalation injury (48 breaths of cotton smoke). Data are expressed as a percent. Open bars represent animals that had no-injury, no-treatment, but were chronically instrumented, placed on ventilator, and fluid resuscitated in the same manner. Closed bars represent animals that were exposed to combined burn and smoke inhalation injury, but with no treatment.

[24]. The aerosolization of tissue-type plasminogen activator (t-PA) into the airways of these sheep significantly reduced the degree of airway obstruction and improved pulmonary function; of note, t-PA also markedly decreased the size of airway cast *in vitro*. The above results suggest that local (airway) hypercoagulability may also contribute to the pathophysiology of ALI after burn, especially when it is accompanied by smoke inhalation. Nevertheless, carefully designed prospective clinical and/or large animal studies should be conducted to clarify more precisely the coagulation state in early and late phases of the post-burn period. Possible adjustment of the pathological alterations should also be considered according to the coagulation state.

There have been a few attempts to restore depleted levels of antithrombin in burn patients. In 2000, Kowal-Vern et al. reported that infusions of antithrombin concentrate in burn patients were safe and well tolerated [4]. In 2001, the same group showed that restoration of plasma levels of antithrombin in burn patients (~120%) improved pulmonary gas exchange and reduced airway resistance [3]. The antithrombin treated patients also had fewer episodes of pneumonia compared to controls. Murakami et al. reported that intravenous administration of recombinant human antithrombin significantly improved pulmonary function following smoke inhalation and pneumonia in sheep [25]. In this study, the authors maintained the plasma levels of antithrombin at baseline values by infusing recombinant human antithrombin at a rate of 0.6 mg/kg/h. The restoration of plasma levels of antithrombin in these sheep resulted in less lung water content, lower lung histology changes (congestion, inflammation, edema, and hemorrhage), and better hemodynamic responses (attenuated hypotension) [25]. Interestingly, high dose of intravenously administered heparin in the same model of sepsis failed to attenuate the cardiopulmonary morbidity [26]. Although the reason that high doses of heparin had no effect in this model is not completely clear, the antithrombin deficiency in these animals may be a possible explanation, since heparin exerts a potent anticoagulant effect only as an antithrombin/heparin complex.

As mentioned above, we have reported an important role of airway fibrin formation in the pathogenesis of ALI following burn and smoke inhalation injury. To prevent fibrin formation, we have aerosolized both recombinant human antithrombin and heparin into the airways of sheep subjected to combined burn and smoke inhalation injury. The combined treatment markedly improved pulmonary gas exchange reducing the degree of airway obstruction and ventilatory (peak and pause) pressures [27]. The advantage of this treatment strategy was to reach a maximum local (airway) anticoagulant effect without significant effects on systemic coagulation. Since, combined burn and smoke inhalation injury is associated with both coagulopathy (local–airway) and systemic inflammation (tissue injury due to activated neutrophils), a different route of administration of these anticoagulants may be useful in order to obtain both systemic anti-inflammatory and local anticoagulant effects. For this purpose, we have administered recombinant human antithrombin intravenously and aerosolized the heparin into the airways assuming that part of the intravenously administered recombinant human antithrombin will leak into the airways along with increased plasma exudates and exert a potent local anticoagulant effect by binding to aerosolized heparin. This combination resulted in even better cardiopulmonary responses [28]. Pulmonary microvascular hyperpermeability was almost reversed by this treatment strategy. We have previously reported that aerosolization of heparin into the airways in septic sheep (smoke inhalation and pneumonia) ameliorated ALI without noticeable systemic effect [29]. Therefore, we also assume that aerosolized

heparin will not significantly interfere with the systemic anti-inflammatory effects of intravenously administered recombinant human antithrombin.

There are many animal studies reporting the beneficial effects of antithrombin on reducing sepsis-related mortality and preventing multiorgan dysfunction. These observations have been confirmed in patients with severe sepsis in phase II clinical trials and a recent meta-analysis. However, a multinational, phase III pivotal trial (KyberSept) that evaluated high doses of antithrombin concentrate in comparison with placebo for the treatment of severe sepsis could not demonstrate a significant reduction in 28-day all-cause mortality in the overall study population [30]. It is worth noting, however, that the subgroup of patients not receiving concomitant heparin had a lower mortality rate [30]. Recently, there was another prospective phase III study, which reported that treatment with high-dose antithrombin may increase survival time for up to 90 days in patients with severe sepsis and high risk of death [31]. Moreover, the authors noted that the beneficial effects of antithrombin treatment may be even stronger when concomitant heparin is avoided.

Taken together, it is apparent that antithrombin deficiency contributes to the pathogenesis of cardiopulmonary morbidity in multiple trauma, including burn injury.

Conclusion

In summary, results of previous studies suggest that restoration of plasma antithrombin may be an efficient and novel treatment strategy for the management of burn patients. Future studies testing the effects of high-dose antithrombin concentrates without concomitant heparin administration should be conducted in large animal models. Multicenter clinicals trial in burn centers should also be considered.

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Hematological Alterations

The Critically Ill Red Blood Cell

E. Almac and C. Ince

Introduction

Anemia is one of the most common problems suffered by critically ill patients and occurs early during their intensive care unit (ICU) stay. Despite alternatives, blood transfusion is still the most common treatment of anemia in ICUs around the world. In the last decade, a number of studies have observed the transfusion practices in North America and Europe. These studies have also provided information regarding the efficacy and the negative consequences of blood transfusion therapy.

Current issues in transfusion medicine, i.e., comparisons between transfusion strategies, leukodepletion, the efficacy of blood transfusions, preservation and rejuvenation of red blood cells (RBCs), are still open for debate. These issues are important because of evidence showing the loss of RBC function during blood storage and beneficial effects of conservative use of blood transfusions in anemia and sepsis. These topics are currently of special interest due in recent new findings relating to RBC (patho)physiology in sepsis, transfusion, and hypoxia. These new insights may help in understanding how RBC function is affected by storage, inflammation, and hypoxemia, and, thereby, optimize its use for transfusion. For these insights to be applied, however, clinical techniques are needed to assess the efficacy of RBCs in perfusing the microcirculation and transporting oxygen to the tissues.

In this chapter, we will review recent developments in RBC transfusion therapy as well as some of the new techniques available which may allow more precise evaluation of RBC function in the environment of the microcirculation.

Anemia in the Critically Ill

Critically ill patients include a wide range of patient groups, but they have several common problems. One of these problems is that critically ill patients often develop anemia. Anemia is among the most common problem in critically ill patients admitted to ICUs. In both of the recent ABC and CRIT multicenter studies, studying blood transfusion practice in Europe and the USA, respectively, two thirds of the patients admitted to the ICU had a hemoglobin concentration lower than 12 g/dl [1, 2]. Both studies also showed that anemia occurred early in the ICU course and worsened with increasing length of stay. This worsening was more significant in non-transfused compared to transfused patients. By ICU day 3, 95% of critically ill patients are anemic [2].

The course of anemia, nevertheless, may be different in the presence of different underlying conditions. In a recent study, it was shown that while other non-bleeding

critically ill patients only exhibit a decrease in hemoglobin values in the first 3 days of admission, hemoglobin concentrations in septic critically ill patients kept on continuing to decrease even after 3 days [3]. This difference in the course of anemia in septic patients could be explained by repeated blood sampling, RBC rheologic alterations, and increased RBC destruction.

The etiology of anemia in critically ill patients is multifactorial. Among the many causes of anemia, blood loss due to bleeding (surgical procedures and gastrointestinal bleeding) remains the main cause. Blood loss in critically ill patients occurs often due to emergency and trauma, surgery, and gastrointestinal bleeding [2]. Several studies have reported that approximately 25% of blood transfusions performed in critically ill patients are given for hemorrhage [4, 5]. Interestingly, in the ABC study, 55% of transfusions were used for hemorrhage [1]. Hemoglobin concentration, however, may also decrease in non-bleeding critically ill patients. Under normal physiological conditions, erythropoietin (EPO) production is induced by hypoxia, as occurs when RBC mass decreases and with anemia, which in the end corrects the tissue hypoxia. In critically ill patients, however, the EPO response to anemia is blunted due to cytokines and the inflammatory response [6]. Disturbances in iron and B12 metabolism may also contribute to the anemia in critically ill patients. Iron metabolism is crucial for the development of erythroid precursors and mature blood cells. In critically ill patients, iron metabolism may be altered by the inflammatory process, which ultimately will lead to anemia [7].

Iatrogenic anemia is another factor contributing to anemia in critically ill patients. Around 40 ml per day of blood is taken from critically ill patients for medical reasons [1, 3] and this volume is even higher in septic patients, due to repeated blood tests [3]. As a result of the repeated blood sampling, phlebotomy may account for almost one fourth of the total blood transfusions in ICU patients [8]. Additionally, increased blood volume secondary to vasodilation may cause a decrease in hematocrit, in the presence of constant RBC mass [9].

Finally, alterations in RBC rheology, leading to increased destruction of RBCs by the reticuloendothelial system, may be another factor contributing to anemia in critically ill patients [10–12]. RBC membrane alterations may occur due to the production of inflammatory mediators by white blood cells (WBCs), by bacteria, or by the RBC membrane itself. Alteration in sialic acid distribution is one of the possible mechanisms that may account for the RBC membrane alterations in septic patients. Indeed, decreases in RBC membrane sialic acid were reported in the first 24 hours of sepsis by Piagnerelli et al. [13]. The pathogenesis of the decrease in hemoglobin concentrations in non-bleeding patients is likely to be a combination of all these factors.

Transfusion in Critically Ill Patients

As discussed above, critically ill patients are often anemic and irrespective of the etiology of anemia, blood transfusion is the most common choice of treatment for these patients. Hence, a large number of ICU patients receive a blood transfusion at some point during their ICU stay. The reported proportion of patients who receive one or more blood transfusions during their ICU stay has varied between 20% and 53%; this percentage rose to 73% to 85%, when the length of ICU stay exceeded 7 days [1, 8].

In the last decade, several studies have investigated transfusion practices in North America and Europe. In 1999, in the Transfusion Requirements in Critical Care

(TRICC) study, Hebert et al. [14] compared the outcomes of 5,298 patients admitted to Canadian ICUs, managed with a restrictive transfusion practice (with a transfusion trigger of 7 g/dl and target hemoglobin concentration maintained at 7–9 g/dl) to patients managed with a liberal blood transfusion strategy (with trigger 10 g/dl and target hemoglobin concentration kept at 10–12 g/dl). Patients in the liberal group received a mean of 5.6 units of RBCs, compared with 2.6 units in the restrictive group. Both 30-day ICU and hospital mortality rates were lower in the restrictive group (19% versus 23%), but the differences were only significant for hospital mortality (22% versus 28%). Mortality rates were significantly lower in the restrictive group than in the liberal group in the subgroups of patients with lower (≤20) Acute Physiology and Chronic Health Evaluation (APACHE) II scores (9% versus 16%) and younger (<55 years) age (6% versus 13%) The authors concluded that "hemoglobin concentrations should be maintained between 7.0 and 9.0 g/dl" [14]. Indeed, this study was the first to demonstrate the beneficial effects of restrictive transfusion in such a big population and raised the question whether our transfusion strategies in the ICU were indeed beneficial for patients. Further fuelled by other observational studies, this study suggested worse outcomes in patients who received blood transfusions.

In a cohort study involving 1,247 critically ill patients in London, UK, Rao et al. reported a mean pre-transfusion hemoglobin concentration of 8.5 g/dl and that 53% of the patients received a RBC transfusion during their ICU stay [4]. The indications for RBC transfusion were very similar to those identified in the TRICC study [5] with around 25% being given for hemorrhage and 72% for a 'low hemoglobin' [4].

In the ABC study, a European cohort study carried out over 2 weeks in 3,534 patients admitted to 146 western European ICUs, Vincent et al. [1] reported a mean pre-transfusion hemoglobin concentration of 8.5 g/dl for patients without bleeding. In this study, the overall transfusion rate during the ICU stay was 37%. The number of patients who received RBC transfusions increased with longer ICU stay and increased to 73% in patients who stayed in the ICU for more than a week. This result was slightly lower than the rate found in a study by Corwin et al. [2]. The ABC study reported higher ICU (19 versus 10%) and overall (29 versus 15%) mortality rates in patients who had received a blood transfusion than in those who had not. Additionally, in matched patients in a propensity analysis, the 28-day mortality rate was 23% among transfused patients and 17% among non-transfused patients. However, the study found that 55% of transfusions were for acute bleeding and only about 45% for reasons equivalent to reduced physiological reserve which did not confirm the findings of Rao et al. [4].

In a smaller study involving 176 patients, Chohan et al. examined the transfusion practice in a large Scottish teaching hospital [5]. They found that the median RBC transfusion threshold in the absence of clinical hemorrhage was 7.8 g/dl [5]. Fifty-two per cent of patients remaining in the ICU for longer than 24 hours received a RBC transfusion. More than 70% of RBC transfusions were administered for reasons other than hemorrhage. Patients remaining for longer than 24 hours in the ICU received a mean of 3.1 RBC units per admission, including 1.6 units per admission for reduced physiological reserve and 1.2 units for hemorrhage. This represented an overall RBC requirement of 0.47 units per ICU day. These investigators showed that conservative transfusion triggers have been adopted by clinicians, but that these deviated from the restrictive practice used in the TRICC protocol where transfusion triggers mostly ranged between 7 and 9 g/dl of hemoglobin, and clinicians usually administered 2 unit RBC transfusions for each transfusion episode.

In 2004, in the CRIT study [2], in which 4,892 patients admitted to ICUs in the USA were observed, the mean pre-transfusion hemoglobin concentration was found to be 8.6 g/dl, which is similar to the TRICC trial and the ABC study, and 44% of the patients received one or more RBC units during their ICU stay. The CRIT study confirmed the results of the TRICC and ABC study by showing that that the number of RBC transfusions was an independent predictor of worse clinical outcome. The CRIT study showed that the number of RBC transfusions a patient received during the study was independently associated with longer ICU and hospital lengths of stay and an increase in mortality. In the CRIT study, a baseline hemoglobin concentration of 9 g/dl was a predictor of increased mortality and length of stay.

Finally, the recent Sepsis Occurrence in Acutely ill Patients (SOAP) study, which included 3,147 patients from 198 European ICUs, reported that 33% of the patients received a blood transfusion during their stay [15]. Mortality rate after RBC transfusion was higher but these patients were in general older and had more co-existing diseases. After propensity matching, transfused and non-transfused patients showed no difference in mortality rates, which was in contrast to the other studies. The authors hypothesized that this may be due to the fact that the RBC transfusions were leukocyte reduced. Indeed, leukodepletion was more common in the SOAP study than in the ABC or CRIT studies. However, results of studies regarding leukodepletion remain controversial. Hebert et al. showed in a large retrospective analysis of 14,786 patients before and after the implementation of universal leukoreduction in Canada, that there was a decrease in mortality rate in cardiac surgery patients and also a reduction in post-transfusion fever, a similar finding to most other studies [16]. Bilgin et al. reported, in a prospective, randomized, double blinded study of patients undergoing cardiac valve surgery that there was a reduction in infection rates and in hospital mortality [17]. Fung et al. showed the beneficial effects of leukoreduced RBCs for cardiac surgery patients with a decrease in post-operative length of stay [18, 19].

A controlled trial in the Netherlands was carried out by van de Watering and compared leukodepleted and buffy-coat depleted RBC transfusions in patients undergoing coronary artery bypass grafting (CABG), with or without valve replacement [20]. These authors found a significant decrease in post-operative infections in patients receiving more than four units of blood. Surprisingly, in this study mortality was reduced only in patients who received leukodepleted erythrocytes but not those who received buffy-coat depleted erythrocytes. That may be explained by the differences in these two methods. Buffy-coat free RBCs contain about 10^9 leukocytes, whereas leukodepleted RBCs contain about 10^6 leukocytes. In addition, Izbicki et al. have shown that storage for more than three weeks may play an important role in the development of post-transfusion leukocytosis in transfusion of non-leukodepleted RBCs by accumulation of interleukin (IL)-8 [21]. Cytokines and inflammatory mediators are known to be produced by WBCs during blood storage and interfere with immune function. Therefore, theoretically prestorage leukoreduction should prevent the accumulation of these products.

The above findings supported the hypothesis that leukocytes are associated with a worse outcome in patients receiving blood transfusions and supported universal leukoreduction. However, in a recent meta-analysis of 12 clinical trials, Fergusson et al. [22] evaluated the efficacy of leukodepleted blood in reducing post-operative infections. They showed that the beneficial effects of leukodepleted blood were only evident in subgroups (cardiac surgery and only transfused patients). The majority of these studies were performed in patients undergoing colorectal or cardiac surgery

and used prestorage leukodepletion. However, most of the trials in this meta-analysis were performed using buffy-coat depleted blood and did not demonstrate an improvement in outcome.

In summary, these studies represented the current transfusion practices in North America and Europe between the years 1990–2002. Restrictive transfusion policies and leukodepletion started to influence transfusion practices during this period. Leukodepleted RBC transfusion seems to be beneficial in specific groups, such as cardiac surgery patients. The differences in the ABC, CRIT, and SOAP studies may be explained by these findings. However, the question as to whether there is enough evidence to justify this costly program has not yet been answered. Europe and Canada have already implemented universal leukoreduction. New results from this implemented program are expected in the near future.

The Age of Blood at the Time of Transfusion

In addition to the presence or absence of leukocytes in transfused blood, a second important factor which may affect the function of transfused blood to correct anemia is the age of storage. In this context 'storage', because it is the most common blood preservation technique, refers to liquid preservation only. Several clinical and pre-clinical studies have shown that storage has deleterious effects on RBC function and storage [23, 24]. However, the clinical importance of this so-called 'storage lesion' is not well known. Questions remain as to exactly when storage diminishes the structural and functional properties of RBCs and how often more harm than good is done when transfusing relatively longer stored RBCs in daily practice.

In the large epidemiological studies, the mean age of blood was 16.2 day ($\pm$7 days) [1] and 21.2 ($\pm$11.4 days) [2]. Interestingly, the age of blood was found not to be related to any clinical outcome. There was a trend to increased mortality with older blood in the CRIT study [2], but this did not reach the significance level. However, in both of these studies the number of transfusions was relatively small (12,000 and 4000 units, respectively). In another study, Raat and colleagues analyzed the age of stored RBC concentrates in 74,084 units in an academic hospital in Netherlands between the years 1997–2001, for a period of 5 years. They found that the mean storage time was 19.4$\pm$7 days and 37% of units were older than 3 weeks [25].

In conclusion, the data above, in a total number of 90,000 RBC units, show that most RBCs being used in critically ill patients are aged between 16 to 21 days. One third of patients receive blood transfusions that are older than 21 days, which indeed may be a clinical problem that needs to be addressed if storage-related RBC dysfunction indeed occurs in these RBC units.

Efficacy of Red Blood Cell Transfusions in Critically Ill Patients

Although conflicting results have been published in clinical studies, in experimental studies storage has so far not been related to beneficial effects. In clinical studies, the cause of these controversial results may be due to technical limitations and lack of appropriate techniques to measure the ultimate goal of RBC transfusion which is to provide adequate microcirculatory perfusion and tissue oxygenation.

Studies by Marik and Sibbald [24] in septic patients and Fitzgerald and co-workers [26] in septic oxygen supply-dependent rats, were the initial studies that raised the question of whether RBC transfusion actually increases tissue oxygenation or is associated with adverse effects. Purdy and colleagues [27] showed in septic patients, a

relation between the age of transfused RBCs and patient mortality. Messana et al. [28] showed, in an experimental study, that storage causes a progressive loss of metabolic modulation probably due to band 3 proteins which may be restored by rejuvenation.

Vamvakas and Carven [29], on the contrary, could not find any deleterious effects of transfusion of aged RBCs in patients undergoing cardiac surgery. Supporting these data, Walsh et al., in a prospective, double blinded, randomized study using leukodepleted RBCs stored for <5 days or for >20 days did not observe any significant adverse effects in critically ill anemic patients [30].

In another study, van Bommel et al. [31], in rats, showed that RBCs stored for 28 days were not successful in restoring microcirculatory oxygenation of the gut. They reported, however, that with the exception of the citrate-phosphate-dextrose (CPD)-stored group, the storage lesion was not severe enough to impair intestinal oxygen consumption. However, 'd Almeida et al. and Raat et al. [23, 32] indicated some limitations in the type of rat model where stored rat RBCs are used for transfusion: Rat RBCs age faster and stored rat RBCs do not regenerate 2, 3-diphosphoglycerate (2, 3-DPG) unlike human RBCs. Raat et al. developed a rat model able to accommodate human RBC transfusions. In a randomized, controlled study the ability of fresh (2–6 days), intermediate (2–3 weeks), and old (5–6 weeks) stored human RBCs to improve gut microcirculatory oxygenation in anemic oxygen-dependent rats, was diminished in the 5–6 week group compared to the fresh and intermediate groups. Based on their findings, the authors above suggested that a limit of 18–28 days could be used to define a RBC unit as 'old'. If such a threshold were applied in clinical practice, approximately two thirds of all transfused RBCs would be classified as old. Therefore, storage related RBC dysfunction, if clinically important, could have far reaching consequences.

In conclusion, however it must be kept in mind that the only available alternative to blood transfusion is to tolerate anemia and apply hemodilution. This procedure may, however, be even more deleterious than transfusion of 'old' blood [33]. This clinical dilemma led to an editorial by Spiess [34] summing up this problem in transfusion medicine in the title as "Damned if you do/damned if you don't".

Storage

The development of blood storage produced a dramatic change in transfusion practice from when two people, a patient and a healthy donor, had to wait directly connected to each other during the transfusion, to today where blood can be stored in solutions for up to 35–42 days with a 24-hour survival of 75–80%. Nowadays, liquid preservation is the most common way to preserve blood for transfusions practice. Liquid preservation, however, has been shown to affect RBC structure and its functional properties, which may interfere with its oxygen transporting capacity [24, 35]. This has been the rationale of limiting the age of stored blood to 42 days. To prevent transfusion of dysfunctional RBCs and standardize transfusion practice, several criteria have been set. However, these criteria are based on the physical properties of RBC units, such as the mean hemoglobin mass per unit, 24-hour survival of 75%, and 1% hemolysis and do not reflect the clinical oxygen efficacy of blood transfusion. [35]. If these criteria cannot differentiate between good and bad functioning red cells *in vivo*, updating and re-evaluating these criteria may be necessary.

Storage Lesion

RBCs are affected by two different mechanisms: first, the microcirculatory condition of critically ill patients that limits the adequate circulation of transfused blood, and second, storage lesions. Storage results in a number of physical and biochemical changes which together are described as the storage lesion.

Biomechanical changes

Hemorheological alterations, such as changes in RBC shape, decreased membrane deformability, and increased aggregability, are effects that can occur during storage and which may possibly disturb RBC flow through the microcirculation and influence its functional activity of transporting oxygen to the tissue cells. The loss of phospholipids from RBCs is seen both in storage and in RBC aging and may contribute to the formation of echinocytes with protrusions and spheroechinocytes during storage [36, 37]. These changes can occur parallel to decreases in surface-volume ratio, increased mean cell hemoglobin concentration and osmotic fragility and decreased deformability. The storage-related decrease in RBC membrane deformability is thought to be associated with reduced adenosine triphosphate (ATP) levels. However; it has been shown that the membrane alterations preceding the reduction in ATP may not have such a major role in storage related lesion, if any.

Other mechanisms, such as membrane phospholipid loss or redistribution, protein and lipid oxidation, have been suggested to contribute to the storage-dependent alterations in RBC membranes. The formation of microvesicals, causing the loss of membrane phospholipids, was identified by Rumsby et al. [38]. An alternative mechanism that has been proposed is the internalization of phosphotidylserine and phosphoethanolamine from the membrane into the cytosol [39] and loss of asymmetry in the RBC membrane. These biomechanical alterations may account for less deformable RBCs, and may cause more problems for a microcirculation already under stress. However, biomechanical alterations are probably not the only problem occurring during storage. This suggestion is supported by a recent study by Verhoeven et al. in which they compared two different methods to change RBC symmetry. They compared flippase, which moves phosphotidylserine from the outer to the inner leaflet of the membrane, to phospholipid scrambling which moves phosphotidylserine from the inner leaflet to the outer leaflet. They showed a decrease in flippase activity starting after 21 days of storage in saline-adenine-glucose-mannitol (SAGM) and further decreasing over time. The authors also showed that the correction of storage induced metabolic changes and restored flippase activity [40].

Biochemical Changes

2,3-DPG: The initial studies about loss of ability of RBCs to deliver oxygen after storage focused mostly on 2,3-DPG. 2, 3-DPG is a metabolite and allosteric modifier of hemoglobin and decreases to very low levels during the first 2–3 weeks of storage. This decrease leads to an increase in hemoglobin oxygen affinity, which may be an explanation for the decrease in RBC ability to deliver oxygen during storage. However 2, 3-DPG levels recover within hours after transfusion [41]. Additionally, a recent experimental study showed that although RBCs were stored for 2–3 weeks and were completely devoid of 2, 3-DPG, their capacity to deliver oxygen to the intestinal microcirculation was not different to that of fresh (2–6 days) RBCs [23].

ATP: An additional biochemical change which occurs in stored RBCs is the decrease in intracellular ATP levels. ATP, in addition to playing a secondary role in membrane deformability, is crucial for RBC function due to its role as a vasodilator under hypoxic conditions [42, 43].

Raat et al. showed that ATP levels remained unchanged in RBCs stored for 2–3 weeks, but dropped to 60% in RBCs stored for 5–6 weeks. This finding was also associated with the ability of RBCs to deliver oxygen and old (5–6 weeks storage) RBCs had a reduced oxygen delivery (DO_2) compared to fresh (2–6 days) and intermediate (2–3 weeks) groups. This finding supports the idea that ATP, suggested to be a vasodilator released by RBCs in the presence of hypoxia, is related to the DO_2 capacity of RBCs and may be negatively affected by storage duration [25].

Nitric oxide: Another mechanism that may account for alterations in the oxygen transport capabilities of transfused RBCs involves nitric oxide (NO). NO and its products, besides their many other roles inside the organisms, can be regarded as one of the major compounds accounting for vasodilatory regulation of blood vessels. Recent studies have shown that RBCs are able to release NO in the presence of hypoxia and that this nitrite-mediated function accounts for hypoxia induced vasodilation [44, 45]. The further identification of functional endothelial NO synthase (eNOS) on RBC membranes has made the RBC a central player, not only in oxygen transport, but also in vascular control mechanisms [46]. It could well be that this NO-mediated function of RBCs may be affected during storage.

Oxygen Delivery in Normal Physiological Conditions and Pathologic Conditions

In general, DO_2 to the tissues is simply calculated as the product of blood flow and arterial oxygen content. This can be described as follows:

$$DO_2 = Q \text{ (flow)} \times CaO_2 \text{ (arterial oxygen content)}$$
$$CaO_2 = (Hb \times SaO_2 \times 1.31) + (PaO_2 \times 0.003)$$

where 1.34 represents the oxygen binding capacity of hemoglobin (ml O_2 per gram Hb) and 0.003 the solubility coefficient for oxygen in blood (0.003 ml O_2 is dissolved for each mmHg of partial O_2 pressure). However, oxygen flux into the tissue and finally into the cells also depends on many other factors such as blood flow distribution between organs and within the microcirculation, functional capillary density, RBC transit times, tissue diffusion coefficient, oxygen transport across the cell membrane, and finally mitochondrial function and oxygen requirement.

RBC Physiology, Microcirculation, and Oxygen Delivery in Critically Ill Patients

RBCs are primarily responsible for oxygen and carbon dioxide exchange and transport from the lungs to the tissues. This exchange is facilitated through the synergistic effects of hemoglobin, carbonic anhydrase, and band 3 protein, and followed by carbon dioxide delivery to the lungs for release. Within the organs, in order to deliver oxygen to the tissues, RBCs need to travel through a fine network of vessels with diameters smaller than 100 micrometer: The so called microcirculation. Normally, erythrocytes have a flexible membrane and can reversibly alter their bicon-

cave, discoid shape, which allows them to pass through capillaries smaller in diameter (2–6 micrometer) than a RBC (8 micrometer). It is obvious that RBC membrane properties are of great importance for entering the capillaries and, thereby, delivering oxygen to the tissues.

Under normal physiological conditions, this finely regulated system of capillaries, arterioles, and venules is able to supply oxygen in excess of oxygen demand, so that cells can continue their function under changing metabolic demands. The microcirculation has an oxygen-dependent regulation system which is connected to the systemic circulation but is also able to regulate and direct blood flow to the tissues where it is needed. The flow of blood in the microcirculation, even under normal conditions, has a heterogeneous nature which actually ensures the even distribution of oxygen to the tissues so that each cell receives the oxygen it needs. Therefore, hypoxia detecting mechanisms are required in the tissues to produce vasoactive compounds affecting blood flow and, thereby, oxygen transport. In addition to endothelial factors, as discussed above, the RBC plays a central role in this process.

A decrease in hemoglobin concentrations during anemia is tolerated to certain levels by the action of compensatory mechanisms. Paradoxically, a moderate decrease in hematocrit may improve oxygen transport by lowering blood viscosity and, thereby, improving microvascular perfusion. With this in mind, an optimal hematocrit is predicted to be lower than physiological hematocrit.

The increase in cardiac output over a wide range of hemoglobin concentrations compensates for a decreased oxygen transport capacity. When it is needed this will be compensated by increased blood flow. This was shown by van der Linden et al. [47] who found that fresh RBCs were as efficient as blood flow increases in relieving oxygen supply-dependent conditions. However, the increased cardiac output is redistributed in favor of organs in most need of oxygen, i.e., the heart and brain. In the vascular bed of other organs, for example, the gastrointestinal tract, blood flow does not increase following hemodilution, and oxygen consumption is preserved by more efficient oxygen extraction. Similarly, the brain has a wide residual extraction and can increase the extraction ratio to higher values. On the contrary, the heart has a very limited extraction residue. If the decrease is more than the compensatory mechanisms can handle, further decrease in arterial oxygen content can lead to an increase in the oxygen extraction ratio ($O_2ER=VO_2/DO_2$). From this point, further reductions in hemoglobin concentration produce oxygen supply dependency of oxygen uptake (VO_2). Further decreases in DO_2 will result in decreases in VO_2 and leave the tissues under hypoxic stress, which if not corrected may lead to irreversible damage.

In critical illness, in addition to anemia, alterations in the physical and functional properties of RBCs occur, similar to storage lesion. These alterations may impair RBC deformability and increase the adhesion of RBCs to endothelial cells [13] or may cause increased intermittent flow or flow stasis and alterations in erythrocyte velocity. All these factors contribute to altered oxygen transport to the tissues and oxygen availability to cells and, thereby, can contribute to the development of organ dysfunction in ICU patients. Endothelial dysfunction or decreased vascular contractility can occur due to ischemia/reperfusion injury after ischemia or after the inflammatory process in sepsis. The release of cytokines during trauma interferes with vascular regulation and oxygen consumption. Reduced RBC deformability in different states of shock and inflammation can be induced by a wide range of factors, such as lipid peroxidation, oxidative stress, spectrin-hemoglobin cross linking, decreased intracellular ATP, loss of membrane surface sialic acid and NO. Apart

from their adverse effects on the microcirculation, pathologic alterations in RBCs may also modulate the inflammatory response of the mononuclear phagocyte system, as these damaged erythrocytes are cleared from the circulation by phagocytosis. In addition, release of cytokines by primed leukocytes, as well as storage-induced lesions of the RBCs themselves may play a role in the diminished tissue oxygenation and patient outcome. As a consequence, microcirculatory function can be decreased, which may contribute to organ dysfunction in critically ill patients [48]. A major limitation in being able to assess the efficacy of blood transfusion to correct microcirculatory DO_2 is the lack of suitable bedside techniques for measuring the determinants of microcirculatory function and tissue oxygenation. A further complication is the insensitivity of systemic hemodynamic and oxygen-derived variables in being able to sense microcirculatory alterations [49].

These considerations underscore the need to directly measure microcirculatory function at the bedside. Three techniques are available, none of which has as yet been applied to the study of the effects of blood transfusions at the bedside of critically ill patients: orthogonal polarization spectral (OPS) imaging or side-stream dark field (SDF) imaging techniques, tissue capnography, and reflectance spectrophotometry.

Visualization of the microcirculation has been a very important issue in the last 10 years especially in critical care medicine. The limitations of using *in vitro* microscopy to assess the microcirculation in the clinical situation led to the development of advanced optical imaging techniques which can be used non-invasively at the bedside. The development of OPS imaging allowed for the first time observations of the microcirculation of internal human organ systems, such as the brain. This development was followed by the development of SDF imaging, with higher magnification and reduction of surface reflection. These imaging techniques, applied sublingually to view blood flow in the microcirculation, have proved to be a highly sensitive marker of outcome in septic patients [50]. Measurement of oxygen availability in the microcirculation is achieved by fiber reflectance spectrophotometry. Here an optical fiber is placed on the tissue surfaces and the optical spectrum of reflected light analyzed. From this analysis the percent microcirculatory hemoglobin saturation can be calculated. This measure, as we showed in a clinically relevant porcine modal, is a precise measurement of the microcirculatory oxygen availability [51]. With the exception of a case study in pediatric patients, none of these bed-side applicable

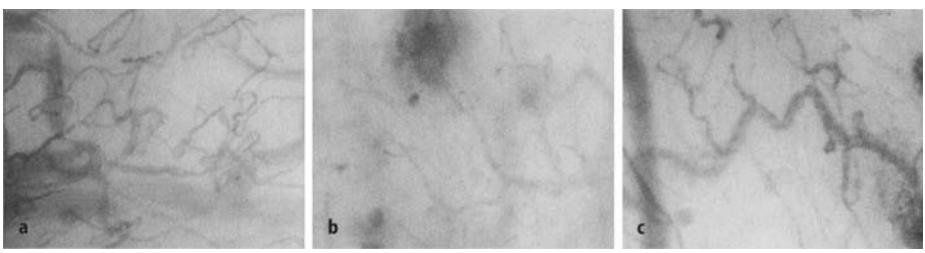

Fig. 1. A side-stream dark field (SDF) measurement in a cardiac patient during extracorporeal circulation showing microcirculatory anemia. Subsequent blood transfusions show a marked improvement in microcirculatory red blood cell (RBC) availability. Before extracorporeal circulation, systemic hemoglobin was 6.4 mmol/l (10.3 g/dl, panel **a**), whilst in panel **b**, during extracorporeal circulation, this value decreased to 4.0 mmol/l (6.4 g/dl). Decreased grayness of the image and fall out of capillaries are seen in panel **b**. In panel **c**, after blood transfusion, increased numbers of RBCs can clearly be seen as a significant increase in darkness as well as in vessels.

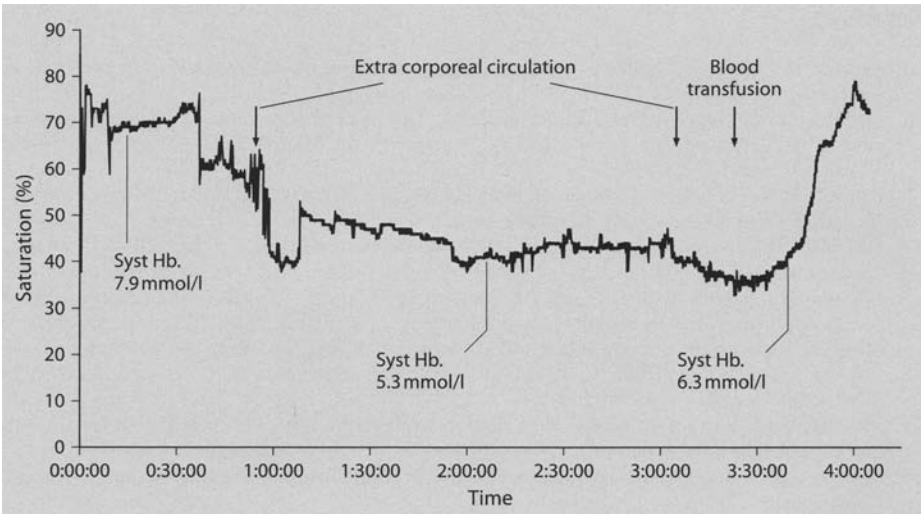

Fig. 2. Sublingual fiber reflectance spectrophotometry showing the effects of blood transfusion on microcirculatory hemoglobin concentration in an anemic cardiac surgery patient on extracorporeal circulation. The first arrow shows the aortic clamp being placed on and the extracorporeal circulation being initiated. The subsequent effects of hemodilution can be seen by a decrease in sublingual microcirculatory hemoglobin concentration. The second arrow indicates the release of the aortic clamp and restoration of the patient's own circulation. At the third arrow, a blood transfusion is given to correct the anemia. As can be seen from this case, sublingual spectrophotometry clearly measures the increased microcirculatory hemoglobin concentration following blood transfusion. 1 mmol/l = 1.61 g/dl

techniques have been applied to studies of blood transfusions in critical illness. Their application, however, may be very promising for the functional assessment of the ability of transfused blood to transport oxygen to the microcirculation and tissue cells. We have performed a number of measurements to explore the feasibility of these techniques to study the microcirculatory impact of blood transfusions. Two examples are presented in Figures 1 and 2 using SDF imaging and reflectance spectrophotometry, respectively, during blood transfusions given during cardiac surgery.

Conclusion

In conclusion, transfusion as the main treatment of anemia in critically ill patients should be revisited and transfusion triggers be re-evaluated. Leukodepletion, especially pre-storage leukodepletion, is probably beneficial in terms of patient mortality and morbidity. However, the final verdict on this issue awaits a large, multicenter, international study. Critically ill patients have many other factors, which may affect the RBC and its functions, making the impact of blood transfusions even more uncertain. It is clear that for proper individual evaluation of whether blood transfusions have been affective in correcting microcirculatory function and tissue oxygenation, these parameters will have to be measured at the bedside. OPS/SDF imaging or spectrophotometry may provide suitable techniques for this purpose.

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Red Blood Cell Transfusion in the Pediatric ICU

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Introductin

The decision process leading to red blood cell (RBC) transfusion should be based as much as possible on available evidence. Risks and benefits of transfusion as well as the risks attributable to anemia must be taken into account. In this chapter, we will discuss what prompts pediatric intensivists to prescribe a RBC transfusion, what should guide this decision, and what is missing to really make a decision based on an evidence-based approach.

Four questions should be raised when considering administration of a RBC transfusion:

- Is there hemorrhagic shock?
- What is the hemoglobin level?
- What is the severity of illness of the patient?
- Is there any other factor that might affect the decision to prescribe a RBC transfusion?

Is there Hemorrhagic Shock?

It is inappropriate to base a decision to transfuse RBCs on the hemoglobin concentration in patients with hemorrhagic shock. The rationale of this statement is easy to understand. Hemoglobin concentration does not drop immediately after an acute bleeding event: the intravascular blood volume decreases, but the hemoglobin concentration will begin to drop only after a few hours, when the circulating blood volume is diluted by fluids given for resuscitation, or after some liquid has moved from the interstitium to the intravascular space. The most important intervention is to stop the acute bleeding and to immediately transfuse if the blood loss is life-threatening. The amount of packed RBCs to transfuse should be based on the amount of blood lost, and on the clinical response of the patient.

What is the Hemoglobin Concentration?

When there is no hemorrhagic shock, the hemoglobin concentration is the most important marker to guide practitioners in the prescription of RBC transfusion. This was confirmed by respondents to questionnaires addressed to intensivists working with critically ill adults [1] and children [2, 3]. When asked at the bedside what drove them to give a RBC transfusion, intensivists responded that the hemoglobin

concentration was the first element in their decision process [4]. Therefore, it makes sense to study what hemoglobin threshold should be used in critically ill patients, taking into account the severity of illness of the patient and the presence or absence of diseases like congenital cardiopathy or sickle cell anemia. A few studies have been published on hemoglobin concentration and what should be the right threshold for RBC transfusion, given the severity of illness and the disease of the patient [5].

What is the Severity of Illness?

A safe hemoglobin concentration in stable ICU patients

There is strong evidence that it is safe not to give RBC transfusion to critically ill children with a hemoglobin concentration between 70 and 95 g/l if they are hemodynamically stable (by stable, we mean that the mean arterial pressure is not less than two standard deviations below normal mean for age and that cardiovascular support has not been increased for at least two hours).

In 1999, Hébert et al [6] published the Transfusion Requirements In Critical Care (TRICC) study, which involved critically ill adults who were randomized to a restrictive or a liberal strategy (threshold hemoglobin of 70 and 90 g/l, respectively). The results of this clinical trial did not show any benefit with a higher transfusion threshold and, in fact, suggested a possibly increased risk with a RBC transfusion threshold of 90 rather than 70 g/l. An adjusted score estimating the severity of multiple organ dysfunction syndrome (MODS) was significantly lower in the restrictive group ($p = 0.03$). There were also more hospital deaths in the liberal than in the restrictive group ($p < 0.05$). The incidence rates of intensive care unit (ICU) deaths and nosocomial infections were also higher in patients who received more transfusions.

In 2000, there were no available data on the safety of a restrictive or liberal RBC transfusion strategy in critically ill children. We therefore undertook the Transfusion Requirement In Pediatric ICU (TRIPICU) study, a multicenter, randomized, controlled non-inferiority clinical trial designed to determine whether a restrictive transfusion strategy is not inferior to a liberal transfusion strategy in usual clinical pediatric ICU practice when only pre-storage leukocyte reduced, packed RBC units are used. The hypothesis was that the risk of adverse outcome that can be caused by anemia in a restrictive strategy group (threshold for RBC transfusion: hemoglobin concentration of 70 g/l) would not be greater than the risk of adverse outcome attributable to more RBC transfusions in a liberal strategy group (threshold: 95 g/l). We enrolled 637 stable critically ill children who had a hemoglobin concentration below 95 g/l within 7 days after admission to the ICU. We randomly assigned 320 patients to a restrictive group and 317 patients to a liberal group. Hemoglobin concentrations were maintained 21 ± 2 g/l lower in the restrictive compared to the liberal group ($p < 0.0001$). The restrictive group received 54% fewer RBC transfusions, and 174 patients (54%) received no RBC transfusion compared with 7 patients (2%) in the liberal group ($p < 0.0001$). The number of patients who developed new or progressive MODS (primary outcome) was 38 (11.9%) in the restrictive versus 39 (12.3%) in the liberal group (absolute risk reduction: 0.4%; 95% confidence interval: −4.6% to 5.4%). There were 14 deaths (4.4%) in each group within 28 days of randomization. No differences were found in other outcomes. The conclusion of the TRIPICU study was that a RBC transfusion threshold of 70 g/l in stable critically ill children significantly decreased RBC transfusion requirements without increasing adverse events when pre-storage leukocyte-reduced RBCs were used.

Some similarities and some differences between the TRICC [6] and the TRIPICU studies [7] are striking. For example, the proportion of patients with two or more organ system failures was similar at baseline in the two studies: 31% (257/838) in the TRICC study [6] and 37% (233/637) in the TRIPICU study. Yet, there was a difference in the number of in-hospital deaths in the TRICC study (93 vs. 118, $p=0.05$), but not in the TRIPICU study (14 vs. 14). Many explanations for this apparent discrepancy can be considered.

First, a regression to the mean or an alpha error are possible, but the results of the TRIPICU study are consistent and statistically significant. Second, the case-mix and the populations are different: the TRICC study involved critically ill adults, while the TRIPICU study included critically ill children. Adults may be more vulnerable to the adverse consequences of RBC transfusion. For example, it is possible that adults with coronary heart disease do not support anemia as well as other critically ill patients; atherosclerosis is rare in pediatric ICU patients. Another plausible explanation for difference in the adverse effects of transfusion is that RBC units were not pre-storage leukocyte-reduced in the TRICC trial [6], while they were in the TRIPICU study [7]. Blood components with fewer than 5×10^6 white blood cells (WBCs) can be labeled as leukocyte reduced. More cytokines can be detected in older RBC units with a higher number of leukocytes [8–11]. There is some clinical evidence that leukocyte-reduction is effective in decreasing the incidence rate of inflammatory-related complications of RBC transfusion. The data from one randomized clinical trial suggests that leukocyte-reduction improves the postoperative outcome of some patients [12]. Implementation of pre-storage leukocyte-reduction as a standard procedure was associated with reduction in bronchopulmonary dysplasia, retinopathy of prematurity, and necrotizing enterocolitis in premature infants [13], and of post-transfusion fever in older patients [14]. The TRIPICU study does not prove that pre-storage leukocyte-reduction decreases the risk of developing MODS in critically ill patients because it was not designed to address this question, but it suggests that pre-storage leukocyte-reduction may be of some benefit in critically ill patients.

Lowest hemoglobin concentration in stable ICU patients

We can conclude from the evidence described above that it is safe not to give a RBC transfusion to stable critically ill patients if their hemoglobin concentration is higher than 70 g/l. Yet, one can ask, what is the lowest hemoglobin concentration that can be well supported by such patients (Fig. 1).

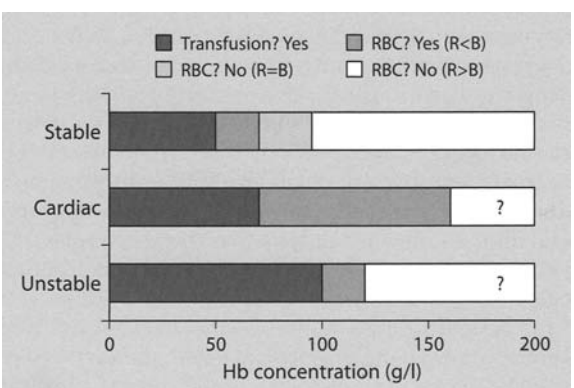

Fig. 1. In stable critically ill children, it is probably safe not to give a red blood cell (RBC) transfusion if the hemoglobin (Hb) concentration is higher than 70 g/l. Higher Hb concentrations are possibly required in children with cardiac disease. The randomized clinical trial completed by Rivers et al [24] suggested that it might be useful to keep the hematocrit over 30% (Hb over 100 g/l) during the first six hours of resuscitation of unstable patients in severe sepsis or shock. B: benefits; R: risks;

There is some evidence that severe anemia can be detrimental to critically ill patients. Oxygen delivery (DO_2 depends heavily on hemoglobin concentration, and there is a risk that severe anemia causes cellular dysfunction [15]. Anemia was associated with a poorer outcome in the CRIT trial, a descriptive prospective study that included 4892 consecutive critically ill adults collected from 213 American ICUs [16]. A retrospective cohort descriptive study involving 1958 Jehovah's Witness Patients showed that the odds ratio for mortality increased in adults with ischemic heart disease if their pre-operative hemoglobin contentration dropped below 100 g/l [17]. Another prospective descriptive study involving 300 Jehovah's Witness patients showed that the odds ratio for mortality increased in healthy adults without ischemic heart disease if their post-operative hemoglobin concentration dropped below 40 g/l [18]. Three prospective studies run in Kenya involving respectively 2433 [19], 1223 [20], and 1269 [21] hospitalized children showed that the risk of death was significantly higher if their hemoglobin concentration was lower than 50 g/l and if they did not receive a RBC transfusion; these children were not critically ill, but most of them had some respiratory symptoms. In another clinical trial, 100 hospitalized, preterm infants were randomized either to a restrictive or a liberal group (three different threshold levels were considered in each group, the threshold being higher when patients were sicker); the risk of developing intraparenchymal brain hemorrhage or periventricular leukomalacia and episodes of apnea was higher in the restrictive group [22].

RBC transfusions are frequently given to critically ill children if their hemoglobin concentration is low. A retrospective study undertaken in 240 critically ill children with a hemoglobin concentration ≤ 90 g/l and conducted in five American pediatric ICUs showed that most pediatric intensivists would transfuse if the hemoglobin concentration drops below 64 g/l [23]. In fact, all patients with a concentration ≤ 53 g/l and 38 out of 41 with a concentration ≤ 64 g/l received at least one RBC transfusion while only 33 of the 105 patients with a Hb concentration ≥ 80 g/l were transfused.

Thus, severe anemia increases the risk of poorer outcome, at least in severely ill patients. The available evidence suggest that a RBC transfusion should be given if the hemoglobin concentration drops below 50 g/l. It is probably appropriate to do so if the hemoglobin concentration is between 50 and 70 g/l, but the evidence to support this is not so strong. On the other hand, stable critically ill children do not require a RBC transfusion if their hemoglobin concentration is above 70 g/l.

Threshold hemoglobin concentration for RBC transfusion in sicker ICU patients

The randomized clinical trial by Rivers et al [24] in the emergency room of a university-affiliated american hospital showed that a rapid (<6 hours) protocol-driven aggressive therapy with a specific goal decreased the mortality of adults with severe sepsis and septic shock. The primary goal was to attain a central venous oxygen saturation ($ScvO_2$) greater than 70%. The rationale was that providing enough DO_2 should prevent the appearance of more cellular insults in critically ill patients and decrease the severity of organ dysfunction that could occur thereafter. In this randomized clinical trial, standard treatment was compared to a protocolized approach and all the following actions that could improve DO_2 and/or decrease oxygen uptake (VO_2) were considered: early endotracheal intubation and mechanical ventilation, aggressive infusion of crystalloids and colloids (up to 80 ml/kg within six hours), RBC transfusion to maintain the hematocrit over 30%, inotrope administration (dobutamine), and vasoconstrictive therapy (epinephrine, norepinephrine, dopamine). The results were spectacular, with a mortality rate of 46.5% in the standard

treatment group (133 patients) compared to 30.5% in the 'early goal-directed therapy' group (130 patients, $p=0.009$). The importance of maintaining the hematocrit above 30% in this success story is unknown, but it might be substantial. Actually, Rivers [25] believes that critically ill patients should receive more aggressive treatment at the beginning, while less agressive therapy may be required once the patient becomes stable. There are no other data supporting such an approach, but it makes sense to treat anemia more aggressively in sicker patients until they improve. Therefore, it may be appropriate to keep the hemoglobin concentration over 100 g/l (hematocrit of 30%) during the first hours of treatment of severely ill patients, while later on, a hemoglobin concentration of 70 g/l may be high enough to fulfill the oxygen requirements of stable patients.

Children with cardiac diseases

Myocardial protection may be the most important goal to attain in children with congenital heart disease. Patients with impaired ventricular function cannot increase their cardiac output as efficiently as other patients. Moreover, even at rest, oxygen extraction by myocardial cells is elevated, which implies a lessened coping capacity when anemia occurs. Thus, increasing the hemoglobin concentration may be the only way to increase DO_2 and adequately support cardiac function in these patients.

The heart is clearly very sensitive to the hemoglobin concentration: Weiskopf et al [26] showed that the heart rate increases steadily in healthy adults at rest when hemoglobin concentration is decreased from 140 to 50 g/l. In healthy animals undergoing acute hemodilution, evidence of heart dysfunction appears only once the hemoglobin concentration drops below 33 to 40 g/l [27]. However, animals with 50% to 80% coronary artery stenosis can show evidence of ischemic insult to the heart with a hemoglobin concentration as high as 70 to 100 g/l [28]. There is some evidence that prevention of organ dysfunction, including 'myocardial protection', with a higher hemoglobin concentration may be a good goal in adults with coronary disease. A retrospective study involving 1475 adults showed that the risk of developping renal injury after coronary bypass surgery was higher if the hematocrit dropped below 24% [29]. In adult patients with cardiovascular disease who refused blood products for religious reasons, Carson et al [17] showed that the risk of mortality after elective surgery increased significantly when the hemoglobin level dropped below 100 g/l whereas in healthy patients who accepted transfusion, the risk of mortality increased only with a hemoglobin concentration below 40 g/l [18].

Is it appropriate to apply these data to children? Coronary atherosclerosis is rare in the pediatric population while it is the most frequent cardiovascular problem in adults. In spite of this, it seems reasonable to hypothesize that anemia can cause more damage to a sick heart. A randomized clinical trial reported that a lower hematocrit (21.5% vs. 27.8%) during the cardiopulmonary bypass of pediatric cardiac surgeries was associated with poorer neurodevelopmental outcome [30]. There is almost no other evidence that higher hemoglobin concentrations protect children with congenital heart disease. Nonetheless, many experts in pediatric cardiology believe in maintaining elevated hemoglobin levels in children without cyanotic heart disease and advocate levels of 120–130 g/l in neonates and 100 g/l in infants and children [15]. Not surprisingly, a bedside survey showed that the hemoglobin concentration before RBC transfusion was indeed higher during the postoperative period of cardiac surgery than for other patients in the pediatric ICU [31]. In spite of this, experts in the United Kingdom advocate low hemoglobin thresholds of

70–80 g/l in stable children with non-cyanotic heart disease [32]. There is indeed almost no 'hard evidence' that 'myocardial protection' with higher hemoglobin concentrations can be a good goal to direct therapy in children with cardiac disease. Thus, given that definitive evidence describing the optimal hemoglobin threshold for transfusion is lacking, it seems reasonable to assume that critically ill children with non-cyanotic cardiac disease should be maintained at a hemoglobin concentration above 70 to 100 g/l.

Equally unproven is the optimal hemoglobin concentration for children with cyanotic heart disease. Many textbooks in pediatric cardiology and pediatric cardiac surgery advocate elevated hemoglobin levels and several recommend specific thresholds that range from 100 to 180 g/l [15]. In fact, there are no good clinical studies that adequately address the question. A case series that included seven children with cyanotic congenital heart disease reported a decreased right to left shunt when increasing the hemoglobin concentration from 130 to 164 g/l; the authors specifically attributed the benefit seen to the decreased shunt and did not think that the benefit could be attributed to an increased VO_2 [33]. Interestingly, experience with bloodless surgery for complex cyanotic defects suggests that cardiac surgery can be safely performed with a lower concentration of hemoglobin without evidence of increased risk. Thus, as stated by Beekman and Tuuri in 1985 [33], it is still true in 2007 that "the optimal hemoglobin concentration for children with cyanotic heart disease has yet to be determined". Presently, it seems appropriate to consider a hemoglobin level above 100 or 120 g/l as reasonable for hemodynamically stable children with a cyanotic heart disease.

Other determinants should be considered in critically ill children with cardiac diseases. For example, in a prospective cohort study of 548 children undergoing cardiac surgery, RBC transfusions were more frequent not only when the preoperative hematocrit was considered low, but also in younger patients, if the surgery was complex, and with longer duration of hypothermia [34].

In summary, the optimal hemoglobin concentration in critically ill children with cardiac diseases is not well defined. Good clinical studies are required before any strong statements can be promoted about when these patients should receive a RBC transfusion.

Other diseases

There is good evidence supporting the point of view that a high hemoglobin concentration (above 90 or 100 g/l) is required in critically ill children with sickle cell disease [35, 36]. This may be true for other diseases, but hard data on this are lacking.

Other Factors that may Modulate Decisions about RBC Transfusion

Determinants other than the hemoglobin concentration can modulate the decision to prescribe a RBC transfusion in pediatric ICU [2, 3], as confirmed by two studies

1st study: An observational cohort study that included 303 children consecutively admitted to an academic pediatric ICU reported that 45 children (15%) received between 1 and 33 RBC transfusions, for a total of 103 transfusions. The stated reasons for administering a RBC transfusion included not only a low hemoglobin concentration, but also the presence of respiratory failure (84/103), active bleeding (67/103), hemodynamic instability (50/103), blood lactate level >2 mmol/l (10/103) or sub-optimal DO_2 (6/103) [4].

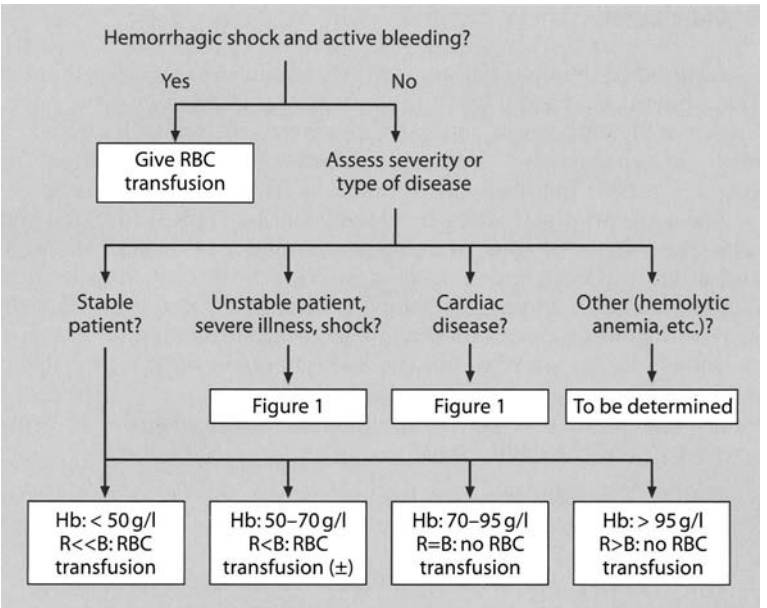

Fig. 2. Algorithm for RBC transfusion in critically ill children. In stable patients, RBC transfusion is usually mandatory if the hemoglobin (Hb) concentration is lower than 50 g/l (definition of 'stable': the mean arterial pressure is not less than two standard deviations below normal mean for age and cardiovascular support has not been increased for at least two hours). A RBC transfusion is probably useful if the Hb concentration is between 50 and 70 g/l; It makes no difference if the Hb is between 70 and 95 g/l. A transfusion may be detrimental if the Hb concentration is higher than 95 g/l. These thresholds for transfusion are probably different in unstable patients and in patients with cardiac disease. The right threshold Hb concentration for RBC transfusion in patients with other conditions, like some hemolytic anemias, is not well determined. B: benefits; R: risks;

2nd study: In a prospective cohort study of 985 consecutive pediatric ICU admissions at Sainte-Justine Hospital, the four most significant determinants of a first RBC transfusion included not only the presence of anemia (defined by a hemoglobin level <95 g/l) during the pediatric ICU stay (13.26; 95%CI: 8.04–21.88; $p<0.001$), but also an admission diagnosis of cardiac disease (8.07; 95%CI: 5.14–14.65; $p<0.001$), an admission Pediatric risk of Mortality (PRISM) score >10 (4.83; 2.33–10.04; $p<0.001$) and the presence of MODS during the pediatric ICU stay (2.06; 95%CI: 1.18–3.57; $p=0.01$) [31].

These studies show that many host-related and disease-related characteristics, as well as the hemoglobin concentration, account for the practice pattern variability observed in pediatric ICUs with respect to RBC transfusion. For pediatric intensivists, it seems generally that more severe illness requires a lesser tolerance of anemia in order to insure adequate DO_2. Yet, it is essential to obtain solid evidence on this subject because it is unclear what degree of anemia, what severity of illness, what type of disorders, and what specific goals should dictate therapy with RBC transfusion.

Conclusion

'Goal-directed therapy' can improve the outcome of critically ill patients. Rivers et al [24] showed that early goal-directed aimed at keeping $ScvO_2$ g/eater than 70% improves the outcome of patients with severe sepsis or septic shock. Van den Berghe et al [37] showed that tight blood glucose control between 4.44 and 6.1 mmol/l (80–110 mg/dl) improves the outcome of critically ill patients.

