INTENSIVE CARE MEDICINE

ANNUAL UPDATE 2006

JEAN-LOUIS VINCENT
EDITOR

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Table of Contents

Cellular Responses in Sepsis

Cellular Dysfunction

Cardiovascular Dysfunction

Hemodynamic Monitoring

Coagulopathies

Blood Transfusions

Cardiopulmonary Resuscitation

Acute Lung Injury

Respiratory Support

Monitoring in Respiratory Failure

Bacteriological Problems

Fungal Infections

Hepatosplanchnic Failure

Renal Failure

Sleep and Delirium

Contemporary Issues

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Common Abbreviations

ALI	Acute lung injury
APACHE	Acute physiology and chronic health evaluation
aPPT	Activated partial thromboplastin time
ARDS	Acute respiratory distress syndrome
ATP	Adenosine triphosphate
BAL	Bronchoalveolar lavage
COPD	Chronic obstructive pulmonary disease
CPAP	Continuous positive airway pressure
CT	Computed tomography
DIC	Disseminated intravascular coagulation
EKG	Electrocardiogram
EVLW	Extravascular lung water
FFP	Fresh frozen plasma
FRC	Functional residual capacity
GCS	Glasgow Coma Score
HIV	Human immunodeficiency virus
HRS	Hepatorenal syndrome
HSP	Heat shock protein
ICG	Indocyanine green
ICP	Intracranial pressure
ICU	Intensive care unit
IL	Interleukin
LPS	Lipopolysaccharide
MET	Medical emergency team
MOF	Multiple organ failure
NF-κB	Nuclear factor-kappa B
NIV	Non-invasive ventilation
NO	Nitric oxide
NOS	Nitric oxide synthase
PAI	Plasminogen activator inhibitor
PAOP	Pulmonary artery occlusion pressure
PEEP	Positive end-expiratory pressure

PSV	Pressure support ventilation
PT	Prothrombin time
SAPS	Simplified acute physiology score
SIRS	Systemic inflammatory response syndrome
SOFA	Sequential organ failure assessment
SvO_2	Mixed venous oxygen saturation
TLR	Toll-like receptor
TNF	Tumor necrosis factor
VAP	Ventilator-associated pneumonia
VILI	Ventilator-induced lung injury

Cellular Responses in Sepsis

The Role of Toll-like Receptors in Sepsis

W. J. Wiersinga and T. van der Poll

Introduction

The recently discovered class of Toll-like receptors (TLRs) has emerged as the central line of defense against invading pathogens. The TLR are the first to detect host invasion by pathogens, initiate immune responses and form the crucial link between the innate and adaptive immune systems. In general, the immune activation that follows TLR activation is sufficient to combat the wide variety of pathogens that daily invade the human body. However, in the case of sepsis, which can be defined as the disadvantageous systemic host response to infection [1], these TLR-mediated responses may exceed the threshold to maintain homeostasis of the immune system. This review focuses on new insights into the pathogenesis of sepsis gleaned from the impressive amount of research that has been conducted in the TLR research field and their potential clinical implications for intensive care medicine.

The Toll-like Receptor Family

The innate immune system discriminates potential pathogens from self through a series of receptors that recognize conserved motifs on pathogens that are not found in higher eukaryotes. These motifs have been termed 'pathogen-associated molecular patterns' or PAMPs, and their cognate binding partners on host cells involved in the innate immune response have been named 'pattern-recognition receptors' or PRRs. Examples of PAMPs include lipopolysaccharide (LPS) from the outer membrane of Gram-negative bacteria, peptidoglycan (present in most bacteria), lipoteichoic acid (in many Gram-positive bacteria) and mannans in the yeast cell wall.