The concept of goal-directed therapy can be applied to blood transfusion medicine. However, what goal to use is still a matter of debate. Many 'goals' have been suggested in the literature, such as mixed venous oxygen saturation (SvO_2), blood lactate level, DO_2 and/or VO_2; none has been validated [38]. Currently, maintaining the hemoglobin concentration above a given threshold and taking into account the severity of illness and the etiologic disease remain the most reliable determinants of RBC transfusion in critically ill children.

In Figure 2, we propose an algorithm for RBC transfusion in pediatric ICU; three questions should be addressed:

1. The first question concerns the presence or absence of hemorrhagic shock.
2. The second question asks if the patient is stable or not, whether he/she is severely ill, and whether he/she presents some specific disease.
3. The third question concerns the hemoglobin concentration.

A large randomized clinical trial [7] suggests that a hemoglobin concentration between 70 and 95 g/l is safe in most stable critically ill children if pre-storage leukocyte reduced packed RBC units are used. Some published data suggest that a transfusion is probably required in pediatric ICU patients if their hemoglobin concentration is lower than 70 g/l, and that it is mandatory if their hemoglobin concentration is lower than 50 g/l. Higher thresholds are probably appropriate in more severely ill patients (for example, patients in shock) and in patients with cardiac disease, but more investigations are required before any strong recommendations can be made in such instances.

There is evidence that algorithm-driven prescription of RBC transfusion can decrease the number of transfusions given to critically ill adults [39]. The efficacy, the safety and the usefulness of the flow chart suggested in figure 2 remain to be determined by prospective studies; meanwhile, it makes sense to base the decision to give RBC transfusion to critically ill children on the available evidence, as is done in this figure.

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Thrombocytopenia in Intensive Care Patients

M. Levi, J.J. Hofstra, and S. Opal

Introduction

Thrombocytopenia is a common feature in intensive care patients. Similar to other settings in which thrombocytopenia may occur, the decrease in platelet count may be caused by impaired production, increased consumption, or enhanced degradation of these cells. In this chapter, we will discuss the epidemiology and differential diagnosis of a decreased platelet count in critically ill patients, First, we will briefly introduce platelet function and platelet vessel wall interaction in the normal situation and during severe infection and/or inflammation.

Platelet Function

Platelets are circulating blood cells that will normally not interact with the intact vessel wall but that may swiftly responding to vascular disruption by adhering to subendothelial structures, followed by interaction with each other, thereby forming a platelet aggregate [1]. The activated platelet (phospholipid) membrane may form a suitable surface on which further coagulation activation may occur. These processes are part of the first line of defense of the body against bleeding but may also contribute to pathological thrombus formation in vascular disease, such as thrombus formation on top of a ruptured atherosclerotic plaque. In case of systemic inflammatory syndromes, such as the response to sepsis, disseminated intravascular platelet activation may occur, which will contribute to microvascular failure and, thereby, play a role in the development of organ dysfunction. In addition, in this situation platelets may be directly involved in the inflammatory response by releasing inflammatory mediators and growth factors.

Under normal conditions, platelets continuously flow along the vascular surface in the human body without adhering or aggregating. However, upon disruption of the integrity of the vessel wall, a swift and complex interaction between circulating platelets, endothelial cells, and subendothelial structures occurs [2]. The result of this interaction is platelet adhesion to the vessel wall and formation of aggregates with each other, thereby creating a first line of defense against blood loss. The interaction between platelets and the vessel wall is mediated by cellular receptors on the surface of platelets and endothelial cells, such as integrins and selectins, and by adhesive proteins, such as von Willebrand factor and fibrinogen.

Platelet-vessel Wall Interaction

Briefly, platelets attach to the subendothelium by molecular bridges between platelet glycoprotein receptors, GPIb/V/IX, and ligands, most prominently von Willebrand factor, that bind subendothelial matrix proteins, such as collagen [3]. Upon this binding, platelets become activated and change their shape, thereby releasing the contents of their storage organelles, including fibrinogen and adenosine diphosphate, which will further promote platelet activation. The shape change will also result in the expression of active glycoprotein IIb/IIIa on the platelet surface, which will allow fibrinogen to form bridges between activated platelets, resulting in a platelet aggregate. During platelet activation and shape change, the platelet membrane turns into a phospholipid surface that is highly suitable for assembly of complexes of activated coagulation factors required for the formation of thrombin, thereby firmly linking the processes of platelet activation and thrombin generation. In recent years, detailed information on the respective roles of the various cell receptors and adhesive proteins in the interaction between platelets and the vessel wall has been accumulated.

Cellular Adhesion Molecules in Health and Disease

Cellular adhesion receptors are integrated membrane proteins that recognize adhesive proteins in plasma or in the extracellular matrix or connect to other cellular adhesion receptors (counter-receptors) [2]. In the interaction between platelets and the vessel wall integrins, selectins and members of the Ig-gene superfamily, which can be found on both platelets and endothelial cells, are most important. The regulation of cellular adhesion by these receptors relies on the ability to rapidly change the affinity of the receptor for its ligand [4]. Cellular adhesive receptors are grouped in several families.

Integrins consist of a non-covalently associated $\alpha\beta$ heterodimeric complex. In humans, there are 18 α and 8 β subunits, which can form up to 24 combinations [5]. Most of the subunits contain 750–1000 amino acids and form transmembrane proteins, for the major part extracellular and with a short cytoplasma tail. Integrins can bind to ligands in plasma (such as von Willebrand factor, fibrinogen, or fibronectin), whereas in the extracellular matrix adhesive proteins such as vitronectin, collagen, laminin, elastin, fibronectin, and von Willebrand factor act as ligands. Besides binding to adhesive proteins, integrins may also serve as signaling receptors, affecting the cytoskeletal apparatus (contributing to platelet aggregate stabilization) and triggering other processes, such as thromboxane A2 generation, increasing cytoplasma calcium, and phosphorylation of platelet proteins [6]. The most important integrin related to platelet aggregation is αIIb/β3 (GPIIb/IIIa), which is the receptor that binds to fibrinogen to form molecular bridges between activated platelets. Another important integrin is the $\alpha 2\beta 1$ (GPIa/IIa) receptor, capable of binding collagen. The role of $\alpha 2\beta 1$ in platelet-collagen interaction is likely to be limited to low shear stress situations [7].

The superfamily of selectins consists of L-selectins and E-selectins, expressed on leukocytes and endothelial cells, respectively, and P-selectins, expressed on both platelets and endothelial cells. E-selectin and P-selectin mediate attachment of neutrophils on cytokine-activated endothelial cells. P-selectin is stored in platelet granules and in Weibel-Palade bodies in the endothelium [8]. On stimulation of platelets

and endothelial cells, it is released and integrated into the cell membrane. P-selectin may form a molecular bridge between activated endothelial cells, platelets, and neutrophils. P-selectin also forms a bridge from platelet-vessel wall interaction to fibrin formation by enhancing the expression of tissue factor on monocytes [9]. Tissue factor is the main initiator of thrombin generation and subsequent fibrinogen to fibrin conversion. P-selectin can be relatively easily shed from the surface of the platelet membrane and soluble P-selectin levels have been shown to be increased during acute coronary syndromes and systemic inflammation [9].

Cell adhesion receptors with leucine-rich motifs form a distinct group of receptors. The most important member of this group is glycoprotein Ib (GPIb), which is the receptor for von Willebrand factor, involved in platelet adhesion to the endothelium at high shear stress. GPIb, consisting of two subunits, GPIbα and GPIbβ, forms a transmembrane complex with GPV and GPIX, two other receptors in this family with leucine-rich motifs and this complex is firmly anchored in the platelet membrane. Relatively recent data indicate that GPIb/V/IX is involved in platelet tethering to and rolling on the endothelium mediated by endothelial expression of E-selectin [10]. GPIb/V/IX can also bind the neutrophil receptor Mac-1, thereby mediating platelet-neutrophil interaction [11].

The Ig-gene superfamily comprises a large family of molecules involved in the recognition of adhering cells (such as cellular adhesion receptors) and of non-self antigens (such as T-cell receptors, antibodies and MHC molecules). The cellular adhesion receptors intercellular adhesion molecule (ICAM) 1–3, vascular cell adhesion molecule (VCAM), and platelet-endothelial cell adhesion molecule (PECAM) belong to this group and play an important role in leukocyte-endothelial cell interaction. Regarding platelet-endothelial cell interaction, PECAM acts not so much as a direct adhesive receptor but rather as a negative regulator of platelet activation. Another adhesive receptor in the Ig-gene superfamily is GPVI, which is a platelet receptor for collagen [12]. Although GPVI may be directly involved in platelet adhesion to collagen, it is likely that it predominantly acts as an activator of the α2β1 receptor.

GPIV (CD36) is a glycoprotein expressed in platelets, endothelial cells, mononuclear cells, and specific epithelial cells. On macrophages it acts as a scavenger for oxidized low-density lipoprotein (LDL). Platelet GPIV binds to thrombospondin and plays a role in the interaction between platelets and mononuclear cells [13].

Interaction between Cells and Adhesive Proteins

There are several pathways that play a role in platelet adhesion to the vessel wall but all are exemplified by cellular receptor-adhesive protein interactions. Most of these interactions have been precisely characterized using experiments with perfusion chambers containing for example de-endothelialized blood vessels. Although the mechanism by which platelets adhere to the vessel wall to achieve hemostasis is fairly well understood, the exact pathways that contribute to platelet adhesion and activation in many disease states, including infection and inflammation, are still unclear. Although essential aspects may be similar, factors like altered shear stress and local dysfunction of endothelial cells, potentially in association with inflammatory mechanisms are probably important in pathological thrombus formation [14].

Von Willebrand factor-mediated adhesion is the most prominent route of platelet adhesion. Von Willebrand factor is a large polymer of disulfide-linked subunits, each comprising 2050 amino acid residues and up to 22 carbohydrate chains [15]. The

molecular masses of the multimers range from about 500 kDa to more than 10.000 kDa. The multimers may form coiled molecules or thin filaments up to 1250 nm long (which means as large as a platelet). Data from clinical studies indicate that large von Willebrand factor multimers may be hemostatically more active than smaller molecules [16]. The biochemical basis for this observation probably relies on the fact that they contain a relatively large number of the domains that will support multiple interactions between the vessel wall, subendothelial matrix, and cellular receptors on platelets. Besides playing a role in platelet vessel wall interaction, von Willebrand factor may also be a ligand between platelet receptors IIb/IIIa, thereby competing with fibrinogen.

Collagen may be considered as another adhesive protein in platelet vessel wall interaction. Collagen types I and IV may directly bind to the integrin $\alpha 2\beta 1$ (GP Ia/IIa) [17]. The relevance of this pathway is underlined by studies with platelets from patients that are deficient in this glycoprotein, which show significantly decreased adhesion. Another platelet receptor for collagen is GPVI, although it is less likely that direct binding of this receptor to collagen is physiologically important [18]. The function of GPVI is rather related to activation of the $\alpha 2\beta 1$ receptor upon binding to collagen and consequent intracellular signaling [19]. In addition, GPIB-V-IX may be considered as a collagen receptor acting via von Willebrand factor.

Other adhesive proteins involved in platelet vessel wall interaction are fibronectin thrombospondin, laminin, and vitronectin. Fibronectin is largely a dimer, composed of subunits with a molecular mass of 220 kDa. Fibronectin is produced by megakaryocytes and stored in α-granules of the platelet and is secreted upon thrombin-induced platelet activation. Fibronectin can serve as a ligand for platelet-platelet interaction through the GPIIb/IIIa receptor. Thrombospondin is released from α-granules on platelet activation and binds to the platelet membrane, where it can interact with fibrinogen, fibrin, fibronectin, collagen, or other platelets. Binding of thrombospondin to the platelet is mediated by the GPIV receptor (CD36) and possibly by integrin $\alpha 5\beta 3$, whereas recently a role for GPIb has been proposed [20]. Both thrombospondin and CD36 can bind erythrocytes infected with *Plasmodium falciparum* (causing malaria tropica), which may account for the microvascular complications of severe malaria [21], and a similar mechanism has been described for thrombospondin binding to sickling cells, which may contribute to microvascular thrombosis in patients with sickle cell disease [22]. Laminin is a large glycoprotein (920 kDa) and is located in the extracellular matrix and the basement membrane. Laminin can bind to platelets but this interaction does not appear to result in platelet activation [23]. Vitronectin is functionally similar to fibronectin and may bind to platelet GPIIb/IIIa or to a specific integrin ($\alpha v\beta$ 3) [24]. Its affinity to artificial surfaces, such as glass, may play a role in platelet deposition on such objects. The role of vitronectin in platelet-vessel wall interaction is unclear. Vitronectin can bind and stabilize the fibrinolytic inhibitor plasminogen activator inhibitor type 1 (PAI-1), which may render fibrin clots less susceptible for lysis, but simultaneously vitronectin provides PAI-1 with thrombin-inhibitory properties.

Platelets in Critically Ill Patients

Critically ill patients often present with thrombocytopenia [25]. The incidence of thrombocytopenia (platelet count $< 150 \times 10^9$/l) in critically ill medical patients is 35–44% [26–28]. A platelet count of $< 100 \times 10^9$/l is seen in 20–25% of patients,

whereas 12–15% of patients have a platelet count $< 50 \times 10^9$/l. In surgical and trauma patients, the incidence of thrombocytopenia is higher with 35–41% of patients having less than 100×10^9/l platelets [29, 30]. Typically, the platelet count decreases during the first four days on the intensive care unit (ICU) [31]. The primary clinical relevance of thrombocytopenia in critically ill patients is related to an increased risk of bleeding. Indeed, severely thrombocytopenic patients with platelet counts of $< 50 \times 10^9$/l have a 4 to 5-fold higher risk for bleeding compared to patients with higher platelet counts [26, 28]. The risk of intracerebral bleeding in critically ill patients during intensive care admission is relatively low (0.3–0.5%), but in 88% of patients with this complication the platelet count is less than 100×10^9/l [32]. Moreover, a decrease in platelet count may indicate ongoing coagulation activation, which contributes to microvascular failure and organ dysfunction. Regardless of the cause, thrombocytopenia is an independent predictor of ICU mortality in multivariate analyses with a relative risk of 1.9 to 4.2 in various studies [26, 28, 29]. Several studies have shown that the number of platelets in critically ill patients is inversely related to survival. In particular, sustained thrombocytopenia over more than four days after ICU admission or a drop in platelet count of >50% during the ICU stay is related to a 4 to 6-fold increase in mortality [31, 26]. The platelet count was shown to be a stronger independent predictor for ICU mortality than composite scoring systems, such as the Acute Physiology and Chronic Health Evaluation (APACHE) II score or the Multiple Organ Dysfunction Score (MODS). A platelet count of $< 100 \times 10^9$/l is also related to a longer ICU stay but not to the total duration of hospital admission [28].

Differential Diagnosis of Thrombocytopenia in Critically Ill Patients

There are many causes for thrombocytopenia in critically ill patients. Table 1 summarizes the most frequently occurring diagnoses recognized in intensive care patients with thrombocytopenia.

Sepsis is a clear risk factor for thrombocytopenia in critically ill patients and the severity of sepsis correlates with the decrease in platelet count [33, 34]. The principal factors that contribute to thrombocytopenia in patients with sepsis are impaired platelet production, increased consumption or destruction, or sequestration of platelets in the spleen or along the endothelial surface. Impaired production of platelets from within the bone marrow may seem contradictory to the high levels of platelet production-stimulating pro-inflammatory cytokines, such as tumor necrosis factor (TNF)-α and interleukin (IL)-6, and high concentrations of circulating thrombopoietin in patients with sepsis. These cytokines and growth factors should theoretically stimulate megakaryopoiesis in the bone marrow [35]. However, in a substantial number of patients with sepsis marked hemophagocytosis may occur. This pathologic process consists of active phagocytosis of megakaryocytes and other

Table 1. Differential diagnosis of thrombocytopenia in critically ill patients

Sepsis
Disseminated intravascular coagulation
Massive blood loss
Thrombotic microangiopathy
Heparin-induced thrombocytopenia
Immune thrombocytopenia
Drug-induced thrombocytopenia

hematopoietic cells by monocytes and macrophages, hypothetically due to stimulation with high levels of macrophage colony stimulating factor (M-CSF) in sepsis [36]. Platelet consumption probably also plays an important role in patients with sepsis, due to ongoing generation of thrombin (which is the most potent activator of platelets *in vivo*), in its most fulminant form known as disseminated intravascular coagulation (DIC). Platelet activation, consumption, and destruction may also occur at the endothelial site as a result of the extensive endothelial cell-platelet interaction in sepsis, which may vary between different vascular beds in various organs [37].

In patients with DIC, the platelet count is invariably low or rapidly decreasing [38]. DIC is the most extreme form of systemic coagulation activation, which may complicate a variety of underlying disease processes, including sepsis, trauma, cancer, or obstetrical calamities, such as placental abruption. It is important to emphasize that DIC is not a disease in itself but is always secondary to an underlying disorder. DIC is a syndrome caused by systemic intravascular activation of coagulation, which may be secondary to various underlying conditions. Formation of microvascular thrombi, in concert with inflammatory activation, may cause failure of the microvasculature and, thereby, contribute to organ dysfunction. Ongoing and insufficiently compensated consumption of platelets and coagulation factors may pose a risk factor for bleeding, especially in perioperative patients or patients that need to undergo invasive procedures. The trigger for the activation of the coagulation system is nearly always mediated by several of the pro-inflammatory cytokines, expressed and released by mononuclear cells and endothelial cells. Thrombin generation proceeds via the (extrinsic) tissue factor/factor VIIa route. Tissue factor may be expressed on activated and inactivated mononuclear cells and endothelial cells and is capable of binding factor VIIa, which then activates downstream coagulation cascades. Concomitantly, impaired function of inhibitory mechanisms of thrombin generation, such as antithrombin and the protein C and S system, occurs. Antithrombin appears to be incapable of adequate regulation of thrombin activity in DIC for several reasons. Antithrombin levels are continuously consumed by the ongoing formation of thrombin and other activated proteases that are susceptible to antithrombin complex formation and antithrombin is degraded by elastase released from activated neutrophils. In addition, impaired synthesis of antithrombin, because of liver failure and extravascular leakage of this protease inhibitor as a consequence of capillary leakage, further contributes to low levels of antithrombin. There are several reasons for severe injury to the protein C system in DIC. Similar to antithrombin, enhanced consumption, impaired liver synthesis, and vascular leakage may result in low circulating levels of protein C. Second, activation of the cytokine network, in particular high levels of TNF-α, results in a marked downregulation of thrombomodulin on endothelial cells, thereby prohibiting adequate protein C activation. In addition, the anticoagulant capacity of activated protein C is reduced by low levels of the free fraction of protein S. In plasma, 60% of cofactor protein S is complexed to a complement regulatory protein, C4b binding protein (C4bBP), and increased plasma levels of C4bBP, as a consequence of the acute phase reaction in sepsis, may result in a relative protein S deficiency. A third mechanism contributing to the enhanced fibrin deposition in DIC is impaired fibrin degradation, due to high circulating levels of PAI-1, the main physiological inhibitor of fibrinolysis. Recent studies have shown that a functional mutation in the PAI-1 gene, the 4G/5G polymorphism, not only influenced the plasma levels of PAI-1 but was also linked to clinical outcome in sepsis and DIC. In other clinical studies in patients with DIC, a high plasma level of PAI-1 was one of the strongest predictors of mortality.

Patients with DIC have a low or rapidly decreasing platelet count, prolonged global coagulation tests, low plasma levels of coagulation factors and inhibitors, and increased markers of fibrin formation and/or degradation, such as D-dimer or fibrin degradation products (FDPs). Coagulation proteins with a marked acute phase behavior, such as factor VIII or fibrinogen, are usually not decreased or may even increase. One of the often advocated laboratory tests for the diagnosis of DIC, fibrinogen, is, therefore, not a very good marker for DIC, except in very severe cases, although sequential measurements can give some insight. There is no single laboratory test with sufficient accuracy for the diagnosis of DIC. However, a diagnosis of DIC may be made using a simple scoring system based on a combination of routinely available coagulation tests [39]. In a prospective validation study, the sensitivity and specificity of this DIC score was found to be more than 95% [40]. Furthermore, this DIC score was found to be a strong and independent predictor of mortality in a large series of patients with severe sepsis and identifies patients who will have most benefit of interventions on the coagulation system [41].

The group of thrombotic microangiopathies encompasses syndromes such as thrombotic thrombocytopenic purpura, hemolytic-uremic syndrome, severe malignant hypertension, chemotherapy-induced microangiopathic hemolytic anemia, and the HELLP syndrome [42]. A common pathogenetic feature of these clinical entities appears to be endothelial damage, causing platelet adhesion and aggregation, thrombin formation, and impaired fibrinolysis. The multiple clinical consequences of this extensive endothelial dysfunction include thrombocytopenia, mechanical fragmentation of red cells with hemolytic anemia, and obstruction of the microvasculature of various organs, such as kidney and brain (leading to renal failure and neurologic dysfunction, respectively). Despite this common final pathway, the various thrombotic microangiopathies have different underlying etiologies. Thrombotic thrombocytopenic purpura is caused by deficiency of von Willebrand factor cleaving protease (ADAMTS-13), resulting in endothelial cell-attached ultra-large von Willebrand multimers, that readily bind to platelet surface GPIb and cause platelet adhesion and aggregation [43]. In hemolytic uremic syndrome, a cytotoxin released upon infection with a specific serogroup of Gram-negative microorganisms (usually *E. coli* serotype O157:H7) is responsible for endothelial cell and platelet activation. In case of malignant hypertension or chemotherapy-induced thrombotic microangiopathy, presumably direct mechanical or chemical damage to the endothelium is responsible for the enhanced endothelial cell-platelet interaction, respectively. A

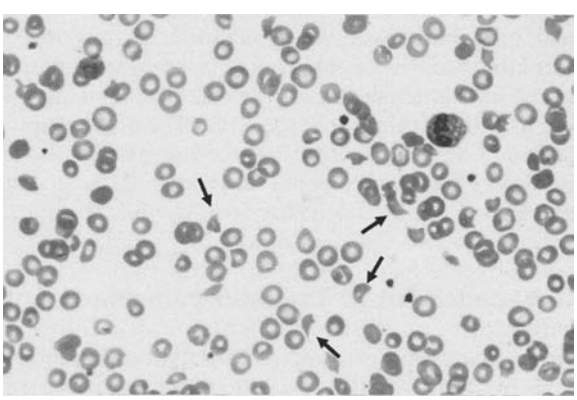

Fig. 1. Blood smear from a patient with thrombocytopenic thrombotic purpura, due to deficiency of ADAMTS-13. The arrows indicate schistocytes generated by mechanical damage to red cells. Also note the reduced number of platelets, indicating thrombocytopenia. (Giemsa staining, x40). Courtesy of Dr. J. van der Lelie, Academic Medical Center, Amsterdam, the Netherlands.

diagnosis of thrombotic microangiopathy relies upon the combination of thrombocytopenia, Coombs-negative hemolytic anemia, and the presence of schistocytes in the blood smear (Fig. 1). Additional information can be achieved by measurement of ADAMTS-13 and autoantibodies towards this metalloprotease and culture (usually from the stool or urine) of microorganisms capable of cytotoxin production.

Heparin-induced thrombocytopenia (HIT) is caused by a heparin-induced antibody that binds to the heparin-platelet factor IV complex on the platelet surface [37]. This may result in massive platelet activation and as a consequence a consumptive thrombocytopenia and arterial and venous thrombosis occurs. The incidence of HIT may be as high as 5% of patients receiving heparin and is dependent on the type and dose of heparin and the duration of its administration (especially when given for more than four days). A consecutive series of critically ill ICU patients who received heparin revealed an incidence of 1% in this setting [44]. Unfractionated heparin carries a higher risk of HIT than low molecular weight (LMW) heparin [45]. Thrombosis may occur in 25 to 50% of patients with HIT (with fatal thrombosis in 4–5%) and may also become manifest after discontinuation of heparin [46]. The diagnosis of HIT is based on the detection of HIT antibodies in combination with the occurrence of thrombocytopenia in a patient receiving heparin, with or without concomitant arterial or venous thrombosis. It should be mentioned that the commonly used ELISA for HIT antibodies has a high negative predictive value (100%) but a very low positive predictive value (10%) [44]. A more precise diagnosis may be made with a 14C-serotonin release assay, but this test is not routinely available in most settings [47]. Normalization in the number of platelets 1–3 days after discontinuation of heparin may further support the diagnosis of HIT.

Drug-induced thrombocytopenia is another frequent cause of thrombocytopenia in the ICU setting [29]. Thrombocytopenia may be caused by drug-induced myelosuppression, such as is caused by cytostatic agents, or by immune-mediated mechanisms. Examples of drug-induced immune-mediated thrombocytopenia are HIT or quinine-induced thrombocytopenia. A large number of other agents may cause thrombocytopenia by similar mechanisms, including medications that are frequently used in critically ill patients such as antibiotics (including cephalosporins or trimethoprim-sulfamethoxazole), benzodiazepines, or non-steroidal anti-inflammatory agents (NSAIDs). Novel inhibitors of platelet aggregation, such as GPIIb/IIIa antagonists (e.g., abciximab) or thienopyridine derivatives (clopidogrel) are increasingly used in the management of patients with acute coronary syndromes and may also cause severe thrombocytopenia [48]. Drug-induced thrombocytopenia is a difficult diagnosis in the ICU setting as these patients are often exposed to multiple agents and have numerous other potential reasons for platelet depletion. Drug-induced thrombocytopenia is often diagnosed based upon the timing of initiation of a new agent in relationship to the development of thrombocytopenia, after exclusion of other causes of thrombocytopenia. The observation of rapid restoration of the platelet count after discontinuation of the suspected agent is highly suggestive of drug-induced thrombocytopenia. In some cases, specific drug-dependent anti-platelet antibodies can be detected.

Management of Thrombocytopenia in Critically Ill Patients

As there are many causes for thrombocytopenia in critically ill patients and each of these underlying disorders may require specific therapeutic or supportive manage-

ment, it is of utmost importance to establish the underlying etiology of the thrombocytopenia. It is evident that the primary focus of attention in the management of thrombocytopenia should be directed towards the management of the underlying condition. In addition to proper treatment for this underlying disorder, further supportive measures to correct the coagulation defects are often required.

Most guidelines advocate a platelet transfusion in patients with a platelet count of $<30-50\times10^9$/l accompanied by bleeding or at high risk for bleeding, and in patients with a platelet count $<10\times10^9$/l, regardless of the presence or absence of bleeding. After platelet transfusion, the platelet count should rise by at least 5×10^9/l per unit given. A lesser response may occur in patients with high fever, DIC, or splenomegaly, or may indicate allo-immunization of the patient after repeated transfusion. Platelet transfusion is particularly effective in patients with thrombocytopenia due to impaired platelet production or increased consumption, whereas disorders of enhanced platelet destruction (e.g., immune thrombocytopenia) call for alternative therapies, such as steroids, immunoglobulin, or splenectomy. Thrombocytopenia due to HIT requires immediate cessation of heparin and institution of alternative anticoagulant treatment regimens such as direct thrombin inhibitors (argatroban or lepirudin) [49]. The importance of starting treatment with direct thrombin inhibitors is underlined by a recent overview showing that the incidence of new thrombosis in patients with HIT who were treated by discontinuing heparin alone or with warfarin was 19% to 52% [49]. Vitamin K antagonists should be avoided in the initial treatment of HIT, since these agents may cause skin necrosis. In patients with a classic thrombotic microangiopathy due to low levels of ADAMTS-13, plasmapheresis and immunosuppressive treatment should be initiated [42].

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Point-of-care Coagulation Monitoring: Current Status of Viscoelastic Techniques

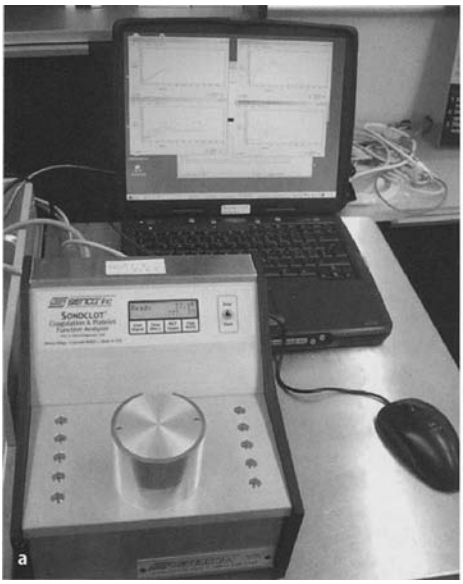
M.T. Ganter and C.K. Hofer

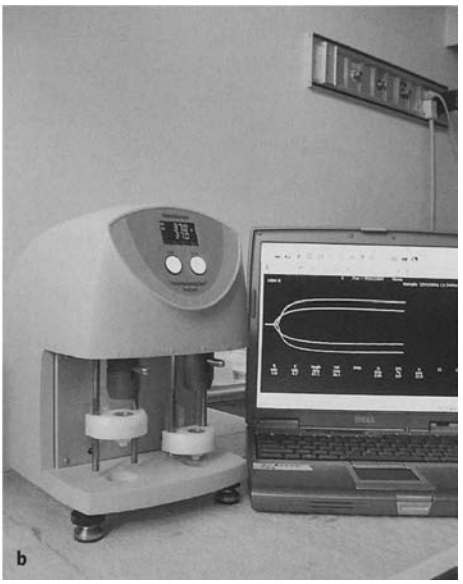
Introduction

Hemostasis monitoring is becoming increasingly important in the management of bleeding patients in the operating room (OR) and the intensive care unit (ICU) in order to improve outcome and reduce costs of treatment. It has been shown in cardiac surgery that frequent reassessment of the coagulation status and transfusion according to well-structured algorithms reduced blood loss and blood component use when compared with transfusion regimens based on clinician discretion [1, 2]. Routine laboratory based coagulation tests (e.g., prothrombin time [PT]/international normalized ratio [INR], activated partial thromboplastin time [aPTT], fibrinogen) measure clotting times and factors in recalcified plasma after activation with different coagulation activators. Platelet numbers are given to complete overall coagulation assessment. Although the values obtained by routine coagulation testing are accurate, standardized, and have been used for a long time, their use has been questioned in the assessment of a severely bleeding patient because values are measured in plasma, no information on platelet function is available, and there is a time delay of 30–60 min from sampling to obtaining the results.

Point-of-care coagulation monitoring may overcome several limitations of routine coagulation testing. Blood is analyzed at the 'bedside' close to the patient and not necessarily in the central laboratory. The coagulation status is assessed in whole blood, better describing physiological clot development by letting the plasma coagulation system interact with platelets and red blood cells (RBCs). Therefore, these techniques may also provide useful information on platelet function. Furthermore, results are available earlier and clot development can be visually displayed real-time using certain devices. According to their main objective and function, point-of-care coagulation analyzers can be classified as follows: Instruments analyzing plasmatic coagulation (e.g., activated clotting time [ACT] or heparin management devices [3]), platelet function (e.g., Platelet Function Analyzer [PFA]-100® [4]), and techniques assessing combined plasmatic coagulation, platelet function, and fibrinolytic system (viscoelastic techniques: Sonoclot® and TEG®/ROTEM®).

This chapter focuses on viscoelastic techniques for perioperative coagulation monitoring of the critically ill patient. The basic principles and properties of the different techniques are summarized, their clinical use is outlined, and the specific ability to monitor different pharmacological substances that interact with hemostasis is presented. Viscoelastic techniques for measuring coagulation have also been used in the hemostasis laboratory for coagulation testing of certain hemostatic disorders or syndromes, but this goes beyond the scope of the current chapter.





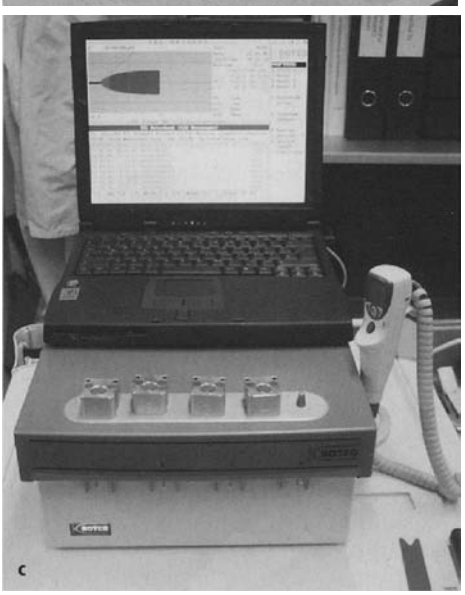


Fig. 1. Viscoelastic point of care coagulation devices **a** Sonoclot®; **b** TEG®; **c** ROTEM®

Sonoclot Analysis

The Sonoclot Analyzer (Fig. 1a, Sonoclot® Coagulation & Platelet Function Analyzer, Sienco Inc., Arvada, CO) was introduced in 1975 by von Kaulla et al. [5]. The principle of the Sonoclot analysis has been described recently in detail [6]. Briefly, Sonoclot measurements are based on the detection of viscoelastic changes of a whole blood or plasma sample. To start a measurement, a hollow, open ended, disposable plastic probe is mounted on the transducer head. Then, 360 µl of test sample is added to the cuvette containing different coagulation activators/inhibitors and calcium (to recalcify citrated blood samples). After an automated mixing procedure, the probe is immersed into the sample and oscillates vertically in the sample. The

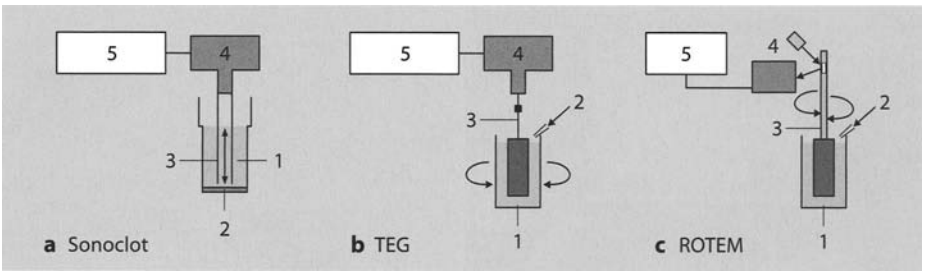


Fig. 2. Working principles of viscoelastic point of care coagulation devices. **a** Sonoclot®: Blood sample in cuvette (1) containing activator (2), disposable plastic probe (3) oscillating in blood sample mounted on electromechanical transducer head (4), data processing (5). **b** TEG®: rotating cup with blood sample (1), coagulation activator (2), pin and torsion wire (3), electromechanical transducer (4), data processing (5). **c** ROTEM®: Cuvette with blood (1), activator added by pipetting (2), pin and rotating axis (3), electromechanical signal detection via light source and mirror mounted on axis (4), data processing (5). For detailed description see text.

changes in impedance to movement imposed by the developing clot are measured (Fig. 2a). Different cuvettes with different coagulation activators/inhibitors are commercially available (Table 1). Normal values for tests run by the Sonoclot Analyzer depend largely on the type of sample (whole blood versus plasma, native versus citrated sample) and type of cuvette used (Table 2).

The Sonoclot Analyzer provides information on the entire hemostasis process, both in a qualitative graph, known as the Sonoclot Signature (Fig. 3) and as quantitative results: The ACT, the clot rate (CR) and the platelet function (PF). The ACT is the time in seconds from the activation of the sample until the beginning of fibrin formation. This onset of clot formation is defined as a certain upward deflection of the Sonoclot Signature and is detected automatically by the machine. Sonoclot's ACT corresponds to the conventional ACT measurement, provided that cuvettes containing a high concentration of typical activators (celite, kaolin) are being used [7–9]. The CR, expressed in Units/min, is the maximum slope of the Sonoclot Signature during initial fibrin polymerization and clot development. Values representing physiologic condition as a function of the activator used are listed in Table 2. PF is reflected by the timing and quality of the clot retraction. PF is a calculated value, derived by using an automated numeric integration of changes in the Sonoclot Signature after fibrin formation has completed (see manufacturer's reference). In order to obtain reliable results for PF, cuvettes containing glass beads for specific platelet activation (gbACT+) should be used [10]. The nominal range of values for the PF goes from 0, representing no PF (no clot retraction and flat Sonoclot Signature after fibrin formation), to approximately 5, representing strong PF (clot retraction occurs sooner and is very strong, with clearly defined, sharp peaks in the Sonoclot Signature after fibrin formation).

The Sonoclot Analyzer has been criticized because its results are influenced by age, sex, and platelet count [11]. Additionally, studies showed poor reproducibility of some of the measured parameters, especially CR and PF [12, 13]. However, others found the Sonoclot Analyzer to be valuable and reliable in patients undergoing cardiac surgical procedures [14, 15] and the Sonoclot Analyzer has even demonstrated a precision close to that of thrombelastography [16]. In more recent studies, test variability of ACT values determined by Sonoclot were comparable to other established

Table 1. Commercially available tests for viscoelastic point-of-care coagulation devices.

Assay	Activator inhibitor	Proposed indication
Sonoclot® Coagulation and Platelet Function Analyzer		
SonACT	Celite	High dose heparin management without aprotinin
kACT	Kaolin	High dose heparin management with/without aprotinin
aiACT	Celite + Clay	High dose heparin management with aprotinin (aprotinin-insensitive ACT)
gbACT+	Glass beads	Overall coagulation and platelet function assessment
H-gbACT+	Glass beads + Heparinase	Overall coagulation and platelet function assessment in presence of heparin; detection of heparin
microPT*	1:1000 TF	Extrinsic pathway; monitoring recombinant activated factor VIIa
Native	None	Non-activated assay Also used to run custom hemostasis tests
Thrombelastograph Hemostasis system (TEG®)		
Kaolin	Kaolin	Overall coagulation assessment and platelet function
Heparinase	Kaolin + Heparinase	Specific detection of heparin (modified Kaolin test adding heparinase to inactivate present heparin)
Platelet Mapping	ADP Arachidonic acid	Platelet function, monitoring antiplatelet therapy (aspirin, ADP-, GPIIb/IIIa inhibitors)
Native	None	Non-activated assay Also used to run custom hemostasis tests
Rotation Thrombelastometry (ROTEM®)		
EXTEM	TF	Extrinsic pathway; fast assessment of clot formation and fibrinolysis
INTEM	Contact activator	Intrinsic pathway; assessment of clot formation and fibrin polymerization
FIBTEM	TF + GPIIb/IIIa antagonist	Qualitative assessment of fibrinogen levels
APTEM	TF + Aprotinin	Fibrinolytic pathway; fast detection of fibrinolysis when used together with EXTEM
HEPTEM	Contact activator + Heparinase	Specific detection of heparin (modified INTEM test adding heparinase to inactivate present heparin)
ECATEM	Ecarin	Management of direct thrombin inhibitors (e.g., hirudin, argatroban)
TIFTEM*	1:1000 TF	Extrinsic pathway; monitoring recombinant activated factor VIIa
NATEM	None	Non-activated assay Also used to run custom hemostasis tests

ACT: activated clotting time; TF: tissue factor; ADP: adenosine diposphate; GPIIb/IIIa: glycoprotein IIb/IIIa receptor. *For research use only (not yet on the market in 2006).

Assay	Activated clotting time (ACT)	Clot Rate (CR)
SonACT	85–145 sec	15–45 Clot Signal Units/min
kACT	94–178 sec	15–33 Clot Signal Units/min
gbACT+	119–195 sec	7–23 Clot Signal Units/min
aiACT	62–93 sec	22–41 Clot Signal Units/min

Table 2. Reference values for Sonoclot® tests (native whole blood).

For specific details on assays, see Table 1.

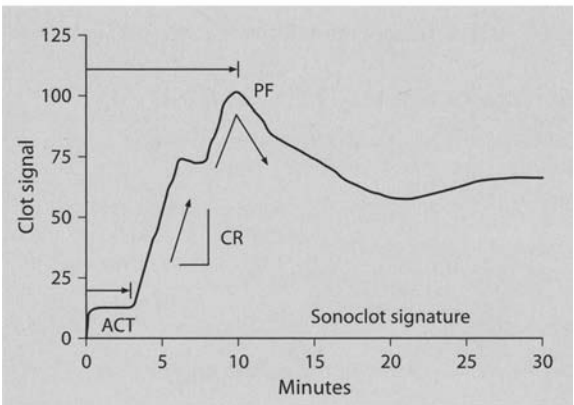


Fig. 3. Typical Sonoclot® Signature ACT: activated clotting time; CR: clot rate; PF: platelet function.

ACT analyzers (8–9% on average) [7–9]. Furthermore, test variability for PF determined by gbACT+ and H-gbACT+ (heparinase glass-bead test) was 6–10% in a recent study assessing PF after administration of the glycoprotein IIb/IIIa (GPIIb/IIIa) antagonist, tirofiban, with or without heparin [10].

Thrombelastography, Thrombelastometry

Thrombelastography is a method to assess overall coagulation function and was first described by Hartert in 1948 [17]. Because the thrombelastograph measures the shear elasticity of the blood sample, thrombelastography is sensitive to all interacting cellular and plasmatic components such as coagulation and fibrinolysis. The thrombelastograph measures and graphically displays the time until initial fibrin formation, the kinetics of fibrin formation and clot development, and the ultimate strength and stability of the fibrin clot as well as fibrinolysis. In the earlier literature, the terms thrombelastography, thrombelastograph and TEG were used generically. However, in 1996, thrombelastograph® and TEG® became a registered trademark of the Hemoscope Corporation (Niles, IL, USA) and from that time on these terms have been employed to describe the assay performed using Hemoscope instrumentation only. Alternatively, Pentapharm GmbH (Munich, Germany) markets a modified instrumentation using the terminology rotation thrombelastometry, ROTEM®.

The TEG® (Fig. 1b) measures the clot's physical property by the use of a stationary cylindrical cup that holds the blood sample and is oscillated through an angle of 4°45'. Each rotation cycle lasts 10 seconds. A pin is suspended in the blood by a torsion wire and is monitored for motion (Fig. 2b). The torque of the rotation cup is transmitted to the immersed pin only after fibrin-platelet bonding has linked the cup and pin together. The strength of these fibrin-platelet bonds affects the magni-

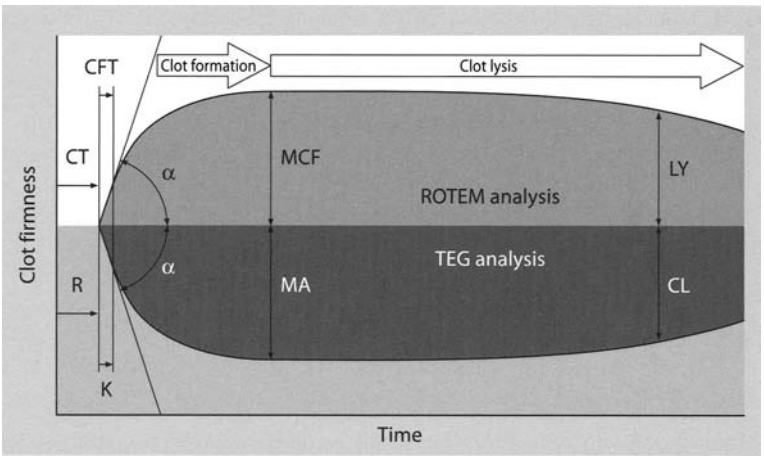


Fig. 4. Typical TEG/ROTEM® tracing. R: reaction time; K: kinetics; α: slope between R and K; MA: maximum amplitude; CL: clot lysis; CT: clotting time; CFT: clot formation time; α: slope of tangent at 2 mm amplitude; MCF: maximal clot firmness; LY: Lysis. For detailed description and reference values please see Tables 2 and 3.

tude of the pin motion. Thus, the output is directly related to the strength of the formed clot. As the clot retracts or lyses, these bonds are broken and the transfer of cup motion is again diminished. The rotation movement of the pin is converted by a mechanical-electrical transducer to an electrical signal finally being displayed as the typical TEG® tracing (Fig. 4). The ROTEM® technology (Fig. 1c) avoids some limitations of traditional instruments for thrombelastography, especially the susceptibility to mechanical shocks. Signal transmission of the pin suspended in the blood sample is carried out via an optical detector system, not a torsion wire and the movement is initiated from the pin, not the cup (Fig. 2c).

Most common tests for both technologies are listed in Table 1. The TEG® and ROTEM® technology are comparable and show similar tracings (Fig. 4) but nomenclature and reference ranges are different (Table 3). The repeatability of measurements by both devices has been shown to be acceptable, provided they are performed exactly as outlined in the user manuals. For example, coefficients of variation using kaolin activated TEG® were 13% for reaction time (R), 4% for kinetics (K), 3% for α, and 6% for maximum amplitude (MA) (TEG® 5000 User Manual) and 3–12% for coagulation time (CT) and clot formation time (CFT, intrinsic-, extrinsic-TEM), 1–5% for α and maximum clot firmness (MCF, intrinsic-, extrinsic-TEM), and 6–13% for MCF (fibrinogen [FIB]-TEM) [18].

Comparing Viscoelastic Techniques with Standard Hemostatic Laboratory Tests

Conventional laboratory coagulation tests are usually performed in plasma and most typically end when fibrin strands are formed. However, viscoelastic tests are whole blood assays measuring the entire clotting process from fibrin formation to clot retraction and lysis. Several studies have compared results from viscoelastic techniques to laboratory coagulation data. It is not surprising that point-of-care

Table 3. Nomenclature and reference values of thrombelastography (TEG®) and thrombelastometry (ROTEM®).

	TEG®	ROTEM®
Clotting time (period to 2 mm amplitude)	R (reaction time) N (WB) 4–8 min N (Cit) 3–8 min	CT (clotting time) N (Cit, INTEM) 137–246 sec N (Cit, EXTEM) 42–74 sec
Clot kinetics (period from 2 to 20 mm amplitude)	K (kinetics) N (WB) 1–4 min N (Cit) 1–3 min	CFT (clot formation time) N (Cit, INTEM) 40–100 sec N (Cit, EXTEM) 46–148 sec
Clot strengthening (alpha angle)	α (slope between r and k) N (WB) 47–74° N (Cit) 55–78°	α (slope of tangent at 2mm amplitude) N (Cit, INTEM) 71–82° N (Cit, EXTEM) 63–81°
Amplitude (at set time)	A	A
Maximum strength	MA (maximum amplitude) N (WB) 55–73 mm N (Cit) 51–69 mm	MCF (maximum clot firmness) N (Cit, INTEM) 52–72 mm N (Cit, EXTEM) 49–71 mm N (Cit, FIBTEM) 9–25 mm
Lysis (at fixed time)	CL30, CL60	LY30, LY60

TEG®: N = normal values for kaolin activated TEG® in native whole blood (WB) or citrated and recalcified blood samples (Cit) (Hemoscope Corp.). ROTEM®: N = normal values for contact (INTEM), tissue factor (EXTEM) and tissue factor plus GPIIb/IIIa inhibitor (FIBTEM) activated citrated and recalcified blood samples [18]. Reference values depend on reference population, blood sampling technique, other pre-analytical factors and coagulation activator.

clotting times (ACT, R, CT) showed a trend in the same direction as laboratory based clotting times, depending on the activator used. Therefore, a whole blood sample from a heparinized patient or a patient with hemophilia (factor VIII or IX deficiency) will show a significantly prolonged CT if a contact activator is used. However, there is a more obvious association between the maximum strength MA/MCF of the TEG/ROTEM® signature and both platelet count (or function) and fibrinogen concentration [19, 20]. To finally determine the fibrinogen influence, tests can be performed eliminating platelet function by a GPIIb/IIIa inhibitor (e.g., fibTEM). This concept has been proven to work and a good correlation of this modified MA/MCF with fibrinogen levels determined by the Clauss method has been shown ($r = 0.85$ [TEG® 5000 User Manual] and $r = 0.75$ [21]).

Cardiac Surgery and Postoperative Care

Coagulation management of patients undergoing cardiac surgery is complex because of a delicate balance between anticoagulation for cardiopulmonary bypass (CPB) and hemostasis after CPB. During CPB, optimal anticoagulation dictates that coagulation is antagonized and platelets are prevented from activation so that microvascular clots do not form on the extracorporeal circuit. After surgery, coagulation abnormalities, platelet dysfunction, and fibrinolysis can occur, creating a situation whereby hemostatic integrity must be restored. The complex process of anticoagulation with heparin, antagonism with protamine, and postoperative hemostasis therapy cannot be performed without careful and accurate monitoring.

ACT is currently used in clinical practice to monitor heparin therapy during CPB correlating well with heparin concentration, mainly before going on CPB [7–9, 22]. The Sonoclot Analyzer, measuring the ACT, has been used to guide heparin management for CPB in the presence or absence of aprotinin and the accuracy and performance has been shown to be comparable to standard ACT machines [7–9]. Furthermore, the Sonoclot Analyzer has been shown to reliably detect pharmacological GPIIb/IIIa inhibition [10, 23] and successfully used to assess the coagulation status and platelet function in patients undergoing cardiac surgery [14].

Viscoelastic point-of-care coagulation devices have been applied, with limited success, to predict excessive bleeding after CPB [24, 25]. However, large prospective [1] and retrospective studies [26] have demonstrated a significant decrease in perioperative and overall transfusion requirement if hemostasis management was guided by TEG®/ROTEM® based algorithms. Interestingly, a recent study by Avidan et al. showed little advantage of a combined transfusion algorithm using TEG® (global coagulation), PFA-100® (platelet function), and Hepcon® (heparinization), over a well-structured laboratory-guided algorithm. Both approaches were able to reduce postoperative blood component usage compared with clinical discretion alone [2].

To detect non-heparin related hemostatic problems even in the presence of large amounts of heparin during CPB, tests with heparinase have been developed for each instrument (Table 1) and an algorithm based upon heparinase-modified TEG® resulted in a significant reduction in hemostatic products [27].

Hepatic Surgery and Postoperative Care

Patients undergoing hepatic surgery and, particularly, orthotopic liver transplantation may have large derangements in their coagulation making point-of-care coagulation monitoring highly desirable. Problems associated with the defective organ (decreased synthesis and clearance of clotting factors, platelet defects) lead to impaired hemostasis and hyperfibrinolysis. Furthermore, systemic complications like sepsis and disseminated intravascular coagulation (DIC) further complicate a pre-existing coagulopathy. Finally, marked changes in hemostasis in orthotopic liver transplantation occur during the anhepatic phase and immediately following organ reperfusion, mainly a hyperfibrinolysis resulting from accumulation of tissue plasminogen activator due to inadequate hepatic clearance and a release of exogenous heparin and endogenous heparin-like substances.

One of the first clinical applications of TEG® was in the hemostatic management of orthotopic liver transplantation and TEG® guided component replacement [19]. Although the value of TEG/ROTEM® in management of patients undergoing orthotopic liver transplantation has been established in the literature [28, 29], only a third of all orthotopic liver transplantation programs in the United States used TEG® routinely according to a national survey in 2002 [30]. In addition to the hemorrhagic risk associated with hepatic surgery and orthotopic liver transplantation, hypercoagulability and thrombotic complications have been described in the postoperative period and can be adequately assessed with TEG/ROTEM® [31, 32]. Only a few studies are available on the use of the Sonoclot Analyzer in hepatic surgery and orthotopic liver transplantation; however, this technique has been found to be useful in the perioperative coagulation management of these patients [33].

Other Applications of Viscoelastic Point-of-care Coagulation Monitoring

Viscoelastic techniques have been used to assess blood coagulation in multiple clinical situations outside the cardiac and hepatic units, but experience is limited. For example, TEG® has been applied to measure the coagulation status in trauma patients [34]. Furthermore, TEG/ROTEM® and Sonoclot® have been used to assess a hypercoagulable state in several clinical settings, e.g., after major abdominal surgery [35], in obstetrics [36], and in uremic patients undergoing a surgical procedure [37]. Finally, there is a long list of publications on the successful use of TEG/ROTEM® and Sonoclot® in other clinical areas, summarized in recent reviews [6, 38, 39].

Monitoring Anticoagulants

ACT measurements to guide heparin therapy and the use of modified point-of-care coagulation tests with heparinase to assess the coagulation status in the absence of the anti-coagulatory effects of heparin have been described above. However, besides the monitoring of unfractioned heparin, studies have shown that treatment with low molecular weight heparin (LMWH) and heparinoids (e.g., danaparoid) can also be assessed with point-of-care viscoelastic tests [40]. Both standard and heparinase-modified tests have to be performed in order to increase the sensitivity of TEG/ROTEM® for the effects of LMWH and heparinoids.

Direct thrombin inhibitors are increasingly being used for different indications. Point-of-care viscoelastic techniques, especially the ecarin clotting time (ecarin directly activates thrombin) are helpful in the assessment of the effects of direct thrombin inhibitors [41].

Platelets play a key role in overall coagulation and assessment of their function is highly desirable (more than the platelet number). Anti-platelet agents typically act to inhibit cyclo-oxygenase 1 (e.g., aspirin [acetylsalicylic acid]), ADP receptors (e.g., clopidogrel), or GPIIb/IIIa receptors (e.g., abciximab, tirofiban). As mentioned above, the Sonoclot Analyzer has been shown to reliably detect pharmacological GPIIb/IIIa inhibition [10, 23]. Furthermore, the MA/MCF from TEG/ROTEM® gives some information on overall platelet function (and fibrinogen levels), but is not sensitive to targeted pharmacological inhibition. Therefore, a more sophisticated and laborious test has been developed recently for the TEG® (PlateletMapping) using arachidonic acid and ADP to selectively activate platelets and determine platelet function in the presence of anti-platelet therapy [42].

Monitoring Pro-Coagulant Therapy

Maintaining an adequate coagulation status is one of the goals in patients with severe hemorrhage besides preserving sufficient blood volume and oxygen carrying capacity. Strategies to support coagulation are based on the underlying cause of bleeding and range from prevention of hypothermia and acidosis, re-warming, transfusion of blood products, selective administration of coagulation factors, and the use of pharmacological agents. Interactions of administered crystalloids and colloids with coagulation have to be considered. For example, progressive hemodilution

with current hydroxyethyl starch solutions still compromises blood coagulation more than gelatin or albumin solutions [43].

Modern practice of coagulation management is based on the concept of specific component therapy and requires rapid diagnosis and monitoring of the pro-coagulant therapy (i.e., clotting times, clot kinetics, and clot strengthening). Fibrinogen is a key coagulation factor (substrate to form a clot) and isolated fibrinogen substitution in severe models of dilutional coagulopathy has been shown to improve clot strength and reduce blood loss [44]. Supplementary administration of prothrombin complex (concentrates of factor II, VII, IX, X, antithrombin III, protein C) additionally improved initiation of coagulation and reversed the dilutional coagulopathy [45]. Fibrinogen levels can be assessed by measuring clot strength (MCF/MA) in presence of platelet inhibition by a GPIIb/IIIa inhibitor (e.g., FIBTEM) [21] or by assessing Sonoclot's CR [46]. Fibrinogen substitution should be considered in a bleeding patient if MCF levels are lower than 9 mm in a FIBTEM test.

Recombinant activated factor VII (rFVIIa) treatment is currently approved for patients with congenital or acquired hemophilia, factor VII deficiency, and Glanzmann's thrombasthenia. However, factor VIIa is increasingly used in off-label indications to control severe bleeding (e.g., major trauma, surgical interventions, intracerebral hemorrhage). The concept is to locally activate the coagulation at sites of tissue factor exposure. The resulting thrombin burst then leads to the formation of a fibrin clot, provided there are sufficient fibrinogen levels. Consensus guidelines have been published for these off-label indications, but it is still unclear how to reliably monitor patients receiving recombinant factor VIIa (rVIIa) [47]. In order to study thrombin generation, modified TEG/ROTEM® parameters (based on the original tracing) have been introduced recently: Maximum velocity of clot formation (maximum rate of thrombus generation, MaxVel), time to reach MaxVel (time to maximum thrombus generation, tMaxVel), and total thrombus generation (area under the curve, TTG) [48]. These parameters are supposed to be more sensitive to rVIIa than standard TEG/ROTEM® parameters and dilute tissue factor should be used as coagulation activator for best sensitivity [39]. In a preliminary study, we were able to monitor the effects of rVIIa *in vitro* after severe hemodilution using the new diluted tissue factor activated tests from Sonoclot (microPT) and the ROTEM® (TIFTEM) [46, 49].

Factor XIII is needed for cross-linking fibrin, therefore, stabilizing the clot, increasing clot strength and resistance to fibrinolysis. There are case reports on patients with unexplained intraoperative bleeding due to decreased factor XIII and subsequent stabilization after substitution. Impaired clot strength and increased lysis have been observed [50].

Antifibrinolytic drugs (aprotinin, tranexamic and epsilon aminocaproic acid) are used mostly in complex cardiac surgery to reduce bleeding and transfusion requirements. Aprotinin may interact with point-of-care coagulation assays, prolonging for example celite-activated ACT tests. Therefore, kaolin or aprotinin-insensitive ACT should be used to guide heparin therapy in these patients [8, 9]. Antifibrinolytic therapy may be predicted *in vitro* in TEG/ROTEM® with certain tests already containing an antifibrinolytic agent (e.g., APTEM). APTEM predictive of a good patient response would then show a significantly improved initiation/propagation phase compared to EXTEM and or disappearance of signs of hyperfibrinolysis. There are no conclusive studies on monitoring desmopressin (DDAVP) therapy so far.

Problems with Point-of-Care Coagulation Monitoring

Several concerns have been raised using viscoelastic point-of-care coagulation tests because these tests are hard to standardize. The blood collection site, processing of the sample (native versus citrated samples, time delay between collection and measurement – for citrated samples a minimum rest time of 30 min is required), patient age and gender may significantly affect the results of these tests [38]. Furthermore, equipment, activators, and other modifications will alter the assay specificity. All these factors have to be considered when interpreting results in the literature and have to be known and standardized when running tests in a single center.

As with all point-of-care devices, there is a concern that the devices are not adequately maintained and that quality controls are not done on a regular basis. Using such an instrument for decision making in patient care may harm the patient because of the possibility of incorrectly measured data. Furthermore, non-laboratory personnel are running these point-of-care tests, which may lead to further errors, if not adequately trained. In an effort to minimize these problems and release the OR/ICU personnel from the burden of maintaining their devices, point-of-care devices have to be at least supervised by the central laboratory. Alternatively, point-of-care coagulation analyzers have been moved into the central laboratory – a trained person runs the viscoelastic coagulation test and the results (evolving signatures) are submitted real-time to the patient's bedside.

Conclusion

Viscoelastic point-of-care coagulation analyzers are being used in certain clinical situations, especially in the management of patients undergoing cardiac and liver surgery. Furthermore, they provide useful information in a large variety of clinical scenarios, e.g., massive hemorrhage, assessment of hypo- and hypercoagulable states, and monitoring of pharmacological treatment with anti- and pro-coagulant agents. The advantage of these techniques is that they have the potential to measure the entire clotting process starting with fibrin formation and continuing through to clot retraction and lysis at the bedside with minimal time delays. Furthermore, physiological clot development is better depicted as a result of whole blood analysis of the coagulation status. However, several problems regarding quality standards have to be considered when using viscoelastic techniques.

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Monitoring of Hemostasis in Emergency Medicine

S. Kozek-Langenecker

Introduction

Exsanguination is still a major cause of death in severely injured patients [1]. Trauma-associated bleeding diathesis, overt at admission to the trauma unit, correlates with the severity of trauma and mortality [2, 3]. Sufficient hemostatic management is critical to the successful resuscitation of the severely injured patient, second in importance only to adequate ventilation. Despite intense efforts to elucidate the pathomechanism and control the process, trauma-associated coagulopathy remains a challenge in the treatment of trauma patients. In this light, monitoring of hemostasis should confirm and specify the clinical diagnosis of bleeding diathesis, guide goal-directed therapy, and possibly predict consecutive transfusion requirements at admission. The present chapter reviews routine laboratory tests and viscoelastic point-of-care hemostasis monitoring as a means of hemostasis monitoring in the emergency setting, as well as relevant pathomechanisms, and therapeutic approaches.

Pathomechanisms of Trauma-associated Coagulopathy

In massively bleeding patients, coagulopathy is complex in origin, correlating with the severity of injury [3, 4]. Figure 1 summarizes the multiple pathomechanisms leading to trauma-associated coagulopathy. Uncontrolled bleeding initially leads to

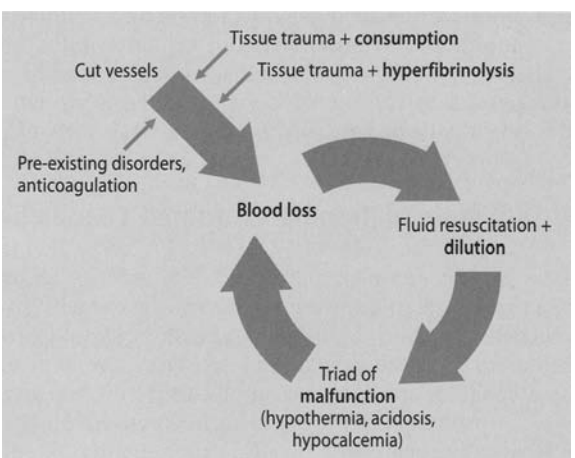

Fig. 1. Pathomechanism of trauma-associated coagulopathy: a "bloody vicious cycle"

loss of coagulation factors and platelets [5, 6]. Trauma-induced exposure of the thromboplastin-rich subendothelial tissue to flowing blood induces the activation of coagulation [7] which may trigger consumptive coagulopathy [8–10]. The majority of blunt trauma and brain injury victims are hypercoagulable early after trauma with tissue trauma being the key stimulus for coagulation [8, 11, 12]. In hypocoagulable patients, however, the remaining procoagulatory potential is reduced by dilution during fluid resuscitation required to restore intravascular volume and to maintain hemodynamic stability. The degree of dilutional coagulopathy also depends on the type of fluid used: Hydroxyethyl starch solutions, gelatins, and dextrans impair platelet function, inhibit fibrin polymerization, and induce an acquired von Willebrand syndrome to varying degrees, depending on the physicochemical characteristics of the colloidal solution [13]. Resuscitation with hypertonic saline appears to aggravate bleeding by its potent anticoagulatory and anti-platelet effects [14], while other hypertonic solutions (glycine, glucose, sorbitol) exhibit a significantly reduced impairment. Additionally, massive transfusion inevitably leads to coagulopathy, although dilution often is not an issue until more than one blood volume (10–12 units packed red blood cells [RBCs]) is given. Tissue injury in trauma may lead to the exposure of tissue plasminogen activator resulting in hyperfibrinolysis if the delicate balance between coagulation and fibrinolysis is lost [15]. Coagulopathy is confounded by hypothermia, acidosis, and pre-existing disorders: Trauma patients are prone to hypothermia, which slows down enzymatic reactions [16], modifies platelet function [17], and stimulates fibrinolysis. Acidosis worsens fibrin polymerization and strengthening of the clot [18]. In a study including both blunt and penetrating injuries, the vicious cycle induced by severity of tissue injury (Injury Severity Score [ISS] >25), progressive core hypothermia ($<34\,°C$), and ongoing cellular shock ($pH<7.0$ and low arterial blood pressure) predicted life-threatening coagulopathy in massively transfused patients [10]. Since trauma is not restricted to previously healthy people, the increasing number of patients taking oral anticoagulants and platelet-inhibiting drugs poses a rapidly increasing problem [19]. Patients with inherited coagulation defects may exsanguinate with trauma unless specific factor replacement is provided. Low hematocrit ($<30\%$) and low ionized calcium (after massive packed RBC transfusions containing citrate) further aggravate bleeding diathesis. This vicious cycle of trauma-associated coagulopathy results in: 1) a defect in clot firmness due to fibrinogen deficiency and thrombocytopenia, 2) impaired clot stability due to hyperfibrinolysis and factor XIII deficiency (being a late phenomenon), and 3) prolonged clot generation due to various coagulation factor deficiencies. The coagulopathy causes uncontrolled bleeding requiring massive transfusion, which is commonly defined as the replacement of one blood volume over a period of 24 h or transfusion of at least 4 units of packed RBC within 1 h.

Diagnosis of Trauma-associated Coagulopathy

Due to the complex nature of hemorrhage in emergency medicine, physicians require coagulation monitoring strategies sensitive to all these major possible pathomechanisms. Initially, a bleeding history should be assessed from the patient if conscious or from accompanying relatives. The clinical examination in trauma-associated coagulopathy may reveal bleeding from mucosal lesions and serosal surfaces, as well as prolonged bleeding at catheter insertion sites and wounds in the absence of a surgically correctable bleeding site. Finally, coagulation tests are required to verify

the clinical diagnosis of coagulopathy, to differentially diagnose the leading pathomechanism for bleeding, to guide coagulation therapy, and to predict the risk for bleeding during the consecutive surgical procedures and hospital stay. In contrast to massive bleeding in elective surgery, monitoring of hemostasis occurs late in traumatic hemorrhage [4] when coagulopathy is already installed and treatment becomes more difficult. At present, several routine coagulation monitoring tests are available for these purposes. Recently, point-of-care coagulation monitoring devices have become available and are likely to overcome several limitations of routine testing.

Principles of Routine Coagulation Monitoring

Even though these tests were not developed to predict bleeding or guide coagulation management in the emergency setting, most centers in clinical practice draw blood at the patient's arrival on the trauma unit for the following routine coagulation tests ('trauma panel'):

Activated partial thromboplastin time (aPTT): The aPTT was developed to monitor heparinization in the treatment of thromboembolic disorders, to characterize clotting factors, and for research purposes on hemophilia. Activation of coagulation factors, formerly known as the 'intrinsic coagulation cascade', is performed by incubating plasma with partial thromboplastins, calcium, and kaolin powder at 37 °C at a standardized pH. The endpoint of measurement is the formation of fibrin strands. Standardization, however, is difficult due to the large variation in calibration constants and methods of endpoint detection, as well as the wide range of pro- and anticoagulant factors affecting aPTT results. The aPTT is sensitive to coagulation factors VIII, IX, XI, XII, V, II, and I, heparin, fibrinogen degradation products, inhibitors, hypothermia, and hypofibrinogenemia. Multiple factor deficiencies tend to show a greater prolongation for a given factor level than single factor deficiencies.

Prothrombin time (PT): This test was developed to monitor and adjust the doses of coumarins. Activation of coagulation factors, formerly known as the 'extrinsic coagulation cascade' is performed by incubating plasma with tissue thromboplastin and calcium at 37 °C at a standardized pH. The time until fibrin strand formation occurs is determined. This test is sensitive to coagulation factors II, VII, X, V, and I. Standardization of the PT for laboratory control of oral anticoagulant treatment is based on the responsiveness of one type of thromboplastin, measured by its international sensitivity index, and conversion into the international normalized ratio (INR). Direct INR determination is performed by local calibration using plasma of certified levels of PT. A PT activity above 30–40% generally ensures normal coagulation within a safe margin.

Platelet count: Platelet counting is routinely performed by automated machines. The number of platelets, however, does not reflect the quality of platelet function.

Fibrinogen concentration: Fibrinogen plays a major role in routine coagulation tests such as PT and aPTT. There are two methods used in specific fibrinogen assays: 1) determination of the amount of fibrinogen molecules per se, e.g., by immunologic, gravimetric, or heat precipitation, and 2) determination of clottable fibrinogen. In the conventional Clauss method, where thrombin is added to plasma, the fibrinogen

concentration is proportional to the coagulation time measured. This test is affected by heparin and fibrinogen degradation products. Excessive bleeding has been reported at fibrinogen levels below 50 mg/dl [20].

Second level coagulation tests: Because of long turnaround times and limited availability in many laboratories, coagulation factor levels and molecular markers of the coagulation and fibrinolytic system are rarely assayed in the acute trauma setting.

Routine Coagulation Monitoring: Predictor of Bleeding and Mortality in Trauma

Severe aPTT prolongation >1.8 times normal is associated with bleeding [20, 21]. Similarly, INR elevations in trauma patients are only indicative for risk of generalized bleeding if they are >1.5–1.8 times normal and are associated with an elevated aPTT [20, 22]. A severely prolonged activated clotting time (ACT) may indicate exhaustion of the coagulation system's reserve [6].

In trauma victims, an initially abnormal PT increases the adjusted odds of dying by 35%, a prolonged aPTT by 326% [3]. Although severely abnormal PTs and aPTTs are predictors of trauma-related mortality, the poor predictive power of moderately impaired routine coagulation tests has repeatedly been argued as a major limitation of these tests. In a multiple regression model, platelet count was not an independent predictor of mortality in emergency medicine [3]. The decline in platelet count is a highly individual phenomenon, some patients are even able to recruit platelets from storage pools. Most patients approach the critical platelet count of $50 \times 10^3/mm^3$ after losing two blood volumes [23].

Limitations of Routine Coagulation Tests

In trauma settings where events proceed at a fast and dramatic pace, real-time monitoring of the patient's coagulation profile and repeated laboratory tests are vital in administrating proper replacement therapy. However, test results of routine coagulation monitoring performed at the hospital's central laboratory are generally only available with a delay of at least 30 min (sample preparation including centrifugation and buffering, transportation of blood samples and test results) [24]. In this light, it seems frustrating that it is recommended that patients should be transfused empirically, based on clinical probability and dogmatic guidelines for massive transfusion, before routine coagulation test results are availabe [19, 25]. The bedside determination of PT and aPTT in whole blood using the CoaguCheck® (Roche Diagnostics, Switzerland), aimed at overcoming this limitation, however, correlation with central laboratory test results is inadequate.

Routine coagulation tests are performed in plasma at a standardized temperature of 37°C, without the presence of platelets and other blood cells. Accordingly, routine laboratory tests cannot assess the effect of hypothermia on hemostasis in hypothermic traumatized patients. Furthermore, fibrinolysis and platelet dysfunction pose diagnostic gaps. Since the hemostatic response to injury is a complex interaction of plasma proteins, platelets, and the vessel wall (cell-based model of hemostasis) it cannot be pictured by tests performed in plasma.

Routine tests pick up abnormalities in hemostasis due to single or multiple deficiencies in coagulation factors, but do not identify them. The PT is a more reliable marker of critically low coagulation factor levels than the aPTT, possibly due to the high rate of false negative aPTT results when acute phase reactant factor VIII is ele-

vated [22]. Several studies demonstrate a poor correlation between the amount of blood products given and the severity of coagulation defects [20, 21]. Obviously, simplistic formulas for predicting factor deficiencies from blood loss are not applicable.

The most important limitation of routine coagulation tests is the fact that the predominant pathomechanism of bleeding in the complex scenario of trauma-associated coagulopathy cannot be differentiated: prolonged aPTT may be due to 'intrinsic coagulation factor' deficiency requiring specific substitution, fibrinogen deficiency requiring fibrinogen substitution, hypothermia requiring rewarming, heparinization requiring protamine reversal, or hyperfibrinolysis requiring antifibrinolytic drugs. Thus, a false differential diagnosis may lead to therapeutic misadventures in trauma patients.

Although aPTT, PT, fibrinogen concentration, and platelet count determination are well validated, methodological problems include variable sensitivity of test reagents, high variability between labs and investigators, as well as insufficient standardization.

Near-patient coagulation monitoring

Thrombelastography (TEG) and rotation thrombelastometry (ROTEM)

The viscoelastic whole blood test was invented by Hartert in 1948 [26]. TEG/ROTEM measure the viscoelastic properties of non-anticoagulated or (citrate) anticoagulated blood after induction of clotting under low shear conditions, resembling the rheologic properties in venous vessels *in vivo*. The pattern of changes in viscoelasticity reflect the kinetics of all stages of thrombus formation (R and K time, clotting time [CT] and clot fomation time [CFT]), the stability and firmness of the clot, which is a function of platelet-fibrin interaction and fibrin polymerization (maximum aplitude [MA], maximum clot fimness [MCF]), as well as dissolution (fibrinolysis). A normal trace is shown in Figure 2.

TEG/ROTEM is a fibrinolysis-sensitive assay and allows for diagnosis of hyperfibrinolysis in bleeding patients [27]. Validation of ROTEM in a model of systemic coagulation activation has recently been published [28]. Standardized operating procedures required for quality control testing are available. A multi-center investiga-

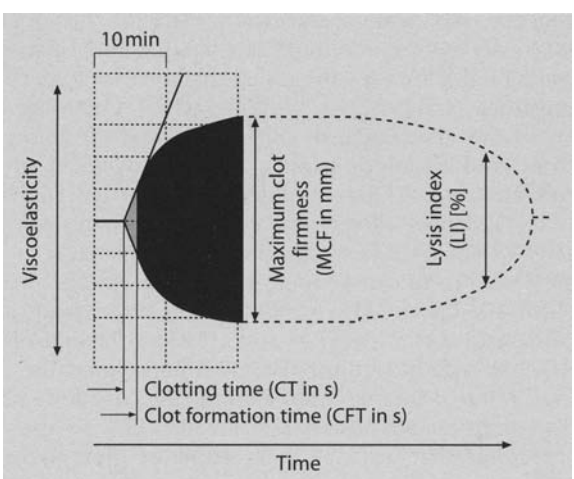

Fig. 2. ROTEM parameters, normal trace.

tion yielded consistent values between centers and provided general orientating reference ranges for the ROTEM [29]. TEG/ROTEM are easy to use by non-laboratory personnel in the emergency unit or the operating room (OR). Interpretation of TEG/ROTEM results is simplified by both graphical and numerical presentation of results and highlighting of abnormal results. While conventional TEG has been described as insufficient monitoring in trauma patients because of unclear interpretation and limited run-to-run variation [30], the ROTEM (Pentapharm GmbH, Germany) improved the original TEG (Hemoscope Inc., USA) procedure by reducing the interference with vibrations and limited transportability, and allowing a computerized analysis of the trace. Addition of different coagulation-activating agents and/or platelet inhibiting agents allows the detection and quantification of specific coagulation defects such as hypofibrinogenemia, factor deficiency, thrombocytopenia, heparin effect, and hyperfibrinolysis. All these aspects of the coagulation scenario come into play when an individual is injured. Thus, ROTEM not only provides a global picture of the injured patient's hemostatic status but also permits differential diagnosis of the major underlying pathomechanism of trauma-associated coagulopathy: EXTEM is a baseline test that uses recombinant tissue factor to activate coagulation (comparable to the PT) which causes rapid generation of the clot. The clotting time (EXTEM CT) gives information about the initial activation and dynamics of clot formation, thus allowing analysis of factor deficiencies (and the detection of anticoagulants). The critical cut-off value for CT, indicating the necessity to administer prothrombin concentrates (PCC) or fresh frozen plasma, appears about 80 s after test initiation. Practical considerations in an emergency setting are that viscoelastic tests are initiated immediately after blood withdrawal. The maximum clot firmness (EXTEM MCF) gives information on the maximum clot strength and stability, which is largely dependent on platelet count and fibrinogen level. Prepared disposable wells containing cytochalasin D, a platelet inhibitor, are used in the FIBTEM test. FIBTEM MCF represents the contribution of fibrinogen to the clot strength. Critical MCF cut-off values appear within 10–15 mins after test initiation (depending on hemostatic function). A low FIBTEM MCF is indicative for administration of fibrinogen concentrates. A normal FIBTEM MCF in the presence of a low EXTEM MCF indicates the need for platelet substitution. Thus, comparing EXTEM MCF to FIBTEM MCF permits differentiation of a low platelet count from dys- or hypofibrinogenemia. Practical consideration: FIBTEM and EXTEM should be performed simultaneously as first line ROTEM tests in trauma patients.

EXTEM allows for the visual diagnosis of hyperfibrinolysis when a typical tapering trace is shown. In addition, wells containing aprotinin (APTEM) permit the quantitive assessment of fibrinolysis and the estimation of the therapeutic benefit from antifibrinolytic agents. Any improvement in CT, CFT, and MCF in APTEM compared to EXTEM demasks low grade hyperfibrinolysis.

INTEM uses ellagic acid contact activator (comparable to the reagent used for aPTT) to analyze the general coagulation status. Wells containing heparinase (HEPTEM) or ecarin can be used to detect specific anticoagulant effects. The comparison of INTEM CT and HEPTEM CT permits the quantification of heparinization. Practical consideration: INTEM and HEPTEM should only be performed as second line ROTEM tests in trauma patients if heparinization is suggested to complicate bleeding. Point-of-care ROTEM monitoring, including EXTEM, FIBTEM, APTEM (and depending on the results and medical history also INTEM, HEPTEM) theoretically represents the best option available at present for monitoring hemostasis in the emergency setting of massive bleeding.

TEG/TEM measurements can be performed at the actual body core temperature of the injured patient and at adjusted test temperatures between 22 °C and 42 °C, thus allowing quantitative analysis of the anticoagulant effect induced by hypothermia. Test temperature adaptions, however, are impracticable in the emergency room because physicians may be tempted to treat abnormal test results with coagulation factor substitution while only rewarming is indicated.

In contrast to routine coagulation testing in plasma, TEG/ROTEM can be performed at the bedside, relevant information can be obtained within a few minutes, and, therefore, goal-directed coagulation therapy can be readily initiated. Each routine test is specific for some portion of the hemostatic mechanism and none can stand alone. Similarly, ROTEM test combinations (EXTEM + FIBTEM) are required as a basic panel in massive bleeding. The approximate cost of such test combinations of routine coagulation and ROTEM are equivalent (at the author's institution). However, if ROTEM helps to shorten surgical procedures, lowers the frequency of re-openings, shortens the stay in the ICU, and minimizes the direct costs of blood products by also avoiding costly adverse effects of transfusion, the ability of ROTEM coagulation monitoring to save costs is significant in clinical practice.

TEG/ROTEM: predictor of (surgical) bleeding

Many articles using TEG/ROTEM have been published so far (PubMed search > 2500 hits), however, the evidence for its usefulness in emergency medicine is scarce. TEG was found to be an early predictor of transfusion in blunt injury patients [12].

Normal viscoelastic test results are unlikely to coincide with bleeding (high negative predictive value). As a consequence, another important implication of TEG/ROTEM monitoring is the immediate initiation of surgical re-exploration if no hemostaseological cause of bleeding is observed.

Management-algorithm for coagulation therapy

Indications for management algorithms are 1) the correction of bleeding, and 2) the prevention of bleeding before invasive procedures. The pure optimization of coagulation parameters in the absence of bleeding, however, is no indication. Although blood transfusion can be life-saving, its numerous negative effects have been well documented. Our transfusion behavior should aim at minimizing the occurrence of indiscriminate transfusion and empiric hemostatic intervention. Indiscriminate blood transfusion and hemostatic agent use may be due to lack of sensitive and timely coagulation data. Because of the multifactorial nature of bleeding especially in trauma, a point-of-care-guided transfusion algorithm should definitely support the clinician's discretion. Although large-scale prospective studies in emergency medicine have not been performed yet, extrapolations from elective surgery are intriguing: The institution of transfusion algorithms based on TEG parameters reduces transfusion requirements (and in some study designs also blood loss) in both routine and high-risk cardiac surgery and liver transplantation [30–36]. TEG-guided administration of clotting factors was superior to routine coagulation testing [37]. Transfusion requirements before and after the implementation of ROTEM were statistically significantly lower and clinically more accurate [38]. Point-of-care monitoring with ROTEM is still an evolving field. In contrast to the published literature in elective surgery, the decision tree has to include the pathway leading to the indication for fibrinogen administration and platelet transfusion (EXTEM-FIBTEM) (Fig. 3) [39]. Testing should be performed

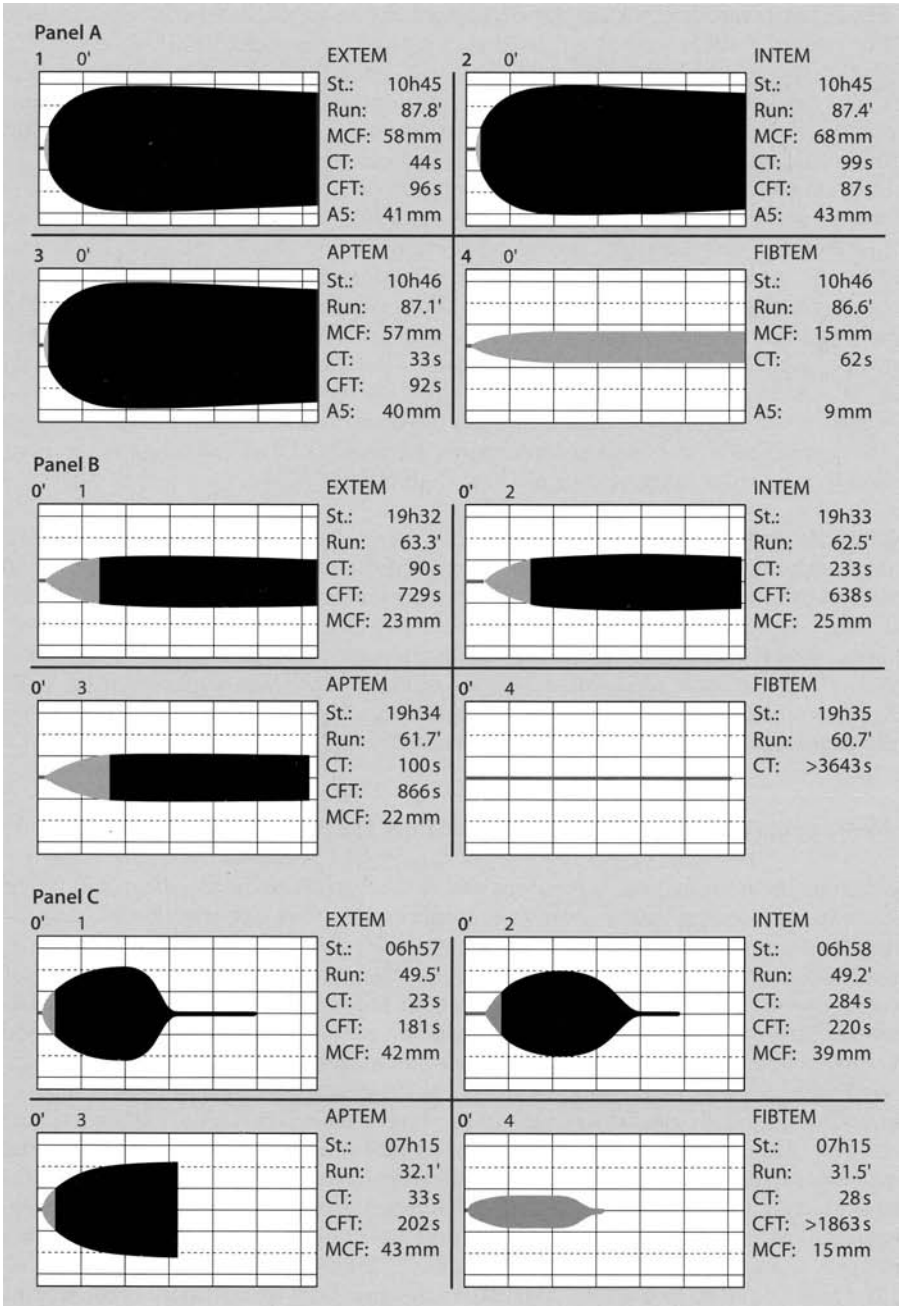

Fig. 3. Panel A: normal ROTEM trace. Panel B: Severe coagulopathy with deficiency in: 1) fibrinogen (FIBTEM MCF< 12 mm), 2) platelets (weak clot in all TEMs), 3) coagulation factors (EXTEM CT > 80s), but no hyperfibrinolysis. Therapeutic consequence: substitution of: 1) fibrinogen concentrate, 2) platelets, 3) prothrombin complex concentrate (PCC) concentrate, Panel C: Hyperfibrinolysis with a typical tapering trace in EXTEM (+FIBTEM) and a stable clot in APTEM. Therapeutic consequence: 1) antifibrinolytics and ROTEM-control after 10 mins.

Table 1. Recommended time points for hemostasis monitoring in emergency medicine

- at admission to the trauma unit or at baseline of surgery with high risk of bleeding (according to the amount of bleeding or critical site such as cerebral, spinal bleeding)
- when relevant bleeding occurs (overt or not surgically correctable bleeding)
- after each blood volume exchange
- after procoagulant therapeutic intervention
- postoperatively to detect hypercoagulability

repeatedly (Table 1). Despite the obvious limitations of routine laboratory tests, they are recommended as a guide to massive transfusion because a routine laboratory-based transfusion algorithm is superior to treatment solely based on the clinician's experience [4, 19].

Blood products and hemostatic agents

When interpreting transfusion guidelines changes in blood products over time have to be considered. The contemporary use of packed RBCs instead of fresh whole blood affects the management of bleeding patients. According to international recommendations and/or hospital-internal agreements, massively bleeding patients receive packed RBCs, plasma (e.g., fresh frozen plasma [FFP]), and platelet concentrates (e.g., apheresis concentrate), as well as coagulation factor concentrates (e.g., fibrinogen concentrate, PCC, purified factor concentrates, recombinant activated factor VII), and hemostatic agents (e.g. antifibrinolytic drugs, desmopressin). Aside from attempts to control the source of hemorrhage, therapeutic options in perioperative coagulation management are summarized in Figure 4 and have been reviewed recently [4, 24, 40–42].

Fibrinogen, the final effector of the clotting system, is vulnerable in trauma-associated coagulability because it reaches critical values before several other coagulation factors [43]. Replacement of only one blood volume may lead to a clinically relevant fibrinogen deficiency [20, 23]. The administration of fibrinogen concentrates is faster and more effective in reversing a fibrinogen deficiency than the administration of FFP [37]. Empiric transfusion triggers in bleeding trauma patients are fibrinogen < 100 mg/dl or alternatively, in a ROTEM-based transfusion algorithm, EXTEM MCF < 50 mm plus FIBTEM MCF < 12 mm (Fig. 5). Due to the dynamic nature of the hemostatic defect, these cut-off values are lower for traumatized patients with ongoing bleeding than for patients undergoing elective surgery with expected bleeding. Fibrinogen substitution partially reverses the dilutional coagulopathy induced by crystalloids and colloids in vitro and in vivo [44]. Clinical TEG/ROTEM experience indicates that the addition of fibrinogen can increase clot strength even in the presence of reduced platelet counts and function.

Replacement of 2.5 blood volumes may lead to clinically relevant thrombocytopenia [23]. Empiric indicators for transfusion of platelet concentrates are platelet counts < 50 (-100) $\times 10^3/mm^3$ [45] or EXTEM MCF < 45 mm combined with FIBTEM MCF > 12 mm. Bearing in mind the individual response, recommendations for fixed transfusion ratios for platelet concentrates versus packed RBCs (ratio of 0.5–0.8) are useless [24].

In contrast to FFP, PCCs are rapidly available in the trauma unit or OR. Empiric transfusion triggers are aPTT or PT > 1.5 (-1.8) times normal or EXTEM CT > 80 s, which indicates clinically relevant loss of coagulation factors II, VII, IX, X. Co-

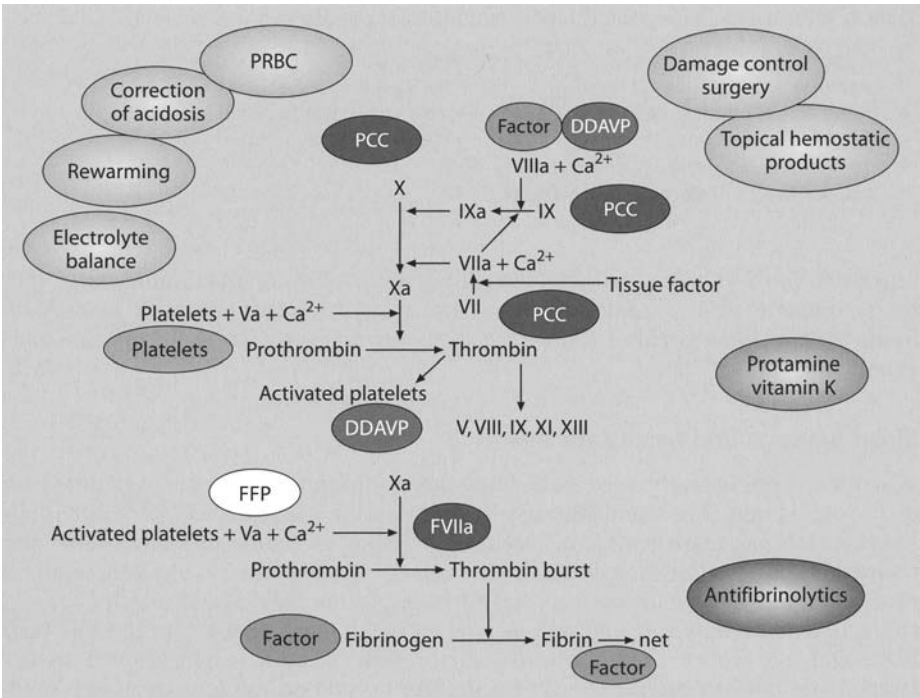

Fig. 4. Coagulation management in trauma patients. FFP: fresh frozen plasma; PCC: prothrombin concentrated complex; DDAVP: desmopressin acetate

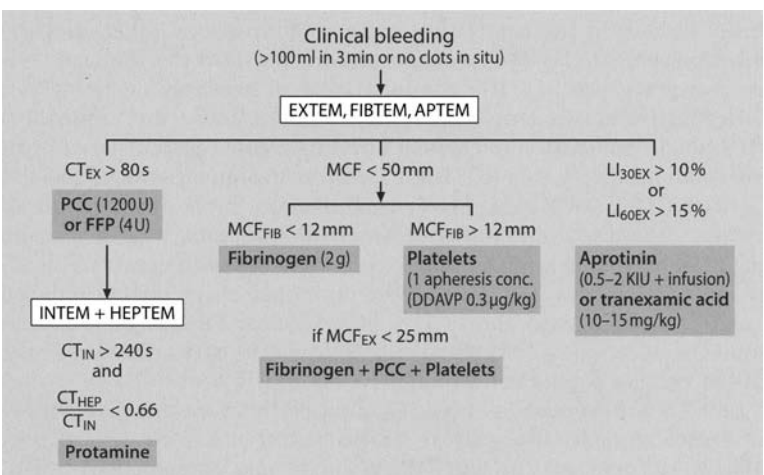

Fig. 5. ROTEM-based algorithm in trauma patients (expert advice). PPC: prothrombin concentrated complex; FFP: fresh frozen plasma; MCF: maximum clot fimmness; CT: clotting time; DDAVP: desmopressin acetate

administration of PCC and antithrombin is not required in traumatized bleeding patients. The content of heparin in PCCs has to be considered in patients with (suspected) heparin-induced thrombocytopenia. Adequate doses to increase coagulation factor levels by FFP are >20–30 ml/kg [37].

The efficacy of recombinant activated factor VIIa (rFVIIa) appears to extend to non-hemophilic patients with severe trauma. Data on modified TEG monitoring using low tissue factor activation have been described in hemophilia [46]. Additional TEG/ROTEM parameters (maximum velocity of clot formation and time to reach maximum clot formation) appear to be more sensitive to rFVIIa than standard kinetic parameters [47]. The most consistent effect of rFVIIa on TEG/TEM is the shortening of r/CT and k/CFT, as well as increased MA/MCF [48]. Current data do not support the use of PFA-100 in monitoring rFVIIa therapy in trauma [48]. Simple reductions of prolonged PT and aPTT are not useful in identifying responders to rFVIIa, however, the lack of reduction may identify non-responders [48]. Activation of factor X appears to predict hemostatic efficacy [49]. However, it must be kept in mind that patients, if over-supported in the pre- and intraoperative period, may rapidly swing back to a thrombotic state postoperatively with the risk of myocardial infarction, pulmonary embolism, or deep vein thrombosis. Monitoring of factor XIII levels (or EXTEM assay with *ex vivo* spiking with factor XIII) may guide factor concentrate supplementation.

Limitations of TEG/ROTEM

When performed by anesthesiologists or nursing personnel within the trauma unit or OR, the possibility for handling mistakes has to be considered. Another limitation of these point-of-care tests is their lack of robustness. Future studies in emergency medicine are warranted to validate critical cut-off values for procoagulant therapy and transfusion. Not only the amount of bleeding, but also the site will determine cut-off values. Acceptance of the method not only by anesthesiologists but also biologists has to be gained and, most important, the improvements in the patient's outcome as well as health cost reductions have to be verified. It needs to be determined whether goal-directed coagulation management based on a point-of-care-algorithm can help to prevent trauma-associated coagulopathy. Because of the inability to detect platelet function disorders, such as von Willebrand syndrome and antiplatelet drug effects (except for the novel TEG aggregation test, platelet mapping) it is recommended to perform more specific platelet function tests.

Platelet Function Tests

Widespread adoption of antiplatelet agents into everyday clinical practice has revolutionized contemporary care of cardiovascular patients. The bleeding risks these drugs pose perioperatively will become increasingly important [50]. There is still no simple reliable method for measuring platelet function. Bleeding times are not predictive of bleeding [51]. Several modern platelet function analyzers on the verge of clinical implementation, including the PFA-100 (Dade) and impedance aggregometry, have recently been reviewed [31]. These assays are not validated for low platelet counts and, therefore, have no use in hemorrhagic thrombocytopenia. Platelet function tests are second level tests if anti-platelet therapy, inherited or acquired platelet defects, or extracorporeal circulation are involved, and if ROTEM and routine 'trauma panel' tests cannot reveal a defect in hemostasis responsible for bleeding. It

may be helpful to assign a hematologist or transfusion specialist to a multidisciplinary team treating acutely bleeding patients, if proper blood component therapy cannot be achieved by the trauma unit team including anesthesiologists trained in coagulation management and point-of-care monitoring.

Conclusion

Trauma-associated coagulopathy is complex in origin and a life-threatening complication in trauma patients. Appropriate use of blood products and hemostatic agents should depend on clinical judgment and the results of timely coagulation tests. The time lapse for reporting results and insufficient identification of the hemostatic defect are obstacles for conventional laboratory coagulation tests. Much interest exists for rapid, near-patient coagulation testing using ROTEM/TEG in emergency medicine. ROTEM permits timely recognition of the underlying pathophysiology and hence goal-directed therapy in elective surgery. Although likely, it has yet to be proven that improvements in hemostatic outcome lead to welcome reductions in morbidity and mortality of trauma patients.

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Oral Anticoagulant Overdose and Bleeding Risk

C.J. Wiedermann

Introduction

The major complication associated with the use of warfarin is bleeding due to excess anticoagulation. The risk of major bleeding episodes in patients treated with warfarin is related to the degree of anticoagulation. An exception to this general rule is retroperitoneal hemorrhage, which may be more common in patients taking anticoagulants, even when levels are within the therapeutic range [1]. Intracranial hemorrhage (ICH) is the most serious and lethal complication of antithrombotic therapy. Over one-half of patients with warfarin-associated ICH die within the first 3 months, a substantially higher mortality rate than that of spontaneous ICH in those not receiving anticoagulants [2, 3].

Anticoagulation with oral vitamin K inhibitors is based upon clinical trials that have defined their benefits relative to associated bleeding risks. Patients presumed to be at high risk for hemorrhage, including ICH, were excluded from participation in many of these trials. Because of underrepresentation in clinical trials, determining the relative efficacy and safety of anticoagulation in patients at varying increased risks is problematic. For ICH, predictors for bleeding during anticoagulation with warfarin and related oral vitamin K antagonists, have been defined and include: Advanced age, defined in different studies as 70 years [2] or 85 years [4]; hypertension, especially a systolic pressure >160 mmHg; prior history of cerebrovascular disease; and increased intensity of anticoagulation (e.g., international normalized ratio, INR >3.0) [4].

This chapter will discuss the general principles underlying the complications of the clinical use of oral vitamin K antagonists, including information on correcting excess anticoagulation after oral vitamin K antagonists.

Clinical Use of Warfarin and Related Oral Vitamin K Antagonists

Warfarin and related oral vitamin K antagonists are metabolized primarily by the CYP2C9 hepatic microsomal enzyme system. This system is inducible by many drugs and has a number of genetic variants, both of which may profoundly alter warfarin's *in vivo* activity. Warfarin is strongly protein-bound, primarily to albumin; only the non-protein-bound fraction is biologically active. Accordingly, any agent that is also bound to albumin may displace warfarin from its binding sites and increase its biological activity.

Different forms of vitamin K antagonists vary in their half-lifes, e.g., acenocoumarol – short, warfarin – intermediate, and phenprocoumon – long [5]. In conse-

quence, moderate overdosage of acenocoumarol may be more easily treated by omitting a single dose of this agent, in comparison to a similar overdosage of warfarin [6]. Because of its longer-half life, phenprocoumon produces more stable anticoagulation than acenocoumarol [5].

The anticoagulant effect of warfarin is mediated through inhibition of the vitamin K-dependent gamma-carboxylation of coagulation factors II, VII, IX, and X [7] resulting in the synthesis of biologically inactive forms of these coagulation proteins; warfarin also inhibits the vitamin K-dependent gamma-carboxylation of the endogenous anticoagulants, proteins C and S [8]. The ultimate anticoagulant effect of warfarin is delayed until the normal clotting factors, especially factor II (prothrombin; half-life of approximately 3 days), are cleared from the circulation. Equilibrium levels of factors II, IX, and X are reached about one week after the initiation of therapy. For this reason, heparin and warfarin treatment should overlap by four to five days when warfarin is initiated in patients with thrombotic disease [7].

The one-stage prothrombin time (PT) is the laboratory test most commonly used to measure the effects of warfarin, and is sensitive to reduced activity of factors II, VII, and X but is insensitive to reduced activity of factor IX. For measuring the PT, the World Health Organization (WHO) developed an international reference thromboplastin from human brain tissue and recommended that the PT ratio be expressed as the INR.

Bleeding

Serial monitoring of the INR will detect many but not all patients who are overanticoagulated before they have had a bleeding episode: in a review of 32 patients with a warfarin-related hemorrhage, the mean INR at the time of the event was 5.9 ± 5.9; in contrast, the mean INR at the last prior measurement, which was obtained an average of 12 days before the bleeding event, was 3.0 ± 1.2 [9]. Studies in patients with atrial fibrillation indicate that the risk increases substantially at INR values 5.0 [10].

In general, patients at high risk of bleeding with warfarin have one or more of the characteristics listed in Table 1. The risk strongly increases in the presence of a combination of risk factors. As the importance of blood pressure control is magnified in patients otherwise at high risk of ICH, control of hypertension is a key to reducing the increased risk that accompanies anticoagulant therapy. An index positively validated for out-patient risk prediction included the following four adverse factors: age

Table 1. Risk factors of bleeding in patients with oral vitamin K antagonists*

Age >75 years
Hypertension (ie, systolic >180 or diastolic >100 mmHg)
Acute or chronic alcoholism, liver disease
Poor drug compliance
Presence of bleeding lesions (e.g., gastrointestinal blood loss, peptic ulcer disease, intracranial hemorrhage)
Bleeding disorder (coagulation defects, thrombocytopenia)
Concomitant use of non-steroidal antiinflammatory drugs (NSAIDs) or antibiotics
Instability of INR control+
INR >3.0§

See references *[11], +[12], §[13]

>65 years; history of stroke; history of gastrointestinal bleeding; one or more of the following – recent myocardial infarction, hematocrit <30%, serum creatinine concentration >1.5 mg/dl (>133 μmol/l), diabetes mellitus [14]. The authors concluded that major bleeding may be preventable in many high-risk patients by avoidance of over-anticoagulation and non-steroidal anti-inflammatory agents [14].

The excess mortality associated with high INR values supports the use of less intensive treatment and a small therapeutic window, with INR close to 2.2–2.3 irrespective of the indication for anticoagulant treatment. More preventive actions should be taken to avoid episodes of high INR [13].

Excessive anticoagulation

The most common causes for excessive anticoagulation are interactions between warfarin and other drugs and superimposed diseases that may interfere with oral vitamin K antagonist pharmacokinetics. Patients frequently become excessively anticoagulated, even those who have been stable for many months. An incidence of an INR ≥6.0 of 22.5 per 10,000 treatment days was noted (Table 2) [15].

Table 2. Factors significantly associated with an international normalized ratio (INR) 6.0*

Diarrhea
Worsened heart failure
Fever
Impaired liver function
Use of acenocoumarol versus phenprocoumon
Stable heart failure

* See references [15, 16]

Correcting Excess Anticoagulation

The optimal method for returning the INR to the desired range depends upon its degree of initial elevation and whether or not clinically significant bleeding is present. Recommendations have been elaborated by the American College of Chest Physician Consensus Conference [7, 17] which are based on studies that use the elevated INR as a surrogate marker for the risk of bleeding. Most of the evidence has been obtained for anticoagulation with warfarin with evidence for phenprocoumon being less well established [18]. Evidence on treatment of patients who present with elevated INR and who have major bleeding are based mainly on observational studies. Treatment includes withholding oral anticoagulants, administering intravenous vitamin K, and transfusion of coagulation factor concentrates (Table 3) [18, 19].

Conclusion

Warfarin or related vitamin K antagonist-associated coagulopathy is commonly encountered and is associated with bleeding. Avoiding over-anticoagulation and reducing periods of overdosing in the course of oral anticoagulant treatment may help to minimise the risk of bleeding. Evidence on treatment of patients who present with elevated INR and who have major bleeding is based mainly on observational studies. Randomized clinical trials that measure the usefulness of interventions by

Table 3. Management of elevated international normalized ratio (INR) or bleeding in patients treated with vitamin K antagonists targeted at an INR range of 2.0–3.0*

3.0 < INR ≤ 3.5 (no bleeding)	No dose reduction may be required Monitor INR again before lowering the dose
3.5 < INR ≤ 5.0 (no bleeding)	Omit dose Monitor daily and resume at lower dose when INR in therapeutic range
5.0 < INR < 9.0 (no bleeding)	Omit dose Give oral vitamin K_1 (phytomenadione) 1–2.5 mg. Monitor daily and resume at lower dose when INR in therapeutic range
INR ≥ 9.0 (no bleeding)	Hold vitamin K antagonist therapy Give oral vitamin K_1 at higher dose (5–10 mg) with the expectation that the INR will be reduced substantially in 24 to 48 hours Monitor daily and give additional vitamin K_1 if necessary Resume at lower dose when INR in therapeutic range Hospitalization may be considered if patient at higher risk of bleeding
Major bleeding at any elevation of INR	Hospitalization Hold vitamin K antagonist therapy and give prothrombin concentrate complex supplemented with vitamin K_1 (10 mg by slow intravenous infusion) Monitor from the fouth hour after prothrombin complex concentrate

* Modified from [17, 18]

clinical mortality and morbidity (bleeding and thrombosis) endpoints are required. Optimal treatment, including the use of oral vitamin K in the asymptomatic patient, and intravenous vitamin K in concert with coagulation factors in the bleeding patient, should reduce the mortality associated with this condition

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Does Sex Make a Difference?

Insight into the Mechanism of Gender-specific Response to Trauma-hemorrhage

M.A. Choudhry, K.I. Bland, and I.H. Chaudry

Introduction

Gender-based differences in patient response to injury/disease have long been recognized both in clinical and experimental settings [1, 2]. Despite this, some remain skeptical on the role of gender in the overall outcome of patients [1, 3]. From an analysis of more than 150,000 trauma patients, it was concluded that male patients are at higher risk of death as compared to female patients following blunt trauma [4]. Similarly, other studies have also indicated that females are more resistant to sepsis as compared to males [5, 6]. However, gender was not found to be a significant factor in the outcome of trauma patients in some other studies [7, 8]. Thus, the role of gender in the outcome of trauma patients remains somewhat controversial. In contrast, the findings from experimental studies clearly indicate that gender plays a critical role in the host response to major injury [1, 2]. These studies have shown that immune and cardiovascular functions are suppressed following trauma-hemorrhage in mature males, ovariectomized and aged females, while both immune and cardiac functions are maintained in proestrus females under those conditions [1, 3, 9, 10]. Similarly, liver functions following trauma-hemorrhage were depressed in males, but were maintained in proestrus females. Moreover, the survival rate of proestrus females subjected to sepsis after trauma-hemorrhage is significantly higher than age-matched males or ovariectomized females. In this chapter, we will review studies delineating the potential mechanisms by which male and female sex hormones influence immune and other organ functions following trauma-hemorrhage.

Gender and Immune Responses

A suppression of the immune response is apparent immediately after injury in males and persists for a prolonged period of time, despite fluid resuscitation [1, 3, 9–14]. Studies have shown that macrophage antigen presentation, T cell proliferation, and cytokine production (Th1 cytokines, interleukin [IL]-2, and interferon [IFN]γ) are decreased in male animals following trauma-hemorrhage. This is accompanied by an augmented release of Th2 cytokines (IL-4 and IL-10) and increased mortality from subsequent sepsis [1, 9, 15, 16]. In contrast to males, females in the proestrus stage of the estrus cycle have normal/maintained immune responses following trauma-hemorrhage [1, 9, 15, 16]. However, depletion of estrogen by ovariectomy prior to trauma-hemorrhage resulted in suppressed immune responses similar to that observed in male mice following trauma-hemorrhage [1, 9, 17]. Studies have also demonstrated that treatment with the androgen receptor antagonist, flutamide,

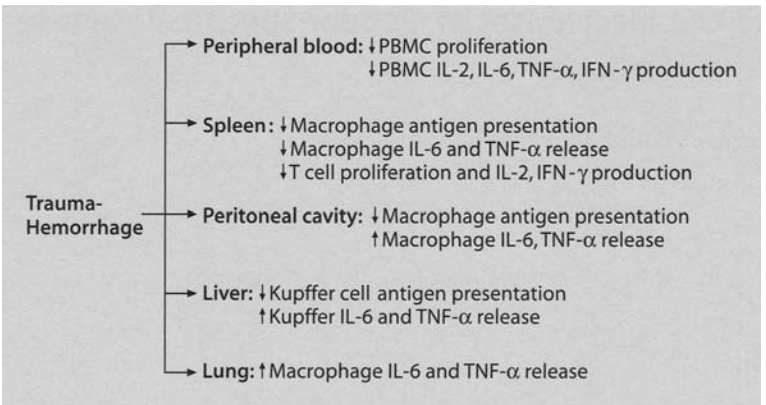

Fig. 1. Influence of trauma-hemorrhage on immune cell function in various compartments. TNF: tumor necrosis factor; IL: interleukin; PBMC: peripheral blood mononuclear cell; IFN: interferon

following trauma-hemorrhage in male mice restored immune functions [9, 18]. Alternatively, the depletion of male sex hormones by castration prior to trauma-hemorrhage also prevented the suppression in immune functions [9, 18, 19]. The role of male sex steroids in suppressed immune response following trauma-hemorrhage is further supported by findings suggesting that administration of 5α-dihydrotestosterone (DHT) in castrated males or in female mice prior to trauma-hemorrhage resulted in the depression of splenic and peritoneal macrophage cytokine production as well as suppressed Th1 cytokines following trauma-hemorrhage [9, 18, 19]. In contrast, treatment of male or ovariectomized female mice with the female sex steroid, 17β-estradiol, prevented the depression in splenic, peritoneal macrophage and Th1 cytokine production following trauma-hemorrhage [9, 18, 19]. These findings, therefore, indicate that male sex steroids cause immune depression while female sex hormones maintain immune response following trauma-hemorrhage.

In addition to suppressed immune responses, another significant observation in these studies is the finding of differences in immune cell effector responses in various tissue compartments of the body following trauma-hemorrhage [9, 20–23]. As shown in Figure 1, the cytokine production (IL-6, tumor necrosis factor [TNF]-α) capacity of peripheral blood mononuclear cells, splenic macrophages, and peritoneal macrophages was significantly decreased following trauma-hemorrhage. On the other hand, cytokine production by Kupffer cells and alveolar macrophages was significantly increased under the same experimental conditions [9, 20–23]. The precise mechanism by which such compartmentalized immune cell responses occur following trauma-hemorrhage in males, but not in proestrus females remains to be established.

Gender and Organ Function

Similar to immune response, other organs such as heart, liver, lung, and intestine, are also severely affected following trauma-hemorrhage in males but not in proestrus females. Studies have shown that cardiac output, stroke volume, rate of pressure

development (+dP/dt), and total peripheral resistance were markedly depressed after trauma-hemorrhage in males despite fluid resuscitation [3]. Our results also indicate that these parameters of cardiac function are significantly depressed in estrus, metestrus, diestrus, and ovariectomized females following trauma-hemorrhage and resuscitation. However, females in the proestrus stage of the estrus cycle maintained cardiac function following trauma-hemorrhage [3, 24].

Similarly, development of edema in lung, liver, and intestine tissue was observed within a few hours after trauma-hemorrhage and persisted for a prolonged period despite fluid resuscitation. However, edema following trauma-hemorrhage was not observed in proestrus females or in males treated with estrogen or other estrogenic agents (e.g., androstenediol). In a recent study, we have determined that the female reproductive cycle is an important variable in the regulation of lung injury following trauma-hemorrhage [25]. The findings from this study suggested that lung myeloperoxidase (MPO) activity (a marker for neutrophil infiltration) was significantly increased in the diestrus, estrus, and ovariectomized animals following trauma-hemorrhage. However, no difference in lung MPO activity following trauma-hemorrhage was observed in proestrus females compared to shams. Furthermore, the increase in lung MPO activity in ovariectomized females was found to be higher compared with female rats during the diestrus, estrus, and metestrus phases of reproductive cycle. This was accompanied by increases in lung neutrophil chemokines (e.g., cytokine-induced neutrophil chemoattractant [CINC]-1, CINC-3) and intercellular adhesion molecule (ICAM)-1 expression in the diestrus, estrus, and ovariectomized animals [25]. The proestrus females did not exhibit an increase in CINC-1, CINC-3, or ICAM-1 expression following trauma-hemorrhage compared to shams. Consistent with the MPO activity, the levels of CINC-1, CINC-3, and ICAM-1 were also significantly higher in ovariectomized females compared to female rats during the diestrus, estrus, and metestrus phases of reproductive cycle [25].

The maintenance of cardiac function and lung injury markers following trauma-hemorrhage in proestrus females was associated with the highest levels of estradiol, whereas all other stages of the estrus cycle had significantly lower plasma levels of estradiol [3, 24, 25]. Although estradiol levels were relatively higher in estrus and metestrus cycles compared to ovariectomized females, the findings of decreased heart function or increased lung injury markers in those animals suggest that the levels of estrogen in the estrus and metestrus cycle were not sufficient to prevent cardiac depression or lung injury following trauma-hemorrhage.

Additional findings suggest that administration of 17β-estradiol in ovariectomized females preventes the depression in cardiac function following trauma-hemorrhage [3]. Similar administration of 17β-estradiol in males also prevented cardiac and other organ dysfunction following trauma-hemorrhage [3, 10, 26]. On the other hand, trauma-hemorrhage in pre-castrated animals did not influence cardiac function in males [3, 10, 26]. However, administration of the male sex hormone, 5α-DHT, in castrated males and in females suppressed cardiac function following trauma-hemorrhage. These findings further support the suggestion that female hormones maintain organ functions while male hormones adversely affect those functions following trauma-hemorrhage.

Potential Mechanisms of Altered Immune and Organ Function Following Trauma-Hemorrhage

The mechanism of the beneficial effects of female sex steroids or harmful effect of male sex steroids after trauma-hemorrhage remains to be established. We have reviewed the studies suggesting the role of inflammatory mediators, heat shock proteins (HSP), and other genomic and non-genomic signaling mechanisms of estrogen-mediated beneficial effects following trauma-hemorrhage in experimental settings.

Sex Hormone Receptors

There are two major subtypes of estrogen receptors (ER), ER-α and ER-β. However, several isoforms of ER-α and ER-β have been reported to-date. For example, ER-α can further be classified into ER-αA, ER-αC, ER-αE, and ER-αF, and ER-β into ER-β1, ER-β2, ER-β4, and ER-β5 [2, 3, 10, 27, 28]. It has been suggested that ER-α and ER-β are encoded by different genes located on different chromosomes and thus do not represent splice variants [27]. Similarly, androgen receptors (AR) are also suggested to exist in more than one subtype; however, these subtypes have not been well studied, especially in the mammalian system. Moreover, it is also not clear whether they are isoforms or subtypes and whether these two subtypes/isoforms are derived from the same or distinct genes [29].

The distribution of AR and ER may vary from organ to organ. Similarly the distribution of AR and ER subtypes is likely to be cell- or organ-specific. In a recent study, we found that heart is primarily rich in ER-β, intestine in both ER-α and -β, lung has more ER-β than α and liver was found to be rich in ER-α [30, 31]. Studies have also shown that the distribution may further be affected by trauma-hemorrhage [32]. Thus, although the effects of 17β-estrogen on immune and other organ functions appear to be mediated via estrogen receptors, it is likely that these beneficial effects are mediated via organ-specific ERs. Recently our studies have utilized the ER-α- and -β-specific agonists, propyl pyrazole triol (PPT) and diarylpropionitrile (DPN), and examined the role of ER-α and -β in different organs following trauma-hemorrhage [30, 31, 33]. The findings from these studies have shown that treatment of animals with the ER-α agonist, PPT, prevented increased MPO activity in the liver following trauma-hemorrhage, while DPN normalized MPO activity in the lung [30, 31, 33].

Inflammatory Mediators

Trauma-hemorrhage results in increased production of pro-inflammatory cytokines, which includes IL-1, IL-6, and TNF-α, as well as the anti-inflammatory cytokine, IL-10 [3, 9, 10, 21]. The elevation in IL-6 and TNF-α has been correlated with poor outcome following major injury including trauma-hemorrhage. Studies have reported that IL-6, which controls multiple cell functions, may have a role in gender-specific alterations in organ functions following trauma-hemorrhage, burn, and sepsis. In this regard, we found that plasma IL-6 levels were significantly elevated within a few hours after trauma-hemorrhage and remained elevated at 24 h following trauma-hemorrhage; however, administration of estradiol during resuscitation downregulated the trauma-hemorrhage-induced increase in plasma IL-6 [3, 9, 10, 21]. Although our previous studies suggest that Kupffer cells are the primary source

of circulatory IL-6 levels, cardiomyocytes can also synthesize IL-6 and the local production of IL-6 is likely more critical in regulating cardiac function following trauma-hemorrhage [3, 9, 10, 21, 26].

TNF-α is another pro-inflammatory cytokine that has been studied in great detail in animal models of trauma. The findings from these studies suggest that TNF-α is increased within a few hours after injury and that treatment of animals with 17β-estradiol prevents the increase in TNF-α following trauma-hemorrhage [3, 9, 10, 20, 21, 34]. Thus, these findings suggest that 17β-estradiol may mediate its salutary effect on cardiac function via modulation of pro-inflammatory cytokines, such as IL-6 and TNF-α.

In addition to cytokines, 17β-estradiol has also been shown to prevent the decrease in nitric oxide synthase (NOS) and to increase leukocyte infiltration [35]. Both diminished NOS activity and increased leukocyte infiltration potentially contribute to altered cardiac function following trauma-hemorrhage [25, 31, 33, 34, 36–38]. Thus, estradiol may also mediate its beneficial effects on organ function by modulating NOS and/or leukocyte infiltration following trauma-hemorrhage.

Nuclear Factor-kappa B

Nuclear factor-kappa B (NF-κB) is a pleiotropic transcription factor implicated in the regulation of diverse biological phenomena, including the cellular responses to stress, hypoxia, ischemia, and hemorrhagic shock [24, 39–41]. Studies have shown that NF-κB is activated in various heart diseases, such as myocarditis, congestive heart failure, dilated cardiomyopathy, and heart transplant rejection. NF-κB is also activated following burn, ischemia/reperfusion, and hypoxia. Moreover, studies have demonstrated that induction of IL-6 by hypoxia, a condition associated with trauma-hemorrhage, is mediated by NF-κB and NF-IL-6 in cardiomyocytes [24, 41]. NF-IL-6 and NF-κB are known to synergistically activate the transcription of inflammatory cytokines. Studies have also shown that estrogen represses IL-6 gene expression through inhibition of the DNA-binding activities of the transcription factors NF-IL-6 and NF-κB by the estrogen receptors [24, 41]. Thus, administration of estrogen following trauma-hemorrhage may inhibit NF-κB binding activity and, thereby, suppress IL-6 production. Altogether, there are multiple possibilities through which estrogen may mediate its beneficial effects on the heart and other organs following trauma-hemorrhage and resuscitation.

Heat Shock Proteins

Estrogen can induce the expression of HSP and several lines of evidence suggest that HSP upregulation plays a major role in the preservation of organ function after ischemic and low-flow conditions [25, 30, 42–45]. Multiple mechanisms have been proposed that HSP may utilize in mediating their protective effects. For example, HSP60 and HSP70 serve as molecular chaperones and maintain protein structures under stress conditions. HSP60 is localized in the mitochondria and is reported to be helpful in maintaining electron chain integrity [42].

HSP70, the most intensively studied member of the HSP family, similar to HSP60, is shown to assist the transfer of newly synthesized proteins into the mitochondria. HSP70 also plays a role in maintaining overall mitochondrial integrity [42]. In addition, there is a growing body of evidence that HSP70 can block the pro-inflammatory cascade via the suppression of NF-κB activation [42–44, 46].

HSP32, also referred to as heme oxygenase 1 (HO-1), has been shown to play a protective role following trauma-hemorrhage [25, 30, 43, 44]. While a definitive mechanism by which HSP32 mediates its beneficial effects remains to be established, studies have shown that it participates in heme elimination. The accumulation of free heme under hypoxic conditions becomes toxic and, therefore, elimination of free heme from the cellular milieu is necessary. Carbon monoxide which is a by-product of heme degradation can activate soluble guanylate cyclase and induce vasodilatation via cGMP. Another potential mechanism of HSP32-mediated tissue protection may be the carbon monoxide-mediated activation of Ca^{2+}-dependent potassium channels. Since the activation of Ca^{2+}-dependent potassium channels leads to hyperpolarization of the smooth muscle cells, their stimulation results in decreased vascular contractility. Bilirubin, another product of HSP32 enzyme activity, has been shown to have potent antioxidant activity. A recent study from our laboratory has shown that HSP32 upregulation inhibits the expression of adhesion molecules and prevents subsequent leukocyte-endothelial cell interactions [25, 30, 43, 44]. Furthermore, it has also been reported that HSP32 upregulation protects mitochondrial function and prevents ATP-depletion after oxidative stress.