The Toll family of receptors, which is conserved throughout evolution from flies to humans, has been implicated as playing a central role as PRRs in the initiation of cellular innate immune responses [2]. First discovered in the fruit fly [3], at present 11 human homologs of *Drosophila* Toll have been identified. This human receptor family has been designated 'Toll-like receptors' [2, 4]. TLRs are distinguished from other PRRs by their ability to recognize, and more significantly, discriminate between different classes of pathogens. Ligands for 9 human TLRs have been described (see Table 1). Of note, one of the TLR mysteries relates to TLR11, a receptor present in mice, but not humans, and known to recognize uropathogenic *Escherichia coli* [5]. Recently, the first defined ligand for TLR11 has been described as a profilin-like protein from *Toxoplasma gondii* [5]. It has to be emphasized, however, that the TLRs function as one system; different components of one microorganism are recognized by different TLRs. *E. coli* for example is a Gram-negative

Table 1. Toll-like receptor ligands in infectious diseases

Receptor	Pathogen/PAMP	Origin of ligand
TLR1	Triacyl lipopeptides	(Myco)bacteria
(with TLR2)	Soluble factors	*Neisseria meningitides*
TLR2	Lipoproteins	Various pathogens
	Peptidoglycan	Gram-positive bacteria
	Lipoteichoic acid	Gram-positive bacteria
	Modulin	*S. epidermidis*
	Atypical LPS	Leptospira, *P. gingivalis*
	Porins	Neisseria, *H. influenzae*
	AraLAM	Mycobacteria
	19 kD antigen	*M. tuberculosis*
	STF	*M. tuberculosis*
	Zymosan	Fungi
TLR3	Ds RNA	Viruses
TLR4	LPS	Gram-negative bacteria
	Fusion protein	Respiratory syncytial virus
	Taxol	Plants
	Hyaluronic acid	Host
	Fibrinogen	Host
	HSP70*	Host
	HSP60*	*Chlamydia pneumonia*
TLR5	Flagellin	Various bacteria
TLR6	Diacyl lipopeptides	Mycoplasma
(with TLR2)	Lipoteichoic acid	Gram-positive bacteria
	Zymosan	Fungi
TLR7	ss RNA	Viruses
	Imidazoquinoline	Synthetic compound
TLR8	ss RNA	Viruses
	Imidazoquinoline	Synthetic compound
TLR9	CpG DNA	Bacteria and viruses
TLR10	Unknown	Unknown
TLR11	Largely unknown	Uropathogenic bacteria
	Profilin-like protein	*Toxoplasma gondii*

PAMP: pathogen-associated-molecular-patterns; LPS: lipopolysaccharide; CpG: cytosine phosphate guanine dinucleotides; STF: soluble tuberculosis factor

* heat shock proteins, or stress proteins, are present in all cells and function as transport proteins within the cell. They are released under stressful conditions such as heat, cold or hypoxemia. When they are expressed at the cell surface, they play a role as signaling proteins in the recognition of diseased cells by the immune system. They are endogenous ligands of TLRs

bacterium expressing several PAMPs (peptidoglycan, LPS, flagellin en bacterial DNA), which are all recognized by different TLRs (TLR2, TLR4, TLR5 and TLR9, respectively).

TLRs: The Essential Link between Innate and Adaptive Immunity

It has become clear that activation of the innate immune system is a prerequisite for the induction of the adaptive immune system [4]. TLRs form the bridge between these two systems and play an essential role in the coordination of the adaptive immune response [4]. TLRs control induction of T cell responses at two levels, first by induction of co-stimulatory molecules which mark the associated peptide as foreign and second by secretion of cytokines that are necessary to overcome the peripheral tolerance induced by regulatory T cells [4, 6]. In addition, TLRs are responsible for the induction of dendritic cell maturation, which is necessary to initiate adaptive immune responses. It has to be said, however, that other components of the innate immune system, such as the complement system and natural killer (NK) cells, are also capable of influencing the adaptive immune system [7].

TLR Signaling

The discovery that TLR4 is the long sought after LPS receptor was a major breakthrough in immunology [8]. However, TLR4 is not the only protein important in the recognition of LPS (Fig. 1.). First, LPS binds to LPS-binding-protein (LBP), which transfers LPS to CD14. CD14 is expressed on the outer membrane of monocytes, some granulocytes and activated B-lymphocytes. MD-2, another TLR4-associated protein, is required to activate the CD14-MD-2-TLR4–complex (Fig. 1). Binding of LPS to CD14 leads to the association of CD14 with MD-2 and TLR4. So far, TLR4 is the only known TLR that requires an extra protein next to the ligand to be activated. After stimulation of the TLR, the adapter molecule myeloid differentiation primary-response protein 88 (MyD88) is recruited. MyD88 associates with interleukin (IL)-1R-associated kinase (IRAK) 4. This results in the phosphorylation of IRAK-1 which forms a complex with tumor necrosis factor (TNF)-receptor-associated-factor (TRAF) 6. This then interacts with another preformed complex (consisting of TAK1, TAB1 and TAB2 or TAB3) which leads to the activation of the inhibitor-of-nuclear-factor-κB (IKK) complex. The IKK complex phosphorylates the IBs. Subsequent release of nuclear-factor-κB (NF-κB) results in the transcription of a whole range of inflammatory genes [9]. In addition, is was recently shown that the transcription factor IRF-5, which forms a complex with MyD88 and TRAF6, also functions as a general signal tranducer that mediates MyD88-dependent gene induction of pro-inflammatory cytokines [10].