HSPs are also known to regulate the process of programmed cell death/apoptosis [42, 46]. One major pathway of apoptosis involves the release of cytochrome C from mitochondria. In turn, cytochrome C binds to a protein known as apoptotic peptidase activating factor (Apaf)-1 and triggers its oligomerization. This complex then attracts the inactive unprocessed pro-form of the proteolytic enzyme, caspase-9, which is then cleaved to its active form, thereby initiating apoptosis. HSPs have been shown to inhibit this process at various points such as preventing the binding of cytochrome C to Apaf-1. Furthermore, HSP70 prevents oilgomerized Apaf-1 from recruiting pro-caspase-9 [42, 46]. Studies have also suggested that over-expression of HSP60 inhibits myocardial apoptosis in response to ischemic injury. Furthermore, a recent study has shown that reducing HSP60 expression with antisense oligonucleotides is associated with an increase in Bax and a reduction in Bcl-2, which induces apoptosis of cardiomyocytes [42, 46]. In addition, HSP90 has been shown to bind to endothelial NOS (eNOS) and stimulate its activity [36, 42, 46]. Thus, HSPs protect cells via multiple mechanisms which target key cellular components and regulatory process.

The overall mechanism by which estradiol upregulates HSP after trauma-hemorrhage remains to be established; however, studies have shown that HSP synthesis is controlled by a family of transcription factors, the heat shock factors (HSF). Although four HSF have been identified, HSF-1 has been shown to regulate the expression of HSP in response to ischemia, hypoxia, heat, stretch, or injury [36, 42, 46–48]. It is suggested that in the absence of ligand, the ER in the cytoplasm of E2-target cells associate with the HSP (e.g., HSP90), which maintains the receptors in an inactive state. Studies have shown that HSP90 also forms a complex with HSF-1 [42, 47, 48]. Interactions involving HSP90 and ER as well as the binding between HSP90 and HSF thus represent an important element in the activation of HSF-1 by E2 [42, 47, 48]. HSF-1, normally present in the cytoplasm in an inactive, monomeric form, migrates to the nucleus upon activation where it binds to the heat shock element (HSE). HSE is present in the promoter of the stress response gene and initiates HSP transcription and synthesis.

Genomic Signaling

It is a widely held belief that steroid receptors (AR and ER) are mainly localized in the cytoplasm and nucleus of the cell. Thus, both androgens and estrogens mediate their actions by activating the transcription factors and accordingly are expected to alter signaling at the nuclear level (i.e., the genomic mechanism). This genomic effect of estrogen last from hours to days. As presented in Fig. 2, upon binding to estrogen, ER becomes activated, dimerized and the complex translocates to the nucleus. In the nucleus, it binds a specific target DNA sequence within estrogen-responsive genes called an estrogen response element (ERE). This leads to the enhancement of transcription. Studies have also indicated that unbound ER can also bind to ERE consensus sequences and activate transcription, but interaction of the receptor with estrogen stabilizes dimerization and enhances its interaction with target sequences within estrogen-responsive genes. It has been suggested that several soluble growth factors, including epidermal growth factor and insulin-like growth factor-1 can activate ER and thus promote gene transactivation in the absence of estrogen [36, 42, 47, 48].

Non-genomic Signaling

In addition to the genomic action, there is evidence indicating that ERs are also localized on the plasma membrane and thus estrogen can also influence cell function by inducing nongenomic effects via plasma membrane ERs. Studies have shown that these so-called nongenomic effects or cell membrane-initiated signals are activated quickly within minutes (Fig. 2). Utilizing a cell-impermeable estrogen conju-

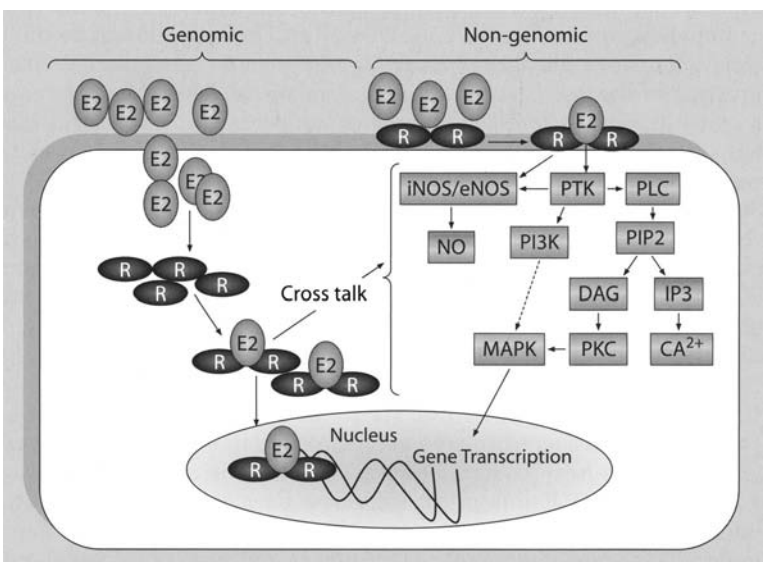

Fig. 2. Genomic and non-genomic actions of estrogen. E2: estrogen; R: receptor; iNOS: inducible nitric oxide synthase; PTK: protein tyrosine kinases; NO: nitric oxide; PI3K: phosphatidylinositol 3-kinase; PIP2: phosphatidylinositol 4,5-bisphosphate; PLC: phospholipase C; IP3: inositol 3-phopshate; DAG: diacyl glycerol; PKC: protein kinase C; MAPK: mitogen activated protein kinase

gated with bovine serum albumin (E2-BSA), studies have shown that many of the signaling pathways (e.g., PLC/PKC; p38/MAPK; PI3k/AKT, NO, Ca^{2+}) are turned on after stimulation of cells within E2-BSA [27, 36, 47, 49, 50].

The PI3K/AKT signaling pathway can also be activated by estrogen in different cell lines (e.g., vascular or epithelial cells). Engagement of membrane ERs results in rapid endothelial NO release through a PI3K/AKT-dependent pathway. The non-genomic effects of estrogen can regulate different cellular processes, such as proliferation, survival, apoptosis, and differentiated functions in diverse cell types, including breast cancer cells [27, 36, 47, 49, 50]. The nature of the plasma membrane binding sites for estrogens is currently under intense debate and investigation. However, to date, both classic ER-α and ER-β and non-classical ER (e.g., GPR30) have been identified at the membrane. It has been shown that GPR30 binds only high concentrations of 17β-estradiol.

We have used E2-BSA to delineate the role of cell surface versus nuclear membrane receptors in estrogen-mediated restoration of organ function following trauma-hemorrhage. Our results indicate that male rats treated with E2-BSA display improvement in cardiac functions at 2 hours following trauma-hemorrhage. We further found that the biologic effects of E2-BSA on cardiac function are receptor-dependent since the administration of ICI 182,780, a selective ER antagonist, along with E2-BSA abolished the E2-BSA-induced cardioprotection in trauma-hemorrhage rats. However, it is to be noted that the restoration of cardiac function following E2-BSA treatment was not complete as compared to the rats treated with estrogen alone. Thus, it is likely that the activation of both surface and nuclear ERs is required for full restoration of cardiac function following trauma-hemorrhage. Our studies also demonstrated that PI3K/Akt pathway plays a major role in mediating the non-genomic effects of estrogen on cardiac function. Similar findings were obtained by other investigators indicating the role of PI3K/Akt signaling in non-genomic estrogen effects [27, 36, 47, 49, 50]. The activation of the PI3K pathway protects organs or cells against ischemia-reperfusion injury and hypoxia through suppression of the cell death machinery. One of the downstream targets of PI3K pathway is Akt and studies have shown that an increase in Akt activity leads to improved left ventricular contractile recovery following transient ischemia. PI3K/Akt has been reported to play an important role in the cell survival pathway [27, 36, 47, 49, 50]. Studies have also reported that the PI3K/Akt signal has an anti-apoptotic activity through different mechanisms, e.g., by phosphorylation of Akt, it induces BAD phosphorylation and hence inhibits its translocation into the mitochondria and binding to Bcl-2 [27, 36, 47, 49, 50]. While it is likely that E2-BSA-induced Akt phosphorylation prevents DNA fragmentation in cardiomyocytes and thus maintains cardiac functions following trauma-hemorrhage, the role of other molecules in mediating the nongenomic effects of estrogen has not been ruled out. In view of this, additional studies are needed to delineate the role of both genomic and non-genomic pathways in estrogen-mediated actions on immune and other organ functions following trauma-hemorrhage. However, it should be noted that although the genomic and non-genomic actions of estrogen have been studied separately, these actions are interdependent and should be considered as synergistically acting aspects of the molecular response to estrogen, leading to the final physiologic outcomes such as survival versus apoptosis, growth regulation and cell motility to name a few.

Conclusion

Clinical and experimental studies suggest that gender is an important factor which should be considered in the overall management of trauma patients. Experimental findings suggest that trauma-hemorrhage results in profound alterations in immune and other organ functions in males and ovariectomized females, but these functions are maintained in pre-castrated males and proestrus females. However, administration of estrogen restored both immune and other organ functions in males and ovariectomized females following trauma-hemorrhage. While more studies are needed to fully understand the precise mechanism by which estrogen mediates its beneficial effects, the studies reviewed in this chapter suggest that estrogen modulates a number of factors including inflammatory mediators and HSPs, and thereby protects organ functions following trauma-hemorrhage.

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Sex-Related Differences in Response to Global Ischemic Insult and Treatment

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Introduction

There are obvious anatomical, physical, and genetic differences between men and women that have always been a central focus of human life. Such differences have become the target of humor, politics, and legal issues and many other aspects of the human experience. At the same time, though receiving less notoriety, there are also striking sex-related differences in the presentation, outcomes, and responses to many disease processes and therapies.

In fact, outside of the overt differences in terms of obstetrical and gynecological practice, breast cancer, and a few other classical diagnoses that may occur more often in women than men, sex-related differences in the presentation, treatment, and response to diseases have not been a major issue in mainstream medicine until recently. Over the past two decades, for example, some reports have begun to address major differences between women and men with acute coronary artery syndromes. In most of these studies, women were found to have worse outcomes [1–3]. However, more recently, it has been suggested that the differences may be more sociologic than biologic [2, 4, 5].

Some investigators have argued that serious illnesses may sometimes be overlooked in women or they may, for various reasons, receive less aggressive intervention than men. Moreover, when excluding such sociological factors, some investigators are now demonstrating that women actually fare better than their male counterparts in most instances, even though they usually present with common diseases at older ages with a number of other co-morbid conditions [10–30]. Naturally, these findings have prompted scientists to investigate the biological bases for these differences.

Again, though not achieving mainstream notoriety, from a historical perspective, male-female variances in response to disease have been conjectured for centuries. For example, in 1786, Dr. Clarke of Dublin, Ireland, proposed a possible explanation:

> *"Observations which have been made on the laws that govern human mortality prove that the mortality of males exceeds that of females in almost all stages of life ... such as a male foetus's requiring more nutrition than female foetus's, because larger, and also for this reason more liable to injury in delivery, are brought into the world less perfect ..."*
>
> Clarke J (1786) Observations on some causes of the excess of the mortality of males above that of females. Communicated by the Rev. Richard Price, D. D. F. R. S. in a letter to Charles Blagden, M. D. Sec. R. S., by Joseph Clarke; B. H. Register Philosophical Transactions of the Royal Society of London.

While such generalized observations have continued to emerge from time to time in the medical literature, many of the details and potential mechanisms have remained under-investigated up until the past quarter century. Over the last two decades, sex-related differences have been found not only in cardiac disease [6–9], but also in cardiac arrest [10–15], trauma [16–20], stroke and cerebral injury [21–23]. More importantly, differences have also been demonstrated in terms of the response to various pharmacotherapies [24–28] as well as the susceptibility and related response to infections and sepsis [29, 30]. It is now clear in the emerging age of genomics that these variations between the sexes are of growing importance. Not only can they provide clues to the biological mechanisms that lead to sex-differences in disease response, but they can also lead to innovative treatment modalities that may soon improve outcomes for both men and women facing a variety of serious disease processes. As an example, the following discussion will address sex-related differences in the arena of cardiac arrest and the evidence pointing toward a potential hormonal mechanism for those variances.

Rationale for Studying Sex-related Differences in Cardiopulmonary Arrest

As stated previously, one of the few instances in which women appear to fare worse than their male counterparts is in the case of outcomes following acute myocardial infarction [1–3]. Again, in this instance, it is now thought that the sex-related differences in the response to this disease process and related therapies may actually be due to the differences in presenting symptoms, history provided, clinical characteristics, and less aggressive therapy [2, 4, 5]. They may also be due to psychological and social factors leading to denial, stoicism or lack of illness recognition.

One associated complication of coronary artery disease and acute myocardial infarction in which such sociological and psychological matters are less of a factor is sudden cardiac arrest. In sudden cardiac arrest, the presentation (cardiac arrest) is straightforward and the treatment provided is, more than likely, always the same. Specifically, for such cases, there are international treatment algorithms which are exactly the same for both adult men and women, regardless of age [31]. Therefore, studies of cardiac arrest are more apt to provide differences in biology and the resulting response to therapy.

Investigations of Sex-Related Differences in Sudden Cardiac Arrest

To delineate differences between males and females in the special subset of cardiac arrest patients, a group of 4,147 consecutive patients experiencing sudden cardiac arrest in the out-of-hospital setting were examined prospectively over a six-year period in a population-based study [15]. The main end-point of this investigation was to see if men or women, treated identically, achieved sustained return of spontaneous circulation and were admitted to a critical care unit with different frequencies. Although it would be preferable to also follow the patients to hospital discharge, the main concern in this particular study was to see which group was more likely to be resuscitated by paramedics. While paramedics follow a fairly rigid standardized protocol, in-hospital therapy can be more variable and also be affected by sociological and psychological factors, particularly in the elderly [32].

In this prospective, population-based study conducted in the 8th largest city in the USA (Dallas, Texas), 42% were women and the women were significantly older than the men by approximately seven years (mean age, 68.7 ± 18 vs. 61.7 ± 17 years for men). There were no significant differences between women and men in terms of arrests that were witnessed or un-witnessed, and no differences were noted in terms of the frequency of bystander cardiopulmonary resuscitation (CPR) or emergency medical service (EMS) response intervals. Importantly, there were no significant differences in the pre-hospital time intervals during which the patients received standard advanced cardiac life support (ACLS) interventions [31]. Also, the number and types of interventions and drugs infused were also identical [15].

There were, however, significant differences between the two groups with regard to the presenting electrocardiographic (EKG) rhythms found by paramedics upon arrival at the scene. Women initially were found to be in asystole (42% vs. 37%) or pulseless electrical activity (24% vs. 18%) significantly more often than the men ($p < 0.001$). Women also presented with ventricular fibrillation/ventricular tachycardia (VF/VT) significantly less often than the men (30% vs. 41%, $p < 0.001$)

However, despite the fact that women were older, with a lower frequency of VF/VT on presentation, significantly more women were resuscitated and admitted to the intensive care unit (ICU) than were men (13.5% vs. 10.7%; $p = 0.005$). While women presenting with VF/VT still achieved return of spontaneous circulation and were admitted to the ICU more often than men (16.3% vs. 12.6%; $p = 0.05$), this advantage was even more pronounced is women with non-VF/VT rhythms (12.6% vs. 9.6%; $p = 0.016$).

Although certain pharmaco-biological, genomic, or even anatomical differences (e.g., volume of distribution, chest wall compliance during CPR) might account for the women's response to therapy, the issue of hormonal differences also arises as an obvious potential factor. At first glance, the issue of hormone influence would seem less of a factor because the great majority of female patients in these studies were post-menopausal with an average age of 69 years. Still, the number of patients taking supplemental hormones was not tabulated and the groups were not initially stratified according to age. Therefore, based on these considerations, the investigators performed a larger follow-up study to further examine the results of the initial observational study. Specifically, the purpose was to delineate the return of spontaneous circulation and short-term survival outcomes in men and women 50 years of age and younger [33].

Examining Sex-Related Differences in Younger Cardiac Arrest Patients

In order to delineate potential hormone-related differences in the same patient population, an updated cohort of nearly 10,000 consecutive out-of-hospital cardiac arrest patients was followed ($n = 9,445$). In this case, the groups were stratified in terms of men and women 50 years of age and younger, versus those over 50, in order to help better capture a subset of women who are more likely to be pre-menopausal (Table 1). Again, the primary endpoint of the study was to compare the young men and women in terms of short-term survival (survival to ICU admission) following out-of-hospital sudden cardiac arrests. Secondary endpoints were to compare these outcomes to men and women in the older groups and to examine the sub-categories of the various presenting EKG rhythms.

The results of this study were even more striking. When examining out-of-hospital cardiac arrests for all ages ($n = 3,926$ women and 5,519 men), the arrest was wit-

Table 1. Tally of 9,445 consecutive subjects entered into a population-based study of out-of-hospital cardiac arrests, stratified by sex and by age for those ≤50 years versus those over 50 years.

	Younger (≤50 years)	Older (>50 years)	Total
Male	1,653	3,866	5,519
Female	839	3,086	3,926
Total	2,492	6,953	9,445

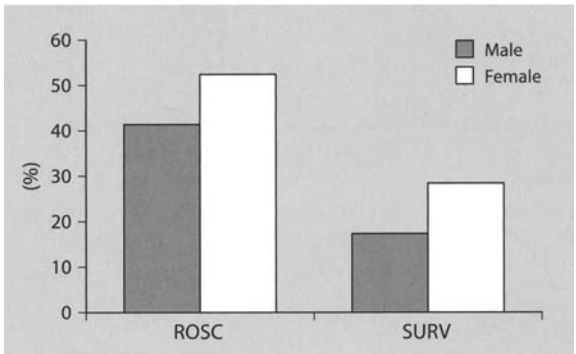

Fig. 1. Comparison of the rates of sustained return of spontaneous circulation (ROSC) and rates of short-term survival (SURV) to admission to an intensive care unit (ICU) among 1,653 men and 839 women (age ≤50 years) with out-of-hospital cardiac arrest showing significant differences for both outcomes ($p < 0.001$).

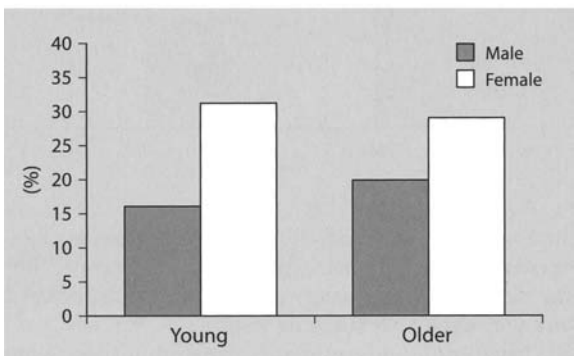

Fig. 2. Comparison of the rates of short-term survival to admission to an intensive care unit (ICU) among men and women with out-of-hospital cardiac arrest who presented with ventricular fibrillation or ventricular tachycardia showing significant differences between the men and women, particularly for those 50 years of age or younger ($p < 0.001$).

nessed less often in women and they had a lower frequency of bystander CPR. They also had fewer VF/VT presentations (21% versus 29% for men). Nevertheless, overall, women still achieved return of spontaneous circulation more often (46% versus 39%; $p < 0.001$) and had a higher rate of short-term survival (21% versus 17%; $p < 0.001$) (Fig. 1). Most importantly, when examining men (n = 1,653) and women (n = 839) who were 50 years of age or less, women achieved return of spontaneous circulation and short-term survival significantly more often (return of spontaneous circulation = 52% for women versus 41% for men, $p < 0.001$; survival rate = 28% versus 17%; $p < 0.001$). Most impressively, the short-term survival rate for younger women with VF/VT was double that of the men (31% vs. 16%; $p < 0.001$) while it was still 29% vs. 20% for the older cohort (Fig. 2).

Furthermore, while there were no significant differences in short-term survival between older women and men presenting with pulseless electrical activity (18% versus 19%), the differences between the younger women and men were striking (Fig. 3) in that women survived to ICU admission in 29% of cases versus 18% of the men ($p < 0.001$). Likewise, the short-term survival rate for those presenting with asy-

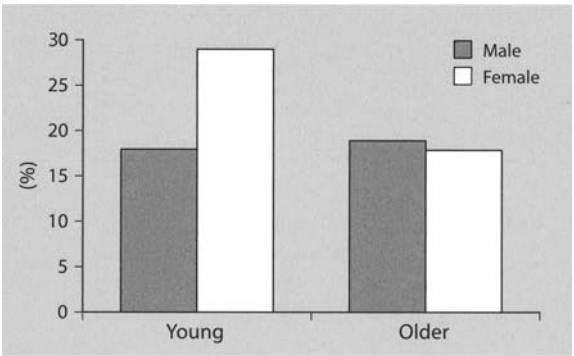

Fig. 3. Comparison of the rates of short-term survival to admission to an intensive care unit (ICU) among men and women with out-of-hospital cardiac arrest who presented with pulseless electrical activity (PEA) showing significant differences for those men and women 50 years of age or younger ($p < 0.001$), but no significant differences between the older men and women.

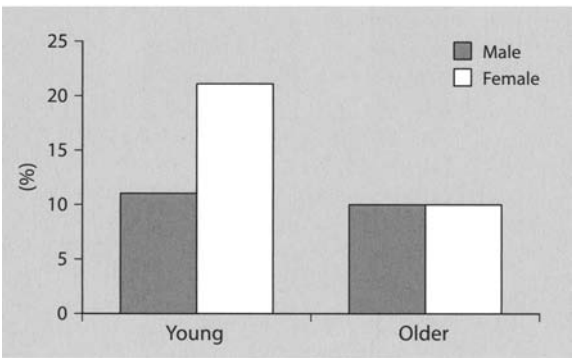

Fig. 4. Comparison of the rates of short-term survival to admission to an intensive care unit (ICU) among men and women with out-of-hospital cardiac arrest who presented with asystole showing significant differences for those men and women 50 years of age or younger ($p < 0.001$), but no significant differences between the older men and women.

stole was 10% for both older men and women (Fig. 4), but 21% for younger women versus 11% for the men ($p < 0.001$). In essence, the main factor in the differences in the rates of resuscitation and short-term survival between men and women was a younger age of 50 years or less.

Other confounding factors explaining these findings were unlikely. For example, men and women received exactly the same interventions during their treatment by paramedics and they received those interventions for identical periods of time. If anything, response time intervals were slightly longer for the women.

Sex Hormones as a Factor in Resuscitation and Neurological Outcome

On the basis of these observational results, it appears that women, and particularly women more likely to be pre-menopausal, may have some physiological advantage over men, particularly in terms of their response to therapy (sustained resuscitation) for this major cause of mortality for both sexes. The inference is that those under the influence of higher levels of endogenous sex steroids had improved chances of resuscitation and short-term survival, either innately as a direct protection against the insult, or through some hormone-facilitated improvement in the response to the standardized therapies delivered.

It is clear that several limitations of the previous studies of cardiac arrest should be noted. First, the studies did not examine the use of exogenous hormones (i.e.,

hormone replacement therapy or birth control pills) or serum hormone levels in this patient population. Also, because of the lack of readily-available records due to privacy regulations, the patients' discharge status and long term neurological outcome were not routinely tracked. Nevertheless, most traditional studies of out-of-hospital cardiac arrest do show a fairly proportional overall discharge rate when one tracks the rate of survival to ICU admission and compares it to the rate of hospital discharge with good neurological outcomes.

Also, the simple end-point of hospital admission, the primary outcome examined for the purpose of this study, has different implications from the end-points of survival to hospital discharge and return of good neurological status. The end-point of hospital admission deals with return of spontaneous circulation and sustained resuscitation while the hospital discharge and neurological outcomes have implications in terms of neuro-protective properties, be they directly from the resuscitation itself or from intrinsic brain protection. In this study of younger men and women, the investigators were most interested in the ability to resuscitate patients. Nevertheless, the concept of ultimate long term recovery is still key.

Three previous studies [12, 13, 34] focusing on out-of-hospital cardiac arrest patients with VF/VT found either no differences or better long-term survival for the men. However, all studies still found more women being resuscitated and admitted to the hospital, even those with VF/VT. Therefore, it has been suggested that the in-hospital therapy may be a factor. In turn, such studies examining the hospital courses and neurological outcome of women and men under the age of 50 years would be of great value and they may help to delineate resuscitation effects from neuro-protection effects.

Experimental Evidence for the Role of Sex Hormones in Resuscitation

There actually is strong experimental evidence to support the concept that female sex steroids improve outcomes after sudden cardiac arrest. Recent animal studies in rodent and feline models have studied estrogen and progesterone as acute resuscitation drugs in models of global cerebral ischemia and the majority have had promising preliminary results [35–39]. Interestingly, testosterone has been noted to have a potential *negative* (anti-estrogenic) effect in times of cerebral ischemia [40, 41].

While the use of estrogen and progesterone, and possibly the blocking of testosterone, have been postulated as potential adjuncts to improve neurological outcomes in neuro-resuscitation, only one study to date has specifically looked at the use of an acute dose of a sex steroid as a resuscitative drug during the conditions of cardiopulmonary arrest [42]. Noppens et al. recently published a rodent study in which male mice were randomized to receive either placebo or 17-β estradiol [42]. Cardiac arrest was then induced in these animals by potassium chloride injection. Ten minutes after the induction of cardiac arrest, CPR was initiated, followed by treatment with estrogen or placebo 1.5 minutes after return of spontaneous circulation. They found that a single low dose of estrogen given after reperfusion was neuro-protective in male mice after cardiac arrest and resuscitation. Nevertheless, studies confirming the ability of sex hormones to actually affect the initial rates of return of spontaneous circulation still need to be performed.

Conclusion

Recent epidemiological studies, as well as recent studies in the animal literature, give rise to the hypothesis that many sex steroids, including estrogen, progesterone, and testosterone, may be responsible, at least in part, for the sex-related differences in the presentations and outcomes of many varied disease states. Such differences have been most striking in terms of the ability to resuscitate women and men in out-of-hospital cardiac arrest, particularly when one examines those patients 50 years of age or less. While more observational studies evaluating differences in the in-hospital courses of patients and their long-term neurological outcomes would be extremely important, further animal studies examining the role of sex steroids in resuscitation are indicated as well. Meanwhile, in the clinical setting, investigators should attempt to control for factors such as elapsed intervals of cardiac arrest before resuscitation, the frequency, duration and quality of CPR provided prior to advanced techniques, and other factors that could affect the extent and degree of global ischemic insults and thus obscure hormonal effects. It is hoped that the scientific information gleaned in related future studies will help to identify mechanisms for significantly improving resuscitation outcomes, not only for both sexes, but also for patients of all ages.

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Influence of Gender on Outcome of Severe Sepsis

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Introduction

Whether gender influences the outcome of severe sepsis remains a matter of debate. Because many confounding variables may affect observed associations between gender and mortality, high-quality statistical analyses are essential to carefully adjust the two groups of patients. About 55% to 65% of patients with sepsis have chronic co-morbidities associated with immune dysfunction (e.g., chronic renal failure, diabetes mellitus, human immunodeficiency virus [HIV] infection, and alcohol abuse), which increase the susceptibility to sepsis [1]. Genetic polymorphisms that affect the susceptibility to infection and/or the severity of the systemic response to infection [2] may lead to variability among individuals and between males and females [3]. Access to healthcare, another determinant of the incidence and outcome of sepsis, varies according to age, ethnic group, and gender, although a recent study conducted in the USA found only relatively small quality-of-care differences between males and females or across income groups compared to the gap for each subgroup between observed and desirable quality of health care [4]. Here, we review the data on the existence of, and reasons for, associations between gender and outcome of severe sepsis (Fig. 1).

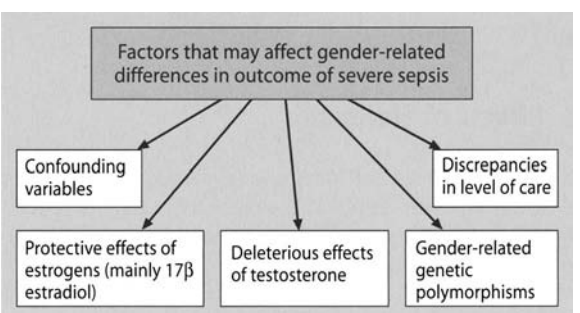

Fig. 1. Diagram showing the main factors that may lead to discrepancies in data from clinical studies into the influence of gender on survival of patients with severe sepsis. Confounders include differences in age, case-mix, nature of the injury preceding sepsis development (e.g., trauma, hemorrhage, or burns), infection source, co-morbidities, and menopausal status. In addition, level of care may differ between men and women. Finally, gender-related genetic polymorphisms that affect innate immunity have been identified. Estrogen (17β estradiol) may exert beneficial effects and testosterone deleterious effects.

Epidemiological Data

Severe sepsis remains among the leading causes of death in western Europe and North America [5, 6]. Mortality rates in patients with severe sepsis range from 20% to 50% depending on the nature and number of organ dysfunctions. The incidence of sepsis has increased over the last two decades [5], and disparities have been identified across ethnic groups and between genders. In the US, the incidence of sepsis was lower in females than in males for all sources of infection except the genitourinary tract. After adjustment for gender, men were more likely to have sepsis than women in every year of a 22-year study, the mean annual risk being 1.28 [5]. Similarly, other studies showed that men had a higher risk of infection [6] and were more likely to develop sepsis after surgery or trauma [7, 8]. Acute lung injury (ALI) was more common, and was associated with worse outcomes, in men compared to women [7, 9–11]. In a study of 545 patients older than 15 years who were admitted for trauma, male gender was associated with a dramatically increased incidence of major infection in all age groups, and the difference between genders was greatest for moderately severe trauma (Injury Severity Score 16–25) [8]. The lower incidence in women of pneumonia, sepsis and multiple organ failure after trauma has been confirmed in other clinical studies [8, 12–14]. Thus, there is general agreement that the incidence of sepsis is higher in males than in females. However, the influence of gender on the outcome of established sepsis is far more difficult to assess.

Assessment of the influence of gender on the outcome of established sepsis in clinical studies has produced conflicting results, perhaps as a result of differences in age, case-mix, nature of the injury (e.g., trauma, hemorrhage, or burns), infection sources, co-morbidities, and menopausal status [7, 8, 11, 13, 15–18]. For instance, in surgical units, survival has been reported to be better in women [11], better in men [18], and independent of gender [7]. Although differences in sample size and case-mix probably contributed to these discrepancies, the main factor may be imperfect matching of males and females. By using a propensity score computed for patients with severe sepsis included in the Outcomerea database®, we were able to adjust carefully for confounding variables. We found that hospital mortality was significantly lower in women [19]. This difference was present in older patients but not in pre-menopausal patients (<50 years of age).

Effects of Hormones

Sex hormones [20] or gender-related gene polymorphisms [3, 21] may protect women against sepsis and death from sepsis. Differences in hormone profiles have been widely suggested as the cause of gender-based differences in the incidence and outcome of sepsis.

Experimental evidence suggested a protective role for female sex hormones [20]. Female mice tolerate polymicrobial sepsis better than do male mice [22]. In "two-hit" models, animals are exposed to hemorrhagic shock or trauma, which is expected to alter or suppress the response to a second insult, such as sepsis [23–25]. In murine two-hit models with sepsis as the second insult, survival was improved in males after testosterone receptor blockade [26] or administration of the inactive testosterone metabolite, dehydroepiandrosterone [27]. Suggested explanations for these findings include better preservation of innate immunity [28], the endothelium [29] or the gut barrier via improved splanchnic perfusion [25]. Cristafaro et al. [30]

reported that oral administration of WAY-202196, a selective estrogen-receptor-β agonist, preserved gastrointestinal barrier function and improved outcomes in three different models of murine sepsis (*Listeria* infection, *Pseudomonas aeruginosa* infection in neutropenic rats, and cecal ligation and puncture in mice).

Thus, estrogens may exert beneficial effects and testosterone detrimental effects in experimental sepsis. However, clinical studies have produced conflicting results. And in a more recent study, mortality in elderly patients with severe sepsis was independent from gender but correlated with higher serum levels of 17β-estradiol and progesterone in males and of 17β-estradiol and testosterone in females [31].

The most extensively studied estrogen is 17β-estradiol, which is the most active. It suppresses major histocompatibility complex II (MHC II) proteins in a tissue-specific manner [32–34] and acts centrally on the immune system by helping to activate $5HT_{2A}$ receptors in the thymus [35]. Estrogen treatment also indirectly suppresses MHC II protein expression via serotonin production. Specifically, increased $5HT_{2A}$ activity causes decreased MHC II production and decreased selection against self-reactive helper T cells (Th1) [36, 37]. Interplay between estrogen and serotonin has also been demonstrated in the vascular system, one important result being alteration of coagulation [38], which is also closely linked to inflammation [39]. Other estrogen effects that protect against acute injury include p38 mitogen-activated protein kinase (MAPK) activation, antioxidant effects, increased nitric oxide (NO) production, modulation of calcium influx and release, activation of KATP channels, and decreased apoptosis [20].

The above-described potential mechanisms of gender-related differences in responses to sepsis would lead to expect better survival in pre-menopausal women than in men. However, this was not usually the case in clinical studies. In post-menopausal women, estrogens are produced outside the ovaries, presumably within the adrenal cortex, although other sources such as T cells, macrophages, and fat tissue may contribute to the high sex-steroid levels observed in severe sepsis. The metabolism of the adrenal hormone, dehydroepiandrosterone, is a major determinant of sex-steroid status in post-menopausal women. Dehydroepiandrosterone is a very weak androgen but can be converted either to more potent androgens or to estrogens by peripheral tissue enzymes (5-reductase for conversion to dihydrotestosterone and aromatase for conversion to 17-estradiol) [40]. The higher body mass index observed in women than men may lead to better protection as a result of greater aromatase activity in fat tissue. Both obesity and advancing age are known to increase aromatase activity [40]. In a recent study [31], survival in elderly patients with severe infection was similar in men and women but varied with the sex-steroid profile. The absence of a gender difference may be ascribable to the small sample size and to the inclusion of patients with sepsis with or without organ dysfunction [31]. Furthermore, confounding factors were not correctly taken into account [31]. Moreover, sex hormone profiles during severe sepsis may fail to reflect baseline hormone production, since severe sepsis is often preceded by several days of systemic inflammation, a process known to decrease testosterone levels [41–43] and to increase 17β-estradiol synthesis via an increase in aromatase activity [44, 45].

Testosterone may be deleterious in patients with sepsis. Testosterone exerts immunosuppressive effects [46], chiefly through activation and repression of transcription [47]. Testosterone administration causes death in female mice with *Plasmodium chaboudi* blood-stage malaria, which is normally self-limited [48]. Interestingly, the detrimental effects of testosterone occurred in the liver rather than the spleen [48]. Testosterone administration altered the activity of the reticular endothe-

lial system in the liver, dramatically suppressing both the malaria-protective gene encoding plasminogen activator inhibitor (PAI-1) and the gene encoding hydroxysteroid sulfotransferase (STA2). Moreover, testosterone increased p38 MAPK activation, upregulated adrenergic receptors and calcium-channel expression, and induced apoptosis (for a review see [20]). These effects were mainly observed in acute myocardial inflammatory response to acute injury, and were also present, at least in part, in a trauma-hemorrhage mouse model [49]. Nevertheless, whether these mechanisms are operative in severe sepsis remains to be determined.

In pre-menopausal women, 17-estradiol produced by the ovaries is the chief circulating estrogen. Serum estradiol concentrations are low in prepubertal girls and increase at menarche. In women, they range from about 100 pg/ml (367 pmol/l) in the follicular phase to about 600 pg/ml (2200 pmol/l) at ovulation. Serum estradiol may reach nearly 20,000 pg/ml (70,000 pmol/l) during pregnancy. After the menopause, serum estradiol concentrations fall to values similar to or lower than those in same-age men (5 to 20 pg/ml; 18 to 74 pmol/l). The protective effect of estrogen on the cardiovascular system has been widely studied [50], and many of the pathophysiological mechanisms underlying atherosclerosis are also involved in the inflammatory process that characterizes severe sepsis. However, in our study mortality was lower in older post-menopausal women [19]. Moreover, mortality is also higher in male children [16], who have extremely low levels of testosterone and other sex hormones. These data suggest a role for other factors, such as gender-related genetic polymorphisms.

Gender-related Genetic Polymorphisms

Gender-related genetic polymorphisms may contribute to the higher mortality from sepsis in males compared to females. Data from a case-control study by Hubacek et al. [21] suggest that common polymorphisms in the gene for lipopolysaccharide binding protein (LBP) may increase both the risk of sepsis and the risk of death from sepsis in men, but not in women. TNFβ NcoI restriction fragment length polymorphism affected the amino acid at position 26 of the TNFβ sequence, which was aspariginase with one allele (TNFB1) and threonine with the other (TNFB2) [51]. The genotype distribution of patients homozygous for TNFB1 and heterozygous or homozygous for TNFB2 was comparable between men and women with severe sepsis in a surgical ICU [3]. In women, no difference in survival rate was found between the different genotypes, whereas mortality was significantly higher in men homozygous for TNFB2 than in men with the other genotypes. The survival rate was higher in women overall but was not significantly different between men and women with the TNB2/B2 genotype [3]. The IL-1 receptor-associated kinase (IRAK-1) variant haplotype is functionally significant in patients with sepsis, being associated with increased nuclear translocation of nuclear factor-κB (NF-κB), greater severity of organ dysfunction, and higher mortality [52]. Because the IRAK-1 haplotypes are located on the X chromosome (at position q28), a larger percentage of men than women are functionally homozygous. Therefore, men are more likely than women to exhibit functional effects of the variant IRAK-1 haplotype, which may lead to an increased risk of sepsis-associated multiple organ dysfunction and death. There was no significant increase in adverse outcomes in heterozygous females [52], indicating that the effects of the IRAK-1 haplotype are not dominant.

Levels of Care

Studies in the USA showed that patients received about half the recommended level of healthcare, with remarkably little variation across geographic regions [53, 54]. However, differences in quality of care have been found among population subgroups. Differences in the level of care may lead to differences in survival between men and women. In one study, for instance, women received better care than men overall; however, higher quality scores for preventive and chronic care masked lower scores for acute care [4]. Similarly, poorer acute care in women has been reported for cardiovascular disease [55]. In several studies, women were less likely than men to undergo intensive evaluation and invasive treatment for cardiovascular disease [56–58]. A large cohort study conducted in Austria in critically ill patients [59] showed greater use of invasive procedures in men compared to women, in all age groups. After adjustment for age, men were more likely than women to receive multiple invasive procedures, even in the youngest age groups. Although disease severity was greater in women, survival was not significantly different, suggesting either an inappropriately high level of care in men or a better potential for survival in women masked by an inappropriately low level of care. However, resource use according to gender may vary across healthcare systems, indicating a need for studies in countries that use different systems. In addition, this study [59] enrolled all ICU patients rather than only those with severe sepsis. In our study, the Nine Equivalents of Nursing Manpower Score (NEMS-9) was similar in men and women, suggesting level of care was identical in this particular subgroup of patients with severe sepsis in a French ICU database (Outcomerea Database) [19].

Conclusion

Men are at greater risk for sepsis than are women. Numerous experimental data suggest better outcomes in females with severe sepsis, compared to males. Nevertheless, clinical data in humans are conflicting. Further work is needed to determine the influence of gender on outcomes of severe sepsis. Studies must pay careful attention to matching the males and females for the many potential confounding variables.

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Prognosis and Long-term Outcomes

The Changing Prognostic Determinants in the Critically Ill Patient

R. Moreno, B. Jordan, and P. Metnitz

"It's more important to know what sort of person this disease has, than what sort of disease this person has." William Osler 1849–1919

Introduction

The science and art of risk stratification appeared in early 1953, when Virginia Apgar [1] published a simple physiological scoring tool to evaluate the newborn child. This system, still commonly used worldwide, evaluates only two physiologic systems: Cardiopulmonary and central nervous system (CNS) function. Several years later, in the early 1980s, several researchers applied the same concept to critically ill patients, through the introduction of the acute physiology and chronic health evaluation (APACHE) and the simplified acute physiological score (SAPS), both physiologically based classification systems [2, 3]. These instruments, named general severity scores, are tools that aim at stratifying patients based on their severity, assigning to each patient an increasing score as their severity of illness increases. Initially designed to be applicable to individual patients, it became apparent very early after their introduction that both systems could in fact be used only in large heterogeneous groups of critically ill patients.

Later on, between 1985 and 1993, several groups added to this function of risk stratification the possibility of predicting a given outcome probability [4–8]. These improved models, now called general outcome prediction models or prognostic models, apart from their ability to stratify patients according to their severity, aim at predicting a certain outcome (usually the vital status at hospital discharge) based on a given set of prognostic variables and a certain modeling equation. Although the methods for selecting the predictive variables varied, all of them used standard logistic regression to develop the equation relating the predictive variables to the probability of the outcome of interest. These models allow the clinician, and particularly the researcher, dealing with severe patients the possibility to adjust for the underline characteristics of the admitted population (case-mix) and the indirect standardization of the outcome of different groups of patients, irrespective of the treatment received in the intensive care unit (ICU). Designed to be applied only in heterogeneous groups of patients, these models predict what would be the aggregated mortality at hospital discharge of a group of patients, with a certain degree of physiologic dysfunction, if they were treated in a *virtual* ICU used to develop the model; their use in individual patients is not recommended [9].

Interestingly, during this process of evolution of the models, the main prognostic determinants of outcome changed, with variables reflecting prior health status and

the degree of physiological reserve slowly but consistently having more importance than variables reflecting the presence and degree of acute physiological derangement.

The Lack of Calibration of General Outcome Prediction Models

Since the early 1990s, when these instruments were subjected to their last revisions [6–8], the performance of these instruments began to slowly deteriorate with time. Differences in the baseline characteristics of the admitted patients, in the circumstances of the ICU admission, and in the availability of general and specific therapeutic measures have all been recognized as introducing an increasing gap between actual mortality and predicted mortality [10]. Overall, during this period, we have witnessed an increase in the mean age of admitted patients, an increase in the number of chronically sick patients and immunosuppressed patients and an increase in the number of admissions due to certain diseases, such as sepsis [11, 12]. Although most of the models kept an acceptable discrimination, their calibration (or prognostic accuracy) deteriorated to a point where major changes where needed. An inappropriate use of these instruments outside their sampling space was responsible also for some misapplication of the instruments, especially for risk-adjustment in clinical trials [13, 14].

A further question is whether the relative impact of changes in the baseline population characteristics, in different ways of delivering care to patients, and in the acute physiological derangement has remained constant since the 1980s.

Changing the Old Models

Since most systems loose calibration with time while maintaining discrimination, several attempts have been made over the years to improve calibration of old models. The first approach to improve the calibration of a model when the original model is not able to adequately describe the population is to customize the model [15]. Several methods and suggestions have been proposed for this exercise [16], based on one of two strategies:

- the customization of the logit (first level customization), modifying logistic equation coefficients (without changing the weights of the constituent variables), such as proposed by Le Gall or Apolone [17–19];
- the customization of the coefficients of all the variables in the model (second level customization) as we described for the mortality prediction model (MPM) II_0 model [15]. This method seems also to have been performed for the APACHE III system in the late 1990s, between the original publication of the system [8] and their so-called independent validation by the same group [20], which resulted in a series of different models, all with the name of APACHE III, but know by different versions (H, J). The details of this process have only recently started to be known and were never fully published [21], but this can explain why this model seems to have kept a reasonable calibration over time (while in fact it was replaced regularly by an updated version with the same designation).

Both of these methods have been used in the past with partial success in increasing the prognostic ability of the models [15, 22]. However, both fail when the problem

of the score is the discrimination of poor performance in sub-groups of patients (poor uniformity-of-fit) [23]. This fact can be justified by the lack of new variables more predictive in this specific context and by a changing importance of the type of prognostic variables in the prediction of outcome.

A third level of customization can be imagined, through the introduction in the model of new prognostic variables and the re-computation of all the weights and coefficients for all the variables, but this technique is closer to building a new predictive model than to customizing an existing model.

In recent years, these approaches have been applied, in particular by Jean-Roger le Gall et al. in France, by Philippe Aegerter et al. also in France, and by David A. Harrison et al. in the United Kingdom. All illustrate quite well the benefits and limitations of these techniques in improving the ability of a general outcome prediction model.

In France, Jean-Roger le Gall et al. tried to improve the SAPS II model, using a retrospective database containing data from 77,490 patients hospitalized in 106 French ICUs between 1 January 1998 and 31 December 1999 [24]. Based on these data, the authors evaluated the goodness-of-fit (calibration and discrimination) of the original SAPS II model, of a customized SAPS II, and of an expanded SAPS II developed in the training set by adding six admission variables: age, sex, length of pre-ICU hospital stay, patient location before ICU, clinical category, and whether drug overdose was present. Interestingly, the introduction of these new variables – all of them reflecting prior patient characteristics and circumstances of ICU admission – significantly improved the calibration of the original SAPS II (that was very poor in this database, with a marked underestimation of observed mortality).

Also in France, in the Paris area, Philippe Aegerter et al., did a retrospective analysis of a prospectively collected, multicenter database with data from 33,471 patients from 32 ICUs belonging to the Cub-Rea database [25]. Based on this dataset, the authors estimated two logistic regression models based on SAPS II. A first model, using first-level customization (having only the SAPS II score as an independent variable and consequently keeping all the original variables and their weights), and a second model, which comprised reevaluation of original items of SAPS II and integration of the preadmission location and more chronic comorbidities, demonstrated a better calibration than the original SAPS II for in-hospital mortality.

Finally, in the United Kingdom (UK), David A. Harrison et al., from the Intensive Care National Audit and Research Centre (ICNARC), used a database with data from 141,106 patients from a total of 163 adult general critical care units in England, Wales, and Northern Ireland, during the period of December 1995 to August 2003 [26]. The authors compared the published versions of the APACHE II [4], APACHE II UK [27], APACHE III [8], SAPS II [6], and MPM II [7], demonstrating that all models showed good discrimination but imperfect calibration. Recalibration of the models was performed by the Cox method and re-estimating coefficients, and led to improved discrimination and calibration, although all models still showed significant departures from perfect calibration.

Designing New Models

During the last few years, two new general outcome prediction models have been developed and published: The SAPS 3 admission model in late 2005 and the APACHE IV in early 2006.

The SAPS 3 Admission Model

At almost the same time as attempts were being made to customize the existing models, other groups tried to update their outcome prediction models by starting from scratch. The first of these efforts to be published was the SAPS 3 admission model, developed by Rui Moreno, Philipp Metnitz, Eduardo Almeida, and Jean-Roger Le Gall on behalf of the SAPS 3 Outcomes Research Group [28, 29]. The project was endorsed by the European Society of Intensive Care Medicine (ESICM), and supported by the Austrian Center for Documentation and Quality Assurance in Intensive Care Medicine (ASDI), the Portuguese Society of Intensive Care (SPCI), and the Medical Economics and Research Centre (MERCS) in Sheffield, UK. An unrestricted educational grant from Merck Sharp & Dohme, Portugal, to the SPCI made it possible to install the Coordinating Centre in Lisbon. iMDsoft (Tel Aviv, Israel) developed and provided the Internet-based data collection software used in the project free of charge.

The study database comprised 19,577 patients consecutively admitted to 307 ICUs all over the world from 14 October to 15 December 2002. This high-quality, multinational database seems to reflect the heterogeneity of current ICU case-mix, typology, and differences in baseline health care systems' in different regions of the world. These can include, but are not limited to, different genotypes, different styles of life, or a heterogeneous distribution of major diseases within different regions, as well as issues, such as access to the health care system in general and to intensive care in particular, or differences in availability and use of major diagnostic and therapeutic measures within the ICUs. Although the integration of ICUs outside Europe and the US surely increased its representativeness, it must be acknowledged that the extent to which the SAPS 3 database reflects case-mix in ICUs worldwide cannot yet be determined precisely.

Based on data collected at ICU admission (± 1 hour), the authors developed regression coefficients by using multilevel logistic regression to estimate the probability of hospital death. The final model, which comprises twenty variables, exhibited good discrimination, without major differences across patient typologies; calibration was also satisfactory. Customized equations for major areas of the world were computed and demonstrate a good overall goodness-of-fit. Interestingly, determinants of hospital mortality have changed remarkably compared to the early 1990s [8], with chronic health status and circumstances of ICU admission now being responsible for almost three-fourths of the prognostic power of the model, an issue that we will discuss later.

The SAPS 3 model is completely free of charge for use in the scientific community and all the data and software needed for its computation are available at the project website (www.saps3.org). A further refinement of the model, now calibrated to the vital status 28 days after ICU admission and complemented by a standardized method for evaluating the use of resources, has been recently presented in abstract form [30, 31].

The APACHE IV Model

Six months after the publication of the SAPS 3 model, Jack E. Zimmerman, one of the authors of the original APACHE model, published, in collaboration with some employees from Cerner Corporation (Vienna, VA), the APACHE IV model [21], recently complemented by a method for standardizing the length of stay in the ICU [32].

The study was based on a database of 110,558 consecutive admissions during 2002 and 2003 to 104 ICUs in 45 hospitals in the USA participating in the APACHE III database. APACHE IV uses the worst values during the first 24 hours in the ICU and a multivariate logistic regression procedure to estimate the probability of hospital death.

Predictor variables were similar to those in APACHE III, but new variables were added and different statistical modeling has been used. The accuracy of APACHE IV predictions was analyzed in the overall database and in major patient subgroups. APACHE IV had good discrimination and calibration. For 90% of 116 ICU admission diagnoses, the ratio of observed to predicted mortality was not significantly different from 1.0. Predictions where compared with the APACHE III versions developed 7 and 14 yrs previously; there was little change in discrimination, but aggregate mortality was systematically overestimated as model age increased. When examined across diseases, predictive accuracy was maintained for some diagnoses but for others seemed to reflect changes in practice or therapy.

More information about the model and the possibility of computing the probability of death for individual patients is available at the web site of Cerner Corporation (www.cerner.com)

The MPM III Model

The MPM III was described last year by Tom Higgins et al. but has been published just as an abstract [33]. It was developed using data from ICUs in the USA participating in the project IMPACT but it is too soon for it to have been adequately evaluated. However, it should be noted that the model incorporates several non-physiological factors.

The ICNARC Model

The failure of the attempt to recalibrate the APACHE system for use in the UK, led the Harrison and the ICNARC group to develop a completely new prognostic model, the ICNARC model, presented as an abstract in the 2006 Meeting of the ESICM in Barcelona [34]. Interestingly again, age, diagnostic category, source of admission, and cardiopulmonary resuscitation before ICU admission have now been combined with physiological information (and some interactions) to better predict the outcome of critically ill patients in the UK.

The Changing Prognostic Determinants in the Critically Ill Patient

The data presented and discussed above seem to indicate that in recent years the prognostic impact of acute derangements in physiology is decreasing; at the same time, chronic health status (from which chronological age is just a small component) and circumstances of ICU admission (patient typology, planned or unplanned admission, reasons for ICU admission, etc.) are becoming more and more important. This fact can be seen very clearly in Figures 1 and 2, when comparing the prognostic impact in the SAPS II versus SAPS 3 models and in the APACHE III and APACHE IV models.

Several hypotheses can explain these facts. One hypothesis to explain this fact, yet to be proved, is that intensivists have learned to deal better with deranged physiol-

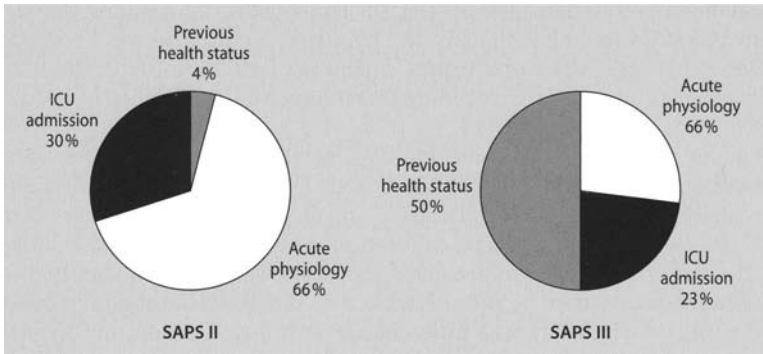

Fig. 1. Explanatory power of the chronic health status, circumstances of ICU admission, and degree and severity of physiologic derangement at ICU admission in the SAPS II model (top) and SAPS 3 admission model (bottom). Adapted from data published in [29]. The impact of chronological age is collapsed on the chronic health status.

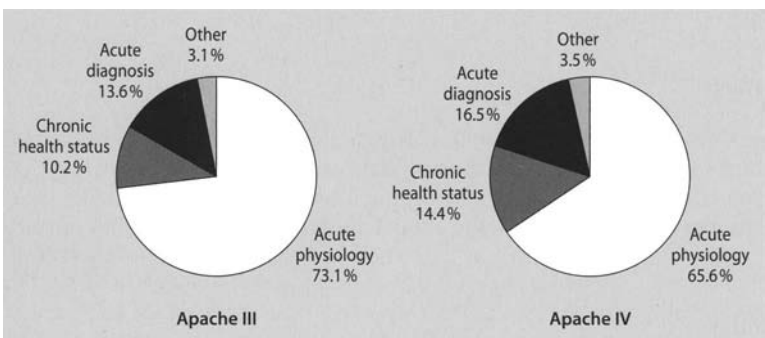

Fig. 2. Explanatory power of the chronic health status, circumstances of ICU admission, and degree and severity of physiologic derangement at ICU admission in the APACHE III model (top) and APACHE IV model (bottom). Adapted from [8, 21]. The impact of chronological age is collapsed on the chronic health status.

ogy over the last decades. In other words, the impact of hypoxemia, bradycardia or tachycardia, low diuresis, etc., is lower because intensive care professionals know better how to stabilize acute physiology and/or to minimize the impact of these deranged values on patient outcome. Consequently, we would expect that in the near future the impact of chronic health and health care system organization will have a higher impact on ICU outcome, with the episode of critical illness becoming increasingly a short period in the evolution of a long term disease, in which prognosis will depend to a larger extent on the underlying diagnosis [35] and the degree of cardiovascular reserve, and the interaction between therapy and the immunological status of the patient will become more important [36].

In addition, the different sampling times of the different models (admission plus/minus one hour for the SAPS 3 admission model versus worst values during the first 24 hours in the ICU for the APACHE IV model could have an influence, by providing different time windows for the collection of data about acute physiology. This seems, however, not to be the case, since the same pattern can be seen in the evolution of the APACHE III model to the APACHE IV model (both using the same time window for data collection).

Table 1. The effect of changing the order of inclusion of the prognostic variables in the SAPS 3 model (Rui Moreno et al., unpublished data).

Order of entrance in the model of the variables	of the explanatory power of the model
1	50.0%
2	22.5%
3	27.5%
2	47.5%
1	25.0%
3	27.5%
3	65.0%
1	22.5%
2	12.5%
1	50.0%
3	37.5%
2	12.5%
2	47.5%
3	37.5%
1	25.0%
3	65%
2	20%
1	25%

1: Chronic health status; 2: Circumstances of ICU admission; 3: Acute physiology

Finally, the way researchers have developed the models can have an impact on the relative importance of the different variables. When developing the SAPS 3 admission model, we tried to follow the path of clinical reasoning in medicine, starting with information on previous health status, then adding information on the circumstances of ICU admission, and finally the information on acute physiology. This strategy resulted in the data presented in Figure 1 (right).

Alternative strategies, changing the order of inclusion of the prognostic variables, are presented in detail in Table 1, but make less sense from a clinical point of view. However, although the importance of chronic health status changes according to the order of entrance of the variables in the model and of the different combinations, it nevertheless always remains very important.

Conclusion: The Future of Outcome Prediction

As science evolves, we should expect that further information about our genotype and phenotype will be incorporated into the process of clinical decision making. This information will certainly be used to stratify patients for the risk of certain diseases such as acute lung injury (ALI) or sepsis [37, 38] and used to choose the best therapy for an individual patient, as already utilized in oncology. Consequently, we will be challenged in the future to incorporate this information in our models, evolving from group predictions to individual predictions, in which our body constitution and chronic disease burden will be more and more important. At the same time, mortality from acute disease (or from acute phases of chronic diseases) will diminish as the science and art of intensive care evolves.

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Chronic Critical Illness

S.L. Camhi and J.E. Nelson

Introduction

Increasing use of intensive care unit (ICU) resources by an aging population has resulted in a large and growing group of adults who are 'chronically critically ill' [1]. Although these patients have survived acute illness, they are profoundly debilitated and have ongoing serious complications with continued dependence on life-sustaining therapies. Chronic critical illness is not simply a prolongation of acute critical illness, but a distinct syndrome consisting of persistent respiratory failure and significant derangements of metabolic, neuroendocrine, neuropsychiatric and immunologic function [1]. The number of patients in the USA with chronic critical illness is estimated to approach 100,000 [2]. As the population ages and ICU treatments are increasingly offered to older, sicker patients, these numbers will increase. In this chapter, we will discuss the definition of chronic critical illness, the scope of this serious health problem, venues of care, outcomes and symptoms, and issues with communication between the health care team and patients and their families. We will end by reviewing an interdisciplinary approach to managing this challenging patient population.

Definition of Chronic Critical Illness

Although prolonged mechanical ventilation is its hallmark, chronic critical illness is a complex systemic illness. Patients survive acute critical illness only to suffer from persistent dysfunction of multiple organs, 'immune exhaustion', and nutritional deficits [1, 3]. They require weeks to months of aggressive life-sustaining therapies, including multiple courses of antibiotics, blood transfusions, hemodialysis, and mechanical ventilation. Common problems include recurrent infections, bone hyperresorption, male hypogonadism, malnutrition, neuropathy/myopathy, brain dysfunction, anemia, pressure ulcers, and distressing symptoms [3–6]. Patients with chronic critical illness typically undergo tracheotomy to facilitate airway management and weaning from mechanical ventilation. Mean length of hospitalization is measured in months [7–10].

No single definition of chronic critical illness has been universally accepted. Some investigators have defined it by a lengthy ICU stay [11] or by the number of days that patients are dependent on mechanical ventilation. However, duration of ventilator dependence has varied widely in these definitions, ranging from as little as >24 hours to as much as >29 days [7, 12–14]. The National Association for Medical Direction of Respiratory Care (NAMDRC) recently offered a consensus defini-

tion of 'prolonged mechanical ventilation' as the need for ≥21 consecutive days of mechanical ventilation for ≥6 hours per day [15]; this definition is consistent with the criteria used by the Centers for Medicare and Medicaid Services to identify patients requiring 'prolonged mechanical ventilation'. We and others have used tracheotomy for failure to wean from mechanical ventilation as a definition of chronic critical illness for clinical, administrative, and research purposes [2, 9, 16]. This approach incorporates clinicians' judgment that the patient is not expected to wean or to die in the near future. It also allows for comparisons within large USA databases under diagnostic-related groups (DRGs) 541 and 542 (formerly 483).

Scope of the Problem

Costs of caring for the chronically critically ill are enormous. In the USA, these costs are estimated to be in the range of 24 billion dollars annually, more than one-third of the total amount spent on all ICU patients [2]. DRGs 541 and 542 (formerly 483) were created to reimburse hospitals for the high costs of treating patients with prolonged mechanical ventilation. Patients are classified in these DRGs if they undergo tracheotomy for reasons other than face, mouth, and neck pathology; DRG 541 (with higher reimbursement) is for surgical patients, whereas 542 is for patients who have not undergone a major operating room procedure (other than tracheotomy). In New York State, where discharges in DRG 483 increased by 60% from 1992–1996, average hospital reimbursement per DRG 483 patient was $111,777. Survivors incurred additional health care expenses for residential nursing facilities and home health services resulting in total costs to the healthcare system of more than 3.5 billion dollars [17]. In North Carolina, the incidence of tracheotomy for prolonged mechanical ventilation increased by 200% in the decade from 1993 to 2002. Although only 7% of ICU patients required tracheotomy for prolonged mechanical ventilation, these patients accounted for 22% of all mechanical ventilation patient charges (1.74 billion dollars) [18].