Next to this so-called MyD88-dependent pathway, a MyD88-independent route, exists which is used only by TLR4 and TLR3 [9]. The MyD88-independent route will result in the delayed activation of NF-κB and the production of interferon (IFN)-β. Furthermore, it is now known that different TLRs use different adaptor molecules. This explains why various TLRs lead to different patterns of gene expression [9]. Next to MyD88, the adapter molecules TIR-domain-containing-adaptor-protein (TIRAP), TIR-domain-containing-adaptor-protein-inducing-IFN-β (TRIF) and TRIF-related-adaptor-molecule (TRAM) have been identified. TIRAP is essential for MyD88-dependent signaling through TLR2 and TLR4 [9]. TRIF is essential for the TLR3 and TLR4-mediated activation of the MyD88-independent

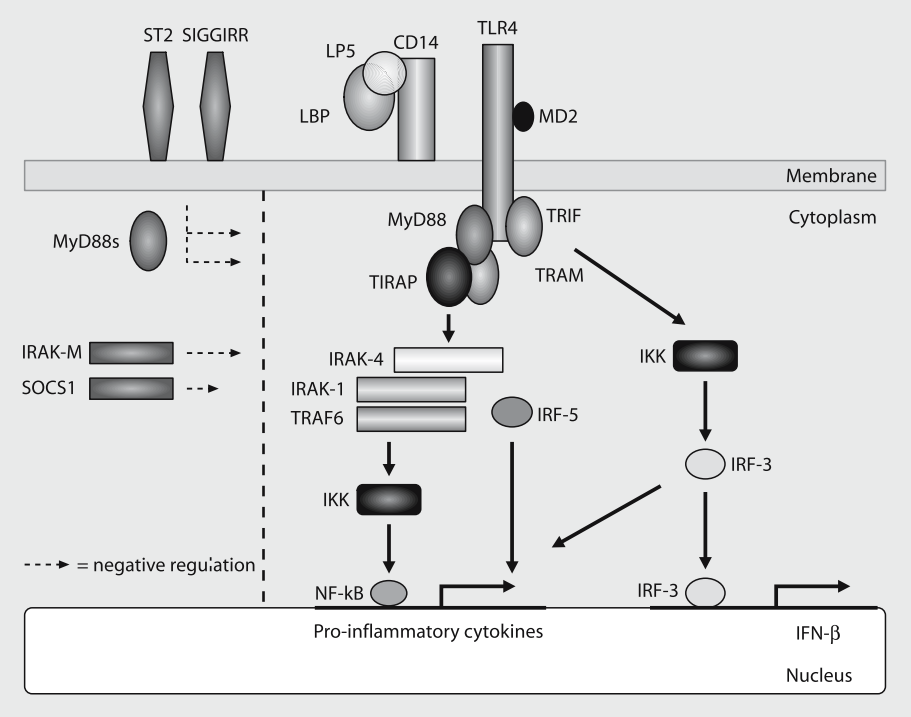

Fig. 1. Toll-like receptor signaling

pathway [9]. TRAM is involved in TLR4 mediated MyD88-independent/TRIF-dependent signaling pathways [9].

At first sight, the TLR signaling pathway seems overwhelming in complexity. However, it is fascinating to see that the innate immune system signals through a channel of relatively low complexity: only 11 TLRs, 4 adapter molecules and a couple of protein kinases are required for the recognition and response to a whole universe of often complex microbial molecules [11]. After this narrow strait there are again thousands of different possible host responses. Beutler has called this the "hourglass" shape of the innate immune response [11].