Venues of Care

Limited ICU resources and the high cost of prolonged ICU stays have led to the development of alternative venues of care for chronically critically ill patients [15]. After tracheotomy, patients may be transferred from the ICU to in-hospital intermediate care or respiratory care units, the latter specifically designed to address the needs of ventilator-dependent patients [19]. In some hospitals the chronically critically ill are managed on general nursing wards. Long-term acute care hospitals receive an increasingly large number of these transfers from ICUs in short-term acute care hospitals. Long-term acute care hospitals are for-profit, stand-alone centers or independent 'hospitals-within-hospitals' designed for long stays and slow weans. Some patients who are stable but cannot be liberated from mechanical ventilation may be discharged to skilled nursing facilities that have ventilator capabilities. Rarely, families have adequate resources to carry the burden of caring for ventilator-dependent, chronically critically ill patients at home. While long-term acute care hospitals do reduce costs of care, there is not yet evidence that these specialized facilities improve long-term outcomes for chronically critically ill patients.[10]

Poor Outcomes for the Chronically Critically Ill

Although expenditures are huge, outcomes for chronically critically ill patients are poor. Hospital and early post-discharge mortality rates are high and return to prior functional status is rare [14, 16, 20–22]. In New York State, from 1992–1996, nearly one half of patients in DRG 483 died in hospital with significantly higher mortality seen in patients >70 years of age. Most older survivors were discharged to residential healthcare facilities rather than to home [17].

Among a recent cohort of 203 chronically critically ill patients (median age of 72 years) cared for in our specialized respiratory care unit, 48% were liberated from mechanical ventilation and less than 10% were discharged to home. Cumulative mortality within 6 months following discharge was 56%, with a median post-discharge survival of 55 days [6]. Overall one-year survival for 1,123 patients with chronic critical illness who were treated at a regional weaning center was 37.9% [20]. The experience of almost all centers across a range of care venues is similar (except outcomes are somewhat better when younger patients with trauma as their initial catastrophic illness are included). In a study of 133 mechanically-ventilated patients admitted to a long term acute care facility, survival at one year was 23% and only 8% of the original cohort had good functionality. Patients 75 years and older and those 65–75 years of age who were not previously independent had a 95% mortality at 1 year [21]. Older age and poor pre-hospitalization status were also associated with high mortality rates in 817 patients receiving prolonged mechanical ventilation at a tertiary care university hospital [14]. Population-based outcomes of patients receiving prolonged mechanical ventilation were recently reviewed by Carson, confirming the scope of the problem and the increasing strain on the US health care system [10].

Burdensome Symptoms and Brain Dysfunction in Chronic Critical Illness

Patients undergoing treatment for chronic critical illness suffer distressing physical and psychological symptoms [16]. In a study of 50 patients at our institution, 36 (72%) were able to self-report symptoms. The overwhelming majority (90%) of patients responding to assessments were symptomatic. Forty-four percent reported pain at the highest levels ("quite a bit" and "very much"). More than 60% reported psychological symptoms (sadness, worry, nervousness) at the highest levels of frequency ("frequently" and "almost constantly"). Ninety percent of patients reported the highest levels of distress due to difficulty communicating. Other common distressing symptoms included dyspnea (on full ventilator support as well as during weaning) and unsatisfied hunger and thirst (Fig. 1).

Another, newly-described burden for the chronically critically ill is severe, prolonged, and often permanent brain dysfunction, including coma and delirium [6]. In our cohort of 203 chronic critically ill patients, of whom less than 15% were admitted to the ICU for a central nervous system (CNS) event, 61 (30%) were comatose throughout the respiratory care unit stay. Of patients who were not comatose, almost half (66/142) were delirious. Patients spent an average of almost 18 days (range 1–153 days), in coma or delirium while in the respiratory care unit (average respiratory care unit length of stay 26 days) and half of survivors were comatose or delirious at discharge. At 6 months, 151 of the 203 original study patients (75%) were dead or institutionalized and of 85 survivors, 58 (68.2%) were too profoundly impaired to

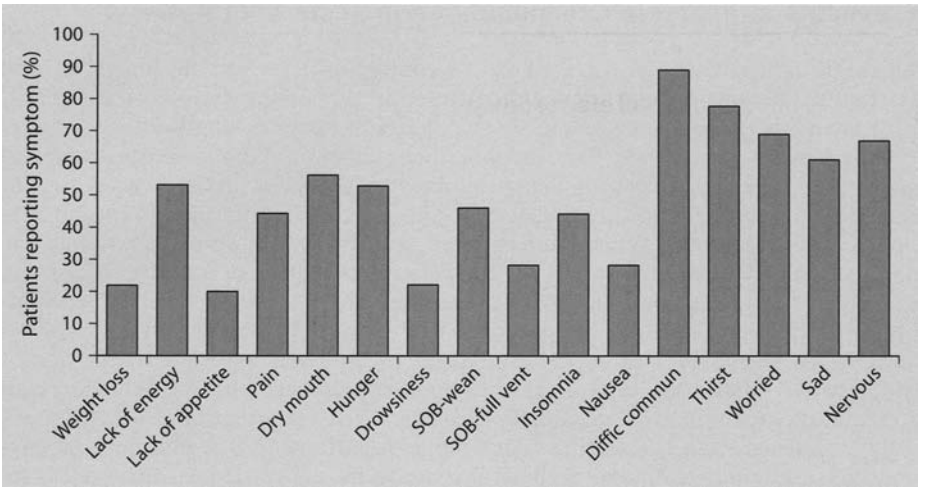

Fig. 1. The figure shows the percent of patients providing self-reports who experienced symptoms at the two highest levels of the physical symptom scale ("quite a bit" or "very much" distressed) and of the psychological symptom scale ("frequently" or "almost constantly" distressed). SOB-wean = shortness of breath during weaning; SOB-full vent = shortness of breath during full ventilator support; Diffic commun = difficulty communicating. Adapted from [16] with permission

respond to telephone cognitive assessment and 53 (62.4%) were dependent in all activities of daily living. Prior to the acute critical illness, most of these patients had lived independently in the community and had no history of cognitive impairment.

Lack of Effective Communication with Patients/Families

Despite poor outcomes and high symptom burdens, many patients and their families choose to continue life-prolonging therapies when critical illness enters a chronic phase. Evidence suggests that this may be due in part to a lack of understanding of critical illness resulting from inadequate communication between health care providers and patients [23]. Among almost 1500 patients in the Study to Understand Prognoses and Preferences for Outcomes and Risks of Treatments (SUPPORT) who were treated in ICUs for more than 2 weeks, less than 40% reported discussion with their doctor about prognosis or preferences for life-sustaining treatment. Almost 50% preferring palliative care thought that the treatment they received was contrary to their preference, and approximately 25% did not know the team's approach to their care [24]. Other evidence shows inadequacies in communication between clinicians and family members of ICU patients [25–27]. In a prospective study at a university-affiliated ICU, 54% of families failed to comprehend the diagnosis, prognosis, or treatment after meeting with a physician [28]. Another study reported that more than two-thirds of family members visiting patients in the ICU demonstrated symptoms of anxiety and depression that could adversely affect their ability to make decisions and that were associated with variables reflecting inadequate communication from clinicians [29]. 'Usual care' in a major academic medical center consisted typically of communication in the absence of senior clinicians and without coordination among multiple members of the critical care team [30].

Benefits of Improved Communication in Acute Critical Illness

Although data remain limited even in the context of acute critical illness, several systematic attempts to improve communication in that setting have been associated with favorable outcomes including shorter ICU and hospital length of stay, greater comprehension of relevant information, high levels of family satisfaction, and appropriate discontinuation of life-sustaining therapies for patients who are not expected to benefit [31–33]. Azoulay et al. showed that distribution of an information leaflet (containing general information about the ICU and the hospital, the name of the ICU physician caring for the patient, a diagram of a typical ICU room naming of the devices, and a glossary of terms commonly used in the ICU) to families of ICU patients reduced the proportion of family members with poor comprehension of the patient's condition and treatment and was associated with significantly better satisfaction in families with good comprehension [34]. Schneiderman and colleagues studied ICU consultations by an ethics team that facilitated communication during acute critical illness and led to reductions in ICU and hospital days and life-sustaining treatments without increasing mortality [35]. A nurse-led communication intervention was reported by Medland and Ferrans, who found that discussion with family members within 24 hours of ICU admission, together with distribution of an informational pamphlet and daily telephone calls from the patient's primary nurse increased satisfaction with care and reduced the number of telephone calls from family members to ICU staff during the acute phase of illness [36]. Using a before-and-after study design, Lilly et al. observed the effects of an intensive communication strategy including meetings between ICU staff and families of acutely critically ill patients; this intervention was associated with a one-day reduction in mean length of ICU stay, without an increase in mortality [30].

Unique and Challenging Communication Issues in Chronic Critical Illness

In the specific context of chronic critical illness, both care and clinician-patient/family communication are particularly challenging [23, 37]. Because patients have survived the acute phase of critical illness, they and their families, and even many clinicians, often have misplaced optimism about the outcome of ongoing treatment. Hemodynamic stability and lower levels of sedation after tracheotomy raise false hopes of meaningful recovery, masking the persistence of life-threatening illness and the overwhelming likelihood of early death or extreme functional dependence. We have found in our qualitative research that many families misunderstand the placement of tracheotomy as a hopeful sign rather than an ominous predictor, and go on to experience disappointment, frustration, and anger as patients remain dependent on mechanical ventilation and other life-sustaining therapies [37]. Families may have moral or religious concerns that lead them to choose aggressive therapies despite the patient's progressive deterioration. In many hospitals, there is no specialized unit to care for the chronically critically ill; these patients are scattered across ICUs and regular wards, without comprehensive or coordinated care by clinicians with experience addressing the unique problems of this group of patients and families. Communication is further complicated by the typical involvement of multiple sub-specialists addressing dysfunction of different organs. Discussion among members of the clinical team is often lacking, as is effective coordination by a primary

care physician. As a result, communication with patients and their families is fragmented and inconsistent. Many clinicians lack training in communication skills and find it especially difficult to approach families when, as in chronic critical illness, uncertainty is unavoidable but prognosis is generally poor.

Information Needs of Chronically Critically Ill Patients and Their Families

Recent research has illuminated the information that is considered relevant and important for medical decision-making by chronically critically ill patients, their families, and professional caregivers. Analysis of focus group discussions and structured interviews of survivors of chronic critical illness, surrogates of survivors and of non-survivors, and of clinicians with experience in care of these patients, showed agreement about major domains of decision-related information: a) nature of illness/treatments, b) prognosis, c) impact of treatment, d) potential complications, e) expected care needs after hospitalization, and f) alternatives to continuation of treatment [37]. Participants in these discussions agreed about the value of consistent, cohesive communication from multiple clinicians with a single clinical 'point person', and endorsed the interdisciplinary family meeting as an effective communication strategy. As reported by these families, however, communication was typically scant, fragmented, and conflicting, without coordination by a primary clinician or team.

Inadequate Communication of Relevant and Important Information

These qualitative data formed the basis for development of a questionnaire, which we administered to 100 respondents – patients with chronic critical illness or, more often, their surrogate decision-makers (75% response rate). Preliminary data from this research confirm the importance of the domains of information identified in the previous qualitative study. These data also reveal serious deficiencies in communication and comprehension of such information. Table 1 shows that for most of the

Table 1. Surrogates' responses to questionnaire addressing communication about chronic critical illness [38]. With respect to the specific topics listed, the questionnaire asked respondents whether they considered the information "important to know" and whether they had received information about it. N = no. of respondents for each question; not all respondents answered each question. MV = mechanical ventilation

Type of information	Subjects rating important		Subjects receiving no information	
	N	%	N	%
Reason for mechanical ventilation	65	100	1	1.5
Reason for tracheotomy	65	100	0	0
Symptom burden of treatment	65	100	28	43.1
Likelihood of ventilator independence	65	100	28	41.3
Hospital survival	58	90.6	29	50.0
One-year survival	49	79.0	46	93.9
Cognitive status after discharge	62	98.4	36	58.1
Functional status after discharge	62	98.4	47	75.8
Quality of life after discharge	62	98.4	41	66.1
Care needs after discharge	62	100	51	82.3
Financial burden on family	58	96.7	43	74.1
Alternatives to continuing MV	63	96.9	51	81.0

informational domains under study, respondents reported that they had not received any information from the clinical team [38]. For example, while respondents almost universally agreed that it is important to know about choices other than continuing mechanical ventilation, more than 80% of respondents reported that they were not given this information. With respect to prognosis, the majority of respondents agreed that it is important to know about the chances of survival; however, half had not received information about expected hospital survival and almost none received information about expected survival after hospital discharge, even though these outcomes have been well documented for this patient group. Similarly, nearly all the respondents agreed the patient's expected quality of life after discharge is important information, yet two-thirds stated that they had not been given this information.

Planning an Approach

The growing literature about chronic critical illness reveals the complexity of this syndrome and of the needs of patients and their families. Appropriate care requires understanding of these issues as well as of outcomes, burdens, and benefits of treatment. In addition, optimal delivery of care requires that the issues are understood in the local context, including census, utilization, reimbursement, and available venues for this care. Although no randomized, prospective trial has yet been performed, evidence supports use of a systematized, care-mapped strategy for care of the chronically critically ill. One component is the use of weaning protocols to maximize ventilator liberation and minimize common complications while delivering uniform care. In the respiratory care unit at our hospital, institution of patient-centered management strategies, including an interdisciplinary care plan, weaning algorithm, planned discharge meetings, and acuity-based staffing, resulted in significant improvements in utilization outcomes of chronically critically ill patients, with a current weaning success rate of almost 50% [6, 19].

The recent NAMDRC Consensus Conference report describes the components of a rehabilitative model of post-ICU weaning [15]. Physicians with experience in ventilator care and weaning assess patients daily and coordinate the interdisciplinary team. Clinical case managers facilitate communication between team members and ensure that the care plan is followed. Bedside nurses provide nursing care with focus on protocols and are trained in patient and family education. Respiratory therapists monitor ventilator and related equipment and work with the bedside nurses on patient assessments and management of weaning per protocol. Other important ancillary services in this rehabilitative model include pharmacy support, physical, occupational, and speech therapy, and psychological and social services.

As confirmed by recent literature about high rates of mortality, physical and psychological suffering, and functional dependence with chronic critical illness, the need for integration of palliative care in the treatment of the chronically critically ill is also essential. Such care could include input from specialists in palliative medicine, where these resources are available. With or without specialty consultation, palliative care in chronic critical illness should include a systematic and aggressive approach to assessment and management of common symptoms, including pain, dyspnea, and depression. An important area for future research as well as clinical care is the use of appropriate techniques to facilitate communication by these tracheotomized patients. We have recently begun a program of investigation in this area.

Another central component of palliative care in this context is proactive communication with patients and families. One strategy for communication involves structured conferences attended by patients and/or families and clinicians contributing significantly to the patient's care. Discussion is coordinated by a single clinician and characterized by open, unhurried, sensitive communication about domains of interest, in terms understandable to a layperson. Content can be guided by recent research on informational needs of patients and families, which includes discussion of expected functional status and quality of life, and alternatives to continuation of critical care treatment. Empirical data about family meetings in the context of acute critical illness and protocols developed for ICU communication may also be helpful if appropriately adapted for the specific needs of chronically critically ill patients, their families, and clinicians.

A recent study by Daly et al. reported the effects of a disease management program on hospital readmission patterns of chronically critically ill patients during the first 2 months after acute hospital discharge [39]. Patients in the intervention group received care coordination, family support, teaching, and monitoring of therapies from a team of advanced practice nurses, a geriatrician, and a pulmonologist. Compared to controls, the patients in the disease management program group had fewer mean days of re-hospitalizaton, although there were no differences in other hospital readmission variables including percentage of patients readmitted, mortality during re-hospitalization, and number of days to first readmission. For those patients readmitted to the hospital total cost savings were estimated at $481,811. The disease management program intervention had no impact on caregiver depression, burden, or physical health [40].

Conclusion

In the wake of the steady stream of advances in critical care medicine is chronic critical illness, a serious and growing health problem. These patients have survived but not recovered from critical illness and their profound debilitation, continued dependence on mechanical ventilation and other life-sustaining therapies, and ongoing needs for high-level care in an institutional setting, impose heavy burdens on the patients, their families, clinicians, and health care systems. Communication between clinicians, patients, and families about realistic and appropriate care goals is lacking, despite an expanding body of evidence to inform such communication. Critical care professionals should keep abreast of the emerging scientific literature about chronic critical illness. Those caring for chronically critically ill patients and families should seek guidance in reports of systematic strategies to organize this care, including a rehabilitative model. Close coordination of care, effective communication between clinicians and patients/families, and assiduous attention to symptom management and other palliative needs appear to be essential. Ongoing and future research will help address many unanswered questions surrounding the pathophysiology and management of chronic critical illness.

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To be or not to be ... Vegetative

M. Boly, A.M. Owen, and S. Laureys

Introduction: What is the Vegetative State

The vegetative state is a clinical diagnosis first defined by Jennett and Plum in 1972 [1]. It is a diagnosis based on the absence of clinical signs of awareness of self or environment despite preserved arousal. That is, if a patient repeatedly fails to answer to commands and if all observed behavior is considered reflexive, the patient is considered to be unconscious.

Patients in a minimally conscious state [2] will show more than the mere reflex behavior observed in vegetative state survivors, but they are unable to effectively communicate. However, these distinctions are complicated by the fact that there is no universally-accepted definition of consciousness [3]. Existing definitions often invoke the importance of 'purposeful' or 'meaningful' behavior, but it is not clear

Table 1. Clinical features of the vegetative state according to the report of a working party of the Royal College of Physicians [6].

Compatible features
• Signs of a cycle of sleep and wakefulness.
• Spontaneous movements (made for no discernible reason) including :chewing, teeth grinding, swallowing, roving eye movements, purposeless limb movements; smiling grimacing, shedding tears, grunting, groaning (it would be unusual for a patient to display the entire range of movements).
• Variously preserved brainstem reflexes including: pupillary, oculocephalic (doll's eye), corneal, oculovestibular (caloric), and gag reflex
• Noxious or noisy stimuli may excite a generalized arousal response including: quickening of respiration, grimaces or limb movements, extensor or flexor withdrawal of a limb.
• Patients' eyes may turn fleetingly to follow a moving object or towards a loud sound.
• Grasp reflexes may be present.
Compatible but atypical features (prompting careful reassessment but not negating the diagnosis)
• follow a moving target for more than a fraction of a second
• fixate a target
• react to visual menace
• utterance of a single inappropriate word
Incompatible features
• evidence of discriminative perception
• purposeful actions
• communicative acts
• a smile in response to the arrival of a friend or relative
• an attempt to reach out for an object
• appropriate use of language

what type of evidence is sufficient to demonstrate that a specific motor action is imbued with purpose or meaning [4]. Until an objective index is found, the boundary separating consciousness and unconsciousness remains arbitrary [5]. Recently, diagnostic schemes built around the presence or absence of criterion behaviors have been developed to distinguish minimally conscious state from vegetative state [2, 6, 7]. While behavior-based diagnostic criteria are useful for characterizing patients clinically, they are inherently flawed because motor responsiveness is often an unreliable proxy for consciousness. Movements that appear to be volitional may actually be reflexive in nature and *vice versa*. Complicating matters further, patients may exhibit behavioral signs of awareness during one examination and fail to do so on the next. Fluctuations in arousal and motor responsiveness commonly occur in disorders of consciousness and may result in diagnostic instability [8, 9]. These factors have conspired to produce high rates of misdiagnosis in vegetative state, especially if the diagnosis is not made by trained physicians with the necessary expertise; previous studies have reported diagnostic error in 18% [10], 37% [11] and 43% [12] of patients considered 'vegetative'.

Complimentary Examinations in Vegetative State

Structural imaging such as computed tomography (CT) and magnetic resonance imaging (MRI) are non-specific for the diagnosis of vegetative state. With regard to outcome, corpus callosum and brainstem lesions, identified with MRI, are associated with non-recovery in post-traumatic vegetative state [13, 14]. Post-trauma metabolic (brainstem spectroscopy) and morphological (T2 star and Flair) MRI studies also correlate with long-term neurological outcome, especially in vegetative state and minimally conscious state [15]. In vegetative state of non-traumatic origin, there is no established correlation between structural imaging data and the development of vegetative state or the potential for recovery [16].

Electroencephalography (EEG) is not specific for the diagnosis of vegetative state and classically shows a diffuse slowing of the electrocortical activity (generalized polymorphic delta or theta rhythm) [17]. Background activity only occasionally shows reactivity to sensory (noxious) stimulation [18, 19]. In most patients, transition from wakefulness to sleep is accompanied by changes in the EEG pattern. However, some patients show persistent very low voltage activity and sporadically isoelectric EEGs have been reported [16]. In approximately 10% of long-standing vegetative state patients, a non-reactive alpha rhythm has been observed [18]. The transition from coma to vegetative state is not accompanied by any notable changes in the EEG. In contrast, transition from vegetative state to awareness has sometimes been accompanied by the reappearance of a reactive alpha rhythm [18, 20].

Evoked potentials are a valid tool for assessing the prognosis of patients who are in a coma but are not helpful in confirming the diagnosis of vegetateive state [21]. Somatosensory evoked potentials (SEP) are the most helpful: bilateral absence of cortical responses three days after the insult is highly predictive of failure to regain consciousness (i.e., death or survival in vegetative state). However, patients with normal SEPs may enter a vegetative state and remain in it and visual evoked potentials (VEP), brainstem auditory evoked potentials (BAEP), and passive auditory oddball paradigm are not specific for vegetative state, niether do they help in predicting the outcome [22].

Functional neuroimaging studies in vegetative state have shown that cerebral metabolic activity decreases to about 50% of normal levels [23, 24]. However, in one group of patients who subsequently recovered, global metabolic rates for glucose metabolism did not show substantial changes [25]. Moreover, some awake healthy volunteers have global brain metabolism values comparable to those observed in some patients in a vegetative state [26] and inversely, some well documented vegetative patients have shown close to normal global cortical metabolism [23]. The most characteristic feature of vegetative state is a dysfunction in the frontoparietal network encompassing the polymodal associative cortices: bilateral lateral frontal regions, parieto-temporal and posterior parietal areas, mesiofrontal, posterior cingulate and precuneal cortices [24, 26]. But neither global nor regional measures of resting cerebral metabolism dissociate vegetative state from minimally conscious state at the individual patient level [27].

In response to external sensory stimuli, functional neuroimaging studies have shown that vegetative patients show cerebral activation, but this activation is usually limited to subcortical and 'low-level' primary cortical areas, disconnected from the cortical network considered necessary for conscious perception. Studies using painful stimulation have shown activation in brainstem, thalamus, and primary somatosensory cortex in vegetative state patients, while hierarchically higher-order areas of the pain matrix (that is, secondary somatosensory, insular, posterior parietal, and anterior cingulate cortices) failed to activate [28]. Moreover, the activated primary somatosensory cortex was isolated and dissociated from the frontoparietal network, thought to be required for conscious perception. Similarly, auditory stimulation in vegetative patients activates primary auditory cortices, but usually not higher-order multi-modal areas from which they were disconnected [29, 30].

Surprising Results in Vegetative State: Signs of Consciousness?

Some electrophysiological and functional neuroimaging studies have shown surprising results in patients diagnosed as vegetative. Hinterberger et al. [31] reported results of a five stage electrophysiological assessment of five patients diagnosed as vegetative and five healthy volunteers. Several of the patients showed normal or near-normal event-related brain potential (ERP) responses to some of the tasks, although results were most constant at the lower levels of the suggested processing hierarchy (e.g., semantic oddball). On the basis of these findings, two of the patients were selected for training on a brain computer interface (also called 'thought translation device') with some success in one of these cases. Similarly, Kotchoubey [32] has reported that some patients diagnosed as vegetative may be capable of processing semantic stimuli indicating some comprehension of meaning. Thus, P3 and N400 components were observed but were often abnormal (e.g., slow negative response instead of a P300) in patients considered to be in a vegetative state [33]. Perrin and co-workers [34] have reported a P300 response to salient stimuli such as the patient's own name as compared to other names in minimally conscious state, but also in some vegetative state patients.

Similarly, several case studies using functional MRI (fMRI) have also provided evidence of preserved high-level cortical processing in some patients reported to be in a vegetative state. An auditory paradigm was used in the first oxygen-15-labelled positron emission tomography (PET) study of a patient in a vegetative state. The authors observed activation in the anterior cingulate and temporal cortices when

this patient (in a post-traumatic vegetative state) was told a story by his mother compared with when he heard nonsense words [35]. These authors interpreted this activation as the processing of the emotional attributes of speech or sound. In another widely discussed PET study of a patient in an upper boundary vegetative state after encephalitis (and before subsequent recovery), activation during presentation of photographs of familiar faces was compared with that during meaningless pictures. Although there was no evidence of behavioral responsiveness during presentation of the familiar-face photographs, except occasional visual tracking, the visual association areas encompassing the fusiform face area showed significant activation [36]. Evidence is also building up indicating that non-communicative patients usually respond more to complex emotionally salient stimuli than to simple stimuli, suggesting some response to meaningfulness of information even in these disorders of consciousness [37]. None of these studies, however, demonstrate that finding evidence of residual complex processing predicts further recovery.

In order to most effectively define the degree and extent of preserved cognitive function in vegetative state, Owen et al. [38] have argued that a hierarchical approach to cognition is required; beginning with the simplest form of processing within a particular domain (e.g., auditory) and then progressing sequentially through more complex cognitive functions. To illustrate this point, a series of paradigms in the auditory domain were investigated, which systematically increase in complexity in terms of the auditory and/or linguistic processes required and, therefore, the degree of preserved cognition that can be inferred from 'normal' patterns of activation in disorders of consciousness. For example, speech perception was assessed by comparing cortical responses to spoken sentences with those to acoustically-matched noise sequences. At the next level, phonological processing of speech was assessed by comparing responses to degraded ('less intelligible') sentences versus normal (intelligible) sentences. Finally, speech comprehension was tested by comparing cortical responses to sentences containing ambiguous words (e.g., "the creak/creek came from a beam in the ceiling") and matched unambiguous sentences. Increases in neural activity during ambiguous sentences reflect the operation of semantic processes that are critical for speech comprehension. The authors illustrated this approach in a patient diagnosed as vegetative who showed activation in response to speech relative to signal correlated noise, potentially reflecting some perception of speech. A significant response was also observed to speech of increasing intelligibility suggesting that these perceptual processes are recruited more strongly for speech that can be more readily understood. Finally, ambiguous sentences yielded a partially normal response, interpreted as evidence that some semantic aspect of sentence processing was intact; in other words, not only did the patient's brain recognize speech as speech, but it seemingly was being processed at a level which, in the healthy brain, is equated with comprehension [38].

Rarely, patients meeting the diagnostic criteria for vegetative state have behavioral features that seem to contravene the diagnosis [27]. From a series of multimodal imaging studies of patients in a vegetative state, three with unusual behavioral fragments were identified. Preserved areas of high resting brain metabolism (measured with fluorine-18-labelled deoxyglucose PET) and incompletely preserved gamma-band responses (measured with magnetoencephalography) were fitted to structural data from an MRI and correlated with the behaviors of the patients [39]. Among those studied was a patient who had been in a vegetative state for 20 years who infrequently expressed single words unrelated to any environmental stimuli [40]. MRI images showed severe subcortical damage. Resting 18F-fluorodeoxyglu-

cose-PET measurements of the patient's brain showed a global cerebral metabolic rate of $<50\%$ of the normal range across most brain regions, with small regions in the left hemisphere expressing higher levels of metabolism. Magnetoencephalography responses to bilateral auditory stimulation were confined to the left hemisphere and localized to primary auditory areas. Taken together, the imaging and neurophysiological data seemed to show that the left sided thalamocortical-basal ganglia loops (that support language function in Heschl's gyrus, Broca's area, and Wernicke's area) were partially preserved. Similar observations in two other patients in chronic vegetative state provide evidence that isolated cerebral networks may remain active in rare cases. The preservation of these isolated behaviors does not indicate further recovery in patients in chronic vegetative state who have been repeatedly examined and carefully studied with imaging tools. Reliable observations of such unusual features should prompt further investigation in individual cases [27].

In several of the EEG [31, 34, 41] and functional imaging [38] studies described above, 'normal' evoked potentials or activation patterns in predicted regions of cortex have been used to infer residual cognitive processing in patients diagnosed as vegetative. The question that invariably arises is whether such signs indicate awareness. It is important to stress that there is a wealth of data in healthy volunteers, from studies of implicit learning and the effects of priming, to studies of learning during anesthesia that have demonstrated that many aspects of human cognition can go on in the absence of awareness. In the examples discussed above (including speech perception and the detection of semantic ambiguous sentences), under normal circumstances cognitive processing is relatively automatic. That is to say, it occurs without the need for willful intervention – you cannot choose to not understand speech that is presented clearly in your native language.

A New Paradigm to Assess Consciousness in Vegetative State

Owen et al. [42] have recently addressed this concern by applying an fMRI paradigm where non-communicative patients are asked to perform mental imagery tasks at specific points during scanning. In one exceptional vegetative state patient studied five months after a traumatic brain insult, activation was observed in the supplementary motor area after she was asked to imagine playing tennis. In contrast, when asked to imagine visiting all of the rooms of her house, activation was observed in premotor cortex, parahippocampal gyrus, and posterior parietal cortex (Fig. 1). Similar activation patterns were seen in 34 healthy volunteers studied in Cambridge and Liège. Importantly, because the only difference between the conditions that elicited task-specific activation was in the instruction given at the beginning of each scanning session, the activation observed can only reflect the intentions of the patient (which were, of course, based on the remembered instruction), rather than some altered property of the outside world. In this sense, the decision to 'imagine playing tennis' rather than simply 'rest' is an act of willed intention and, therefore, clear evidence for awareness and command-following in the absence of voluntary motor responsiveness. Of course, negative findings in such patients cannot be used as evidence for lack of awareness, as false negative findings in functional neuroimaging studies are common, even in healthy volunteers. However, in the case described here, the presence of reproducible and robust task-dependent responses to command without the need for any practice or training suggests a novel method by which some non-communicative patients, including those diagnosed as vegetative,

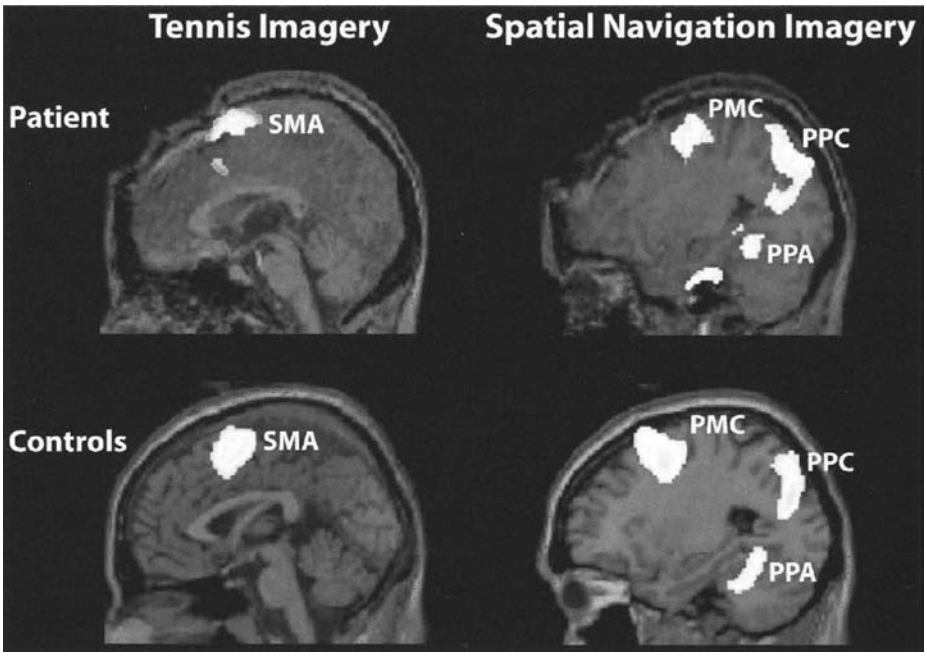

Fig. 1. Supplementary motor area (SMA) activity during tennis imagery in the patient and a group of 12 healthy volunteers (left panel). Parahippocampal gyrus (PPA), posterior parietal-lobe (PPC), and lateral premotor cortex (PMC) activity while imagining moving around a house in the patient and in the same group of volunteers (right panel). Adapted from [42] with permission.

minimally conscious or locked in, may be able to use their residual cognitive capabilities to communicate their thoughts to those around them by modulating their own neural activity [42].

Where Do We Go from Here?

Though enhancing the potential role that functional neuroimaging techniques can play in differential diagnosis in disorders of consciousness, the study by Owen et al. [42] will not challenge current practice in therapeutic decision making. Indeed, the patient studied was in vegetative state following a traumatic brain injury, and at 5 months post injury had a 20% chance of some recovery. Concerns about end-of-life decisions, treatment withdrawal or ending of artificial hydration and nutrition were, therefore, never applicable to this patient. Furthermore, this patient, though meeting established criteria for vegetative state at the time of scanning, showed an atypical clinical presentation. When re-examined six months later she showed inconsistent visual tracking – the most frequently encountered preliminary clinical sign of recovery from vegetative state.

The electrophysiological and functional neuroimaging studies described here further demonstrate the great need to increase research efforts to improve diagnosis in disorders of consciousness and better understand borderline behavior such as fixation, eye tracking, and orientation to pain. In the current context, functional neuroi-

maging can improve our understanding of the neural correlates of these behaviors, as well as their significance in terms of awareness. A transition from case reports to multicenter studies enrolling large number of patients is also crucial.

Like brain death, vegetative state is a clinical diagnosis. In brain death, complementary examinations are used to confirm clinical diagnosis [43]. At present, in vegetative state no such ancillary objective measurements exist. They are, however, needed, given the clinical difficulties of quantifying consciousness based on behavior [44] and the known problem of clinical misdiagnosis.

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Quality and Management

Intermediate Respiratory Care Units

M. Ferrer and A. Torres

Introduction

Unlike the situation in North America, respiratory physicians in most European countries have not been involved in critical care medicine until recently [1], since both specialties have developed separately over time. This separate development has been particularly clear in Spain, due to the following circumstances [2]: 1) When intensive care medicine began in Spain, Spanish pulmonary physicians did not have a strategic vision of the future, unlike cardiologists, who demanded and assumed responsibility for the coronary units; 2) historically, pulmonary physicians have shown little interest in the care of critically ill respiratory patients; and 3) specialists in intensive care medicine have defended their specialty and have avoided others entering it.

In the USA, respiratory specialists have been running respiratory intensive care units (ICUs) since the 1960s [3] and, since the late 1980s, other units with lower levels of resources called non-invasive respiratory care units, intermediate respiratory care units or high dependency units (HDUs) [4, 5]. Despite some concerns in defining these units [6], their effectiveness, both in medical and in economic terms, has already been described [7, 8]. The experiences reported suggest that it is possible to provide effective care for patients with acute on chronic respiratory failure and/or those requiring prolonged mechanical ventilation outside the general ICU, and at a lower cost. In Europe, respiratory care units have only been developed relatively recently [9], but there has been a rapid increase in numbers in recent years [10]. European intermediate respiratory care units tend to serve as specialized single organ units at an intermediate level of care between the ICU and general ward, but their characteristics are not homogeneous.

Several circumstances have contributed to this development. The administrative organs of the medical societies for pulmonary medicine, such as the Respiratory Intensive Care Assembly of the European Respiratory Society (ERS) have shown a growing interest in patients with severe respiratory conditions. In addition, European intensive care physicians are progressively open to the involvement of other specialists in the management of these patients. This has been demonstrated by the creation of a joint consensus document, the appointment of pulmonary medicine specialists for ICUs and, in general, a more open strategic view, which has probably been generated by the problems of growth and the aging of the medical staff. Finally, the training programs of residents in pulmonary medicine are being reviewed, placing greater importance on training in critical care respiratory medicine for postgraduate formation.

The recent report by a Working Group of the ERS [11] elaborated by specialists in pulmonary medicine, anesthesiology, and intensive care medicine stratifies the

Table 1. Levels of care for acute respiratory patients [11].

Level 0	May be treated in a conventional hospital ward
Level 1	Patients with a risk of clinical deterioration or who come from higher levels of care.
Level 2	Patients requiring care for failure of a single organ (respiratory) or for postoperative care, or those from a higher level of care.
Level 3	Patients requiring advanced or basic respiratory support with failure of at least 2 organs.

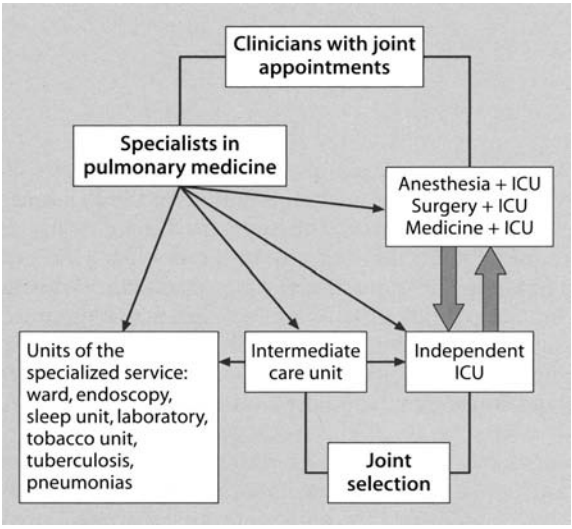

Fig. 1. Model of integration of the Respiratory Medicine specialties in the care of critically ill patients as proposed by the report of the *European Respiratory Society*. Adapted from [11].

grades of medical care to critically ill patients into several levels, justifies why it would be easy for specialists in pulmonary medicine to adapt to one of these levels (Table 1), and describes the difficulties of integration which underline the absence of the specialty of pulmonary medicine in the process agreed upon in Europe for the development of critical care medicine. The document by the ERS proposes a model of integration of critically ill patient care for specialists in pulmonary medicine (Fig. 1), with the intermediate respiratory care unit being of special relevance in this model.

The present chapter will review the most relevant characteristics of intermediate respiratory care units, with emphasis on the definition, organization, and selection criteria.

Definition and Justification for Intermediate Respiratory Care Units

An intermediate respiratory care unit is defined as an area of monitoring and treatment of patients with acute respiratory insufficiency or acute episodes caused by a primarily respiratory disease. The objective of this unit is basically to provide adequate and correct cardiorespiratory monitoring and/or treatment with non-invasive mechanical ventilation (NIV) for patients with respiratory insufficiency. Likewise, the intermediate respiratory care unit allows continuous monitoring of post-thoracic

surgery patients or those receiving mechanical ventilation through tracheotomy, and the management of critically ill patients with difficulty in withdrawal of invasive mechanical ventilation. Non-invasive monitoring techniques and NIV should be the main therapeutic option.

The intermediate respiratory care units are designed to offer respiratory patients an intermediate grade of care between the ICU and conventional hospitalization. The first document referring to the needs and functions of the intermediate respiratory care unit in hospitals proposed the creation of these units for both non-invasive monitoring of severe respiratory patients and to achieve better withdrawal of mechanical ventilatory support [4]. These units have received different names but their activity may be summarized as care for: 1) patients requiring NIV due to acute respiratory insufficiency or acute episodes; 2) patients discharged from an ICU requiring a period of intermediate surveillance; and 3) prolonged weaning with tracheotomy and, if in a medical-surgical unit, patients during the postoperative period following thoracic surgery. The arguments for defending these units are: 1) they are units which require fewer resources for patients who, in another case, would remain in the ICU or would be badly attended in a conventional hospitalization ward; 2) they can provide care for patients with chronic obstructive pulmonary disease (COPD) who may require prolonged withdrawal from artificial ventilation; 3) many of the patients admitted to the intermediate respiratory care unit may be treated with NIV; and 4) the cost/efficacy of NIV has been clearly demonstrated.

NIV is, and should be, the main justification for the presence of these units. This type of mechanical ventilation has shown to be effective in acute hypercapnic respiratory insufficiency in COPD [12;13] and in some types of patients with acute hypoxemia and non-hypercapnia (acute pulmonary edema, immunosuppressed patients, post-thoracic surgery) as well as in the withdrawal of artificial ventilation in patients with chronic respiratory diseases [14–16]. In all these cases, NIV effectively prevents nosocomial respiratory infection, which is one of the most frequent and severe complications of invasive mechanical ventilation.

A key argument in favor of the need for intermediate respiratory care units arises from the consideration that many of the patients who are admitted to a traditional ICU do not require or do not benefit from the high level of care and the monitoring provided by the personnel in these units. Nonetheless, these patients cannot be adequately managed in a conventional hospital ward; thus, the intermediate respiratory care unit is the appropriate setting for their treatment. Indeed, the clinical features of intermediate care area patients are similar to those of less severely ill ICU monitoring patients [17]. Some have undertaken the development of these units based on the reduction of the overload in the ICU, without a decline in the quality of care provided to the respiratory patients.

It has been estimated that up to 40% of patients admitted to an ICU do not require invasive mechanical ventilation. Likewise, only 40% of patients with acute respiratory insufficiency due to lung disease require invasive mechanical ventilation [18–22]. In a study on 99 Italian ICUs, COPD was the most common chronic underlying disease in patients admitted to an ICU and the most frequent cause of admission was cardiopulmonary monitoring [23]. In relation to this study, it may be concluded that the resources available in the ICU for carrying out functions such as monitoring and treatment of patients with acute chronic respiratory insufficiency in whom invasive mechanical ventilation is not indicated are over or inappropriately used. Moreover, when these patients require invasive mechanical ventilation, up to 60% of the duration of ventilation is used in its withdrawal [23–26].

Several studies have suggested that the transfer of patients from the ICU to the intermediate respiratory care unit or direct admission to these units of patients in whom the probability of requiring intensive care is low may be an effective way to reduce costs and improve the use of general ICUs [7]. It has been demonstrated that 40% of patients in medical ICUs and 30% of those in surgical ICUs are admitted with the sole objective of performing continuous 24 hour monitoring and not for carrying out specific therapeutic procedures. The mission of the intermediate respiratory care unit would be not only to reduce healthcare costs but also to favor a more efficient use of the existing resources in the ICU. The correct use of the intermediate respiratory care unit makes the availability of free beds in the general ICU possible and, thereby, facilitates more appropriate use of the general ICU. In addition, an intermediate respiratory care unit offers patients who cannot be admitted to the general ICU an alternative to the potentially insufficient care of a conventional hospital bed [7, 8, 20].

The above considerations justify the creation of the intermediate respiratory care unit whose essential objective is to provide the best quality healthcare together with: 1) reduced needs for healthcare personnel, particularly of nursing and auxiliary staff, and reduced consumption of technical and healthcare resources [7]; 2) better use of ICU resources, with this unit being reserved for patients truly requiring intensive treatment [8]; and 3) the possibility of earlier ICU discharge of patients who have overcome the acute phase of their disease but who still require medical or specialized nursing care or NIV for facilitating weaning, all of which are aspects which cannot be provided in conventional hospital areas.

In addition to the factors related to the consumption of healthcare resources, other advantages should be considered such as providing more privacy to the patient, greater comfort in regard to reduced use of equipment, less environmental distortion (noise and lights), and a more flexible visiting schedule for members of the family. All of these aspects make the intermediate respiratory care unit a more ideal setting for the care of this type of patient, contributing to their improvement and facilitating hospital discharge, particularly in patients requiring continued ventilatory support at home [10, 27].

In summary, intermediate respiratory care units reduce hospital costs, reduce mean ICU stay and occupation, without increasing the mean hospital stay, improve patient and family satisfaction and do not have a negative impact on the final outcome of the process. Thus, intermediate respiratory care units are structured with a very favorable cost/effectiveness relationship if applied to patients requiring specialized respiratory care [28].

Fortunately, pulmonary medicine has been implicated in the use of NIV from the introduction of this technique. Thus, pulmonary physicians working in hospital medicine are familiar with the technique and know when and how to apply it. Nonetheless, in general there are insufficient human resources and space; that is, intermediate respiratory care units are not generally available.

Criteria for Admission to an Intermediate Respiratory Care Unit

The decision to admit a patient to an intermediate respiratory care unit should be individualized, taking into account the age, co-morbidities, and the wishes of the patient. Patients with very severe respiratory insufficiency with a high probability of requiring invasive mechanical ventilation and those with other severe non-respira-

tory organ dysfunction should be considered for admission to an ICU. On the other hand, patients with acute or acute-on-chronic respiratory failure not fulfilling determined criteria of severity may be admitted to a conventional hospital ward. Patients fulfilling any of the following criteria should be considered for admission to an intermediate respiratory care unit:

- Transfer of patients from the ICU who, following stabilization, continue to depend on invasive mechanical ventilation after unsuccessful disconnection from the ventilator by NIV or tracheotomy, with the aim of progressing with the disconnection and/or programming home mechanical ventilation. The patients should be conscious, hemodynamically stable, with no evidence of sepsis, and should have stable renal function without cardiac arrhythmia or uncontrolled bleeding.
- Transfer of patients from the ICU who, after having become stabilized following a severe clinical process or who have required prolonged invasive mechanical ventilation, require nursing care and/or physiotherapy as an intermediate step to conventional hospitalization.
- Use of NIV for the treatment of acute or acute-on-chronic respiratory failure.
- Severe respiratory insufficiency which, although not requiring ventilatory support, does require non-invasive monitoring.
- Patients after thoracic surgery with pneumonectomy or with a foreseen significant reduction in postoperative pulmonary function, relevant comorbidity, or age over 70 years, or when relevant medical respiratory complications appear during the postoperative period. In these patients, the above mentioned criteria indicated for identifying patients requiring ICU admission should be considered.
- Life-threatening hemoptysis.

Localization and Design of Intermediate Respiratory Care Units

There are currently no guidelines or standards on the best location and design or constitution of these units in hospitals [10, 23, 27, 29]. Since these are respiratory patients whose main treatment is the application of NIV [29, 30], the medical personnel in charge should be a specialists in pulmonary medicine and functional dependence should be to a Department of Pulmonary Medicine, similar to the relationship of Coronary Care Units with the Department of Cardiology.

Taking this into account several models for the location of intermediate respiratory care units in a hospital have been proposed [27].

- Independent location: This has the advantage of greater functional independence and of providing adequate support to a large group of patients as case-mix will be better controlled. This location should have good access to the Department of Pulmonary Medicine and/or the ICU. The most important limitation is the loss of efficiency when the intermediate respiratory care unit is small or when the units are large and have low indexes of occupation, as well as less flexibility and integration of personnel and accessory spaces which may lead to higher costs.
- Parallel model: An intermediate respiratory care unit adjacent to the ICU. This provides greater privacy to the patients and allows greater flexibility in regard to both the availability of equipment and healthcare staff rotation with the ICU. The limitations are the lack of occupation if the intermediate respiratory care unit is large.

- Integrated model: An intermediate respiratory care unit integrated within the ICU or in the pulmonary medicine ward with a more or less specific area provides the advantage of continuity of care to the patient within the same unit, facilitating transfers based on a worsening or improvement in the patient's condition as well as a greater flexibility and integration of the medical, nursing, physiotherapy, auxiliary, and administrative personnel. It has the restriction that the work loads vary greatly with respect to complexity, making an adjustment in nursing staff needs necessary. Other limitations may be high equipment costs per bed and training of healthcare personnel.

  One variant of this last model would be an intermediate respiratory care unit adjacent to and functionally integrated with a sleep disorder laboratory. These areas have resources for respiratory monitoring which are normally not occupied during the day. Given the specialization of the intermediate respiratory care unit in NIV, these areas could be used as 'day hospitals' for the initiation of home mechanical ventilation in some chronic patients and those who are already receiving chronic ventilation and require monitoring for identifying and eventually solving problems which arise at home. At the same time, some of the monitoring resources available in the sleep disorders laboratory could be used in patients admitted to the intermediate respiratory care unit [10]. The model would facilitate the unification of NIV in acute situations and home mechanical ventilation.

Although the model of integration of the intermediate respiratory care unit in the pulmonary medicine ward is the most common model [29], location will largely depend on the characteristics of each center.

Physical Structure and Size

The area available for beds should be double or more than those used in conventional hospital wards [10, 31]. Therefore, an open structure (without partitions between the beds) and a central nursing control station, similar to that in most ICUs, provides greater facility of movement and a better view and care than a closed structure (with partitions). However, the latter provides greater patient and family comfort and privacy, as well as better infectious control measures, particularly in limiting the spread of multi-drug resistant respiratory pathogens. Indeed, one of the advantages of the intermediate respiratory care unit over the ICU is the greater patient contact with the family [32]. If the latter design is chosen, it is advisable to ensure continued visualization of the patients from the nursing station, whether direct (a partially glass partition) or with a closed video and audio circuit. Since some patients require wheelchairs for mobility, it is advisable to allow sufficient space for maneuvering of these chairs.

The number of beds in the unit should allow for the needs of the reference area of the hospital. The number of patients who would benefit from NIV and/or monitoring whould be taken into account, being larger during some periods of the year that during others. On the other hand, NIV should also be performed in the pulmonary medicine ward and in the ICU, depending on the disease severity of the patients [10, 13]. Small units (for example three beds) lose some of the cost savings in terms of personnel and infrastructure compared to a conventional ICU [27]. This increase in cost may be covered by integrating the intermediate respiratory care unit in a pulmonary medicine ward. Since NIV has shown to be useful in acute respira-

tory insufficiency in immunosuppressed patients [33], a room with infrastructure for patient isolation may be useful.

The use of ventilation and monitoring requires a greater number of electrical connections, gas outlets (oxygen, compressed air) and vacuum, monitor supports, respirators or drip stands etc. than in a conventional ward. A specific system including all of these requisites at the head of the bed would be useful.

The nursing station should have a structure which allows direct patient supervision as well as monitoring of physiologic parameters. It is also necessary to have an area for office work and another for administrative functions [34]. Other necessary areas are offices for the physicians, the secretary, pharmacy, storeroom, and files, which may be shared in integrated models.

Staffing

These units should be attended by a professional multidisciplinary team, which should be directed by specialists in pulmonary medicine. Likewise, it is necessary to have someone to coordinate and supervise the nursing staff. There should be one physician per six patients [35]. It is not necessary to have medical personnel in the intermediate respiratory care unit 24 hours a day but a physician on duty should be physically available in the hospital [27, 29]. In general, the care provided during the evening and night could be integrated within the medical duties of the pulmonary medicine ward. Nursing staff on each shift should consider one nurse for 3 to 4 patients [10] with 24 h nursing presence and care in the intermediate respiratory care unit. Likewise, personnel from physiotherapy are also necessary with one person for 6 beds, ideally with morning and afternoon shifts. Nursing and physiotherapy personnel should have experience in the management of ventilators, nebulizers, and oxygen therapy, as well as the placement of masks and the control of patients with severe respiratory insufficiency. Nursing auxiliaries are necessary, especially for patient mobilization and posture changes.

Material Needs

The materials required for these units depend on many factors including the specific characteristics of each hospital. These requisites may be grouped into the following schema: infrastructure and furnishings, monitoring and diagnostic equipment, equipment for respiratory therapy, and equipment for thoracic surgery patients.

Infrastructure and furnishings

It is advisable to have a nursing station for all the unit's documents, including the clinical histories of the patients, and at least one computer. Likewise, dressing carts and a unidose medication system as well as a cart with equipment for tracheal intubation and cardiopulmonary resuscitation are required. The latter should also include medication for emergency situations. The beds should, ideally, be electric to allow posture changes. Similarly, anti-sloughing mattresses should be provided. The rooms should have the following:

- Auxiliary bars beside the beds for the placement of fungible material used in patient care.
- Wall connections for oxygen and pressurized air as well as vacuum outlets. A minimum of two oxygen and pressurized air and three vacuum outlets per

patient are advisable. The vacuum outlets should be able to be connected to aspirators and pleural drainage systems and should, therefore, have the corresponding fungible material. Likewise, there should be a manual resuscitation bag.
- An acoustic warning system and/or interphone connected to the nursing station.
- An auxiliary table and telephone with exterior connection.
- Preferably functional armchairs to allow patients to be seated if possible.

The units should have at least one patient-lift/crane to raise patients from bed as well as architectonically barrier-free bathrooms. There should also be enough pumps for intravenous infusion and administration of nutrition.

Monitoring and diagnostic equipment

As a general norm, invasive monitoring should be reserved for the ICU and non-invasive monitoring for the intermediate respiratory care unit. The essential monitoring required for NIV is pulse-oximetry and arterial blood gases after the initiation of ventilation or following changes in ventilatory parameters [30]. Respiratory frequency is another important parameter [36]. At the same time, continuous electrocardiogram (EKG) and non-invasive arterial pressure monitoring, as well as monitoring of ventilator pressure and flow in ventilated patients, are necessary.

Apart from NIV parameters, capnography at the end of expiration, transcutaneous carbon dioxide pressure (PCO_2), measurement of the ventilatory pattern, maximum inspiratory pressure, neuromuscular impulse by occlusion pressure of the airway (Po.1), dynamic pulmonary volume, and peak flow may be monitored.

Respiratory therapy equipment

Since NIV is one of the main reasons for the intermediate respiratory care unit, there should be both pressure-cycled as well as volumetric ventilators. Among others, these ventilators should have an internal and/or external electric battery to facilitate patient transportation. Continuous positive airway pressure (CPAP) apparatus is also necessary for spontaneous respiration. Likewise, all the fungible material necessary for NIV should be available:

- Complete tubing, including the exhalation ports in the case of ventilators without a separated circuit for inspiration and expiration, and oxygen connections when the ventilators do not use pressurized oxygen and bacterial filters.
- Nasal, nasal-oral, or complete facial masks of different sizes and shapes to adapt to the face of each patient should be available. Likewise, harnesses for maintaining the mask on the patient's head are necessary, especially when the patients require home mechanical ventilation.

Since a significant proportion of patients require tracheal intubation and invasive mechanical ventilation, there should be at least one ventilator of this type to maintain patients until they are transferred to an ICU. As in the previous section, fungible material such as tubing, endotracheal tubes etc. should be available.

For the care of patients with a tracheotomy, different sized cannulas are necessary with a balloon which may be inflated by pressure or with a sponge inside, with or without fenestration, to allow phonation. In addition, silver cannulas should be available for patients not requiring ventilation. Other material needed by patients with a tracheotomy includes stoma dilators for cannula changes, and cleaning brushes for internal cannulas or phonation valves.

Humidification is another important aspect of respiratory therapy. Humidifiers usually use hot water, although heat and moisture exchangers are appropriate for patients with tracheotomy. Similarly, aerosol therapy requires nebulizers and their accessories. Nebulizers may be pneumatic, to generate larger particles which are deposited in the bronchial tree, or ultrasonic to create smaller particles able to reach the pulmonary parenchyma.

Equipment for thoracic surgery patients

Specific equipment for these patients includes pleural drainage together with specific sealing systems and aspiration of the pleural cavity, which may be either open or closed.

Interaction of the Intermediate Respiratory Care Unit with Other Departments or Units

Intensive Care Units

The aging of the population with the consequent increase in patients with chronic diseases together with greater knowledge of the pathophysiology of many processes leads to greater therapeutic efficacy, increasing the need for more beds in the ICU [26]. If we consider that the objective of the ICU should be to provide high quality healthcare, then it must be understood that the intermediate respiratory care unit and the ICU should complement each other. Some time ago it was indicated that the intermediate respiratory care unit should optimize the use of healthcare resources by reducing the number of admissions in the already overworked ICU without compromising the quality of care provided [4]. This is based on the fact that around 40% of ICU admissions do not receive intensive treatment, particularly invasive mechanical ventilation, thus, this group of patients would benefit from admission to the intermediate respiratory care unit, especially those with acute respiratory insufficiency due to lung disease. Since these patients are treated with NIV or with CPAP, more effective care may be provided by the intermediate respiratory care unit without reducing the quality of care given [10] while, at the same time, more beds remain free in the ICU for patients with multiorgan failure and/or indications for endotracheal intubation. Thus, it has been reported that the closure of an intermediate respiratory care unit leads to a greater rate of admissions to the ICU of patients with diseases of less severity as measured by the APACHE II score [8].

Chronic patients who have recovered from an exacerbation of their disease but who still require nursing care or monitoring may also benefit from the intermediate respiratory care unit as may individuals who are difficult to wean from the ventilator after recovering from the acute phase of their disease process. This type of action cannot be easily provided in a general hospital ward and, therefore, the ICU stay for these patients is often unnecessarily prolonged [10].

In summary, the following are determined situations in which the ICU may benefit from the activity of an intermediate respiratory care unit:

- Admission for acute exacerbations of a chronic respiratory disease [37], particularly COPD, requiring NIV
- Acute or acute-on-chronic respiratory failure with prolonged ICU stay, motivated by the need for ventilation after recovery from the acute episode.

- Neuromuscular patients for a NIV schedule or in more advanced stages for tracheotomy as invasive mechanical ventilatory support.
- Patients with ventilator weaning difficulties who may benefit from NIV.
- Patients with complex diseases who, on ICU discharge, may benefit from better monitoring in the intermediate respiratory care unit compared to that provided in a general hospital ward, thereby, allowing earlier identification of patients with greater risk and reducing the mortality in this subgroup [11, 37].
- Greater homogeneity of the case-mix in the ICU so that the ICU does not receive admissions with low scores on the severity scales (SAPS, APACHE) [27].

Another advantage of the intermediate respiratory care unit in the management of these patients is the greater comfort and privacy provided in comparison with the ICU. The intermediate respiratory care unit allows a more normal life with greater family contact.

Department of Thoracic Surgery

The opening of an intermediate respiratory care unit is always beneficial for the Department of Thoracic Surgery since these units are covered with a better nurse-patient relationship and greater possibilities of non-invasive monitoring for hemodynamically stable postoperative patients discharged from the ICU or from the areas of postoperative recovery early following extubation. A 24–48 h stay in the intermediate respiratory care unit facilitates stabilization of the patient, thanks to the use of respiratory physiotherapy and, in many cases, NIV. Intermediate respiratory care units are even more effective after upper airway surgery in which ICU stay may be uneventfully shortened. These areas are also useful for performing tracheotomies in neuromuscular patients and for the use of laser in tracheal stenosis. Moreover, the intermediate respiratory care unit can provide a greater union of the specialties of Pulmonary Medicine and Thoracic Surgery when the two Departments share the intermediate respiratory care unit as a hospital resource.