Regulation of TLR Signaling

In order to prevent strong uncontrolled inflammatory reactions tight regulation of the TLR signaling pathway is mandatory. In general, an encounter of the immune system with pathogens will result in the upregulation of a whole spectrum of different TLRs. For instance, in patients with sepsis caused by the Gram-negative bacterium *Burkholderia pseudomallei* increased expression of TLR1, TLR2 and TLR4 on the cell surface of circulating monocytes and granulocytes is seen, together with increased TLR1, TLR2, TLR4, TLR5 and TLR10 mRNA levels in blood cells (our unpublished data). However, the exact consequences of these enhanced TLR expression profiles for host defense remain to be established. Furthermore, it has been

shown that certain cytokines play essential roles in TLR regulation. Besides LPS, inflammatory cytokines, such as IL-2, IL-15, IL-1β, IFNγ and TNF-α, are able to induce TLR2 gene expression in mouse macrophages [9]. Interestingly, this did not hold true for TLR4 gene expression.

Negative regulation of the TLR signaling pathway is essential (Table 2) [9, 12]. In the cytoplasm, IRAK-M inhibits the dissociation of the IRAK1-IRAK4 complex from the receptor, suppressor-of-cytokines-signaling-1 (SOCS1) probably directly inhibits IRAK1 and a short form of MyD88 (MyD88s) blocks the association of IRAK4 with MyD88. On the cell membrane, other members of the TIR-superfamily, such as single-immunoglobulin-IL-1R-related-molecule (SIGGIRR) and ST2, also negatively modulate TLR signaling [9, 13, 14]. More specifically, ST2 is an inhibitor of TLR2, TLR4 and TLR9 signaling [13]. Lastly, the TLR-like molecule RP105, which surface expression is dependent on the co-expression of the MD-2 homolog MD-1, interacts directly with the TLR4 signaling complex, inhibiting its ability to bind a microbial ligand [15].

Other mechanisms by which TLR signaling can be controlled include the reduction of TLR expression by TLR degradation or inhibition by anti-inflammatory cytokines. Furthermore, it has become clear that TLRs can function as death receptors; this TLR-induced apoptosis may be important in the control of a dysregulated TLR response [12].

Table 2. Negative regulators of TLR signaling

Compartment	Molecule	Mode of action
Extracellular	sTLR2	Soluble decoy receptor; TLR2 antagonist
	sTLR4	sTLR4 might block the interaction between TLR4 and MD-2 and CD14, terminating TLR signaling
Transmembrane	ST2	Associates and sequesters with MyD88 and TIRAP
	SIGGIR	Negatively interacts with TLR4, IRAK1 and TRAF6
	TRAILR	Decreases NF-κB translocation by stabilizing IκBα
	RP105	Interacts with the TLR4 signaling complex, inhibiting its ability to bind its microbial ligand
Intracellular	IRAK-M	Inhibits the dissociation of the IRAK1-IRAK4 complex from the receptor
	SOCS1	Associates with IRAK1 and inhibits its activity
	MyD88s	Blocks the association of IRAK4 with MyD88
	TRIAD3A	Induces ubiquitination-mediated degradation of TLR4 and TLR9
	NOD2	Suppresses NF-κB
	P13K	Inhibits p38, JNK and NF-κB function
	TOLLIP	Autophosphorylates IRAK1
	A20	Causes rapid cellular degradation of TRAF6

TLR: Toll-like receptor; SIGGIR: single immunoglobulin IL-1R-related molecule; TRAILR: TNF-related apoptosis-inducing ligand receptor; SOCS: suppressor of cytokine signaling-1; MyD88s: myeloid differentiation primary-response protein 88 short; IRAK-M: IL-1R-associated kinase-M; TOLLIP: Toll-interacting protein

Are the TLRs Central in the Host Defense against Sepsis?

Given their central role in the recognition of microbes, it is rational to hypothesize that TLRs play a central role in sepsis pathogenicity. Indeed, animals lacking the gene encoding TLR4 do not develop septic shock in response to LPS [16]. Although LPS is the best studied and probably most important mediator of sepsis, peptidoglycan, lipoteichoic acid, bacterial CpG motifs and flagella are other important microbial products implicated in the pathogenesis of sepsis. All these PAMPs signal through different TLRs. As a result, the relationship between TLR expression and human sepsis may be complex.

In recent years, TLR2 has been recognized as the Gram-positive TLR because of its ability to sense major Gram-positive cell wall components such as peptidoglycan and lipoteichoic acid, whereas TLR4 – the LPS receptor – is seen as the Gram-negative TLR. However, as more knowledge about the precise role of TLRs in different bacterial infections becomes available, this concept has to be modified. For instance, *Streptococcus pneumoniae* is sensed by the innate immune system not only through TLR2 which recognizes lipoteichoic acid and peptidoglycan, but also through TLR4 which recognizes pneumolysin. It was recently showed that TLR2 is indispensable for alveolar macrophage responsiveness toward *S. pneumoniae* [17]. However, in the same study, TLR2 gene-deficient mice intranasally inoculated with non-lethal to lethal doses of *S. pneumoniae* displayed only a modestly reduced inflammatory response in their lungs and showed an unaltered antibacterial defense and survival in comparison with wild-type mice [17]. These data suggest that the function of TLR2 is limited in the innate immune response to *S. pneumoniae*. Clearly, other PRRs play an important role.

It seems obvious that TLR4 plays an important role in Gram-negative infections. As mentioned, TLR4 deficient mice do not develop septic shock after administration of high doses of LPS [16]. Furthermore, TLR4 deficiency resulted in diminished clearance of *Haemophilus influenzae* and *Klebsiella pneumoniae* in a mouse model of pneumonia [18, 19], suggesting that the recognition of LPS by TLR4 contributes to an effective immune response during these infections. However, not all studies demonstrate the importance of TLR4 signaling in Gram-negative infections. When mice lacking the *TLR4* gene are inoculated with the Gram-negative bacterium *B. pseudomallei* in a model of severe sepsis no differences are observed in terms of inflammatory response (cytokine production, histological organ damage) or outcome (survival) when compared to normal wild type mice (our own unpublished data). In the same model, TLR2 mutant mice show a clear survival advantage over wildtype mice. To make things more complicated, responsiveness of TLR2 to LPS has also been described [20]. In this respect, it is interesting that pretreatment with bacterial lipoprotein, a TLR2 ligand, protected otherwise highly susceptible TLR4-deficient C3H/HeJ mice from *Salmonella typhimurium* induced Gram-negative sepsis via enhanced bacteria clearance [21].

The relationship between TLR expression and human sepsis may be complex, as is suggested by some recent studies in septic patients [22, 23]. Increased cell surface (neutrophils and monocytes) expression and increased mRNA levels of both TLR2 and TLR4 are seen in septic patients [22, 23]. No association with functional outcome could be determined [22]. Interestingly, in one study increased levels of TLR2 mRNA were seen in both Gram-positive and Gram-negative sepsis, whereas TLR4 mRNA was only increased in Gram-positive sepsis [22].

New TLR-mediated Players in the Sepsis Arena

Some recently discovered mediators of sepsis are directly involved in TLR signaling, all of which are regarded as promising new therapeutic targets.

Triggering Receptor Expressed on Myeloid cells-1 (TREM-1)

TREM-1 amplifies the TLR-mediated inflammatory response to microbial products [24]. TREM-1, which signals through the adapter protein DAP12, is strongly and specifically expressed on monocytes and neutrophils from patients with sepsis [24]. In human endotoxemia, monocytes displayed a gradual upregulation of TREM-1, whereas granulocyte TREM-1 expression was high at baseline and immediately downregulated upon LPS exposure along with an increase in soluble TREM-1 [25]. Elevated concentrations of soluble TREM-1 in bronchoalveolar lavage (BAL) fluid can indicate ventilator-associated pneumonia (VAP) in patients receiving mechanical ventilation [26], and high concentrations in plasma can indicate infection in patients with systemic inflammatory response syndrome (SIRS) [27]. Excitingly, blockade of TREM-1 protected mice against LPS-induced shock, as well as microbial sepsis caused by live *E. coli* or cecal ligation and puncture (CLP) [24]. In addition, a synthetic peptide mimicking a short highly conserved domain of sTREM-1 protected septic animals from LPS hyper-responsiveness and death [28].

Intriguingly, although TREM-1 signals through the adapter protein DAP12 [24], a recent study showed that DAP12-deficient mice have – contrary to what would be expected – enhanced TLR responses *in vitro,* as indicated by an enhanced production of pro-inflammatory cytokines by DAP12-deficient macrophages in response to TLR agonists *in vitro* and *in vivo*, as indicated by an increased susceptibility to endotoxic shock [29]. Thus, perhaps certain DAP12-associated receptors function as negative regulators of TLR responses.