Other Departments

The intermediate respiratory care unit may be useful for the Departments of Otorhinolaryngology, Internal Medicine, and even the Department of Pulmonary Medicine since the use of NIV and non-invasive monitoring as well as the better nurse-patient relationship make it possible for the intermediate respiratory care unit to be used as a step between the ICU and general hospital wards in patients not requiring intensive care but who need close monitoring not provided in conventional wards [38]. It has been reported that the mortality of these patients was lower in general hospital wards following opening of an intermediate respiratory care unit [39]. A reduction in readmissions to the ICU has also been reported [40]. Likewise, some authors have described the convenience of admission to the intermediate respiratory care unit for several days after discharge from the ICU for patients undergoing corrective surgery for obesity, for the management of obstructive sleep apneas that often accompany obesity, for patients requiring airway care, and for the prevention of possible bed sores [41].

Conclusion

Intermediate respiratory care units have been developed increasingly since the late 1980s. These units have lower levels of resources compared with ICUs, and can provide effective care for patients with acute on chronic respiratory failure and/or those requiring prolonged mechanical ventilation outside the general ICU and at a lower cost. NIV should be one of the fundamental pillars for the justification of these units. Intermediate respiratory care units may also serve as step-down units from the ICU to the general or respiratory ward when patients still require nursing care, physiotherapy, and/or when withdrawal of invasive mechanical ventilation is difficult and patients require NIV or ventilation through tracheostomy. Severe respiratory insufficiency that requires non-invasive monitoring, postoperative care of thoracic surgery patients with pneumonectomy or with a foreseen significant reduction in postoperative pulmonary function, as well as patients with life-threatening hemoptysis may also benefit from admission to an intermediate respiratory care unit. These units can be independently located, situated parallel to an ICU, or integrated within an ICU or respiratory ward. The units are characterized by a lower nurse to patient ratio, often 1/2.5 – 1/4, compared to ICUs. Intermediate respiratory care units are an area of respiratory medicine and should be part of the Department of Pulmonary Medicine. This requires the constant presence of physicians who are trained in the care of patients with severe respiratory insufficiency.

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The Impact of Noise in the Intensive Care Unit

R.J. Pugh, C. Jones, and R.D. Griffiths

Introduction

Noise may be defined simply as "unwanted sound" [1]. The World Health Organization (WHO) recommends that the average background noise in hospitals should not exceed 30 A weighted decibels (dB[A]), and that peaks during the night-time should be less than 40 dB(A) [1]. Noise in hospitals and particularly in intensive care units (ICUs), frequently exceeds these values [2–4]. The United States Environmental Protection Agency in fact defines noise as "any sound that may produce an undesired physiological or psychological effect in an individual or group". Noise affects both staff and patients. It may impede concentration and cognitive function [5, 6]. It interferes with effective communication and may thus increase the risk of accidents [5, 7]. The critically ill are particularly sensitive to the disruption of sleep by noise [8]. In addition, and especially for the elderly and hard of hearing, noise may hinder communication and impair understanding of their environment. It may also potentially contribute to the abnormal thought processes and behavior associated with ICU delirium [9].

The purpose of this chapter is to draw attention to the impact of noise in the ICU. The physiological and psychological effects of noise on patients and staff will be discussed. Finally, we will examine measures that could limit the disruption caused by noise.

Noise in the Intensive Care Unit

Directive 2003/10/EC of the European Parliament and of the Council on the minimum health and safety requirements regarding the exposure of workers to the risks arising from physical agents (noise) sets the maximum average exposure to noise over 8 hours to 87 dB(A) [10]. Health care workers (and certainly patients) often spend longer continuous periods in the ICU, and this is far higher than the WHO recommendations relating to the hospital setting. Notably, the EC directive also requires that "risks arising from exposure to noise shall be eliminated at their source or reduced to a minimum." The noise in ICUs is significant.

In measuring exposure to noise, A-weighting is used to reflect the relative sensitivity of hearing to higher frequencies. The energy average equivalent level of A-weighted sound over a specified time, LAeq, is recommended for the measurement of continuous sounds [1]. Studies of noise levels within ICUs have not consistently shown a diurnal variation [2–4, 8, 11, 12]. A LAeq of 55 to 70 dB(A) is widely reported from adult and pediatric ICUs [2, 4, 13, 14]. Peaks of noise greater than 80

Table 1. Causes of noise in intensive care [12, 14, 15]

Noise caused directly by behavior	
Items falling onto floor	Up to 92 dB(A)
Equipment movement (e.g. bed)	90 dB (A)
Talking	75–85 dB (A)
Door closure	85 dB (A)
Pager	84 dB(A)
Telephone	70–80 dB (A)
Television	79 dB (A)
Noise due to equipment	
Connection of gas supply	88 dB (A)
Ventilator alarm	70–85 dB (A)
Nebulizer	80 dB (A)
Oximeter	60–80 dB(A)
Monitor alarm	79 dB (A)
Ventilator	60–78 dB (A)
i.v. infusion alarm	65–77 dB (A)
Endotracheal aspiration unit	50–75 dB (A)

dB(A) occur day and night [4, 8], though they are perhaps less common at night [3, 11]. Peaks of greater than 100 dB(A) have been reported from a Scottish pediatric ICU [15], and up to 120 dB(A) from a Brazilian pediatric ICU [14]. These noise levels greatly exceed the WHO guidelines mentioned above. To place these levels of sound into context, 70 dB is equivalent to standing 1 m from an operational vacuum cleaner, 80–90 dB the noise heard on a busy urban street, and 120 dB is equivalent to a train horn heard from 1 m away.

When sources of excessive noise in ICUs are analyzed, the causes of peak sound levels with longest duration are: Talking (associated with mean peak sound 85 dB[A]), television (80 dB[A]), and monitor alarms (79 dB[A]) [11]. Noise from equipment alarms (including monitors, ventilators, and infusion pumps) may range from 60–85 dB(A) [11, 14] (Table 1). High frequency oscillator ventilators produce significantly more noise than conventional ventilators [16]. Telephone ringing and pagers may cause sound levels of 80 dB(A) or greater [11], though the noise should be short-lived. Patients usually cite staff conversation and alarms as the most disruptive or disturbing of sounds [9, 10], but short-lived high intensity noises are found to be especially annoying [8]. Over 50% of sound peaks have been attributed to modifiable human behavior [11], though arguably in a favorable environment noise from some equipment alarms could also be minimized.

Design of the ICU has a bearing on the disruption caused by noise. The acoustics of the unit are important; increased reverberation time enables the propagation of echo and reduces speech intelligibility [7]. ICUs may be open-plan, or may include a number of separate rooms; noise recorded from single rooms occupied by healthy subjects was significantly lower than that recorded from an open ICU [8]. On a surgical non-critical care ward, closing doors to single rooms decreased noise within rooms by 6 dB [17]. However, noise generation and dispersal within single rooms may be problematic in critical care. Noise recorded in a pediatric ICU from smaller two-patient rooms was higher than that in the four-patient rooms and closure of doors to patient rooms actually increased noise within the room [12]. Proximity of the ICU entrance door to patient rooms may also influence the disruption caused by noise [8].

The Effects of Noise on Patients

Sleep

ICU patients sleep poorly: Both medical and surgical ICU patients demonstrated abnormal wake-sleep cycles according to polysomnography (a continuous monitor of physiological functions including electroencephalogram [EEG] capture) [4, 18], The major environmental factors influencing sleep are noise and ambient light [3, 19], but the causes of sleep disruption are multifactorial, and include the acute illness, pain, anxiety, medication, and chronic illness, such as chronic obstructive airways disease (COPD) [4, 20, 21]. Poor ventilator synchrony may also adversely affect sleep [20]. Higher acute physiology score, lower Glasgow coma scale (GCS) score, and level of sedation administration are associated with an increased risk of sleep disturbance [18].

Sleep is classified as non-rapid eye movement (NREM) and rapid eye movement (REM) sleep [20, 22]. NREM sleep may be further sub-divided into stages 1 to 4. NREM stages 3 and 4 (slow wave sleep) and REM are felt to be more restful periods of sleep [20–22]. In the non-septic critically ill patient, sleep is fragmented and distributed throughout the 24-hour period [4]. These patients also experience a relative predominance of stage 1 sleep and decreased or absent stage 2, 3, 4 and REM sleep [4]. Septic patients did not demonstrate clear sleep or wake periods in this study.

Sleep deprivation has important effects in the critically ill. Cognitive function may be impaired, which may contribute to confusion and delirium [20–22]. Slow wave sleep is important for memory formation, and amnesia is also associated with delirium [23]. Sleep disruption in the critically ill may cause increases in blood pressure [20]. In healthy subjects, sleep deprivation is also associated with impaired immune function and development of a catabolic state [20–22].

The role of noise in causing sleep disruption is becoming clearer. Sleep laboratory experiments in healthy subjects indicated that exposure to critical care unit noise is associated with poorer REM sleep [24]. In a small study of patients on an intermediate respiratory care unit, the number of nocturnal peaks in sound level correlated well with the number of arousals from sleep [25]. Studies using polysomnography have found that noise was associated with 26% of awakenings from sleep per subject among 22 medical ICU patients, of whom 20 were mechanically ventilated [4]. However, sound elevations were responsible for only 21% of all arousals and awakenings among 7 ICU patients and 6 healthy subjects with 68% of awakening and arousals in the critically ill being unexplained [8]. This questions the significance of noise as an additional contributor to sleep disruption in the severely ill. Degree of disruption caused by noise seems to be unaffected by age, sex, duration of ICU stay, or severity of illness [8].

Patients report that sleep obtained in the ICU is significantly worse than that normally experienced at home [19]. Measurement of vital signs and phlebotomy are perceived as more important causes of sleep disruption than noise [4], but interventions occur at least hourly to adult ICU patients [3]. It is possible that noise causes arousal, but not awakening, and that the cause of arousal is not recalled [19].

Patients report that staff communication is the most irritating noise [8, 19]. Alarms also seemed to be perceived as disruptive to a degree out of keeping with evidence of disruption from polysomnography. However, the proportion of patients recalling noise post-ICU is variable, possibly depending on case mix, e.g., only 11 out of 298 patients (3.7%) recalled noise in one Australian study [26].

Other Effects of Noise on Patients

Noise has been shown to be associated with cardiovascular stress in some patient groups. Poorer sound acoustics are associated with relative increase in pulse amplitude among coronary care patients and a higher incidence of readmission following acute coronary syndrome [27]. Noise may also increase requirement for analgesia and sedation among ICU patients: A direct relationship was found between analgesia administration and level of ICU noise [9]. For patients sedated with propofol prior to surgical procedures, significant arousal may be seen in bispectral index monitoring as a response to noise [28].

Hearing loss is an under-recognized complication of critical illness, to which noise makes a significant contribution and the elderly are particularly susceptible [29]; a third of American adults over the age of 70 years report hearing impairment [30]. Noise also interferes with communication between patients, relatives, and care providers in the ICU. The effort expended in perceiving speech by hearing-impaired older adults may also impair retention of speech content [31]. Noise causes more disruption of speech processing tasks among the elderly and hearing impaired than in young adults with normal hearing [32]. The elderly and temporarily or permanently hearing impaired are, therefore, among the least likely ICU patients to understand their environment.

Noise is not a recognized risk factor for the development of delirium in the ICU [33], which may be defined as an acute change in mental status, plus inattention and disorganized thinking or altered level of consciousness [34]. Risk factors for delirium include age and pre-morbid cognitive function [34, 35], physiological disturbances (for example, infection, central nervous sytem pathology and metabolic disturbance) and pharmacology (including withdrawal) [33]. However, delirium is a multi-factorial process [35], and factors such as hearing and visual impairment [36] and sleep deprivation may also play a role [35]. Noise may contribute to abnormal perception among critically ill patients, and particularly those with restricted hearing, an increasingly common problem in the elderly patient. In the general population, paranoid delusional thinking is associated with hearing impairment [37]. Psychotic symptoms are found more frequently among psychiatric patients with hearing impairment than among healthy subjects [38]. Deafness is also associated with experience of delusions among patients with dementia [39]. It is at least feasible that noise, which disrupts sleep, memory formation, and cognitive function, and interferes with comprehension of one's surroundings, may contribute to delirium in the critically ill. It is also understandable that the individuals directly responsible for much of the ICU noise, namely health care workers, may be perceived in a somewhat threatening light. ICU patients may close their eyes but it is impossible for them to shut their ears. For some patients hearing sounds that remind patients of the ICU can be a potent trigger for flashbacks and physiological arousal when they go home [23].

Effects of Noise on Staff

In addition to effects on patients, noise may affect the health and performance of health care professionals in the ICU. The prevalence of hearing impairment among health care workers is unknown. In the UK, approximately 2% of working adults report severe hearing difficulty, though tinnitus is reported far more frequently [40].

Hearing impairment is unlikely to occur with an energy averaged LAeq8h of 75 dB(A) [1], which is greater than that previously reported from ICUs. The impulse noise limit for adults has been set by Directive 2003/10/EC at 140 dB(A), again higher than reported peaks from ICUs [5].

However, increases in noise are associated with significant increases in objective (heart rate) and subjective (stress/ annoyance ratings) measures of stress in pediatric ICU nurses [12]. The members of staff in coronary care units have reported feelings of greater work-related pressure in conditions with poor acoustics [7]. Noise-induced stress was identified as a significant risk factor for 'burnout' among critical care nurses [41]. Both within and outside of the health-care setting, the effects of noise on performance include annoyance, anxiety, poor concentration, and impaired execution of tasks, particularly complex tasks [5, 12]. For example, anesthesia residents subjected to noise performed more poorly in tests of mental efficiency and short-term memory [6]. In laboratory experiments, lack of control over noise may diminish a tendency to help out other individuals [42]. Indeed, coronary care patients have reported finding the attitude of staff much better in conditions with good acoustics [27]. Higher background noise may impair communication between health care workers, and may in itself lead to an elevation in amplitude of speech (the 'Lombard effect'). This has implications for patient confidentiality, and may predispose to accidents as a result of a combination of impaired communication and poor concentration.

Minimizing the Impact of Noise in the Intensive Care Unit

Noise can disrupt patient sleep, cause stress in patients and their carers, and significantly disrupt communication, but there are a number of ways in which the impact of noise in the ICU may be diminished.

The largest proportion of noise generated in the ICU seems to result directly from human behavior, for example from talking or television [11]. Theoretically, the most effective change to minimize noise disruption would be an educational program that raised awareness of the problem and was followed by the implementation of measures to limit noise and activity during rest periods. Such programs have failed to show consistent or dramatic results. Conducting teaching sessions intended to minimize staff communication close to patients' rooms failed to reduce noise on surgical wards and in a surgical ICU [17]. Introduction of guidelines to limit noise, light, and patient activity in a surgical ICU (closure of all doors, reduction in alarm intensity, limitation of nursing intervention, television, radio, telephone and conversation between 11 pm and 5 am) led to a slight reduction in energy average sound from 51 to 48 dB [43]. A behavioral modification program, which involved change of nursing and medical routines and the introduction of non-disturbance periods, resulted in a reduction in disturbance activity and noise levels in a neuro-ICU [44]. Reduction in sound peaks greater than 80 dB(A) was also seen as a result of a behavioral modification program introduced to a medical ICU, and followed up by spot checks [11]. In order for behavioral-modification programs to succeed, there needs to be enthusiasm from health care staff for a project. Re-organization of nursing and medical activities may strengthen the educational message, and frequent re-evaluation, education, and feedback may be required to reinforce the behavioral change. However, simple changes such as those instituted by a critical care nurse who was a patient in her own unit [45] may help. She remembered being woken from sleep in ICU by bin

lids crashing down and when she returned to work organized for all the bins to be replaced with ones where the lid came down very slowly. However, each patient is individual in their tolerance for and in how they view noise. Some patients like the reassurance of hearing alarms and having people talking around them because they feel safe.

The design of ICUs may influence disruption caused by noise. Single rooms may reduce the transmission of noise from outside the room, but will also inhibit dispersal of sound from within [12]. However, the needs of critical patients and those in the recovery phase may be different and providing quiet areas, with a clear transition point to remind staff, for longer stay patients in the recovery phase may benefit. Use of single rooms, for example, may be appropriate for the patient who has stabilized following the early stages of his or her critical illness, is trying to re-establish normal circadian rhythm, and for whom requirement for intervention is not particularly intensive. In uncomplicated patients, equipment noise may be minimal and it may be possible for alarms to register at a central nursing station rather than in the room itself. Reducing the sound reflectivity of surfaces and echo propagation, for example by replacing ceiling tiles with sound-absorbing ones, improves speech intelligibility, reduces stress among health care workers and patients, and reduces readmission of patients after acute coronary syndrome [7, 27]. Positioning of entrance doors, central nursing stations and separate hand-over rooms away from patient beds may also reduce noise. Efforts could also be made to reduce noise production by equipment such as high-frequency oscillator ventilators.

In healthy subjects and cardiac patients, other attempts have been made to minimize the impact of noise. Exposure of healthy subjects to white noise increased their arousal threshold in response to ICU noise, the change from baseline to peak seemingly more predictive of arousal than the absolute peak sound level [46]. Again in healthy subjects, use of earplugs attenuated the disruption of the sleep cycle caused by exposure to ICU noise [47]. In patients recovering from cardiac surgery, music intervention reduced annoyance scores and sympathetic response to noise [48]. However, use of these techniques for general medical or surgical ICU patients, in whom the prevalence of cognitive or hearing impairment is likely to differ, is so far unreported. Use of hearing aids where appropriate in daylight hours may in fact improve communication of patients with staff and relatives, though the background noise of the ICU may also be amplified. However, with the increasing use of digital hearing aids, which boost only the frequencies the patient needs, this is likely to be less of a problem.

Conclusion

Noise is a common problem in ICUs. It has significant adverse implications for the health and well-being of patients and staff. All previous studies of ICU noise have reported levels that exceed the WHO and Environmental Protection Agency (EPA) recommended limits for hospitals. The 2003 European directive relating to exposure of workers to noise obliges employers to evaluate and control sources of noise. Efforts to reduce the impact of noise in the ICU should use a multi-faceted approach, modifying staff behavior and practices, minimizing the disruption caused by equipment and alarms, and optimizing design of the ICU. However, probably the most important aspect to this is to highlight to those involved in the care of critically ill patients of the consequences of their behavior on patient comfort.

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Alarms: Transforming a Nuisance into a Reliable Tool

G. Murias, B. Sales, and L. Blanch

Introduction

Up to 77% of admissions to medical intensive care units (ICUs) take place, at least in part, for monitoring purposes, even though only 10% of the patients monitored will subsequently have indications for major interventions [1]. Modern ICU equipment takes advantage of a wide range of technologies to track physiological variables in order to detect changes that could be life-threatening. As response time is a key issue, most of these devices are equipped with a more or less sophisticated set of alarms that alert intensivists, nurses or respiratory therapists about changes that could represent a risk to patients.

Description of Alarm-related Problems

The technologies used for monitoring purposes are variable in terms of accuracy and reproducibility and some of the signals produced are very poor. Even in the operating room – where the patient is usually deeply sedated and muscle relaxed – many of the signals monitored are loud. Kestin et al. found that only 3% of the alarms activated in the operating room represented a risk for the patient [2]. The situation may be even worse in the ICU, where patients are usually awake and sometimes agitated. In a prospective observational study in a pediatric ICU (PICU) [3], Lawless found that less than 6% of over 2176 alarms activated were clinically important. The authors found a positive predictive value of 7% for pulse oximeter alarms, 16% for end-tidal PCO_2 alarms, 3% for ventilator alarms, and 5% for electrocardiogram (EKG) alarms. In another study in a surgical ICU [4] Bentt et al. found that pulse oximeter alarms could sound for 28 min/hour (nearly half of the time). Tsien and Fackler [5] found that 86% of 2942 alarms activated in a pediatric ICU were false alarms and an additional 6% were considered clinically irrelevant. Positive predictive values for the various devices ranged from $<1\%$ for the pulse oximeter to 74% for the arterial catheter mean systemic blood pressure signal.

With a mean of 4.3 devices/patient [6] and up to 10 independent alarms/device [7], there are many concerns about the high rate of false positive alarms in the ICU:

1. The boy who cried wolf. As in Aesop's fable, members of staff become unresponsive after numerous false alarms. In 2002, the Joint Commission on Accreditation of Health Care Organizations [8] reviewed 23 reports of death or severe injury related to mechanical venilation. Alarms were involved in 65%, with delayed or no response or failure to activate or set up equipment correctly

being cited in most cases. This is truly significant, considering that not a single case of ventilator malfunction was found. A survey of 23 cardiac anesthesiologists and cardiac surgeons showed that only heart rate and arterial blood pressure alarms were regularly set, while all other cardiovascular alarms were apparently disabled. Moreover, the alarm limits were so extreme that even definitely dangerous situations would not result in an alarm. Despite these settings, the clinicians would tolerate an alarm for up to 10 minutes before taking action [9].

2. The noise level. Balogh et al. [10] found that the sound pressure level to which ICU patients and staff were subjected was over the permissible noise exposure of 45 db recommended by the US Environmental Protection Agency at all times, both during the day and at night. Alarms were the cause of the most irritating noise with peak levels of up to 90 db (a sound pressure level equivalent to a major road at 10 m distance) [11]. The poor quality of ICU patients' sleep correlates with the noise level [12, 13]. Noise has even been related to burnout among ICU nurses [14].
3. Costs. Dealing with false alarms is a waste of human resources and money. An ICU bed costs up to 8 times the amount of a bed in a regular hospital ward [15]. Noseworthy et al. [16] found that human capital accounts for the main part (64%) of ICU costs, whereas supplies and equipment capital were responsible for less than 13%. So, even when direct monitoring costs are relatively low, the cost of the human resource dedicated to monitoring could be of major importance.

With alarms being so deficient, do we really need them? The answer to this question is supported by only indirect evidence that mainly comes from the operating room. During the 1980s, anesthesia-related deaths went from 25–50 per million [17] to 5 per million [18, 19]. The first international symposium on preventable anesthesia mortality and morbidity was held in Boston in 1984. At that conference, preliminary findings were presented that examined closed malpractice claims against anesthesiologists in the state of Washington. The most common respiratory events that caused permanent brain damage or death were inadequate ventilation, esophageal intubation, and difficult intubation [20]. In the mid-1980s to early 1990s, the pulse oximeter and capnograph monitors came into widespread use [21]. A comparison of the claims for inadequate ventilation and esophageal intubation in the 1980s with those in the 1990s shows a decrease from 25% to 9%, which closely corresponds to the reduction in the number of cases of brain damage or death in the same period. Inadequate ventilation decreased significantly when either a pulse oximeter or capnograph was used. The capnograph reduced the proportion of claims for esophageal intubation. The portion of claims for difficult intubation was unchanged by use of the pulse oximeter and capnograph monitors [22].

The first 2000 incidents reported in the Australian incident monitoring study were analyzed with respect to the role of monitors used during general anesthesia. The authors calculate that, used on its own, the pulse oximeter would have detected 82% of incidents. Capnography alone would have detected 55% of the incidents and in combination with the pulse oximeter 88% would have been detected. The addition of a blood pressure monitor would increase the detectable incidents to 93%, and an oxygen analyzer would produce an additional 2% improvement [23].

The answer obviously does not lie in using fewer alarms to reduce the number of spurious alarms. What we really need is better alarms. Figure 1A shows a typical

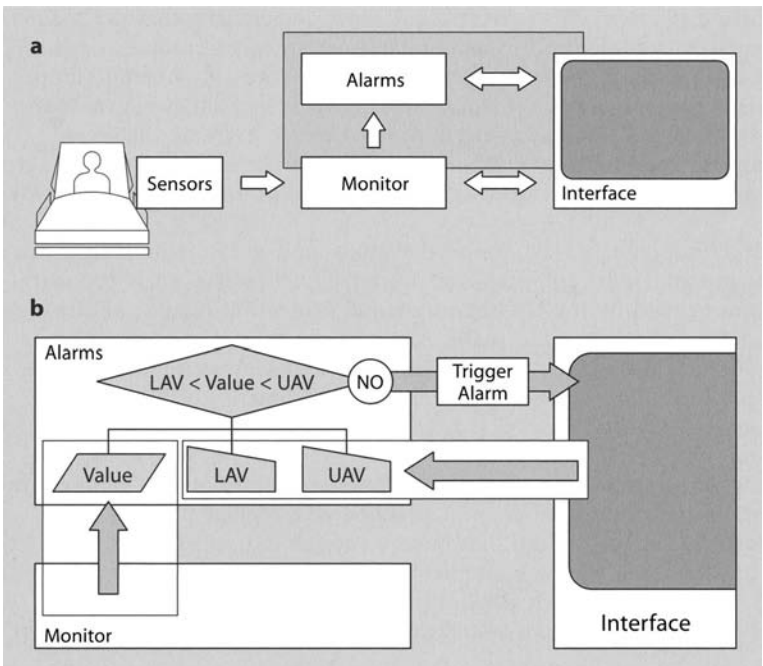

Fig. 1. a Typical topology of the monitoring/alarm system. Monitors track patient status using a variety of sensors and transducers. Signals coming from transducers are conditioned and digitalized, and then presented to the user. **b** Alarm layer behavior in a closer view (see text for details). (LAV: lowest allowable value; UAV: highest allowable value)

topology of the patient-monitor system. Sensors close to (or even inside) the patient modify their physical or chemical characteristics in response to a change in the patient's status. The signal arrives at the monitor where it is usually conditioned, filtered, and digitalized. Finally, the information is presented to the user through an interface.

Alarms represent a layer in monitoring equipment intended to track monitored variables. An algorithm decides when to trigger an alert to the user. Figure 1b represents a closer look at the shaded area in Figure 1a. It shows the typical behavior of an alarm layer: a value coming from the monitor layer is compared with two values that are usually set by the user, the lowest allowable value and the highest allowable value, and the user is alerted each time the measured value is outside the permitted range. Actually, signals are affected by both random (noise) and non-random error (bias). The latter is usually not a problem, as it is overcome by calibration. The former, however, threatens measurement reproducibility.

There are a few ways to overcome random error, although none is completely effective or applicable in all types of alarms. These include: (1) averaging repeated measurements; (2) filtering the signal; (3) fitting signals to known equations; or (4) improving the signal-to-noise ratio. The first method is widely used in low signal monitors, such as those for thermodilution cardiac output. As the values are dumped, random error tends to cancel out and this improves the signal quality; however, the monitor's time response tends to be poor [24]. The second method

Table 1. Possible patient/monitor system states. The 2×2 table shows the four possible system states while monitoring. False negatives (non-detected events) represent a risk for patient safety; they are not frequent, as the algorithms are designed to easily trigger the alarms. False positives (non-risky events detected as alarms) are, however, very common, and signify a concern in many ways (see text).

		Alarm	
		Yes	No
Real problem	Yes	True positive	False negative
	No	False positive	True negative

takes advantage of frequency filters to cut out the 'noisy' portion of the signal. Nevertheless, although sometimes the noise and the signal can be clearly separated, most of the time the frequency of the noise is within the boundaries of the signal frequency, so filters also eliminate a part of the signal and leave a part of the noise. Fitting signals to known equations gives adequate results, but this technique is limited by the availability of a fitting equation. Improving signal-to-noise ratio is the best option, as all the information in the signal is preserved. However, even though some advances have been made along these lines [25, 26], limitations inherent to sensor technology preclude the complete resolution of the problem.

When one of the parameters monitored lies outside of the allowed range, current monitor algorithms 'decide' whether or not to trigger the alarm (the world of current monitors is black and white). Table 1 is a typical 2×2 table showing the possible situations. As the failure to detect a potentially hazardous phenomenon (false negative) is considered to be a serious problem, control algorithms are designed to trigger the alarm easily. However, the combination of low-quality signals and 'soft'-triggered alarms produces an impressive number of false alarms (false positives).

To date, signals have been analyzed as if they were not related. This is far from the reality of the situation. Imagine you are at the bedside trying to determine if something is going wrong with your patient. You find that his heart rate is 110 bpm. Looking at the trends, you see that blood pressure – although still inside the allowed range – has been falling as has pulse oxygen saturation and mixed venous oxygen saturation (SvO_2). You carefully inspect the arterial pressure trace and see a marked pulse pressure variation in phase with ventilatory cycles and consider a fluid challenge. This is the kind of scenario in which intensivists take clinical decisions. The world of the ICU patient contains many shades of gray.

Alarm Troubleshooting

One alternative approach to the current paradigm is the development of expert systems. An expert system has two main components: An interpreter (the software) and a knowledge base (a system of related concepts that enable the computer to approximate human judgment). An expert system uses available information, heuristics, and inference to solve problems. Instead of attempting to define situations in terms of 'True' or 'False', the logic of an expert system analyzes the problem in terms of 'degree of truth'. If the rules can be defined, computers provided with expert systems can do a job in a way quite similar to the way people do it. Some efforts at using expert systems to improve alarms performance have been impressively successful [27–29]. However, the need to define a set of rules has confined expert systems to limited, controlled environments.

A newer approach is the use of data mining techniques. Data mining is the process of automatically searching large volumes of data for patterns. Data mining techniques extract implicit and previously unknown information from data. Data mining can create rules. An example could help to clarify this point. Imagine you are in charge of a supermarket. You wonder how to increase your profits. You consult a marketing specialist (expert systems approach), who tells you to place your high-profit beer close to the high-profit snacks. He/she knows the rule: 'People who buy beer usually buy snacks'. Another approach is to analyze your previous sales (data mining approach). Doing so, you find that chicken buyers usually buy onions. You don't know why; actually, you don't care why. However, you know that it could be a good idea to place your high-profit chicken close to your high-profit onion. Both approaches (expert systems and data mining) can be complementary.

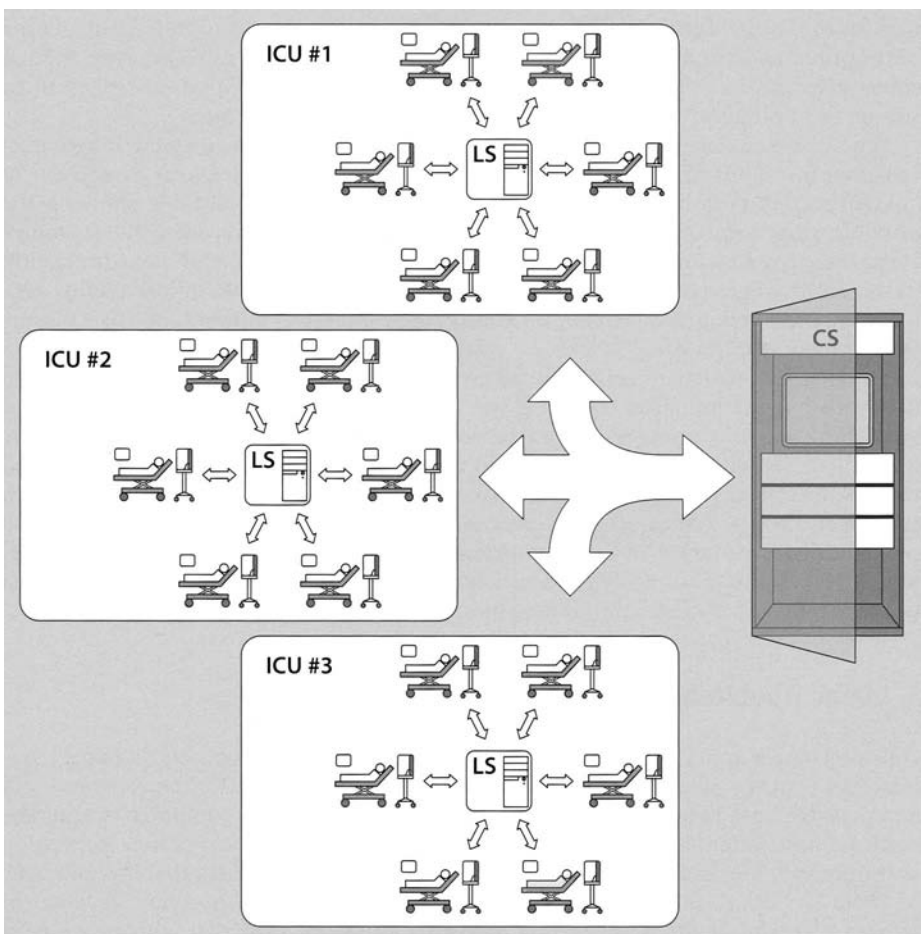

Fig. 2. Alternative topology of the patient/monitor system. A local server (LS) acquires data from monitors and mechanical ventilators where an expert system analyzes signals and, using rules from a knowledge base, decides to trigger or not to trigger alarms. A central server (CS) is committed to create new rules applying data mining to data grabbed by the LSs. The CS feeds the knowledge base from the expert systems with the new rules.

Let us return to the monitoring issue. Figure 2 shows a hypothetical group of ICUs. Each of them has a local server acquiring data from monitors and mechanical ventilators. The local server has an expert system with a basic set of rules that allows it to alert physicians when something is wrong. When an alarm is triggered, an interface allows the attending physician to gain information about whether the alarm is real (signaling a potentially risk situation), artifactual (a low pulse oximetry value due to a misplaced sensor), or factitious (e.g., a high airway pressure alarm in a coughing patient). Furthermore, local servers send information (data from monitoring devices and the attending physician's feedback) to a central server. The central server is devoted to data mining. It finds new rules from data and feeds the local server's expert system. After a while, the expert system has rules that a human expert could never have enunciated. For instance, 'pulse oximeter alarms tend to be false alarms if there is no concomitant change in heart rate'. Moreover, when an event occurs, the central server has the records from the previous occurrences. A rule like 'if heart rate variability decreases, the patient WILL fibrillate' could be stated, allowing the expert system to alert physicians about events that have not yet actually occurred.

Closing Remarks

More than 20 years ago, Hudson [30] pointed out that monitoring must include a human component for data assimilation and proper judgment, that is, for turning crude data into useful information. However, the amount of data present in today's ICU is so huge that it can overwhelm intensivists. Moreover, clinicians need to understand what the data mean to take full advantage of them. An expert system, acting as an interposed layer, could reduce the quantity of data to be analyzed immensely, saving the ICU team time and helping them to solve problems. It can be argued that a highly trained intensivist could perform better than a computer at detecting adverse effects and complications. Even so, there is a dramatic shortage of critical care specialists [31–33] and this situation will probably worsen in the future [34]. Second, this can be a time issue. Most expert systems learn and improve their performance continuously and can, with time, do better than humans. Automobile antilock braking systems (ABS) shorten stopping distance even when the driver is a very skilled professional. In fact, they are so efficient that cars in many countries must have one by law, and even if you are Michael Schumacher, you cannot disable it!

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Ethical and Legal Dilemmas in Accessing Critical Care Services

N.M. Danjoux and L. Hawryluck

Introduction

There is a growing need for critical care services, with increasing demands due to demographic trends, technological advances, growing costs of standard care, unexpected surges in demand, and public expectations. With the practice of critical care medicine evolving so rapidly due to the ability to sustain lives indefinitely, patients are living longer in the intensive care unit (ICU). As a result, patients and caregivers are faced with difficult decisions, often based on differing opinions on the appropriate use of newly emerging, potentially life-sustaining, yet expensive interventions. When conflicting views are held, their resolution can place undue strain on families and caregivers. To help deal with these situations, some institutions have developed guidelines and policies to reflect best standards to help guide difficult decisions about limits to treatment. However, there is no consensus on the use of such guidelines and their application in practice.

Fair, equitable, and reasonable access is a common goal across ICUs and is important in demand forecasting, subsequent planning and investments of healthcare resources. Establishing best practices can help other ICUs deal with similar challenges. Active involvement by all individuals in decision-making processes, resolutions, deliberations, and policy-making at a systems level is important and must be implemented locally based on the specific needs of an institution or community. If this does not occur, we risk reaching a point where marginal gains to individuals threaten the welfare of the majority.

Background

The ability to sustain the lives of critically ill patients poses very important ethical considerations for clinicians and individual members of a multicultural society. Most of these considerations center around one fundamental dilemma – given our capacity and ability to prolong life, does this mean we should? Concepts and notions of dignity, suffering, benefits and burdens, cultural and religious beliefs, individual and collective values and ultimately quality of life issues are central in this widely debated dilemma. These concepts must be weighed into all decision-making processes to ensure that individual patients' values and beliefs are respected. Yet there is another dimension to this central dilemma that is not discussed as often: How is such respect for autonomy and multiculturalism balanced with the need to ensure access to a limited critical resource for others in a just society?

Understanding belief systems held among various cultural and ethnic backgrounds is critical for healthcare providers. Cultural and religious beliefs often impact decisions made regarding advanced treatment options [1] and it is not uncommon for patients or their families to disagree with advice and treatment provided. This issue is particularly important in multicultural societies where clinicians are expected to have insight into the needs of such a diverse population. Addressing concerns among individuals from various backgrounds and belief systems regarding end-of-life care and technology is a complicated and sensitive issue that further impacts the decision-making process.

Questions arise regarding the role of personal values and beliefs and the ability to provide equitable and reasonable access to limited services in a multicultural society. Out of respect for the diversity of views and ideas that surround us, it would seem ethical to mandate a case-by-case approach for determining the appropriateness of using life-sustaining interventions. However, in the light of resource constraints, the feasibility of fully respecting all beliefs, wishes and hopes, no matter their degree of reasonableness, is legitimately brought into question. Yet, is it even realistic to attempt to develop consensus on key values to guide the appropriate use of critical care services given the diversity of opinions, including those grounded in cultural and religious identity?

What do Patients want at the End of Life? Lessons Learned and Remaining Challenges

Advance care planning provides a voice for patients in the event that they become incapable and unable to express their wishes or make their own decisions. While such initiatives have been helpful, they are not commonly used by patients [2]. Even when a substitute decision-maker is appointed, many do not discuss their values or beliefs nor their wishes regarding medical interventions [3]. Moreover, when it comes to life-sustaining interventions, there is a general lack of understanding of what such interventions entail, the risks and discomforts involved, and what they can and cannot do. Patients' perceptions of life-sustaining treatments differ from providers with different understandings of outcomes and care goals [4]. Efforts are being made to develop tools to help improve public education on life-sustaining and palliative interventions [5]. However, the effectiveness of these interventions in making more informed decisions and their indirect ability to contribute to subsequent utilization of critical care services still remain to be seen.

Research has shown that quality end-of-life care and advance care planning serve as a means for patients to avoid unnecessary prolongation of dying, to achieve a sense of control, alleviate burden, and strengthen relationships with loved ones [6]. More recent studies have also highlighted the importance of trust in the treating physician, avoidance of unwanted life support, effective communication, continuity of care, and life completion [7]. The importance is in individualizing such care and decision-making [7].

The emphasis in these studies seems to indicate patients' desires to avoid life support and not to use such interventions to sustain life at the cost of quality nor to solely prolong the dying process. What remains unknown is whether these beliefs regarding life-sustaining interventions change as death approaches, how cultural and religious beliefs influence such perceptions regarding technology and end-of-life care, and how these views are altered in a broader multicultural society. If changes

regarding wishes for life-sustaining interventions do occur, their timing in a given person's illness, what drives them, and how they are negotiated with healthcare teams, remain unknown.

Advanced care planning has also given rise to other ethical concerns [8]. Medical teams are mandated to turn to families and substitute decision-makers who are placed in the difficult position of having to assist in complex, high-stake decision-making during emotional and stressful times. This model of shared decision-making is the preferred one among families in North America [7], yet such participation places a tremendous burden on families, often leading to anxiety and depression [9]. Whether faced with the potential or imminent loss of a loved one, substitute decision-makers/families will often cling to any hope for survival and decisions often do not reflect those that would have been taken by patient if he/she was still capable [10].

The Appropriateness of Life-sustaining Interventions

It remains difficult to quantify and define what constitutes an appropriate versus an inappropriate use of life-sustaining interventions despite many previous efforts [11]. What is known is that the proportion of ICU deaths that involve withholding and withdrawing life-sustaining treatments is high and has been increasing in ICUs around the world [12–16]. Such practices are supported by ethical principals of autonomy, beneficence, and non-maleficence [17]. Justice, in contrast, is not mentioned as often. Inappropriate or futile treatments are usually broadly defined as encompassing treatment whose chances of benefit are very poor, treatments whose benefits are outweighed by the burdens involved, and treatments that only serve to prolong the dying process. ICU admission can, therefore, be considered inappropriate when it is deemed to cause additional suffering without providing a reasonable hope of survival (with the corollary caution that what is a reasonable chance of survival remains another source of debate). Such inappropriate treatment is arguably unethical, yet research indicates that 87% of physicians and 95% of nurses recalled providing it in recent practice [18, 19]. It is not uncommon for physicians and other health professionals to lack understanding of the laws that govern withholding and withdrawing life support and how to satisfy legal requirements in their practices [20].

Some clinicians provide care that others consider to be futile due to prognostic uncertainty [18]. Numerous clinical tools exist (APACHE II, SOFA) to help predict the likelihood of mortality for populations of critically ill patients, yet clinicians require more reliable predictors to help them make recommendations for individuals themselves and to guide triage decisions about ICU admissions [21]. While research has indicated that some scoring systems may be effective as triage tools, their use in such a manner is currently not widely accepted in practice. Clinicians are heavily influenced by internal factors when deciding to admit a patient to the ICU: Gender, experience [22], religious beliefs [23], strength of religious conviction [24], personality type [25], pressure from the referring clinician [19, 26, 27] and area of specialization [22] have all been found to be correlated with their likelihood to pursue life-sustaining treatments for their patients.

To date, no study has determined how frontline multi-professional members of an ICU team would define the appropriate use of critical care services. What should be the goals of an ICU admission? What is considered to be a 'good' or a 'bad' outcome

following ICU admission? In a morally pluralistic society, are there any common values that guide our decisions to pursue or withhold life-sustaining treatment? To date, these questions remain largely unanswered.

Conflicts in Decision-making

Conflicts are common in the ICU setting [28] and can be defined as a disagreement related to the management of a patient in the ICU involving more than one individual and requiring some decision or action [28]. Conflicts often occur: a) between healthcare providers and families, characterized by lack of communication, family dissatisfaction and trust [29]; b) among team members related to staff behavior and lack of leadership and coordination; and c) intra-family conflicts, frequently reported at the end-of-life [30, 31]. Conflicts in decision-making over appropriate treatment plans was found to be the single most troubling ethical issue in healthcare [32].

While conflicts that occur in the ICU are common, they have not previously been well documented in the medical literature. In studies describing conflicts in the care of patients with prolonged ICU stay, nearly a third of patients have conflicts associated with their care. Of these, approximately half occurred between the ICU team and family members, while over a third occurred between ICU team members [28, 31]. The most common types of conflicts occur in the decisions to withdraw or withhold treatment [31]. There is inconsistency in the healthcare teams' identification of and responses to such situations, and there is variation from one institution to the next. Conflicts between the ICU team and referring healthcare teams at the institutional level are even less well described.

Questions arise as to whether hospitals should, and, in fact, whether they need, to become more active participants in the future resolution of intractable conflicts. In a study reporting how ethical conflicts are experienced, it was found that there is wide variability in the proportion of conflicts that nurses believed were resolved, ranging from 25–70% [33]. When conflicts occurred between nursing staff and physicians, nurses described their organizations as being non-receptive. In addition to the perceived organizational reluctance to deal with physicians and the expressed lack of access to ethics committees, these conflicts were seen as irresolvable in the nurses' minds [33].

To date, no data are available on exploring the hospitals' perception of their role in these situations, the barriers preventing them from becoming more engaged and/or supporting their staff, and the ways in which they could become more active participants in addressing conflict situations in both the early and late stages. We lack an understanding of how conflicts in the ICU are resolved and the perceived effectiveness of available policies and procedures in contributing to such resolution when there may be discordance between clinicians and families, caregivers, or administration.

The complexities and tensions in today's ICU settings will undoubtedly continue as demand for ICU care increases, and even escalates, providing fertile ground for conflict among healthcare professionals and between these professionals and families. Cases of intractable conflict result in considerable expenditure of resources, both medical and legal, and are emotionally and psychologically draining for those involved. While research into the sources of conflict is lacking, some hospitals have enacted policies that suggest ways to deal with them in the clinical setting, at least

in the early stages. While most of these policies provide suggestions for resolving conflicts between the ICU team and patients or their families [34–36], no guidance is given for resolving conflicts among healthcare teams nor is guidance provided for conflicts that remain intractable. Not surprisingly therefore, if and when conflicts arise regarding initiating or continuing life-sustaining interventions, the process and policies to resolve them at the hospital level and the ways in which such policies advocate that power be wielded or not, are inconsistent.

Hospital Policies

The establishment of institutional guidelines and policies regarding resuscitation and appropriate use of critical care resources may reduce the likelihood of prolonged court battles as well as the imposition of poorly understood professional standards for decision-making at the bedside. But, in general, they do not guarantee protection from legal liability. Efforts at conflict resolution are commendable on many levels, however, little attention has been given to the potential negative effects of such policies upon particular groups. For example, it is proposed that the use of institutional policy to break decision-making barriers will fall negatively on certain groups who may prefer more aggressive interventions [37]. The involvement and application of institutional policies should be evaluated in the context of historical and current experiences of marginalization and disempowerment [38]. Furthermore, most policies, despite their approaches to engage multiple levels of appeals and consensus, typically grant final decision-making to providers and institutional representatives rather than to care seekers [38].

The implementation and use of policies is an ongoing challenge, and some decision tools may in fact impair communication and appropriate decision-making [39]. Ultimately, there is no agreement as to the proper guidelines to follow to help guide appropriate decision-making in the ICU. It is, therefore, important to investigate issues at the local level to determine what issues are common. With indiscriminate use of such policies, the challenge arises when difficult decisions require guidance, and the means to resolve them becomes difficult. Additional research is required to investigate decision tools, their use in practice, impact on clinical practice, quality of care and outcomes.

A Canadian Initiative: Experiences from Ontario

The challenges discussed above are common among ICUs around the world despite differences in demographic, economic and social factors. In Canada, the province of Ontario has embarked on a comprehensive strategy to improve the delivery of critical care services. The Ministry of Health and Long-Term Care is leading several innovative initiatives, one of which is dedicated to investigating ethical issues of access to critical care. To our knowledge, the government of Ontario is the only government providing a platform for critical care professionals to discuss such dilemmas outside of the confines of the critical care field and attempting to provide some initial resolution to these common challenges.

The goals of this initiative include: 1) improving consistency in the use of life-sustaining interventions; 2) understanding current challenges and exploring the means to improve conflict resolution processes; and 3) developing means to ensure fair and

reasonable access to critical care services, respecting multicultural values and beliefs within a just society given the reality of the demographic issues we face.

Methods for the Ontario Approach

To achieve these goals, initial background research is required. Despite the growing body of literature in end-of-life ethical dilemmas, there is a lack of information regarding quality of care issues, decision-making and resource allocation, and current practices and beliefs among frontline workers. Additional considerations relevant to Ontario's diverse population are also required. Ontario is a very multicultural society, with immigrant populations forming a significant and increasing proportion of the province's population [40].

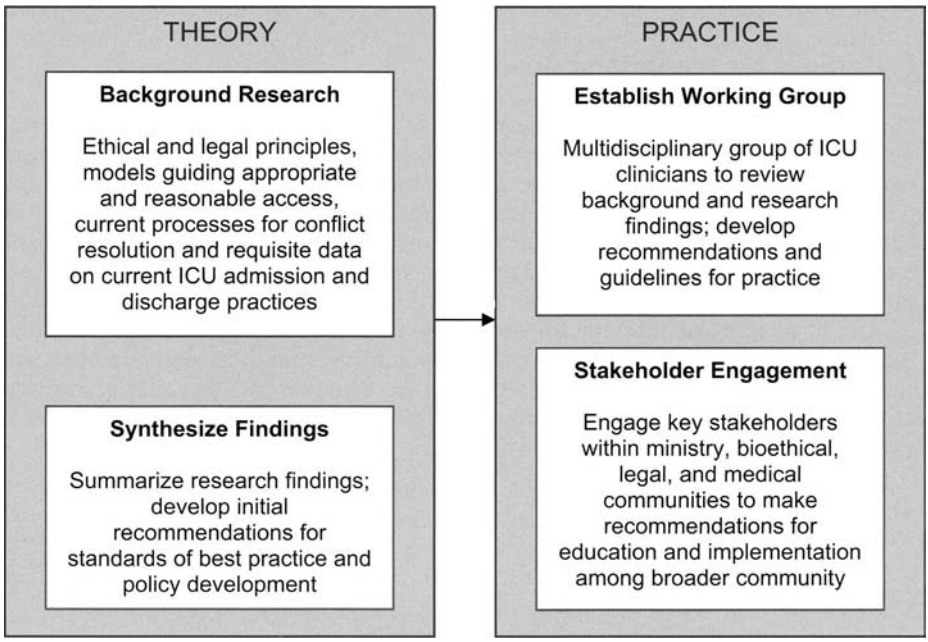
A total of thirteen projects are underway, which taken together, will ensure an understanding of current practices regarding admission and discharge to ICUs and explore ethical and legal principles guiding appropriate and reasonable access to critical care services. In broad terms, these will explore:

1. Existing hospital admission, discharge, and resuscitation policies to ensure a clear understanding of their scope and content.
2. Perceptions of healthcare providers to understand the nature, etiology and outcomes of conflicts in the ICU from the perspectives of clinicians, hospitals, ICU administrators, and risk managers. In Ontario, conflicts in decision-making that cannot be resolved proceed to involve the legal system through organizations that provide patient advocacy and support (Office of the Public Guardian and Trustee) and an independent provincial tribunal (Consent and Capacity Board). Understanding the challenges encountered by these legal professionals is crucial for potential change and improvement; therefore, their perspectives are also being captured in this background research.
3. Definitions of appropriateness of care, and what it means from the perspectives of the multidisciplinary members of the frontline ICU team.
4. Technology utilization and its perceived use by different cultural and religious groups in end-of-life situations. An appreciation of how such perceptions change and are influenced by others living in a multicultural society is also being explored.
5. Common law and Canadian Charter of Rights and Freedoms cases and a review of legislation in other jurisdictions. Implications for future policy development will be considered.

To study the prevalence of conflicts in the ICU, some hospitals in Ontario have joined the international Conflicus® study, designed by the Ethics Committee from the European Society of Intensive Care Medicine to report the incidence of ICU conflicts and identify potential targets for their prevention.

At the conclusion of these research projects, initial recommendations will be made regarding the means to improve consistency, eliminate obstacles and define a standard of care in the use of life-sustaining interventions and to improve the ethical and legal means to resolve intractable conflicts in a fair, timely and practical manner. A working group, composed of critical care practitioners, bioethicists, and legal professionals, is being created to further devise a set of recommendations to propose a practical systematic approach to provide consistency in the everyday triage decisions faced by frontline workers. Further recommendations for implementation

Table 1. Ontario's plan towards the development and implementation of best practices regarding the appropriate use of critical care services



including education and awareness will be identified to facilitate this potential change between key professional and stakeholder groups. Education strategies would include the medical, ethical, and legal communities to teach best practices for the appropriate use of critical care services and the processes for conflict resolution. Investigations will be made into the potential for broader public education strategies to better inform advance care planning by increasing knowledge of life-sustaining, palliative interventions and the appropriate use of limited resources (Table 1).

Implications for the Critical Care Community

To improve patient outcomes and cost-effectiveness, ICU resources should be managed in a way that is most beneficial to the patients that need it most. The most important means to achieve this goal is, and will always be, through clinicians' communication and negotiation skills. Continuing education in such skills through formal continuing medical education programs and institutional staff development programs are key to the provision of quality care and should not be neglected [38].

Sound admission and discharge criteria are another approach to identify patients who will receive the most benefit from intensive care. Appropriate admission criteria caused a reduction in the number of low-risk patients admitted to ICUs by as much as 35% [41]. Their impact, if any, on the frequency of admissions deemed inappropriate is harder to find in current literature.

Once developed, the Ontario initiative to define best practices regarding the appropriate use of critical care services and the means to ensure equitable and rea-

sonable access for all members of a multicultural society can serve as a practical model for other critical care communities. To our knowledge, it is the first strategy to involve government, critical care, bioethics, and legal communities to systematically attempt to address the everyday challenges of triage decisions in the ICU setting.

In the past year, critical care groups have devoted interest in the development of triage policies for mass disaster situations such as the pandemic flu. The creation of such policies is arguably easier when considering pandemic preparedness, due to the extremely limited resources and the need to allocate them in a utilitarian way that ensures the greatest number of survivors. The 2006 Ontario Health Pandemic Influenza Plan (OHPIP) [42] is a collaborative effort that developed a critical care triage policy when critical care resources, especially ventilators, are expected to face scarcities in the event of a pandemic [43]. While OHPIP's ultimate goal is to build pandemic preparedness across the province, the critical care component of its plan is an example of how to most effectively use critical care services when resources are exceptionally restricted. Yet the reality is that these guidelines have been developed for a situation that may never actually arise and little guidance is provided for situations in which front-line practitioners find themselves on a daily basis.

For everyday critical care service delivery, best practices are essential to ensure consistency and facilitate decision-making among healthcare providers and patients. Previous efforts to standardize best practices or develop guidelines for critical care have failed to provide detailed recommendations in the event of intractable conflicts and in light of cultural values. The initiatives currently underway will draw upon various constituencies to ensure a broad range of stakeholders' opinions is incorporated into the recommendations to improve future adherence.

The Ontario initiative will also build increased understanding of cultural differences and beliefs regarding end-of-life care, in particular those beliefs related to the use of life-sustaining interventions. Such information is not easy to find and could serve as a resource for others. Increased understanding and respect for cultural differences and the effect of these beliefs on treatment preferences will lead to improved communication and reduce conflict in the care of patients of all ethnic backgrounds [37]. At present, we do not have a fair and equitable way to provide care for patients in the ICU that respects the diversity of multicultural values. These values are dependent on the individual institution or population, and must also be addressed at that level.

Practical means and models will be provided to ensure that all members of the community are legitimate stakeholders in healthcare. These will describe ways to initiate appropriate collaborations between community members, policy makers, economists, managers, and health care workers. This work will seek to build an understanding and close knowledge gaps among professionals. To this end, the educational needs of legal, bioethical professionals, and community stakeholders around life-sustaining interventions will be explored and educational programs will be developed, which could be adapted for a global audience.

As with all policies and descriptions of best practices, the Ontario initiatives risk not being used or followed. It is suggested that policies include some form of a tracking process to monitor the particular circumstance in which they are utilized [38]. Developing this type of tracking across the province will be a challenge. Integration with current data monitoring systems would seem to be a logical solution, yet the parameters for measurement are difficult to define. Feedback from frontline clinicians regarding the resolution of ethical dilemmas and conflicts in decision-

making would allow an exploration of the moral agency of caregivers, institutional mechanisms for the discussion of ethical conflicts and the ability to address the range of conflicts experienced by health professionals [33]. Such tracking could provide valuable information for future policy refinement and staff education as well as detecting whether utilization of such measures with particular groups is disproportionate. Steps could then be taken to address these concerns.

Conclusion

For everyday challenges encountered in critical care, best practices are required to ensure consistency among healthcare providers and patients. Efforts have been made to standardize care and develop guidelines but have failed in providing detailed recommendations in the event of intractable conflicts and in light of cultural values. In Canada, the province of Ontario has taken leadership in engaging the critical, bioethical, and legal communities to address the everyday challenges of decision-making in the ICU. Best practices regarding the appropriate use of critical care services and the means to ensure equitable and reasonable access for all members of a multicultural society will draw upon various constituencies to ensure the incorporation of a broad range of stakeholders' opinions. Once developed, these recommendations and practice guidelines may serve as a practical model for other critical care communities around the world.

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Emergency Care for the VIP Patient

E.C. Mariano and J.A. McLeod

Introduction

On March 30, 1981, a 70-year-old Caucasian male walked into George Washington Hospital emergency room in Washington, DC, complaining of dyspnea after sustaining a gunshot wound. The patient collapsed upon his arrival and was immediately brought to the trauma room where the emergency room team resuscitated him. Once the patient was stable, he was transferred to the intensive care unit (ICU).

Although this patient's injuries were typical for a gunshot wound to the chest, this was not a typical trauma patient. This patient was American President, Ronald Reagan. What was the experience like for the physicians in the emergency room and ICU who took care of the man leading the United States of America? President Reagan's physicians encountered many challenges not only because of the extent of the President's wounds but also because of the potential political, social, and historical ramifications of the medical care they were providing to him [1]. In many ways, the doctors at George Washington Hospital were taking care of the ultimate 'very important person' or VIP.

Any physician practicing in an emergency room or ICU setting at one time or another encounters a patient who is considered a VIP. A VIP draws special attention based on his or her status in society and level of importance as perceived by the health care professional providing treatment. How does a physician deliver appropriate medical care in the emergency room or ICU to a patient who is considered a VIP?

This chapter examines the unique aspects and challenges involved in the care of a VIP in the emergency room and ICU. The authors consider the treatment of a VIP patient to be unique in the medical realm because it defies the basic tenets and understanding of triage as applied in modern medical facilities. The VIP patient, because of their importance, bypasses the usual triage process and is given top priority over other patients. The special handling of the VIP patient not only induces the potential for inappropriate and ineffective care, but also perpetuates the VIP's expectation of high priority treatment in future medical encounters.

The authors provide a working clinical definition of a VIP patient and dissect the components of the 'VIP syndrome': The dilemma that entraps health care professionals into providing inappropriate medical care to the VIP. Citing historical examples of the VIP syndrome, this chapter offers a prescription for physicians to help them avoid the syndrome and instead to provide appropriate, effective care for the VIP as well as all patients.

Definitions

In lay terms, the acronym 'VIP' stands for 'very important person'. This term is often applied to heads of state, politicians, the wealthy, and celebrities such as movie personalities and rock stars. The VIP is a person who is known and recognized in society, i.e., a person who is considered famous or influential.

The authors believe that in the medical setting, the VIP acronym more appropriately stands for 'very intimidating patient' (Mariano, 1999, Medical Care to VIPs: Challenges and Lessons. Presentation to VIPMAC, Warrenton, VA). Using this definition, the VIP patient is any patient who intimidates the physician. How do you know if the physician is intimidated? Our clinical definition of a very intimidating patient is a patient who 'induces tachycardia in the physician'.

This leads to a symptom-based definition of VIP: Any patient who causes the physician to feel intimidated (i.e., experience anxiety or 'tachycardia'). This definition expands the field of potential VIPs beyond the high-profile or wealthy individuals, star performers and athletes, and current newsmakers. A patient, by this definition, may be a VIP if he or she is a friend, family member, or fellow physician. In truth, almost every physician has experienced a very intimidating patient.

Why is it medically challenging to take care of VIP patients? Because of their position and level of importance in the eyes of the physician, these patients have a high risk for receiving poor care. Thus arises a conundrum: Why are VIP patients at risk for poor care when as very important persons they should be receiving top care? The answer is the VIP syndrome.

The VIP Syndrome

The VIP Syndrome was first described in 1964 by Dr. Walter Weintraub in the *Journal of Nervous Mental Disorders*. In his article, "The VIP Syndrome: A Clinical Study in Hospital Psychiatry," Dr. Weintraub describes the VIP patient as an "influential person [who] was often followed by considerable turmoil within the institution" [2]. Weintraub observes that although such turmoil is expected, there was a "...gradual realization that few of the VIPs were responding favorably to treatment" [2].

What was lacking in VIP care that was present in the treatment of all of the other patients being treated? Putting it bluntly, the answer was: The VIP patients did not receive the usual standard of care appropriate for their condition. The VIP patients were victims of physicians trapped in the VIP syndrome.

The cartoon in Figure 1 entitled "The Doctor's Dilemma," illustrates what the authors identify as the four components of the VIP Syndrome:

- VIP – the presence of a very intimidating patient. In the cartoon case, it is the woman.
- Request (or a demand) for services as dictated by the VIP. In the cartoon case: "I'd prefer to have you examine me at home." The type of care and the timing of care are determined by the patient and revolve around his or her personal schedule.
- Deviation from regular practice or procedure. In the cartoon case: "But I never make house calls!"
- Intimidation. In the cartoon case: "On the other hand, she is my wife." The doctor wants to avoid the VIP's displeasure and strives instead to maintain or earn her approval.

Fig. 1. Illustration of VIP syndrome. By JB Handelsman, 1990, © The New Yorker Collection from cartoonbank.com. Used with permission.

What happens to physicians embroiled in the VIP syndrome? A common example arises when physicians treat fellow physicians. Lipsitt illustrates a poignant historical example of this situation and outcome – where the VIP's demands vitiated the physician's judgment and impaired the medical treatment:

"Rendering proper care to a friend and colleague is no easy task. Even Sigmund Freud, introspectionist extraordinaire, was hesitant to acknowledge his devastating cancer of the jaw, his addiction to cigars, his putative hypochondriacal, cardiac and gastrointestinal symptoms, or his migraines and sinusitis. His personal physician, Dr. Max Schur, provides an account of Freud as a different patient, with all the disagreements, distrust, physician shopping, and problems with professional courtesy that characterize the behavior of physician as patient. Indeed, Schur often reprimanded Freud for smoking against medical advice, but nonetheless entered a corrupt bargain to smoke a cigar with him socially even when this was not Schur's customary habit" [3].

A physician in thrall to the VIP syndrome faces a high risk of providing substandard care to their VIP patient. Ironically then, a VIP patient who expects to receive 'special care' actually receives poorer, even substandard, care. The VIP's celebrity status predisposes him or her to greater risks for morbidity and mortality.

In "Doctoring Doctors and Their Families," Schneck explores the topic of doctors treating other doctors or family members [4]. Dr. Schneck describes the VIP syndrome in action as medical providers intentionally circumvent administrative and medical regimens in order to minimize discomfort and inconvenience to the VIP. Overall, this scenario leads to "poor medical care and outcomes, confusion, and occasional hostility" [5].

History provides numerous examples in which famous people have received infamous care at the hands of physicians caught in the VIP syndrome. In "When Illness Strikes the Leader: the Dilemma of the Captive King", Post and Robins elegantly

illustrate how American as well as European heads of state have received surprisingly poor medical care [6]. The authors describe a prevailing atmosphere of secrecy, deception, and denial of the diagnosis of several American presidents and other national leaders.

- President Woodrow Wilson suffered a stroke while in office. Both his personal physician, Dr. T. Cary Grayson, and First Lady, Edith Wilson, hid the President from public view and repeatedly denied his disability. In President Wilson's case, the First Lady dictated the course of medical care.
- President Franklin D. Roosevelt suffered from hypertensive heart disease. His personal physician, Dr. Ross McIntyre, repeatedly denied his patient's illness and did not confirm this diagnosis with his patient or with members of the first family.
- President Dwight Eisenhower suffered a myocardial infarction while visiting Colorado Springs. His personal physician, Dr. Howard Snyder, instructed the patient to remain in bed and did not send him to a hospital for evaluation.
- Anecdotal articles that appeared in the press in 1999 described a Kremlin hospital surgeon who revealed that she had treated ailments of Soviet leaders – including Stalin's abscessed toe – behind a sheet in the Kremlin. The Soviet physician herself was not allowed to see or talk with her patient [7].
- In *The Private Life of Chairman Mao*, Mao Zedong's personal physician of 22 years, Dr. Li Zhi-Sui, vividly describes his dilemma and difficulties in taking care of his patient under a system in which committees of politicians dictated how he was to take care of his patient [8].

The four components of the VIP syndrome deserve additional consideration to better understand why VIP patients are predisposed to receiving substandard treatment:

1. The VIPs themselves are part of the problem. Because of their position and status, they expect special care. These patients may tend to be cavalier about medical advice that would limit their usual activities. Often these patients are noncompliant because they may seemingly believe that the usual principles of medicine do not apply to them. Some VIP patients give every indication that they think their celebrity confers immunity against the usual diseases. They may also be in denial about their own mortality and thus resist discussions about illness and death. Any physician who renders a medical opinion that conflicts with the patients' views may be dismissed and terminated. These patients often 'doctor shop' for a physician who is more likely to agree with them.
2. The patients try to demand or dictate their own care. The VIP patient (or their spouse or staff) may routinely tell the doctor how to practice medicine and provide health care. Typically the VIP is not a doctor and, therefore, does not have the appropriate medical background to recommend a treatment plan.
3. Deviations from the usual practice of medicine occur. The usual procedures are bypassed or abbreviated. Often when this occurs, details are overlooked that would have prevented more significant problems from developing later in the patient's care. Consider the frightening results of deferring the genital-rectal exam for a patient, when a timely comprehensive physical examination upon admission to the hospital would have identified a carcinoma of the genitourinary or gastrointestinal system.
4. An atmosphere of intimidation prevails. The physician strives to avoid disapproval and may actually be eager to court the approval of the VIP. The physi-

cian and the medical team may be subconsciously attempting to impress and please the VIP. Procedures and tests that are uncomfortable may be postponed or never done, in the effort to avoid subjecting the VIP to inconvenience or pain. The physicians may not want to tell their patients any 'bad' news. The goal becomes keeping the VIP 'happy'.

Therefore, when a physician 'expedites', 'facilitates', and otherwise avoids the routine 'inconvenience' that may be part of the usual standard of care, the VIP patient's risk increases. Any short cuts or abbreviated care can result in substandard and poor care.

Another way to view the issue: When the physician alters or discards the medically relevant procedures, then the VIP is denied the usual care patients receive. In the emergency room and ICU, such treatment can result in injury and even death. The VIP syndrome not only puts the patient at significant risk for morbidity and mortality but results in one additional side effect: It teaches and trains the VIP to become an impatient patient. The VIP syndrome perpetuates the VIP patient's expectations of special care and handling. VIP patients become conditioned to expect the same type of treatment from subsequent physicians.

VIP and Triage

The first step toward solving any problem is to analyze the components that caused it. To dissect the components of the VIP syndrome means squarely facing the underlying premise upon which the VIP syndrome is based: Not all patients are created equal. The VIP syndrome recognizes that there are mainly two classes of patients: (1) The VIP (the 'elite') patients; and (2) all other patients.

In the emergency room and ICU setting, the ideal situation is for patients to be resuscitated and treated regardless of their social status or celebrity. In these life-or-death situations, physicians are trained to rely upon accepted triage methods to determine which patient to treat first when faced with multiple patients. Running counter to standard procedures, the VIP syndrome disrupts the triage protocol because it bases priority not on the patient's medical condition but instead on the patient's social position.

How does a physician avoid the pitfalls of the VIP syndrome? First: Recognize that the VIP syndrome exists. Second: Become 'in tune' with one's own behavior when encountering the VIP patient.

Treatment of the VIP Syndrome

To avoid the VIP syndrome, here is a prescription for physicians and health care professionals:

V: Vow to value your medical skills and judgment.
I: Intend to command the medical aspects of the situation.
P: Practice medicine the same way for *all* your patients.

If despite best efforts a physician feels he or she cannot provide appropriate care, then the authors recommend that the physician decline the opportunity to care for this patient. Refer this patient to another colleague who is more comfortable taking care of the VIP.

Special Handling of the VIP Patient

Many physicians, who have avoided the VIP syndrome successfully, and have appropriately managed the care of these patients, have found their relationship with their VIP patients to be memorable, professionally fulfilling, and emotionally rewarding. Not only do the physicians get a unique glimpse into the personal lives of famous persons, but they also enjoy the trust and respect of a person who may be highly regarded in society.

The special relationship can also benefit the VIP when the physician becomes a source of caring but clear-eyed, honest opinion. Too often VIPs are surrounded by a coterie of people who are eager to please them. This eagerness to please can result in: (a) Premature delivery of good news and positive speculation; and (b) delays, denials, or excessive 'spinning' of bad news. A physician who is confident in the doctor-VIP relationship can offer 'bad news' or disagree with the patient, and the patient may actually view the physician's input as 'refreshingly honest' and sincere.

Having treated numerous VIP patients, including three American Presidents, the authors offer the following principles in dealing with the VIP:

1. When you treat the VIP, remember that there is only one celebrity in the room and it's not you (the physician). Avoid seeking fame based on your contact with a famous person.
2. Do not treat and tell. Do not 'drop names' or brag about your encounter. Respect the privacy of your patient. Be part of the team that protects the patient from unnecessary notoriety and publicity.
3. Do not practice medicine in a vacuum. Seek assistance in rendering health care and work together closely with consultants. Remember that the VIP often compares your advice and conduct with what other physicians tell them. Strive to ensure that you and the consultants and specialists are consistent in your treatment plans and overall medical opinions.

The authors have found these three basic principles to be quite effective in the management of the VIP as well as in the care of all patients.

Post and Robins offer 12 recommendations about ways to approach these patients [6]. Among these recommendations are:

- minimize harm to the patient
- do what is medically best
- tell the truth
- be candid
- encourage maximal patient autonomy

Post and Robins point out that all of their 12 recommendations could easily apply to all patients you treat, and several run parallel to the Hippocratic Oath. These principles are universal to good medical practice.

VIP Shock: Response to Acute Serious Illness or Trauma

Emergency room personnel routinely receive patients who are gravely ill, suffering severe trauma, or near death. The emergency physician becomes accustomed to thinking clearly and acting decisively in such situations.

If the severely sick or injured patient is the king, president, or prime minister of

the nation, however, then even seasoned physicians may experience acute stress reactions. The energy of aides and first responders who accompany the VIP to the emergency room will likely heighten the stress. Added to the climate of panic may be the physician's own personal fears: "Will I kill this patient? What will happen if things go wrong? How could I live with myself if I cannot save this patient? If my treatment is judged by others to have been inadequate, will I lose my job and career?"

Police officers face an analogous kind of stress when forced to make a decision whether to use deadly force on a criminal suspect. When faced with that rare, emergent, life-or-death decision in the field, police officers typically suffer a number of symptoms that impair their senses and judgment, including tunnel vision, heightened visual acuity to certain details, auditory blunting, and a sense of slow motion [9].

To reduce the potential for these acute stress reactions, the authors themselves have conducted scenarios among emergency response teams. Emergency room physicians might consider preparing a personal plan for reacting to an unlikely but crucial event of a VIP patient crisis. At the time of the crisis, the physician needs to self-monitor his or her emotional and physical reactions to the event, and get help from colleagues and support staff on duty to help ensure proper care is delivered.

Conclusion

Taking care of a patient in an emergency room or ICU setting can be difficult because of the life-or-death decisions a physician must make quickly to provide appropriate care. The stress upon physicians in these scenarios can be compounded and complicated particularly if the physician is intimidated by a VIP patient in an emergency room or ICU.

Physicians and other health care providers can take steps to avoid falling victim to the VIP syndrome by: 1) Vowing to value their own medical skills and judgment; 2) Intending to command the medical aspects of the situation; and 3) Practicing medicine as they do for all their patients. By adopting this strategy, physicians can provide the appropriate effective and compassionate care they would offer to all their patients.

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Brain Death: Compliance, Consequences and Care of the Adult Donor

D.J. Powner

Introduction

Critical care physicians often certify brain death and may continue the care of those patients who become organ donors. This chapter will review recent publications and current practices in these topic areas with the intent of encouraging compliance with established policies for brain death determination and promoting investigations needed to establish evidence-based treatment guidelines for donor care.

Brain Death Certification Policies and Practices

Despite appropriate continuing discussion of the moral and ethical bases of the equivalence of brain death and patient death [1], its concept and certification processes have been widely accepted. Similarly, although not directly investigated, the irreversibility of brain death, as defined by current practice, has been indirectly validated through publications in which patients with that diagnosis have been somatically supported without awakening for several months or longer. Diagnostic methods were not always specified in these reports and some were related to maternal brain death wherein care was provided until delivery. However, over 70 such patients have been followed without a single neurological recovery recorded [1, 2].

Criteria for the declaration of death based upon absent neurological function have traditionally been set forth by policies and procedures. Internationally this process may be guided by national standards, individual physician practice or, as in the United States, by local hospital policy [3, 4]. Extensive variability in both policy requirements and physician compliance with those requirements has been documented [3–5].

Specific criteria have been recommended by authoritative groups [6, 7] and generally include: warnings not to test in the presence of several confounding variables (e.g., certain drugs, severe hypothermia, shock), required documentation of unresponsiveness or cause of the coma, and specific testing methods, particularly for cranial nerve and medullary (apnea) function. 'Confirmatory' tests such as nuclide cerebral blood flow, electroencephalography, transcranial Doppler, or four-vessel angiography may be required or recommended when portions of the physical examination might be compromised. Policies may reference 'whole brain' or 'brainstem' criteria and stipulate the number and qualifications of the examiner(s), if and when assessments should be repeated, and specific values for some tests (e.g. $PaCO_2$ during apnea testing).

Recent publications have highlighted: methodological variations in the assessment of apnea [8], indicating equivalent results with T-piece, oxygen catheter, and

continuous positive airway pressure systems during testing; confirmatory testing with Doppler [9] wherein sensitivity for determining brain death was about 88% but specificity was close to 100%, radionuclide SPECT [10] showed a high degree of concordance with 4-vessel angiography, and the partial pressure of oxygen ($PbtO_2$) in brain tissue [11] was diagnostic of brain death when the $PbtO_2$ became zero; and the occurrence of reflex or automatic movements after brain death from presumed spinal cord origins [12]. Thus, policies and procedures for certification of brain death may continue to change as newer techniques develop and interest in this area stimulates research. The critical care physician, therefore, is likely to be responsible for evaluating such technologies and providing leadership in the revision and enforcement of established policies/procedures.

Physiological Impact of Brain Death

Traumatic brain injury (TBI) and other catastrophic neuronal insults in humans release a large quantity of 'stress hormones' (e.g., epinephrine, dopamine, corticosteroids) into the circulation. Superimposed upon this already hyper-adrenergic milieu, further catecholamine release/effects probably occur as anatomical brain death occurs. Animal studies have shown that the rapid induction of brain death stimulates the series of cardiovascular responses shown in Table 1. Hypertension, systemic and pulmonary arterial vasoconstriction and dysrhythmias occur early as the brainstem is compressed, but are soon followed by vasodilation and an abrupt decline in blood pressure. Histopathological contraction bands in the myocardium and pulmonary edema corroborate other physiological signs of acute heart failure.

Postulated mechanisms for these physiological changes during the evolution of clinical brain death include a 'catecholamine storm' of circulating hormone release, an accentuated sympathetic nervous system discharge, and the subsequent loss of autonomic vascular tone, described as 'cerebro-spinal disconnection' similar to changes after spinal cord injury. Pretreatment in animals with calcium channel and beta-receptor blockade before induction of brain death is protective, further implicating catecholamine-induced injury. Detailed recordings of these events in primates were published 18 years ago [13], but have not been duplicated in humans, although the sequence of clinical changes, histopathological findings and laboratory data in patients appear similar.

Table 1. Physiological effects of brain death – Baboon model [13]

Initial: (minutes)
Bradycardia and other dysrhythmias
Increased systemic and pulmonary vascular resistances
Hypertension
Elevated pulmonary artery occlusion (wedge) pressure
Increased cardiac output and left ventricular contractility
Secondary:
Decreased systemic and pulmonary vascular resistances by 15 minutes
Decreased left ventricular contractility and blood pressure by 45 minutes
Reduced right and left ventricular compliance
Decreased coronary artery perfusion
Cardiovascular collapse by 5–7 hours

Genetic induction causes production and release of pro-inflammatory cytokines, particularly tumor necrosis factor (TNF)-α and interleukin (IL)-6 during the evolution of brain death. The precise mechanism for this response remains unclear but appears related to reperfusion injury in donor organs/tissues [14, 15]. Cytokines are released locally in many organs, but increased circulating blood levels confirm a systemic distribution and effect. Tissue based cytokines not only produce alterations in donor organ function but also enhance subsequent graft rejection in the recipient [16].

Milieu for Donor Care

If organ procurement for transplantation is planned after brain death, the clinician is challenged to refocus care toward preservation and improvement of those organs [17]. Some data suggest that some outcome measures from living donors may be superior to those from cadaver sources, indicating that organs are compromised after brain death and during donor care. The etiology of such compromise may be a pre-existing condition (e.g., atherosclerotic cardiovascular disease), direct organ trauma, conditions resulting from the neurological catastrophe precipitating patient admission (e.g., myocardial stunning, coagulopathy), reperfusion injury, pro-inflammatory/catecholamine production/release, or events related to organ removal/ transport/ implantation. However, clinical experience and many observational studies during donor care indicate that organ compromise, especially in the heart and cardiovascular system occurs before explantation.

Clinicians providing donor care, therefore, are often confronted with the several physiological challenges listed in Table 2 very soon after brain death. The traditional practice of rapid organ allocation so as to expedite removal of organs from the unfavorable milieu of the donor has recently evolved toward a more extended treatment time. The intention of continued donor treatment is to allow correction of physiological abnormalities, thus improving organ function so that marginal organs might become transplantable. Recovery of organ function, especially the heart, has been well documented using treatments titrated to targeted physiological parameters [19–21]. These studies demonstrate that although donor organs, especially the heart and lungs, may have been compromised, careful treatment may restore function and provide organs equivalent to those originally deemed 'acceptable' by standard criteria.

Organ Allocation versus Donor Care

Concurrent with the care of the donor is the allocation of his/her organs to recipient transplantation programs. This important selection process combines variables unique to the donor and recipient. Some allocation variables (e.g., age, sex, ABO blood type, body-mass index, cause of death, prior medial/surgical/medication history, length of time after admission, smoking history) are important factors for allocation, but are not amenable to change during donor treatment. Other variables, however, are amenable to change and may affect the potential for transplantation of all organs or those specifically targeted. Those interventions amenable to change during donor treatment, therefore, will remain the focus of this chapter.

One of the most important investigational challenges within donor care is to identify ranges of tolerance within physiological and laboratory variables that will

Table 2. Common physiological conditions present during donor care

Condition	Cause/Comment	Treatment
Hypothermia	Loss of hypothalamic temperature regulation causing a poikilothermic state; infusion of fluids/blood products below normal body temperature	Passive/active rewarming via forced air/water blankets; warm inspired gas from ventilator; warm intravenous fluids or gastric/colonic lavage
Hypotension	Decreased left ventricular contractility ascribed to myocardial ischemia/stunning; vasodilation after cerebro-spinal disconnection; prior dehydration or hypovolemia	Appropriate evaluation and treatment of abnormal preload, afterload, heart rate and contractility – see text
Polyuria	Physiological mobilization of excess fluid; hyperglycemic, osmotic, hypothermic or drug-related diuresis; diabetes insipidus; serum sodium >155 mEq/l harms the donor liver [18]	Sequential assessment of urinary/serum electrolytes; hourly urine replacement with appropriate fluids; anti-diuretic hormone for diabetes insipidus
Infection	Prior donor infection or nosocomial exposure (e.g., ventilator associated pneumonia, urinary/ intravascular catheters)	Continue antibiotics against known pathogens; obtain surveillance cultures; prophylactic antibiotics not recommended
Nutrition	Variable impact depending upon donor's prior nutritional status and length of admission	Continue nutritional support – enteral route preferred; caloric/carbohydrate loading is controversial; glycemic control is controversial – see text
Hypoxemia	Usual causes in critically ill/injured patients; prior goals of PaO_2/FIO_2 ratio >300 challenged by extended donor criteria – see text	Improvement in lung parameters due to donor care documented to 'salvage' lungs for transplantation – see text

still permit transplantation of an organ that will provide acceptable performance for the recipient. These ranges may extend beyond currently applied allocation standards or the 'normal values' of laboratory or physiological testing and constitute 'extended' or 'expanded' criteria. Extended criteria have been supported in many small group series and expand the number of organs available. However, investigations that provide reliable predictions of success from such extended criteria in larger recipient groups are lacking.

Similarly, tolerance ranges for parameters that can be influenced by the clinician must be integrated with those factors not amenable to change to create weighted sets of combined variables that may better anticipate the possibilities of success or complications after implantation. Creating these probability sets will require a large database of reliably obtained physiological and laboratory information and sophisticated statistical tabulations. However, this effort could provide evidence-based guidance to the allocation process that currently does not exist.

Finally, as others have expressed, an 'acceptable' outcome after transplantation may not be free of complications. An appropriately developed database, therefore, should assure with some recognized probability when more benefit than harm will likely occur from a given organ offering. Balancing the intensity of the recipient's 'need' against such a 'benefit/risk' probability will ultimately always be somewhat subjective but could be greatly assisted by a stronger data process.

Goals for Donor Treatment

In the absence of evidence-based data that address acceptable tolerances for those variables amenable to modification during donor care, many 'authoritative' recommendations have been published [22–26]. Guidelines for physiological parameters are shown in Table 3. The current goal for other laboratory variables is to maintain them within hospital "normal" values.

Table 3. Recommended cardiovascular and related parameters [22–26]

Central venous pressure: 4–12 mmHg
Pulmonary artery occlusion (wedge) pressure: 6–12 mmHg
Cardiac index: >2.4 l/min/m^2
Cardiac output: >3.8 l/min
Left ventricular ejection fraction: >40%
Systemic vascular resistance: 800–1200 dynes/s-cm^5
Mean arterial blood pressure: >60 mmHg
Urine output: 1–3 ml/Kg/hr
Temperature: >36°C (97°F)
Hemoglobin: >10 g/dl
Hematocrit: >30%
Other laboratory parameters: within hospital normal values

Special Considerations

Several issues during donor care remain controversial and potentially conflict with other established critical care principles:

Glycemic Control

Rigorous treatment to avoid hyperglycemia is a widely promoted standard of critical care [27]. Hyperglycemia has been proposed to compromise the immune system in patients, but the harmful or possibly beneficial effects of such immunocompromise within the transplantation process soon to follow donor care are unknown.

The contribution to donor polyuria or other effects of serum hyperosmolality produced by hyperglycemia may complicate fluid/electrolyte treatment. Therefore, at least minimal therapy to avoid these effects may be necessary.

Liver glycogen content has been considered important in sustaining the explanted liver through cold storage and the possible reperfusion injury that may occur after implantation. Glycogen stores in the liver can be increased acutely at the time of liver removal using direct portal vein infusions of glucose [28] and may be possible as a function of donor nutrition [29], although a method of assuring glycogen 'loading' during donor care is not published. It seems unlikely that harmful liver steatosis could be induced during the short-term administration of extra carbohydrates, but this has not been investigated.

Islet cell stimulation by elevated serum glucose may be desirable prior to pancreatic organ or islet cell transplantation. However, potentially harmful effects have been noted and the optimal serum glucose concentration to assure a desirable effect is not established [30].

Therefore, several important considerations affect blood glucose tolerance during donor care. Without evidence-based data, the preferences of individual transplant programs/surgeons usually determine glycemic limits.

Hypertension/Hypotension

Hypertension is much less common than hypotension during donor care, but when present, potentially increases myocardial work and oxygen consumption. Goals include mean arterial pressure less than 90 mmHg [24] and systolic blood pressure <160 mmHg [26]. Only short-duration vasodilators, nitroprusside or nicardepine, or the beta-receptor blocker, esmolol, should be used to avoid possible pharmacological suppression of contractility in the recipient.

Hypotension, due to the above noted mechanisms, is common. Resuscitation and maintenance of cardiovascular hemodynamics follow customary critical care practices. A variety of tools has been used to assess measured or derived components of cardiac function, i.e., heart rate, preload, afterload and contractility. Diagnostic and monitoring methods include echocardiography, pulmonary artery catheterization, central venous pressure and esophageal Doppler [24, 26, 31, 32]. Appropriate assessment should direct selection of therapy, i.e., blood product/colloid/crystalloid fluid administration, inotropic drugs or vasopressor medications. Generally, of course, the minimum amounts of intravenous fluid or drugs needed to achieve the hemodynamic goals (Table 3) are preferred.

Mechanical Ventilation/Hypoxemia

No evidence-based data support mechanical ventilation methods different from those customarily practiced in critical care. The traditional allocation criterion of a PaO_2/FiO_2 ratio >300 has been challenged by successful transplantation using expanded donor criteria at a lower ratio. Assertive pulmonary care also improves donor lungs and allows successful transplantation of these 'marginal' organs [33]. *Ex vivo* evaluation and treatment of compromised lungs prior to implantation [34] remain investigational.

Hormonal Therapy

Replacement or supplemental administration of several hormones has been advocated, including glucocorticoids, thyroid, insulin, and vasopressin. A retrospective assessment of such use showed beneficial effects of some hormones in the number and function of some organs, but indications, dosages and concomitant information about donor condition or care were not given [35]. Donor treatment with methylprednisolone has become common practice as a method to increase oxygenation [36], but routine administration of thyroid and other hormones remains controversial [37]. If given, dosing recommendations have been published [23, 26].

Bacterial Contamination

Bacterial, viral, fungal, and protozoan infections may be transmitted from an infected donor to the recipient(s) and may be associated with catastrophic consequences in graft and patient survival [38], although recent case reviews have suggested that certain Gram-positive donor infections may be tolerated after implanta-

tion [39]. Despite such reports, goals during donor care include continuing all preventative measures to avoid nosocomial infections (e.g., established ventilator associated lower respiratory infection prevention protocols, blood stream infection prevention guidelines) and to treat recognized infection. The distinction between a 'colonized' presence of infection and true tissue invasion is often difficult. Further, the latent incubation period before an infection present in the donor becomes manifest may contribute to findings of recipient contamination without evidence of donor infection. These factors have prompted some centers to initiate prophylactic antibiotics during donor care, but recent consensus opinion recommends against such treatment [26].

Surveillance cultures and sampling of material or fluid that appears infected are justified, as is continued treatment of known infections. Best practice guidelines for culturing methods, interpretation of preliminary data, and selection of empiric antibiotics recommend antibiotic selection based upon the sensitivities of organisms encountered in the local hospital followed by modifications when final cultures are known [40].

Coagulopathy

Abnormal coagulation leading to serious hemorrhage during donor care may result from previous medications prior to or during the current hospitalization that affected platelet function or production of coagulation proteins, consumption or dilution of normal coagulation factors, or development of disseminated intravascular coagulation (DIC). Factor replacement therapy or treatment of thrombocytopenia or dysfunctional platelets follows standard critical care practice as titrated against appropriate laboratory measurements. The presence of donor DIC is not considered an absolute contraindication to organ use [41].

Off-label administration of recombinant factor VIIa has been widely reported in patients who may evolve brain death and following transplantation, especially in liver recipients. Its use during donor care, however, has not been evaluated and must be considered investigational. Severe thromboembolic complications in patients are well known [42] although their incidence in a general population of bleeding patients [43] and among donors is not.

Reperfusion Injury and Preconditioning

Cellular injury to an organ after its removal, storage and re-implantation is well documented and considered likely due to production of oxygen radicals or other pro-inflammatory mediators. It is also hypothesized that the accumulation of pro-inflammatory cytokines during the evolution of brain death may similarly stimulate a form of reperfusion injury due to the cardiovascular and hormonal changes previously discussed [14, 44]. Preconditioning interventions during donor care have been proposed as possibly beneficial in preparing organs for such injury and perhaps favorably modifying their response to reperfusion stress [45]. Induced liver ischemia just prior to liver removal in the operating room [46], and an association between donor cardiopulmonary arrest and better liver function in the recipient have suggested a benefit from preconditioning ischemia [47]. Dopamine-induced production of heme oxygenase and other immunologic properties may be protective especially during renal transplantation [48, 49], but routine use of dopamine for this purpose has not yet been recommended.

Anemia

No evidence-based data are available to guide therapy to optimal hemoglobin concentrations. Harmful effects of both anemia and blood transfusion are well recognized within critical care [50]. Donor organ tolerance for anemia after cessation of cerebral oxygen consumption and the effect of immunomodulation induced by transfusion upon subsequent recipient care, however, are unknown. Recommendations for hemoglobin include 9–10 g/dl [23, 26] or hematocrit >30% [24].

Future Challenges

A potentially positive impact of donor care upon subsequent graft and recipient survival has been accepted. However, the range of donor organ tolerance among variables that can be altered during donor care remains largely unknown. Because many of these variables are inter-related the relative weighted importance of each must be investigated. Similarly, the interaction of variables amenable to change and those that cannot be altered and their relative importance must become known. Existing local, national and international databases should be expanded to include more detailed physiological data from donor care so as to create the data platform from which statistical correlation with outcomes may be derived. This complex statistical process may provide quantifiable risk information during organ selection and allocation, and provide better definitions of donor characteristics that are associated with acceptable recipient outcomes. These parameters would allow donor care in the future to be directed toward broader tolerance ranges for physiological parameters and thereby expand the pool of useable organs.

Conclusion

Brain death certification and donor care are commonly the responsibility of the critical care physician. Adherence to applicable policies/procedures and providing leadership in modifying those policies as evidence-based data indicate are important parts of that responsibility.

Physiological changes that evolve during brain death challenge the clinician to recover or maintain organ function that will provide the best possible organ to the recipient. Many factors causing the profound abnormalities in cardiovascular/pulmonary function, fluid-electrolyte/acid-base balance, and hormonal dysregulation are unknown. Treatment is, therefore, often directed toward symptom management rather than pathophysiological mechanism.

Further investigation is required into the extended limits of those variables amenable to change during donor care that will produce an acceptable organ. Integration of these limits with similar expanded criteria for those fixed variables not amenable to change may provide better predictions of outcome during allocation and better goals during donor care.

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Disasters

Update on Avian Influenza for Critical Care Physicians

C. Sandrock

Introduction

Human influenza pandemics over the last 100 years have been caused by H1, H2, and H3 subtypes of influenza A viruses. More recently, avian influenza viruses have been found to directly infect humans from their avian hosts. The recent emergence, host expansion, and spread of a highly pathogenic avian influenza (HPAI) H5N1 subtype in Asia has heightened concerns globally, both in regards to mortality of HPAI H5N1 in humans and the potential of a new pandemic. In response, many agencies and organizations have been working collaboratively to develop early detection systems, preparedness plans, and objectives for further research. As a result, there has been a large influx of published information regarding potential risk, surveillance, prevention and control of highly pathogenic avian influenza, particularly in regards to animal to human and subsequent human to human transmission. This chapter will review the current human infections with avian influenza and its public health and medical implications.

Influenza A Viruses

Influenza A, B and C are the most important genera of the *Orthomyxoviridae* family. Influenza A is responsible for human pandemic outbreaks and seasonal epidemics and influenza B is responsible for increasing cases of seasonal disease. Influenza A viruses are enveloped, single-stranded RNA viruses with a segmented genome. The eight RNA segments of the genome encode for 11 viral proteins, including the polymerase proteins (PB1, PB2), matrix proteins (M1, M2) and the surface glycoprotiens hemagglutinin (HA) and neuraminidase (NA). Influenza A viruses are classified into subtypes on the basis of the antigenic properties of the hemagglutinin and neuraminidase glycoproteins expressed on the surface of the virion [1]. To date, 16 hemagglutinin and 9 neuraminidase subtypes have been identified and are found in 144 different combinations (e.g., H1N1, H3N2, H5N1, etc.) [2].

The hemagglutinin glycoprotein mediates attachment and entry of the virus by binding to sialic acid receptors on the cell surface [3, 4]. The binding affinity of hemagglutinin to the host sialic acid allows for the host specificity of influenza A. More specifically, avian influenza subtypes prefer to bind to sialic acid linked to galactose by α-2,3 linkages, which are found on duck intestinal epithelium and poultry and duck respiratory epithelium [5]. Human virus subtypes, H1, H2, and H3, bind to α-2,6 linkages found in human respiratory epithelium. Swine contain both α-2,3 and α-2,6 linkages in their respiratory epithelium allowing for easy co-infec-

tion with both human and avian subtypes [6]. This reason has been cited as the likely genesis of novel strains, as in the 1968 H3N2 human pandemic, and has given pigs the designation of a 'mixing vessel' for new strains [7, 8]. To a lesser degree, humans have been found to contain both α-2,3 and α-2,6 linkages in their lower respiratory tract and conjunctivae which allows for human infections of avian strains [9]. The hemagglutinin glycoprotein is the main target for immunity by neutralizing antibody.

The neuraminidase glycoprotein allows the spread of the virus by cleaving the glycosidic linkages to sialic acid on host cells and the surface of the virus [10]. The virus is then spread in secretions or other bodily fluids. The neuraminidase glycoprotein is a lesser target for immunity by neutralizing antibodies, but is the target site for the antiviral neuraminidase inhibitors.

In addition to hemagglutinin and neuraminidase classification, influenza A viruses are characterized by their pathogenicity. Highly pathogenic avian influenza (HPAI) is defined by the World Organization for Animal Health (OIE) as any influenza that causes severe disease or death in domestic poultry. HPAI viruses, with very few exceptions, are of the H5 or H7 subtype, but not all H5 and H7 subtypes are HPAI viruses. The potential pathogenicity of H5 and H7 subtypes can be evaluated by sequencing the hemagglutinin gene, since pathogenicity is associated with the presence of multiple basic amino acids at the hemagglutinin cleavage site. A change from a low pathogenic H5 or H7 subtype to a highly pathogenic form may occur upon introduction into poultry and is thought to occur primarily as a result of insertion of basic amino acids in the hemagglutinin cleavage site. Molecular studies have shown that the 1918 human pandemic H1N1 subtype originated as a low pathogenic avian virus in contrast with current human cases of H5N1 worldwide, which are the result of a highly pathogenic avian influenza virus.

Influenza A viruses are highly variable as a result of molecular changes in the RNA segments that occur through a number of mechanisms; the most important of which are point mutation (antigenic drift) and RNA segment reassortment (antigenic shift) [10]. Like other RNA viruses, the influenza A viruses lack proofreading ability, and are, therefore, subject to point mutations [10]. These individual mutations in the viral genome cause minor changes in the antigenic character of virus, with amino acid changes in hemagglutinin and neuraminidase of principal importance. Reassortment occurs when a host cell is infected with two or more influenza A viruses and leads to the creation of a novel subtype containing a new hemagglutinin or neuraminidase that is immunologically distinct from those of the previous circulating strains, as can be seen in pigs, which possess receptors for both the human and avian subtypes [8]. Three major pandemics have occurred in the last century (1918 H1N1, 1957 H2N2, and 1968 H3N2) through reassortment. However, point mutations leading to viral adaptation to a human host can occur with any avian influenza subtype.

Host Range of Influenza A Viruses

Influenza A viruses infect a wide range of hosts including many avian species, and various mammalian species such as swine, ferrets, felids, mink, whales, horses, seals, dogs, civets, and humans [11–13]. Wild birds (ducks, geese, swans, and shorebirds) are important natural reservoirs of these viruses, and all of the known 16 hemagglutinin and 9 neuraminidase subtypes have been found in these birds. In most cases,

these subtypes are found within the gastrointestinal tract of the birds, shed in their feces, and rarely cause disease. Since 2002, however, HPAI H5N1 viruses originating in Asia have been reported from approximately 960 wild bird species, causing disease in some instances and asymptomatic shedding in others [14]. The virus has now spread across Asia, Europe, the Middle East, and some African countries. Additional species, such as tigers, leopards, cats, stone martens, and humans have also become infected with HPAI H5N1. The wide host range of many of these bird species may be one potential mechanism of spread of HPAI H5N1worldwide, thus complicating the potential contact, transmission, and mutability of HPAI H5N1 in animal and human populations.

Epidemiology and Pathogenicity of Avian Influenza Infections in Humans

The incidence of avian influenza infections in humans has increased over the last decade (Table 1) [11, 12, 15–20]. Initially, cases of avian influenza (H7N7) in humans occurred in association with poultry outbreaks, manifesting as self-limiting conjunctivitis [11]. Then, in 1997, a large scale HPAI H5N1 outbreak occurred among poultry in Hong Kong, with 18 documented human cases [17]. Two subsequent poultry outbreaks in Hong Kong in 1999 and 2003 with HPAI H5N1 occurred without human cases until 2003 when two members of a family in Hong Kong contracted HPAI H5N1. In December of 2003, HPAI H5N1 surfaced in poultry in Korea and China, and from 2003–2006 the outbreak stretched worldwide in the largest outbreak in poultry history. Human cases of HPAI H5N1 followed the poultry outbreak, with a total of 256 cases and 151 fatalities thus far. Other limited outbreaks have occurred, causing variable human disease (Table 1) [21]. However, HPAI H5N1 remains the largest and most significant poultry and human avian influenza outbreak.

Epidemiologic investigations of human cases of avian influenza shows that the virus was acquired by direct contact from infected birds. Influenza A is transmitted

Table 1. Avian influenza A outbreaks reported in humans

	United Kingdom 1995	Hong Kong 1997	Hong Kong 1999	The Netherlands 2003	Canada 2004	Worldwide (southeast Asia, Africa, Middle East) 2003-present
Influenza A subtype	H7N7	H5N1	H9N2	H7N7	H7N3	H5N1
Source of infection	Poultry	Poultry and waterfowl	Poultry	Poultry	Poultry	Poultry and waterfowl
Clinical presentation	Conjunctivitis	Conjunctivitis and ILI	ILI	Conjunctivitis pneumonia, ILI	Conjunctivitis, ILI	Conjunctivitis, ILI, pneumonia, multi-organ failure
Number of human cases	1	18	2	89	2	256
Number of fatalities (%)	0 (0)	6 (33)	0 (0)	1 (1)	0(0)	151 (59)

H: hemagglutinin; N: neuraminidase; ILI: Influenza-like illness

Table 2. Person-to-person transmission of avian influenza

	Hong Kong 1997	Hong Kong 1997	Netherlands 2003	Thailand 2004	Vietnam 2004	Indonesia 2006
Influenza subtype	H5N1	H5N1	H7N7	H5N1	H5N1	H5N1
Location	Household	Hospital	Household	Hospital	Hospital	Household
Transmission to	Family member	Health care worker	Family member	Family member	Health Care worker	Family member
No of cases	1	8	3	2	0	7
Clinical presentation	seropositive	seropositive	Conjunctivitis and ILI	Pneumonia, death	N/A	Pneumonia, death

ILI = influenza-like illness

through the fecal-oral and respiratory routes among wild birds and poultry [13, 14]. Human interaction with these infected secretions and birds was the major mode of transmission, with contact including consumption of undercooked or raw poultry products, handling of sick or dead birds without protection, or food processing at bird cleaning sites [11, 12, 15–17, 19, 22–24]. All birds were domesticated poultry or waterfowl, and no transmission from wild birds or contaminated water has been reported. In a few cases, limited human to human transmission was reported among health care workers and family members (Table 2) [24–28]. In each of these cases, no personal protective equipment was utilized and was the major factor in transmission between humans.

Clinical Manifestations of Avian Influenza in Humans

The clinical manifestations of avian influenza in humans have ranged from mild conjunctivitis to severe pneumonia with multiple organ failure (MOF) [11, 12, 15–20, 22, 23, 29]. While the ages of the patients have varied, the majority of cases in both the 1997 and 2003 HPAI outbreaks were young. In 1997, the median age of the cases was 17.2 years, while the cases from 2003–2004 in Southeast Asia had a median age of 16 (range 2 months to 90 years). Nearly all cases were linked with sick and infected poultry, and the incubation period ranged from 2 to 8 days from contact to symptoms. The symptoms in each outbreak have varied with the avian influenza A subtype. In 2003 during the Netherlands outbreak with subtype H7N7, 92% (82 of 89) presented with conjunctivitis [11, 29]. The other cases in Canada and the UK also presented with conjunctivitis [22]. However, with HPAI in Hong Kong in 1997, 18 of the cases had an influenza-like illness [17, 19]. In 11 cases, pneumonia developed with 6 of these progressing to MOF, acute respiratory distress syndrome (ARDS), and death [17]. Reye syndrome, pulmonary hemorrhage, and predominant nausea, vomiting, and diarrhea complicated cases.

Cases from the worldwide outbreak originating in Southeast Asia had similar presentations to the 1997 HPAI H5N1 cases [30–33]. The main presenting symptom was pneumonia with fever and an influenza-like illness. Diarrhea was present in up to 70% of the cases. Many cases had both thrombocytopenia and lymphopenia. Chest radiographic findings included interstitial infiltrates, lobar consolidation, and

air bronchograms. Sixty-eight percent of patients developed ARDS and MOF within 6 days of disease onset. The case fatality rate has ranged from 67–80%, depending on the case series [34]. Once the cases reached the critical care unit, however, the mortality was 90%. The average time of death from disease onset was 9–10 days.

Post-mortem studies have illustrated findings consistent with MOF and overwhelming systemic inflammatory response syndrome (SIRS), including diffuse alveolar damage, acute tubular necrosis and atrophy, disseminated intravascular coagulation (DIC), and multi-organ damage [35]. Interestingly, the virus has been isolated from the lungs, intestine, spleen, and brain, suggesting viremia. However, active replication of the virus was limited to the lungs. This overwhelming inflammatory response, with acute lung injury (ALI) and ARDS as the predominant feature, coincides with the findings of a preferential binding of the avian influenza A viruses to α-2,3 linkages in type II pneumocytes of the lower respiratory tract of humans [36]. Subsequent viral replication, cytokine release, overwhelming host immune response, and the subsequent systemic manifestations then occur.

Diagnosis

The clinical diagnosis of avian influenza infection in humans is difficult and relies on the epidemiological link to endemic areas, contact with sick or dead poultry, or contact with a confirmed case of avian influenza. Since many infectious diseases present with these findings, the only feature significant to the clinician may be contact in an endemic area, through travel or infected poultry, and the clinician should always elicit this detailed history.

The definitive diagnosis is made from isolation of the virus in culture from clinical specimens. This method not only provides the definitive diagnosis, but the viral isolate is now available for further testing, including pathogenicity, antiviral resistance, and DNA sequencing and analysis. Alternatively, antibody testing can be performed, with a standard four-fold titer increase to the specific subtype of avian influenza virus. Neutralizing antibody titer assays for H5, H7 and H9 are performed by a microneutralizaiton technique. Western blot analysis with recombinant H5 is the confirmatory test for any positive microneutralization assay. More recently, rapid diagnosis can be performed with reverse transcription-polymerase chain reaction on clinical samples with primers specific for the viral subtype [37]. This test should only be performed on patients meeting the case definition and with an indirect immunoflorescence or enzyme immunoassay test confirming influenza A.

Any suspected case of avian influenza in a human should be investigated by the public health officials in the province or country of origin [38]. Additionally, governmental laboratories are often equipped with the appropriate biolevel safety 3 laboratories, primer libraries, and associated expertise to confirm the diagnosis quickly and efficiently. Any clinical specimens should be submitted with the assistance of the public health experts

Treatment

Treatment of avian influenza infections in humans includes antiviral therapy, supportive care, and adjunctive therapies [39–43]. Controlled clinical trials on the efficacy of antivirals (neuraminidase inhibitors), supportive therapy, or adjuvant care

have never been performed, so current recommendations stem from the experiences of past avian influenza outbreaks and animal models.

The adamantanes (rimantadine and amantadine) and neuraminidase inhibitors (oseltamivir and zanamivir) are the antivirals used for treatment and prophylaxis of influenza infections in humans [42]. Adamantanes bind to the M2 protein on the viral capsule, inhibiting dissociation of the matrix proteins from the nucleocapsid during viral uncoating. In avian influenza virus infections, adamantanes have no role due to widespread resistance. HPAI H5N1 isolated from Southeast Asia carried the mutation in M2 that conferred resistance to this group of antivirals. In fact, over 90% of isolates of H1 and H3 human subtypes during seasonal influenza have had resistance to the adamantanes, thereby limiting their use in seasonal epidemics with human subtypes. Their role has been limited now to prophylaxis in the community when the circulating strain is known to be susceptible to the adamantanes.

Neuraminidase inhibitors (oseltamivir and zanamivir) have been extensively studied for both treatment and prophylaxis in the human influenza A subtypes, H1, H2, and H3, as well as influenza B [40, 42]. In avian influenza, the efficacy has been well documented in animal models where improved survival has been seen after infection with HPAI H5N1. The timing of treatment is paramount, as earlier therapy is directly related to improved survival. The greatest level of protection was seen if the neuraminidase inhibitors were started within 48 hours of infection, and protection rapidly dropped after 60 hours. In HPAI H5N1 cases from Southeast Asia, survival appeared to be improved in patients who received oseltamavir earlier at 4.5 days compared to 9 days. Both of these times are much longer than documented in the animal models, so the window of optimal therapy is largely unknown. Additionally, therapy with oseltamivir has been shown to decrease the viral level in nasal secretion in patients infected with HPAI H5N1. For oseltamavir, therapy has been at 75 mg twice daily, with 75 mg once daily reserved for prophylaxis. The drug has a 90% oral bioavailability and reaches significant plasma and bronchoalveolar lining fluid levels. Zanamivir is available in a dry powder inhalation at 10 mg twice daily for treatment and 10 mg daily for prophylaxis. Zanamivir has not been used in human avian influenza cases, and some concern exists over treatment with an inhalation powder as plasma levels are significantly lower than with oseltamivir.

Neuraminidase inhibitor resistance has been documented in HPAI H5N1 subtype in a Vietnamese girl treated with 75 mg daily for 4 days for post-exposure prophylaxis [44]. The NA glycoprotein had a histadine to tyrosine substitution at position 274, conveying markedly higher IC 50 for oseltamivir. Zanamivir resistance was not found with this change [39, 41]. Neuraminidase resistance has not been documented in other HPAI H5N1 cases to date.

Combination therapy has not been studied in influenza A viruses. Ribaviron by inhalation has been evaluated *in vitro* with some avian influenza A subtypes and has been found to reduce mortality from influenza B in a mouse model. Further animal model studies are indicated to determine if there is a role for ribaviron or combination therapy with avian influenza A viruses [42].

Supportive care with i.v. rehydration, mechanical ventilation, vasopressor therapy, and renal replacement therapy are required if MOF and ARDS are a feature of disease [43]. Due to the progression of pneumonia to ARDS, non-invasive ventilation is not recommended, and early intubation may be beneficial before overt respiratory failure ensues. Corticosteroids have been used in some patients with HPAI H5N1, but no definitive role for steroids has been determined. Other immunomodulatory therapy has not been reported.

Vaccination

Human vaccination for avian influenza viruses has not been widely used, although multiple vaccination trials are underway. Prior avian vaccines in humans have been poorly immunogenic and thus have limited use [45–48]. An inactivated H5N3 has been tested and was tolerated but with limited immunogenicity [45]. Other H5 vaccines have developed neutralizing antibodies, but to a limited degree. Recently, a large randomized trial looked at an H5N1 attenuated vaccine from the Vietnam strain. Only a modest immunologic response was seen, with microneutralization antibodies being developed at 12 times the dose for seasonal influenza. The side effects were minimal. A number of other industry trials with adjuvent vaccines are currently ongoing. Sandofi-Adventis has recently reported success with a H5N1 attenuated vaccine at low doses, but the results have not been released thus far. Although promising, human vaccination against avian influenza viruses is still under development. Underscoring this development is the uncertainty of a pandemic strain, which may have vastly different antigenic properties of any developed H5 vaccine.

Infection Control and Preventative Measures

Health care infection control is a crucial component in the management of avian influenza infection or a new pandemic strain. Experience with the severe acute respiratory syndrome (SARS) outbreak in 2002 has illustrated that appropriate infection control measures are paramount to reducing spread to health care workers and possibly the community [49]. Therefore, the World Health Organization (WHO) and Centers for Disease Control and Prevention (CDC) recommend contact and airborne precautions for any initial suspected case of avian influenza in a human. In late October 2006, the CDC released an updated interim guidance on the use of masks and respirators in the health care setting (Table 3). In certain high risk procedures, additional protection with an N-95 particulate respirator may be considered given the likelihood of generating aerosol particles that may enhance transmission (Table 4). Respiratory protection should be worn along with an impermeable gown, face shield, and gloves. Initial cases should be placed in a negative pressure isolation room with 6–12 air changes per hour. Hand hygiene with antibacterial soap or alcohol based washless gel should be standard, with appropriate basins at each patient room. Seasonal vaccination of all health care workers should be preformed and emphasized to reduce spread. Visitors and family members should be strictly monitored and limited to reduce the likelihood of spread. Finally, antiviral chemoprophylaxis should be available to any health care workers with exposure to an infected

Table 3. masks and respirators for health care workers

	Surgical Mask	N-95 Respirator	N-95 Cartridge mask	Powered air purifying respirator (PAPR)
Protection	Droplet	Aerosol	Aerosol	Aerosol
Disposable	Yes	Yes	Filter only	No
Fit testing	No	Yearly	Yearly	Yearly
Power source	No	No	No	Battery
Stockpiling	Yes	Yes	No	No
Cost	Very low	Low	High	Very high

High Risk Procedures
Nebulization of medication
Endotrachial intubation
Non-invasive mechanical ventilation
Bronchoscopy
Humidified oxygen delivery
Non-rebreather mask without expiratory filter

Table 4. High risk aerosol procedures in avian influenza

individual. Any symptomatic worker should be taken off duty and workplace surveillance should occur. With these aggressive measures, risk to the health care worker, patients, and family members will be reduced.

Conclusion

Avian influenza viruses have occurred with increased incidence within the human population, reflecting the delicate and tangled interaction between wildlife, domesticated animals, and humans. Disease in humans can be limited to conjunctivitis or an influenza-like illness, but HPAI H5N1 causes mainly severe pneumonia, respiratory failure, and death. Most cases have occurred with direct transmission from infected poultry or waterfowl, with only a few limited cases of human to human transmission. Treatment has been successful with the neuraminidase inhibitors if started early, and vaccine development is underway with a more immunogenic attenuated H5N1 virus preparation. Infection control measures are the mainstay for prevention and disease reduction. Avian influenza viruses may constitute part of the next pandemic, so appropriate knowledge, prevention, and treatment will reduce the likelihood of this occurrence.

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Critical Care Pandemic Preparedness Primer

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Introduction

The first half decade of the 21st century has brought with it infectious outbreaks such as severe acute respiratory syndrome (SARS) [1], bioterrorism attacks with anthrax [2], and the spread of H5N1 influenza A in birds across Asia and Europe [3, 4] sparking concerns reminiscent of the days of the Black Plague. These events, in the context of an instantaneous global-media world, have placed an unprecedented emphasis on preparing for a human influenza pandemic [5, 6]. Although some argue that the media have exaggerated the threat, the warnings of an impending pandemic are not without foundation given the history of past influenza pandemics [7], incidence of H5N1 infections among humans [8], and the potential impact of a pandemic. Reports of the 1918 pandemic vary, but most suggested that approximately one third of the world's population was infected with 50 to 100 million deaths [9]. Computer modeling of a moderate pandemic, less severe then in 1918, in the province of Ontario, Canada predicts 73,252 admissions of influenza patients to hospitals over a 6-week period utilizing 72% of the hospital capacity, 171% of intensive care unit (ICU) capacity, and 118% of current ventilator capacity. Pandemic modeling by the Australian and New Zealand Intensive Care Society also showed that critical care resources would be overwhelmed by even a moderate pandemic [10]. This chapter will provide intensivists with a review of the basic scientific and clinical aspects of influenza as well as an introduction to pandemic preparedness.

Influenza Virology and Pandemic Pre-requisites

Influenza is a RNA virus of the family orthomyxovirus. There are three types of influenza: A, B, and C, although only A and B are pathogenic to humans [11]. Influenza viruses are sub-typed based on two of their surface proteins; hemagglutinin and neuraminidase. Hemagglutinin facilitates viral cell entry via the sialic-acid receptor whereas neuraminidase plays a role in the cleavage of glycosidic linkages allowing release of viral progeny. Influenza A has 15 distinct hemagglutinin subtypes (H1-H15) and 9 neuraminidase subtypes (N1-N9). Human strains of influenza A are referred to by a combination of their hemagglutinin and neuraminidase subtype along with the city and year in which the virus was first identified, i.e., 'A/Sydney/97 H3N2'. Nomenclature for influenza B is much more straightforward as influenza B only has a single subtype of hemagglutinin (H1) and neuraminidase (N1).

Birds, in particular water fowl, are clearly the species with the greatest diversity of influenza A infections. All subtypes of influenza A (H1–15 and N1–9) have been

found in aquatic birds. In contrast, among mammals fewer subtypes have established sustained transmission (humans H1–3/N1,2; pigs H1,3/N1,2; horses H3,7/N7,8) [11]. Avian influenza is further subdivided into 'low pathogenic' or 'highly pathogenic' strains. Low pathogenic infections are less virulent because they are restricted to the respiratory (usually upper) and gastrointestinal (GI) tracts. This is because their hemagglutinin precursor can only be cleaved to produce its active form by extracellular proteases found in the respiratory and GI tracts. Conversely, the significantly increased virulence seen in highly pathogenic viruses is attributable to systemic infection which occurs because alterations in the hemagglutinin allow cleavage by intracellular proteases found in all organ systems [12].

To understand how novel influenza viruses evolve with the potential to cause a pandemic, one must consider the concepts of 'drift' and 'shift' [9, 11, 13]. Drift refers to point mutations occurring in the surface hemagglutinin or neuraminidase leading to a slight modification of the antigenic properties of the virus. Where drift is a minor change in viral genome, shift is a major change in the genome that results from the reassortment of genes from two influenza viruses leading to a 'new' virus with antigenically distinct glycoproteins. Until Taubenberger and his colleagues recently sequenced the genome of the virus responsible for the 1918 influenza pan-

Table 1. Pandemic definitions and resources

Pandemic Conditions	WHO Pandemic Phases	Links to Selected Pandemic Plans
1. a new influenza A virus arising from a major genetic change i.e., an antigenic shift 2. a susceptible population with little or no immunity 3. a virus that is transmitted efficiently from person to person 4. a virulent virus with the capacity to cause serious illness and death.	**Interpandemic period** *Phase 1.* No new influenza virus subtypes have been detected in humans. An influenza virus subtype that has caused human infection may be present in animals. If present in animals, the risk of human infection or disease is considered to be low. *Phase 2.* No new influenza virus subtypes have been detected in humans. However, a circulating animal influenza virus subtype poses a substantial risk of human disease. **Pandemic alert period** *Phase 3.* Human infection(s) with a new subtype, but no human-to-human spread, or at most rare instances of spread to a close contact. *Phase 4.* Small cluster(s) with limited human-to-human transmission but spread is highly localized, suggesting that the virus is not well adapted to humans. *Phase 5.* Larger cluster(s) but human-to-human spread still localized, suggesting that the virus is becoming increasingly better adapted to humans, but may not yet be fully transmissible (substantial pandemic risk). **Pandemic period** *Phase 6.* Pandemic: increased and sustained transmission in general population.	WHO: www.who.int/csr/disease/influenza/pandemic/en Canada: www.phac-aspc.gc.ca/cpip-pclcpi U.S.A.: www.pandemicflu.gov European Union Plans: www.ecdc.eu.int/Influenza/National_Influenza_Pandemic_Plans.php Australia/New Zealand Intensive Care Society: www.anzics.com.au/uploads/influenza_pandemic_report.pdf Ontario, Canada: www.health.gov.on.ca/english/providers/program/emu/pan_flu/pan_flu_plan.html Toronto Academic Health Science Network: http://portal.sw.ca/tahsn

demic [a], it was felt that pandemics resulted from reassorted viruses (shift) whereas small epidemics and mismatches with influenza vaccines resulted from drift [11]. Taubenberger's group showed that the 1918 human H1N1 influenza virus differed from the H1N1 avian influenza virus of the day in only 10 amino acids, the result of drift. Although this new knowledge increases concern that H5N1 avian influenza may drift into a strain that fulfills the conditions necessary to produce a pandemic (Table 1), it also allows the World Health Organization (WHO) to monitor for key changes in H5N1 viral sequence which may enable human-to-human transmissions.

Clinical Presentation and Complications

Influenza presents with a variety of general symptoms familiar to most clinicians. These may include: Fever, headache, malaise, cough, sore throat, rhinitis, nausea and vomiting. Symptoms can vary with the specific strain of influenza [14] or host factors such as age [15]. Avian influenza H5N1 presents with a similar constellation of symptoms which also vary between family clusters [8]. Given this variability, it is difficult to determine *a priori* what symptoms a potential pandemic strain of influenza may produce. Complications produced by influenza are more predictable and include pneumonia (bacterial or viral), myositis, rhabdomyolysis, encephalitis, aseptic meningitis, transverse myelitis, and exacerbation of any underlying chronic condition particularly cardiac, pulmonary, or renal disease. Complications from H5N1 human cases to date are similar to H1N1 only more severe. High risk groups for complications of seasonal influenza include patients with cardiac, pulmonary or renal disease, diabetes, hemaglobinopathies, immunosuppression, and residents of nursing homes or those over 65 years old.

A review of H5N1-infected patients who required intensive care unit (ICU) support suggests a very virulent disease [16], although these data may be skewed by reporting bias. Of the 41 patients reported, 68% developed multiple organ failure (MOF) with a mortality rate of 90%. The time to ICU admission was rapid at 2 days (IQR 0.75 to 3.25 days) with a median time from hospital admission to death of 6 days. The majority of patients developed respiratory failure, but of note 44% developed hemodynamic compromise and 24% renal failure. Pneumothorax occurred in 17%, a rate higher than that noted in most series of acute respiratory distress syndrome (ARDS).

Diagnosis

The diagnosis of influenza can be challenging. While it may be possible to differentiate a viral from bacterial infection based upon features of the history and clinical exam [17], it is difficult to differentiate influenza from other respiratory viral infections, making laboratory diagnostics essential. Immunoflorescent antibodies (IFA), direct immunofluroesence, ELISA and molecular methods such as real-time polymerase chain reaction (PCR) are the most commonly used diagnostic methods in non-pandemic settings. During pandemics, clinical diagnosis may be more useful due to the increased pre-test probability, particularly if few other respiratory viruses are co-circulating at the time [18].

Prophylaxis and Treatment

Prophylaxis for influenza includes vaccination [19] or antiviral use [20, 21], both of which are currently available for seasonal influenza strains. It is unlikely, however, that vaccination will play a significant role in the early days of a pandemic scenario due to the lag time in production once a pandemic strain is identified [5, 22]. Although much effort is being directed toward developing a H5N1 vaccine [23–25], variations in the strain, when hemagglutinin mutations necessary for more efficient human-to-human transmission occur, may decrease the efficacy of the vaccine. Further, it is possible that the pandemic strain may not even be H5N1. A significant focus has, therefore, been placed on the potential role of antivirals for treatment or prophylaxis during a pandemic. The antivirals currently available are the adamantanes (amantadine and rimantadine), which block fusion of the virus and host-cell membranes, and the neuraminidase inhibitors (oseltamivir and zanamivir) which block the release of viral progeny from the infected cell [20]. Computer modeling shows that antivirals could play a role in both containment of an early outbreak through prophylaxis [26] or treatment [27], which has been favored in cost-analyses [28]. The primary limitation of antivirals is the development of resistance [29, 30] against influenza A, particularly for the adamantanes [31] which also lack activity against influenza B.

Transmission and Infection Control

The incubation period for influenza varies with age and ranges from 1–4 days (averaging 2 days) for adults. Adults are typically infectious from the day prior to the onset of symptoms to day 5 of their illness whereas infants and children can be infectious several days prior to symptom onset and continue to shed virus for weeks [32]. Influenza is transmitted primarily via respiratory droplets although the previous 'black and white' distinction made between droplet and airborne transmission of respiratory viruses was oversimplified [33]. There is evidence that airborne (aerosol) transmission of influenza does occur in some circumstances [21, 32]. None the less, except under select circumstances, hand-hygiene and droplet/contract precautions (mask, gloves, gowns, and eye protection) remain the mainstay of infection prevention for influenza [34]. Readers should note that airborne precautions with the use of 'N-95' (EU FFP2) masks are recommended for avian influenza (H5N1) [35]. It remains uncertain what precautions will be most appropriate during a pandemic. The precautions used may in fact change over time as the response evolves from efforts to slow the spread by controlling the very first cases in a region to later in a pandemic when the infection is broadly established in the community.

Some have questioned the utility of personal protective equipment for health care workers during a pandemic given that health care workers' largest exposure risk will not be at work but rather when they are outside of work in the community. This argument neglects to recognize that exposure risk is additive and health care workers who treat patients with influenza would have an additional risk above that of non-health care workers. Reasonable efforts should be made to mitigate this additional risk through the provision of appropriate personal protective equipment, while recognizing that it will not be possible to prevent all transmissions to health care workers. Honest communication and efforts to protect health care workers will be essential to ensure they will continue to report for duty during a pandemic [36].

In addition to protecting health care workers during a pandemic, vulnerable non-influenza patients admitted to hospitals must be protected against nosocomial transmission. Strategies to prevent nosocomial transmission include cohorting infections patients separately from non-infectious patients and surveillance for symptomatic patients. This presents a greater challenge since influenza is infective prior to the onset of symptoms [21, 33, 37]. Thus, cohorting should not be relied upon as a fool-proof method of infection control. However, it can still significantly decrease the exposure of highly susceptible critically ill patients to potential infection.

Pandemic Planning Activities

Governments, organizations and businesses of all sizes have developed or are in the process of developing pandemic response plans. Links to a selection of pandemic plans can be found in Table 1. A review of European Union pandemic plans revealed a strong commitment by governments to the planning process but coordination was lacking between countries [38]. Most pandemic plans are based on several basic assumptions. The first assumption is that a pandemic will occur in a series of waves, each lasting 6–15 weeks, occurring over the span of a year or more. The clinical attack rate assumed by most plans ranges from 15–35% and represents a less severe pandemic then in 1918. Plans also assume that transmission will primarily occur in communities, as opposed to within health care facilities as occurred in SARS [1]. The primary focus of pandemic plans is system capacity, whereas in SARS the objective was containment. Pandemics are by definition widespread, affecting many areas at once, thus plans must focus on self sufficiency since support from neighboring countries or communities is unlikely.

The following sections review key issues for a hospital response to a pandemic, followed by issues specifically related to critical care.

Preparing Hospitals to Respond

Patient flow and clinical pathways

Capacity will be the primary issue during a pandemic. In order to optimize capacity there must be a well coordinated influx and efflux of patients through the system as a whole and through individual hospitals. Figure 1 illustrates the proposed flow of patients with influenza through the Ontario health care system in a pandemic. The premise behind this model is that each decision point differentiates those patients who need to receive advanced care while diverting those who are able to care for themselves thus decreasing the burden on the health care system. Within health care pandemic planning, much attention has been paid to admission criteria, however discharge criteria are even more important [39]. Clinical pathways [39] can facilitate patient flow through hospitals, improve patient safety, and support health care workers performing in expanded scopes of practice.

Communication

Communication is always a challenge in any disaster. SARS highlighted communication challenges within hospitals [40, 41]. Hospitals must develop a communication plan. During a pandemic senior hospital leadership must be visible, supportive, communicate frequently and transparently with staff, patients, family members, and

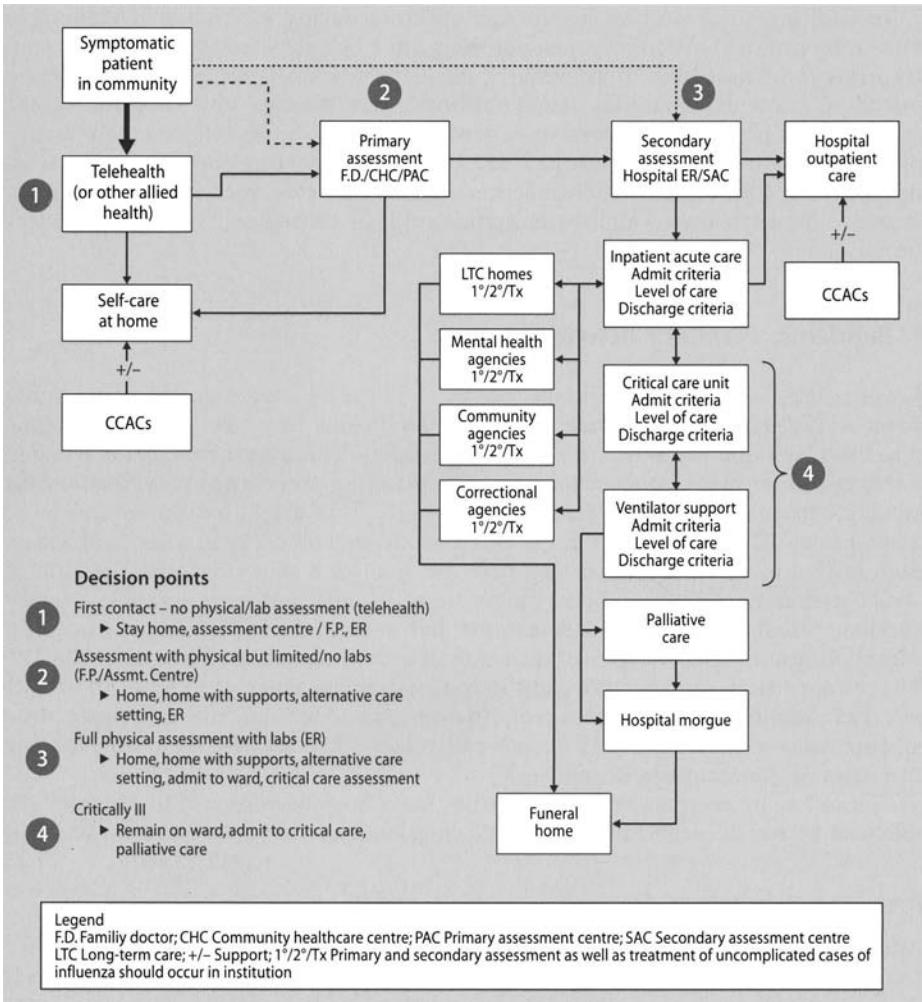

Fig. 1. Proposed flow of influenza patient through health care system

the media. Communication plans should be coordinated with other health care facilities, public health and all levels of government.

Command and control

Clear lines of command are critical to mounting an effective response, but traditional organizational structures used in health care are not intended for managing crises such as a pandemic. The Incident Management System (IMS) is rapidly being adopted by health care systems [42] and is ideal for structuring a response to a pandemic.

Human resources

Human resources shortages pose the greatest threat to a successful pandemic response. With expected absenteeism rates in the private sector of up to one third, health care organizations can anticipate similar if not higher rates at a time when system demands will be several fold higher then normal. Plans must be in place to scale back non-essential work and focus on 'essential work'. In doing so, it is important to remember that every health care worker will be essential and mechanisms must be in place to redeploy staff from non-essential to essential activities. Human resource plans must extend beyond the response phase into recovery. We have learned from SARS that the impact of personal and co-worker illness or death during an outbreak can have lasting effects on health care workers, long after the event [43].

Ethics

Many difficult issues must be confronted during a pandemic. When seeking to address such issues decision makers must be guided by both science and ethics. The complexities of these issues are beyond the scope of this chapter and readers are directed to a very thoughtful review [44].

Critical Care Response to a Pandemic

Accommodating influenza and non-influenza patients

When confronted with an overwhelming situation, people have a tendency to focus only on this issue (i.e., influenza) until it is resolved. However, in a pandemic we will still have an equal duty to care for the patient who happens to have a motor vehicle accident or myocardial infarction as well as those with influenza. A single pool of critical care resources exists that must be accessed by all. Thus, intensivists must plan to deal with both influenza and non-influenza patients during a pandemic which may last 12 months or more. Strategies to increase capacity include transforming non-ICU care areas, such as post-anesthetic care units, step-down units, endoscopy units, into ICUs and then assigning some units to deal with cohorts of either influenza or non-influenza patients. This task can be facilitated by creating and maintaining an inventory of all areas in your hospital that have the key requirements for conversion to an ICU: Oxygen, suction, medical gas, and electrical power, and adequate physical space to accommodate staff, equipment and patient care. Regional coordination is necessary to ensure that all essential health care needs are met within a region when individual hospitals scale back their routine services to meet the surge in patients.

Surge capacity

Plans and processes to deal with surges in critical care patients during a pandemic need to be developed. Involvement of intensivists in this planning process is essential. Most ICUs are capable of dealing with small surges (i.e., $<20-30\%$ above their day to day capacity) without exceeding their ability to cope. Various strategies such as a mass critical care and triage may be required to cope with larger surges. Collectively these concepts are referred to as surge response strategies. Hick and colleagues [45] differentiate between two important concepts: Surge capacity – making available adequate resources to deal with increased number of patients; and surge capability – the ability to manage increased number of patients. These definitions illustrate the need to plan for staff resources in addition to equipment and facilities.

For further information on surge capacity see the article by Hicks et al. [45] and web resources available at the Centre for Excellence in Emergency Preparedness (www.ceep.ca).

Care teams

One strategy that can be utilized to increase capacity is to modify the bedside staffing structure through the use of care teams. In a care team, a group of health care workers work together to care for a defined group of patients, usually in a fixed geographical area. This system makes use of a pyramid supervisory structure with less skilled or experienced staff being supervised and assisted by a small number of more skilled or experienced health care workers. This allows resources to be used more efficiently and allows less skilled health care workers working in expanded roles to function safely and effectively. A 50% increase in the critical care human resources capacity could be obtained by supplementing experienced ICU staff with non-ICU staff in care-teams. This structure is also consistent with the IMS organizational structure. ICU outreach teams for hospital wards and telephone support from academic intensivists for community intensivists may also help to maintain system capacity [40].

Mass critical care

Mass critical care is a different model and a different standard of critical care from what is practiced under normal circumstances. Simply stated, the goal of mass critical care is to provide a few key interventions (those with the highest impact and potential to save lives) to many people rather then providing very resource intense interventions to a few [40, 46, 47]. All processes and procedures are open to modification and must be considered from a new perspective including standards of care, staffing, equipment, and the allocation of resources. Although there certainly is a need to modify the standard of care during a pandemic, one must always keep in mind the primary objective of ensuring that the maximum number of people possible, survive. Thus, caution must be exercised when expanding clinical roles or modifying management to ensure that care is not compromised beyond the point where more harm is being done than good. For instance, it is of little use to move to a ventilation strategy such as long term manual ventilation with bag-valve-masks that may allow many more people to be ventilated but results in an increased number of deaths due to barotrauma than would have occurred if fewer patients were ventilated using a less harmful ventilation strategy. Striking an appropriate balance requires monitoring treatment outcomes during the response. In order to comply with medicolegal and ethical standards, plans to alter the standard of care during a pandemic should be publicly discussed and documented in advance with clear, objective criteria defined for the institution of mass critical care [44]. For these same reasons it is critical that all hospitals within an area adhere to the same standards of care.

Triage

During a pandemic, surge capacity may be maximized, yet resource scarcities will still occur [46, 47]. In such situations it is necessary, and in fact mandated by international law [47, 48], to utilize methods for allocating resources that are both equitable and maximize the benefit to the population at large [45]. Such methods are referred to as triage. Human rights, humanitarian laws [48] and strict adherence to ethical practices, such as transparency and accountability, must be observed when triage protocols are being developed [46, 47]. A full exploration of the ethical issues

Table 2. The prioritization tool for use in the critical care triage protocol. Adapted from [49]

Colour Code	Initial Assessment	48 hour Assessment	120 hour Assessment	Priority/Action
Blue	Exclusion criteria* **or** SOFA >11*	Exclusion criteria **Or** SOFA >11 **Or** SOFA 8–11 no Δ	Exclusion criteria* **or** SOFA >11* **or** SOFA <8 no Δ	Medical management +/– palliate & d/c from CC
Red	SOFA≤7 **or** Single Organ Failure	SOFA score <11 and decreasing	SOFA score <11 and decreasing progressively	Highest
Yellow	SOFA 8–11	SOFA <8 no Δ	SOFA <8 with <3 point decrease in past 72 h	Intermediate
Green	No significant organ failure	No longer ventilator dependent	No longer ventilator dependent	Defer or d/c, reassess as needed

* If exclusion criteria or SOFA >11 occurs at anytime from initial assessment to 48 hours change triage code to Blue and palliate. CC: critical care; d/c: discharge; Δ: change

related to triage can be found in the framework developed by the Joint Centre for Bioethics [44].

Prior to recent pandemic planning initiatives, no triage systems had been developed for use in critical care for medical illnesses. Illness severity scoring systems used in critical care research have a reasonable ability to predict ICU outcome. However, they are not intended to predict mortality in the individual patient and are cumbersome to use and impractical when human resources are scarce. Although validated for predicting outcome, they have not been validated for guiding, or more specifically restricting, treatment. Christian et al, have recently published the first comprehensive triage protocol designed for use during a pandemic [49]. This protocol has been incorporated into the Ontario Pandemic Influenza Plan [39]. The triage protocol utilizes the Sequential Organ Failure Assessment (SOFA) score [50] and has four main components: Inclusion criteria, exclusion criteria, minimum qualifications for survival, and a prioritization tool (Table 2).

A challenge in developing critical care triage protocols for a pandemic is that many prognostic factors, such as the natural history and response to treatment, are unknown. Given the highly complex nature of triage protocols, it is impossible to create a triage system *de novo* during a pandemic [46, 47]. The best way to prepare for critical care triage during a pandemic is to develop general triage guidelines [49] in advance of the pandemic and then modify the protocol once variable factors, such as probability of survival and available resources, are known. The infrastructure and training necessary to allow effective triage must also be addressed.

Conclusion

Although influenza is an illness we contend with every year, a great deal of uncertainty exists as to what an influenza pandemic would have in store for the world. This uncertainty makes specific planning difficult and increases anxiety among both

the public and health professionals. It is important to remember however, that as a pandemic draws nearer our knowledge will increase, dissipating the uncertainty. Although our plans must remain general, we must initiate the planning process now. Firstly, we do not know when the next pandemic may begin. Moreover, once a pandemic does begin, there will be insufficient time to lay the foundation upon which to mount a response. Critical care will play an instrumental role in the response to a pandemic, thus intensivists must be involved in planning the response. Intensivists bring to pandemic planning a unique understanding of treating critically ill patients and managing ICUs. Having read this chapter, intensivists should feel more comfortable engaging their colleagues in public health, infectious disease, and emergency medicine in planning together to prepare their community to respond to a pandemic.

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Pathobiology of Blast Injury

A.M. Dennis and P.M. Kochanek

Introduction

Injury due to explosive detonation has previously been isolated to industrial accidents and soldiers and civilians in areas of armed military action. Substantial data regarding blast-related patterns of injury has come from military reports and research, and there have been significant advances in protective vehicle and body armor, 'far forward' provision of medical care, and evacuation procedures. Despite this, explosive munitions and improvised explosive devices still comprise the majority of combat morbidity and mortality [1–4]. There is also increased targeting of civilians in a global political environment where incendiary devices are a principle instrument of modern terrorism [5–7]. Events in preceding decades indicate a critical need for both civilian and military emergency and intensive care providers to understand the pathophysiology and management of blast-related injuries.

Reviews of military casualties and terror-related bombings reveal that while pulmonary and orthopedic consequences of blast injury are predominant, traumatic brain injury (TBI) is more prevalent than initially thought. Data from Israel and Ireland demonstrate a striking "excess number of head and brain injuries" when anatomic analyses are performed [6, 7]. There is emerging evidence that blast-related TBI may be a unique entity, produced by kinetics specific to explosive force, and exacerbated by a milieu of extra-cerebral consequences.

In this chapter, we will 1) discuss the general pathomechanisms of blast injury, 2) review the current information on this unique mechanism that manifests specifically in blast-related neurotrauma, 3) discuss experimental studies of the cellular and molecular response to blast-induced neuronal and axonal damage, 4) review the concept of secondary injury in blast-induced TBI, and 5) discuss further directives in therapy for this unique brain injury medium.

Blast Injury: Pathomechanics

Blast overpressure occurs as heated and compressed air molecules expand outward from the detonation epicenter. The leading edge of this blast wave forces an instantaneous and extreme rise in surrounding air pressure (positive phase), followed by collapse to a sub-atmospheric level (negative phase) before normalizing. Figure 1 depicts this stereotypic pressure rise and fall, known as a Friedlander wave. Primary blast injuries result from the cussive effect of the leading blast overpressure energy wave as it impacts the body. In open spaces, a single wave is produced, but in closed environments, the energy is deflected off various surfaces, impacting victims repeat-

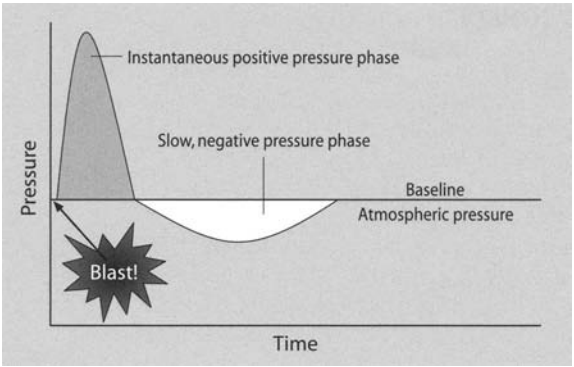

Figure 1. Idealized Friedlander Wave, depicting rise in ambient atmospheric pressure and subsequent fall to subatmospheric levels before equilibration.

edly, and with magnified intensity. Spalling describes the forceful movement of energized fluid into less dense tissues, and differential tissue inertia results in non-uniform acceleration and shearing at interfacing planes. Projectile debris from the device or immediate surroundings inflict blunt or penetrating trauma, referred to as secondary blast injury. This is of particular concern with improvised explosive devices or 'dirty bombs', frequently laden with objects such as nails, glass, or ball bearings in order to maximize destruction. The magnitude of the blast can displace the victim's entire person, resulting in tertiary or displacement injury from collision with stationary objects. Consequences of the detonation such as building collapse contribute to quaternary injury, and a quinary mechanism occurs with exposure to or inhalation of toxic, infectious or radioactive substances [8–15].

Primary injury from blast overpressure predictably involves hollow organs such as the ears, respiratory tract, and abdominal viscera. Hearing loss is extremely common, and in an organ evolved to magnify subtle sound waves, rupture of the tympanic membrane is pathognomonic for significant blast overpressure exposure. The majority of blast-related literature addresses lung pathology (blast lung): Alveolar rupture, pneumothorax, pulmonary contusion, and hemorrhage. Respiratory failure may result, while arterial air emboli from pulmonary disruption sometimes circulate to the cerebral vasculature or coronary arteries with deleterious effects. In the abdominal compartment, rapid gaseous distension and violent compaction of viscera results in rupture or hemorrhage, and can occur without outward signs. In fact, life-threatening internal injuries due to blast overpressure are notable for lack of external evidence of trauma.

Subsequent to primary blast overpressure, secondary and tertiary mechanisms yield a spectrum of life-threatening injuries not unlike more familiar blunt and penetrating trauma incurred in motor vehicle crashes, falls, gunshots, and stabbings. However, serious, simultaneous involvement of multiple anatomic regions is a hallmark of critical explosive injury. Bleeding from shrapnel wounds, traumatic amputations, fractures, and internal injuries frequently leads to hemorrhagic shock and a systemic inflammatory response, while thermal blast energy and fire can cause extensive burns. All of this generally ensues amid mass casualty and chaos, sometimes in austere environments, and often in the context of limited or overwhelmed medical resources.

Blast-related Neurotrauma: 'Shell Shock' Revisited

A retrospective study of terror-related ballistic injuries in Israel (firearm and explosive), found a 17% mortality associated with internal injuries involving the thorax and abdomen, and 80% mortality when similar patients also sustained a head injury [6]. TBI is the most common cause of death in terrorist bombings. Blast-related severe TBI victims, with or without concomitant extra-cerebral trauma, are an important population as the overwhelming majority go on to die despite survival to hospital admission. While previous explanations blamed air embolism for central nervous system (CNS) injury, blast TBI is a more complex entity involving multiple mechanisms and levels of injury. Acutely, victims demonstrate a spectrum of neurologic dysfunction, from transient loss of consciousness to coma. Long-term survivors frequently suffer chronic cognitive impairment or psychological manifestations. The terms 'shell shock' and 'commotio cerebri' were coined decades ago to describe the occurrence of these neurologic phenomena without external evidence of trauma or obvious intracranial pathology [16–18].

Blunt force and penetrating trauma cause skull fractures and discreet mass lesions: Cerebral contusions, hemorrhages, and lacerations. In TBI, inertial and rotational forces also result in global damage with diffuse axonal injury (DAI) and cerebral edema. DAI is observed in acceleration-deceleration mechanisms such as motor vehicle crashes, frequently implicated in traumatic coma, and is distinguished by widespread axonal swelling and retraction balls in cerebral white matter, cerebellum, and brainstem [19]. Other hallmark features include microglial activation and punctate hemorrhages in these locations, as well as lesions in the corpus callosum. Evidence suggests that clinical and experimental blast TBI is strikingly similar to DAI, with abnormal axonal morphology, cerebral edema, punctate hemorrhages in white matter, choroid plexus, cerebellum and brainstem, and occasional subdural or subarachnoid bleed [20, 21]. Injuries resulting from incendiary devices are multifaceted in nature, and the secondary and tertiary mechanisms certainly account for a portion of observed TBI. However, blast TBI as a unique consequence of primary blast overpressure is currently being investigated as the etiology of diffuse brain injury in the absence of obvious external violence, and as a result of "kinetic energy transfer of blast overpressure to the CNS" [24]. This has serious implications in the critically wounded blast victim, as extra-cerebral injuries exacerbate head injury with secondary insults, and TBI can impair appropriate systemic physiologic compensation in hemorrhagic shock [22, 23].

While blast waves may travel directly through the cranial vault, alternately increasing and decreasing intracranial pressure (ICP) and shearing tissues, the relative homogeneity of the brain and its encapsulation in a hard, bony case make direct transcranial injury seem unlikely to explain all of the findings and possibly does not represent the primary mechanism of injury. Soldiers wearing protective helmets still manifest varying degrees of intracranial blast injury [2]. Furthermore, Cernak et al. found similar neuropathology in physically secured blast injured rats, regardless of whether the head was shielded from direct impact or not [24]. One explanation is that as blast overpressure oscillates through the torso, compression of organs and vessels forces blood into the cranium as a surge of arterial flow or increased venous backpressure. Blast waves can propagate in air and water, and blast overpressure may also travel cranially via a path of least resistance out of the thorax in blood vessels and spinal fluid [18]. Water is denser than air, and because a fluid blast overpressure wave is actually more intense, serial Friedlander waves transmitting into

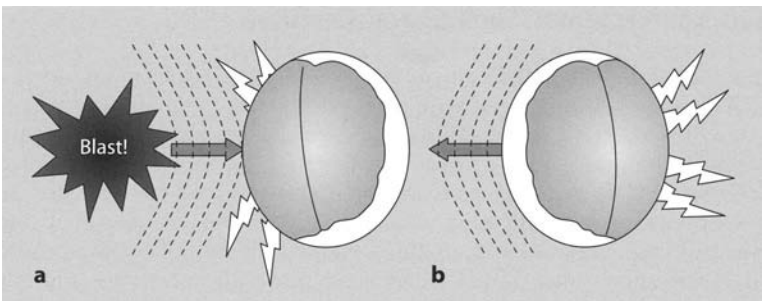

Figure 2. Plausible mechanism for diffuse brain injury due to blast energy. **a** Initial positive pressure phase of the Friedlander wave impacts the rigid skull, while the stationary brain lags behind. **b** The subsequent negative pressure phase causes a whip-lash motion back towards the energy source, while the brain has begun to move in the opposite direction, resulting in coup-contre-coup and DAI spectrums of brain injury.

the cranial vault could certainly generate the observed pattern of injury. Another possible mechanism is that the rapid positive pressure phase first encounters and accelerates the rigid skull. The brain is unattached, suspended in spinal fluid, and lags behind, suffering an initial blow on the blast-facing surface. When the subsequent lower energy negative wind pulls the head in the opposite direction, the brain is struck again, this time on the opposite, non-blast facing surface (Fig. 2). While additional investigation is needed to elucidate whether blast overpressure itself results in altered level of consciousness and unique brain pathology, or whether this is simply another mechanism of acceleration-deceleration injury, there is an emerging body of evidence that suggests that blast injury may produce a new pattern of brain injury in civilian bombings and modern urban warfare casualties. If a unique pathobiology of brain injury is produced by blast injury, a unique and tailored approach to both resuscitation and neurointensive care may be necessary to maximize outcomes.

Experimental Blast-Induced TBI: Implications of Cellular and Molecular Mechanisms of Neuronal and Axonal Injury

Studies in a number of experimental models of blast-induced injury have been carried out, and although many questions remain, evidence is provided supporting contributions of DAI, oxidative stress, cellular stress with immediate early gene activation, calcium accumulation, decreases in tissue magnesium, energy failure, neuronal death, and edema.

A number of clinical and experimental studies have observed evidence of DAI in blast TBI. Using a rodent non-penetrative blast model, Säljö et al. demonstrated failure of heavy chain phosphorylated neurofilament protein (p-NFH) migration out of the neuron perikarya into the axon where they are an integral component of cytoskeletal integrity and axonal transport [25]. This response was found to be dose related, and more pronounced in the blast-exposed hemisphere, though both hemispheres were involved. Accumulation of p-NFH in the perikarya was present from 18 h to 7 days, and normal axonal distribution of p-NFH had returned at 21 days. Kaur et al have explored inflammation in experimental blast TBI and found a significant, widespread increase in activated microglia (assessed immunohistochemically) in the

gray and white matter, as well as in the choroid plexus of ventricles [26, 27]. Additionally, at 7–14 days after injury in cerebral and cerebellar cortices there was non-specific "darkening" of dendrites and axons associated with microglial cells, suggesting neuronal alterations as a consequence of blast.

Oxidative stress is a recognized secondary injury cascade in TBI, induced by the primary insult and also an important consequence of key secondary injury processes such as excitotoxicity, mitochondrial dysfunction/failure, nitric oxide (NO) synthesis, peroxidase activation, and inflammation. Reactive oxygen species (ROS) induce membrane lipid peroxidation, DNA damage, protein oxidation, and lipid and protein nitration, among other mechanisms. Oxidative damage can initiate necrotic, apoptotic, and autophagic cell death cascades. Endogenous antioxidants, including small molecules such as ascorbate, can mitigate the damage, however, there is strong evidence that these pathways are often overwhelmed – including studies in human TBI [28]. In rodents exposed to either whole body or local pulmonary blast, remarkably, important biochemical consequences were noted in brain specifically the highly vulnerable hippocampus [24]. Quantification of levels of both markers of oxidative stress, and endogenous antioxidant proteins such as superoxide dismutase (SOD), and glutathione peroxidase revealed an immediate increase within a few hours of insult, with return to normal levels by 5 days. After whole body blast in rats, the increase in these markers of oxidative stress was greater when compared to local blast rats, except for glutathione peroxidase [24]. The levels were also elevated in direct proportion to injury severity.

Another line of evidence for neuronal consequences of blast is the presence of immediate early genes previously demonstrated to be associated with neuronal damage. Phosphorylated c-Jun is recognized as a marker of neuronal stress, and appears to be a signal for induction of apoptosis. In rodents exposed to impulse noise (a pressure pulse wave approximating that seen when handling heavy weapons), Säljö et al noted immunopositivity for c-Jun in the temporal, cingulate and piriform cortices, as well as CA 1, 2, 3 and dentate gyrus of the hippocampus. Terminal dUTP nick-end labeling (TUNEL) for double stranded DNA nicks co-localized with c-Jun staining, and supports the production of neuronal death in ~2–5% of neurons surveyed in this model, with a relatively modest injury level [29]. The early gene, c-Myc, has been implicated in the activation of caspases, mediators of apoptosis. In this model, neuronal and astrocytic c-Myc immunoreactivity in rats peaked at 18 h after injury in identical regions to c-Jun, as did c-Fos [30]. This suggests a timeline for neuronal death, and possible opportunities for aborting these pathways.

Unregulated calcium influx in injured neurons results in cellular dysfunction and is associated with energy failure. To this end, Cernak et al. compared magnesium, calcium, ATP, and water levels in the lungs and brainstem of rabbits exposed to local pulmonary blast injury only [31]. Immediately following injury, in lung tissue, there were markedly increased amounts of water and calcium, and decreased levels of magnesium and ATP. In the brainstem, the findings were similar, with increased brain water and decreased magnesium and ATP. Calcium levels increased in the brainstem, but not to a significant degree. These findings suggest that blast TBI has many features of other forms of TBI: Edema, calcium cytotoxicity, energy depletion. This study also supports the notion that indirect blast induces brain injury; perhaps through acceleration-deceleration, transmission of blast overpressure out of the thorax, ischemia, or hypoxia from apnea, lung injury or hemorrhagic shock, or extreme excitation of brain tissue from afferent nerve impulses from damaged tissues [24].

Role of Secondary Injury

Critically wounded blast victims pose a distinct therapeutic challenge in expeditious prevention of secondary insults and mitigation of harmful physiologic responses. Despite recognition of the blast-related TBI spectrum, the responsible mechanisms (primary blast overpressure vs secondary and tertiary injuries) are extremely difficult to separate in a real-time patient encounter. However, what is paramount is the substantial risk of secondary insults in this milieu of life-threatening multiple trauma.

Previous publications in civilian TBI have unequivocally established that TBI outcomes are often dismal when hypotension occurs [32]. This relationship is also seen in the setting of blast injury. In a recent combat casualty review of close proximity blast, the single most important predictor of mortality was the presence of hypotension; and when any combination of penetrating head injury, multiple long bone fractures or associated incident fatalities were present, mortality was 86% [33]. Hemorrhagic hypotension from internal bleeding, fracture, traumatic amputation and multiple shrapnel wounds is an important cause of secondary insult: 1) there are often prolonged extraction times in the field, 2) injuries cannot always be definitively managed in the field, 3) adequate volumes of resuscitative fluids may not be available, and 4) administration of crystalloids in the face of ongoing blood loss can result in accelerated hemorrhage and hemodilution with impaired oxygen delivery and coagulation. Reduced cerebral blood flow (CBF) in the face of enhanced metabolic demands is due to TBI related loss of cerebrovascular autoregulation, and inadequate circulating blood volume and perfusion pressure. There is also evidence that TBI blunts an appropriate blood pressure response to volume resuscitation in hypovolemic shock. Additionally, there is experimental evidence that blast overpressure causes immediate bradycardia, hypotension and reduced cardiac index. Irwin et al were able to prevent bradycardia and hypotension in experimental blast injury with bilateral cervical vagotomies and administration of atropine, suggesting maladaptive physiologic responses related to vagal reflexes [34]. Several studies have demonstrated electroencephalogram (EEG) slowing and attenuation after exposure to blast, and the reproducibility of brainstem and white matter pathology also corroborates the occurrence of centrally mediated processes.

Second only to hypotension, hypoxemia is a significant factor predicting poor outcome in TBI patients [32]. Clark et al. demonstrated increased hippocampal neuronal death and decreased early motor function performance in rodents subjected to experimental TBI and secondary hypoxic insult [35]. Apnea, hypoventilation, and impaired gas exchange from brainstem and lung pathology are certainly implicated in this paradigm. Ischemia from hemorrhagic shock also contributes relative tissue hypoxia in a failure of oxygen and substrate delivery.

Free radical production, inflammation, and excitotoxicity occur as direct consequences of TBI, but there is also speculation that extra-cerebral and endothelial injuries elaborate harmful products into the circulation that exacerbate TBI upon reaching the cerebral vasculature and brain tissue. One such hypothesis is that as blast overpressure traverses the body, rupture of blood vessels leads to hemorrhage and endothelial activation. Red blood cells are also disrupted, releasing hemoglobin that is oxidized, catalyzing free radical formation. The ensuing oxidative stress perpetuates cellular injury [36]. In addition, hemoglobin has the ability to bind NO, thereby possibly contributing to ischemia and impaired oxygenation by vasoconstriction in the microcirculation. Further actions of free radicals in the brain con-

tribute to increased blood brain barrier (BBB) permeability and ultimately cerebral edema, as well as enhanced inflammatory cell responses. Extracerebral effects of blast-injury and shock on systemic elaboration of cytokines, endotoxin, and/or other potentially toxic mediators of secondary damage in brain are also possible – but remain to be explored and fully characterized.

The challenge of preventing and/or promptly and optimally treating secondary insults in critically wounded severe TBI patients is a pressing military and civilian concern. Not only could advances in far-forward and pre-hospital resuscitation strategies reduce mortality; novel therapies and resuscitation fluids could significantly impact the morbidity of TBI, resulting in a greater percentage of 'good' or 'excellent' neurologic outcomes.

Future Directions in Therapeutic Interventions

Amidst the reality of civilian mass casualty and hostile battlefield environments, where chaos and limited available resources are commonplace, optimal evacuation, triage and resuscitation of TBI and combined systemic trauma is not always feasible. Effective, practical interventions to both prevent secondary insults and mitigate maladaptive physiologic responses would, therefore, represent a significant breakthrough. Furthermore, if blast-related TBI exhibits unique pathobiology, prehospital and neurointensive care interventions specific to the constellation of physiology and molecular alterations would be invaluable.

Expedient initial treatment of hypotension and prevention of additional hypotensive episodes can be achieved with volume resuscitation and control of hemorrhage sites. Resuscitation fluid may not be readily available, however; for example, a battlefield medic can only carry a finite amount of equipment. Furthermore, the use of crystalloids for volume expansion is only temporizing, as critically wounded patients rapidly require blood products to restore homeostasis. Far-forward providers should be equipped with necessary supplies for abating hemorrhage, and provision of recombinant activated factor VII should be considered for life-threatening bleeding [37, 38].

Fresh whole blood is now being used early in the resuscitation phase by the US Army in the setting of major hemorrhage in the Iraq War (Holcomb, presented at ATACCC, St. Pete Beach, FL, August 2006). A novel resuscitation fluid to expand blood volume in smaller aliquots than conventional fluids would be ideal. If this compact fluid could also provide oxygen delivery, osmotic or oncotic pressure, or other mediators of immediate injury, it would be significant. Hypertonic saline restores blood pressure in much smaller amounts than isotonic fluids as interstitial fluid moves into the intravascular compartment to equilibrate the osmotic gradient. In addition, hypertonic saline avoids hyposmolarity and is effective in treatment of increased ICP [39]. However, its efficacy in this specific setting remains to be definitively proven. Currently Hextend is the standard resuscitation fluid of the US military [40]. Mannitol has long been used as a hyperosmolar therapy for increased ICP, and does cause an immediate rise in blood pressure and drop in ICP; however, this is followed by osmotic diuresis, which is not desirable in patients with hypovolemic shock. Hypertonic saline increases cerebral perfusion by expanding circulating blood volume while at the same time ameliorating intracranial hypertension. Thus, there may be theoretical advantages for its use. There remains concern regarding BBB permeability in injured brain, permitting hypertonic fluid leakage into tissue

and contributing to vasogenic edema. It follows then, that a strategy to maintain BBB integrity would be a desirable adjunct. Immediate BBB breach and tissue damage from the inciting injury cannot be prevented, but further compromise from hypotension, hypoxemia, inflammation, oxidative stress and endothelial activation are reasonable therapeutic targets for future therapies.

Vasogenic edema is only one mechanism of brain swelling. Cytotoxic edema also ensues. To this end, a family of membrane water channel proteins known as aquaporins serve to regulate fluid shifts into and out of neurons and astrocytes. Aquaporin knockout mice exhibit reduced edema and decreased ICP and brain water content after experimental TBI [41]. Manipulation of these transmembrane proteins might be an effective therapeutic option in the amelioration of evolving edema and increased ICP.

Disturbed cerebral blood flow (CBF) is another priority for further study and intervention. Cerebrovascular autoregulation is lost after injury, and in the face of low or high blood pressure extremes, this can be catastrophic. Thrombosis and platelet aggregation, direct vascular disruption, and tissue swelling contribute to impaired blood flow [42]. Vasospasm may be of special importance in blast induced TBI related to the extremely high prevalence of subarachnoid hemorrhage in experimental models of this condition. Whether or not triple H therapy – as used in the treatment of vasospasm after subarachnoid hemorrhage – is beneficial in blast-induced TBI remains to be determined [43]. Vascular perturbations such as reduced endogenous NO or other vasodilators, and increased factors such as endothelin-1 contribute to vasoconstriction [44]. On the other hand, vasodilation and markedly elevated cerebral blood volume can contribute to increased ICP. In the field, it is impossible to discern what the cerebrovascular milieu is; however, judicious application of diagnostic modalities upon arrival to definitive care, such as tissue oxygen monitoring and bedside cranial Doppler ultrasound, might guide optimal blood pressure range or use of vasoactive drugs by providing insight into the presence and nature of vascular dysfunction.

Formation of free radicals (reactive oxygen and nitrogen species) in TBI and systemic trauma as well as experimental blast injury elaborate secondary injury via membrane lipid peroxidation, altered protein conformation and binding, inflammation, endothelial activation and microcirculatory disarray. Antioxidant strategies and inhibitors of lipid peroxidation have the potential to dramatically impact this aspect in both isolated TBI and TBI in the context of severe polytrauma.

Blood substitute formulation has been attempted, but is as yet unsuccessful. Albumin is an attractive colloid molecule for inclusion in such a fluid, however, the albumin molecule can induce oxidative stress when associated metal moieties become redox active *in vivo*. This may explain the increased mortality of critically ill patients in several albumin studies, one of which demonstrated an increased mortality in the subset of TBI patients, although other mechanisms may be involved [45]. The addition of hemoglobin for augmented oxygen delivery in addition to oncotic pressure is promising as well. However, previous formulations have been fraught with multi-organ system failure complications, likely due to the oxidative stress induced by free hemoglobin. Novel formulations that mitigate these adverse effects would be advantageous in the setting of limited or unavailable blood products.

Initially, it appears that electrical brain activity is attenuated after blast. As brain injury evolves, subsequent excitotoxicity exacerbates neuronal damage. Anti-epileptic medications, barbiturates, anesthetic agents, and hypothermia mitigate this response. Other possible therapies include hypothermia and compounds to block N-

methyl-D-aspartate (NMDA) receptors and inhibit accumulation of glutamate, glycine, excitotoxic amino acids, and calcium early after injury. As calcium accumulates in neurons, mitochondrial damage ensues, resulting in energy failure and cell death via necrosis or apoptosis. Cyclosporine-A has been shown to abate the formation of a membrane permeability transition pore, thereby protecting mitochondria and conferring neuroprotection in experimental models [46]. While necrosis occurs due to essential failure of the cell, apoptosis is more complex, requiring initiators and signal cascades. Drugs to block the various steps in this process while homeostasis is disturbed may prevent neuronal death, or at least increase the likelihood that the cell will survive long enough for favorable conditions to return.

Therapeutic hypothermia is neuroprotective in experimental models of ischemia, hypoxemia, cardiac arrest, hemorrhagic shock, and TBI [47]. Hypothermia attenuates excitotoxicity, free radical production and inflammation, reduces metabolic expenditure, and decreases ICP. Results of mild therapeutic hypothermia on survival and neurologic outcome after cardiac arrest have been favorable, while clinical trials of therapeutic hypothermia for TBI patients have failed to demonstrate consistent neuroprotection. However, it is important to recognize that patients with clinically significant hypotension were excluded from TBI trials. Hemorrhagic shock from blast injuries add an ischemic insult to TBI, thus the combination of TBI with associated orthopedic and internal injuries typical of blast mechanisms would seem amenable to treatment with mild hypothermia [48]. Inability to control hemorrhage in this setting, suggests the potential need to combine mild cooling with therapies supplementing coagulation. This combined approach merits future investigation.

When inter- and intra-cellular aberrancies are considered simultaneously, it is abundantly clear that the most effective therapeutic approach will be multipronged and incorporate many different modalities. In addition, advances in genetic testing may allow the identification of persons with susceptible genetic polymorphisms, facilitating individual treatment plans. It is exceedingly unlikely that a single 'magic bullet' will emerge. Rather, the best intervention for each target in injury progression will need to be gauged by pre-hospital and neurointensive care providers. It remains to be seen what variety of therapeutic options will become available.

Finally, current treatment of blast-induced TBI in the military setting involves the use of decompressive craniectomy [2]. This may be an important strategy for controlling raised ICP in this setting. Additional experimental and clinical studies are needed.

Conclusion

Explosive munitions were once an inevitable aspect of armed military action, broaching civilian consciousness only in the context of war. Innocent inhabitants caught up in regional conflict, or victims of abandoned mine fields comprised the civilian portion of blast injuries not related to industrial accidents. Sadly, improvised explosive devices and civilian bombings have become a key aspect of the urban battlefield and politically motivated terrorism incidents. Military and civilian providers alike have been forced to re-examine their approach to victims as more patients are surviving to hospital admission than in previous experiences due to advances in prehospital care. Despite being a relatively crude weapon, improvised explosive devices have evolved a frightening level of sophistication and the indiscriminate and unpredictable nature of this modality is the essence of its effectiveness.

In response to this distinct pattern of injury, the medical research community and care providers are applying previously accumulated knowledge and techniques and moving in new directions to elucidate the precise nature of blast-related injuries, so that ideal therapies may be developed and employed. Ironically, blast-related TBI is similar to previous patterns of TBI such as DAI, traumatic subarachnoid hemorrhage and TBI exacerbation by hemorrhagic shock, aspects that have previously been investigated, but deserve additional study. It is likely that in the face of this new challenge, many questions that have yet to be fully answered will still need to be addressed. Bench research findings should be integrated with bedside observations, and novel therapies and intelligent resuscitation strategies will need to be developed in the context of the complexity of TBI.

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Personal Reflections on Emergency Preparedness and the Response to A Major Natural Disaster: Hurricane Katrina

N.E. McSwain Jr

Introduction

The aftermath/response of a disaster can be divided into four phases. The importance of each depends on the length of time and the resources required. This in turn depends on the length of the disaster, the area involved, the number of the population affected, the re-supply available, the extent of the devastation, and the size of the evacuation. The four phases are discussed using Hurricane Katrina as an example. The phases are:

1. Immediate: The initial time period when the facility has to exist, function, care for patients and staff, utilizing only supplies on hand prior to the emergency
2. Outside response: Plans developed to take advantage of outside resources brought in for the evacuation
3. Backfill: The time period when additional resources to manage the short term conditions become available to the institution
4. Restoration: Long term rebuilding of the medical community to restore the pre-emergency capabilities and function. This last phase is usually the longest and requires the most resources.

Immediate Response

In developing an all encompassing emergency preparedness/disaster management plan it is best to assume the worse case scenario. In other words, "plan for the worst, hope for the best". For a health care facility the worst case scenario is a situation which the medical facility will become an 'island' when the disaster occurs. By this I mean that immediate re-supply will be impossible and although patient evacuation will be required this too may/will not be possible immediately. Furthermore, this isolation could last for several days (as occurred with Hurricane Katrina). Under this scenario, the ability of the medical care providers to function and provide care will be depend on their prior training, the supplies available, the number of providers available, and the number of patients that require care. Only the supplies that were present prior to the onset of the disaster will be accessible. The timeframe for re-supply and/or evacuation will determine how these resources must be allocated. In many situations this dependence can last from 1–5 days. From a medical perspective, three separate assets have to be carefully considered: 1) In-hospital resources; 2) emergency medical services (EMS) resources; 3) resources available to the family of 'trapped' medical providers. Finally, the location of patient care will be determined by the extent of damage to the facility that arises from the disaster.

- **Examples from Hurricane Katrina**
  When New Orleans flooded in the aftermath of Katrina in 2005, the medical center became an island. Resources (supplies and personnel) could not get in and neither could patients or medical care workers get out. Eighty percent of the city of New Orleans was flooded with up to 12 feet of water! Some areas had water for 3 weeks. In the area southeast of the City (St Bernard Parish), 95% of the land area was flooded.

  Provision of medical care and all other functions of the hospital had to be carried out by the personnel in the hospital at the time of the flooding. The medical center command structure had to be reconstituted from the personnel that were present. Resourcefulness and adaptability became essential attributes. For example, as the power began to fail, communication within the hospital was accomplished by runners to the various areas within the hospital building. Every 4 hours the institutional command staff and middle management met. Communication outside of the institution also required 'runners', either using boats or wading through the contaminated waters.

  All operations of the Emergency Departments of both Tulane and Charity Hospitals had to be moved up to the second floors as the water rose and inundated the first floors. The required relocation of medical care and the internal transfers of patients and supplies to higher floors without the benefit of elevators, and in the dark, became a major logistic consideration that required an early solution. It is worth noting that pre-planning scenarios had not considered these events and, thus, there were no policies or suggestions as to how to deal with these challenges. Patients were left in the intensive care units (ICUs), with the equipment and supplies, until the time of their eventual evacuation. At that time they had to be moved down the stairs to the evacuation point.

  The absence of running water for 5 days meant that toilets and sanitation issues became extremely important. The rest rooms very quickly became very stinky. We placed the large (and thick) red biohazard disposal bags under the toilet seats and added some kitty litter waste to the bag. This 'low tech' solution went a long way to addressing this issue.

  Any supplies that will be needed within the 'island' for 5–7 days require secure storage. Security considerations need to include security from 'two-legged animals' (i.e., humans), four-legged animals, six-legged animals, eight-legged animals. In the case of a flooded New Orleans, all manner of animals and insects were looking for food. Careful consideration should be given prior to an emergency regarding what you need to store. How much are you going to have on hand? How long do these supplies need to last until replacement from outside sources? This final consideration may need to be 'fluid', depending on the nature of the individual emergency situation and based on reliable communication about a realistic timeframe for resupply and assistance.

The ability of the medical care providers to function becomes a critical factor when communication is down and the providers are trapped. The stranded health care workers do not have the ability to provide for the needs of their family and loved ones, or even to communicate with them. This reality is an overlooked factor when medical/disaster planners address the needs of their institution. Are the families of the institution's medical care workers prepared both with material resources and mental resources? Have the employees (not only physicians and nurses, but all institutional employees) had a discussion with their spouses, children, and other loved ones of how they can survive when the medical care workers are not present and have no communication?

The loss of communication both within and without both hospitals was the system failure that produced the most significant problems. Communication failure has frequently been cited as a major difficulty in numerous other disasters in the United States and other countries. Hurricane Katrina was no exception. It is an unfortunate reality that despite all the available technology in the United States, communication problems persist. We have heard it over and over and over and over again, but it is not going to be the final time because the problem has not been solved.

- **Examples from Hurricane Katrina**
  In the aftermath of Hurricane Katrina, communications failed; they always fail. No innovative person has yet solved this problem. Let us discuss a few specifics: How about cell phones? Cell phones work great, unless the cell phone towers are blown down, or power is out, or they cannot be recharged. Therefore, in reality, cell phones do not work for long! Twenty-four hours is about all you can get out of them. Ah! There's this wonderful new thing called satellite phones – they work; just hook up to the satellite, talk through a satellite, go all over the world with it. Theoretically a terrific solution, however in the aftermath of Hurricane Katrina the media came in and hooked up all their communication to the satellite and used up all the bandwidth! So satellite phones did not work either.

  If phones go through the hospital circuit boards or your EMS circuit boards, they will not work if there is no power! So, hospital phones do not work either. We had two phones within the hospital that were connected directly to the local telephone network, bypassing the hospital network. These were our only reliable means of communication to the outside.

Under normal circumstances, the command and control structure for an institution as complex as a modern medical center is extremely important. In a disaster, a robust command structure becomes absolutely crucial! Many hospitals do not have proper education for the administrative personnel to 'run a disaster', functioning as commander. The hospital administration incorrectly assumes that commanding a disaster within the hospital is the same as the daily management of a hospital. It is not! The federal and Joint Commission on Accreditation of Healthcare Organizations (JCAHO) mandated disaster plans are designed to deal with a biochemical or biologic 'disaster'. Such disasters do not (have not!) happened in the United States nor in the world. Natural disasters (earthquakes, hurricanes, flooding, tsunami, etc.), explosions, transportation accidents, and even multiple car crashes are the common disasters. Unfortunately most educational courses do not address the needs of these more realistic disaster scenarios.

Hospital administration not only needs to be educated, but also must have the type of personality that can take over and command. In a real emergency it is not sufficient to just think and plan to make a judgment decision next week. Multiple decisions need to be made "now"!

- **Examples from Hurricane Katrina**
  At Tulane in the aftermath of Katrina we were very lucky because both the chief executive officer (CEO) of the hospital, and the President of the Hospital Corporation of America (HCA)/Delta Division(which is basically Louisiana, Mississippi and Texas), stayed with us the whole time. They remained with us – the CEO to make sure that we had re-supply and that the hospital was running right, and the President of Delta Division of HCA to make sure that he got helicopters and

other things in to get us out. The Command Center at Tulane was dark, quiet and it had one light in it and that is where both the telephones were, so that is where we had to stay.

Ham radio stations or operators can be very helpful for communication, unless their towers have blown down. In the absence of conventional communication we resorted to a great method of communicating between Charity Hospital and Tulane Hospital – one person with a bullhorn standing on the top of Charity Hospital, one person with a bullhorn standing on top of Tulane Hospital – they were our relay team.

An important function of the security system is to protect the patients and the medical staff from being overrun by people seeking to use the hospital as a shelter. Clearly if the hospital was designed and designated as a shelter then it should be used as such. On the other hand, if it was not set up to be a shelter, then it should not be used as one. But in most instances hospitals are not designated disaster shelters. Hospitals provide medical care and that is what they have to be kept for. They have to be kept separate from the shelters; this reality can create heart rendering decisions that your security must support!

- **Examples from Hurricane Katrina**
  Thankfully, our security system protected us. Tulane hospital and medical school had 39 hospital police that stayed with us the whole time. They were well armed, well practiced, and we even had a special weapons and tactics (SWAT) team inside the hospital for a 'hostage situation'.

  Some almost comical things occurred. On Wednesday night, two days after the hurricane, a Coast Guard helicopter landed. Earlier in the evening we had been transporting some patients out, but this was not an evacuation helicopter. It stayed about two minutes then departed, leaving a young man standing on the heliport in desert camies, a rifle on his back and carrying his military pack. He came directly to us and asked, "Where's Charity Hospital? I was sent here to defend them." We replied, "Okay, Charity Hospital's right across there. We'll get a boat for you." He got in the boat and made his way over to Charity. About 18 hours later, sometime Thursday morning, he came back and said, "Okay, my mission's through over there. Now I'm here to protect ya'll." We responded, "Ah, okay, well gosh, we're glad to have you." To protect us, he spent the next two days crawling around on the parapet looking for bad guys.

Response from Outside (Evacuation)

There are two types of evacuation from hospitals after a disaster. Each requires triage, but with different priorities, considerations, and conditions. First, is evacuation wherein patients must be evacuated down (or up) within the hospital to the access level of the helicopters, land vehicles, or boats. Secondly, the patients must be transferred from the hospital in distress to a medical facility with the available resources to care for the patient.

Evacuation within the hospital without power means lifting and moving the patients in the stairways with manpower and probably without stairwell lights. Patients who require life support must be moved with their life support devices. There will also be some patients who are able to move themselves, although they

may require some assistance. Triage in this setting means moving the patients in the quickest and most efficient way possible. A group who can move themselves should use one set of stairs so that at the same time another group that requires lifting can use another set of stairs.

- **Examples from Hurricane Katrina**
  At Tulane, we had two patients who were on left ventricular assist devices (LVADs.) at the time of the hurricane. The LVAD weighed about 500 lbs. and needed to be within two feet of the patient at all times. We used emergency medical technicians to direct the patient movement down the stairs, since they had the most experience with such activities.

  The way that we prioritized (triaged) for this 'internal evacuation' was as follows: The most critical patients were moved first. Next, and down another stairwell, we moved the walking patients. This allowed us to move the greatest number of patients in the quickest manner. Third, we moved the bed-ridden patients. They required lifting and a lot of manpower to physically move them down the stairs. Lastly, we transported the difficult to move patients, such as the two patients on the LVADs.

Patients who were evacuated to another facility, required a different triage system. Patients had to be moved on vehicles and with medical care personnel who could handle their condition. Ambulatory patients, referees, medical, and hospital staff could be transferred, *en mass*, to a transfer point. These people required little care en route or while waiting for transportation. Helicopters that could carry walking personnel and those that could carry patients circled in the sky to queue up for the heliport serving the hospital. It was not until the helicopter landed and the load master identified his/her resources that a decision could be made as to who could be loaded. Only at that time could the heliport medical personnel decide who to send.

- **Examples from Hurricane Katrina**
  Our triage priorities for external evacuation were as follows: Walking patients went out first followed by patient families. Third were medical refugees, then medical staff, and lastly the command personnel left. The external evacuation triage area was located in the lower part of the parking lot. Patients could be moved up a ramp by pickup trucks or vans (in many instances using vehicles that had been parked on the ramp). Helicopter transport was the only means of nighttime evacuation, which meant that those vehicles needed to be loaded at night. At night, it was the same kind of a situation except that it was very dark at the bottom of the garage where patients were being taken care of while they were awaiting transportation out.

  The configuration of the parking lot heliport (helicopters landed on the 7th floor of the parking garage, a structure previously stressed for use as a heliport) was to have waiting patients on rolling devices stationed on the 6th floor of the ramp. Ambulatory patients and staff waited in the stairwells on the opposite end of the 7th floor. When a helicopter landed, hand signals were used to indicate which group should come forward and the configuration of the vehicle to be used. Almost all of the landings and loading were 'hot', so there was not time to reconfigure the helicopter. One helicopter might load stretcher patients while the next to land would load walking patients and staff. The objective was to move the largest number of people in the quickest and most efficient manner. Some of the staff and press, not understanding this process, were very critical of 'who evacu-

ated first'. However, we simply did not have the luxury to reconfigure the helicopters to change the type of seating or supine positioning. Patients were loaded who fit the configuration as the helicopter landed.

Louis Armstrong New Orleans International Airport (MSY, formerly Moisant Field) was the closest airport where the large Chinook helicopters (empty weight 10,185 kg, maximum take-off weight 22,680 kg, maximum capacity 30 stretchers with 2 attendants) could land. Unfortunately, the very sick patients could not be sent out on these larger vehicles, because there was no sophisticated medical care at the airport. Because of this reality, all the patient care ambulances, air ambulances, or helicopters had to fly all the way out to receiving hospitals. which agreed to take the patients. Many of these institutions were a considerable distance from New Orleans, making the transport and return times of the helicopter pretty long.

When Hurricane Katrina hit, there were 110 patients at Tulane and 800 staff. Across in the Medical School there were 200 employees, 79 dogs and cats, one parrot, and one parakeet. The animal caregivers refused to leave 'their' animals behind and the helicopters did not want to carry them. So long discussions were had, but the issues were apparently resolved, because all of the animals eventually left. In the 48 hours after the storm and flooding, 250 helicopters and an unknown number of boat trips were needed to evacuate the patients and personnel. A total of 254 patients, 1400 medical care workers and family (and those 81 animals) did get out. There was a considerable disparity between the number of patients and personnel at Tulane Hospital before the storm and the number of people actually transported from Tulane in the storm's aftermath.

Backfill

This is a very important component of disaster management, particularly in dealing with the casualties and victims of the storm (as opposed to the patients being cared for prior to the disaster). Backfill is the process wherein outside equipment, personnel and supplies are delivered to the area of the disaster.

- **Examples from Hurricane Katrina**
  Within a very short time Pre-Hospital Trauma Life Support (PHTLS) personal, under the direction of Jeffrey Guy, MD, a trauma/burn surgeon at Vanderbilt University in Nashville, mobilized 1500 medical and EMS personnel. Within 1–2 days they delivered more than 500 ambulances ready to move into New Orleans and Mississippi and help people out. This incredible outpouring of assistance was based on the recruiting that went on with the PHTLS Committee and personal contacts that we had with the military. Furthermore, as has been well documented in the media, the federal response (Federal Emergency Management Agency, FEMA) to Katrina and the flooding was painfully slow. Even in the ensuing weeks, the backfill process in New Orleans was complicated by the continued presence of large amounts of standing water.

Restoration

Restoration of the city is currently continuing. It will be a long process. In addition to the efforts and funds needed to 'restore' the city, restoration of the medical care system has only begun.

- **Examples from Hurricane Katrina**
  Currently (one year after Hurricane Katrina), there is not one hospital in the City of New Orleans that is completely open. The problems in restoring the medical infrastructure are much greater than re-supply of equipment and cleanup of the facilities. For example, there is not enough housing for the medical care personnel to return to the city. One year after Hurricane Katrina and the New Orleans flood there are only 1500 hospital beds open in the entire city. Before Katrina hit, there were 20 hospitals in New Orleans with 5300 beds. Today, only six hospitals are open and functioning and none of these institutions is completely open.

Conclusion

The outline discussed above describes the important components of a 'new approach' to disaster planning. This approach recognizes that the immediate response will require 2–5 days of self sufficiency. Pre-disaster planning should consider the resources (food, medication, power, personnel, sanitation) that will need to be present to permit ongoing patient care (of the patients already in the hospital). Adequate, secure (!) facilities for storage of these resources must be addressed. A command system should be developed and needs to consider communication (think creatively and realistically!), location of a command center, and identification of an incident commander. Finally, security is crucial to successful implementation of the immediate response.

The duration and resources ultimately needed for the immediate response will depend critically upon the timeliness of the response from the outside. Frequently resupply, especially of critical items, will be the initial outside response. As soon as practical, evacuation of patients and caregivers should commence. Planning for evacuation within the hospital may be needed in addition to the more conventional disaster planning considerations given to external evacuation. Evacuation of critical patients and exhausted personnel takes priority over backfill. Backfill will deliver additional medical systems, equipment and caregivers to relieve the exhausted onsite caregivers. Finally, restoration will be needed to rebuild the medical care system, possibly with loss of some of the resources present before the disaster.

Although the precise nature of the next major disaster remains unknown, it is certain that the world will witness future major disasters. The greatest asset in the face of a major disaster is the professionalism and dedication of the health caregivers. The unfortunate reality is that 'conventional' disasters will continue to be the most likely scenarios well into the future. Investing in the EMS and trauma systems will be the best means of dealing with such disasters. Finally, although I have attempted to provide some insights and anecdotes that may be applicable to future disasters, every disaster is unique! Adaptability, creativity, dedication, and courage will always be needed.

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