Macrophage Migration Inhibitory Factor (MIF)

In recent years MIF has emerged as a pivotal regulator of innate immunity that has been implicated in sepsis pathogenesis [30, 31]. MIF regulates innate immune responses through modulation of TLR4 [30]: when MIF-deficient mice were challenged with LPS they showed a defective response as a direct result of decreased TLR4 expression [30]. In patients, MIF levels correlate with fatal outcome in sepsis [32]. MIF-directed therapies might offer a new treatment opportunity for sepsis. Inhibition of MIF activity with neutralizing anti-MIF antibodies protected mice from septic shock [31]. Furthermore, a specific small molecule inhibitor of MIF, named ISO-1, partially protects mice from sepsis induced by endotoxin or CLP [33].

High-Mobility Group Box 1 protein (HMGB-1)

HMGB-1 is recognized as a cytokine and functions as a late mediator of sepsis and is elevated in the majority of septic patients [34, 35]. It is secreted by activated immune cells and, along with the receptor for advanced glycation end products (RAGE), interacts with TLR2 and TLR4, which may provide an explanation for the ability of HMGB-1 to generate inflammatory responses that are similar to those initiated by LPS [36]. LPS stimulation was found to mediate the release of HMGB-1

from macrophages at a considerably later stage than the release of the pro-inflammatory cytokines TNF-α and IL-1 [35]. Administration of HMBG-1 itself was lethal to mice, whereas the administration of antibodies to HMGB-1 diminished endotoxin lethality [35].

TLR Polymorphisms in Sepsis

Recent phenotype-genotype studies showed that TLR polymorphisms can alter both the susceptibility to and the clinical course of infectious diseases. Mutations in TLR encoding genes are not uncommon. For instance, the reported incidence of the TLR4 Asp299Gly polymorphism lies between 6 to 11% in the Caucasian population [37, 38].

It was first shown that TLR4 mutations (Asp299Gly and Thr399Ile) are associated with endotoxin hyporesponsiveness in humans [39]. Subsequently, it was reported that polymorphisms in TLR4 could predispose people to develop septic shock with Gram-negative microorganisms [37, 40]. Most likely, the increased susceptibility to Gram-negative sepsis is caused by a diminished and thus inadequate response to LPS. Further associations have been found between the TLR2 Arg753Gln polymorphism and increased susceptibility to sepsis caused by *Staphylococcus aureus* [41]. On the other hand, in a series of 1047 patients with culture proven meningococcal disease, the TLR4 Asp299Gly polymorphism was – contrary to the hypothesis – not associated with host susceptibility or severity of disease [38]. In a cohort of 252 critically ill patients, it was shown that single nucleotide polymorphisms (SNPs) in CD14 and TLR2 are associated with increased prevalence of sepsis, but not with altered prevalence of septic shock or decreased 28-day survival [42]. In this study, as was expected, CD14 SNPs were associated with Gram-negative infections and TLR2 SNPs with Gram-positive infections [42]. Interestingly, when these results are taken together, it could well be that certain SNPs in TLRs may alter recognition and clearance of bacteria, but they do not seem to change the outcome of patients with sepsis. In addition, one has to bear in mind the importance of ethnic differences in genetic variations; for example in a recent Japanese cohort of 197 critically ill patients and 214 healthy controls not one participant carried a TLR4 polymorphism and no association was found between CD14 polymorphism and sepsis [43].

New studies that investigate the role of TLR polymorphisms in sepsis are underway. SNP analyses can serve as both an important research tool to further elucidate the complex pathogenicity of sepsis and as a clinical instrument to predict the clinical course of ICU patients and to ultimately individualize treatment [44].

The TLRs as a New Treatment Target in Sepsis

Manipulation of TLR pathways has great therapeutic potential: novel TLR immune-regulatory drugs are being developed to treat a wide range of conditions, such as infectious diseases, asthma, inflammatory bowel disease and cancer [45]. In the case of sepsis, one could think of TLR antagonists, TLR signaling pathway inhibitors or stimulators of the negative regulators of the TLR pathway as new treatment targets.

For example, a recent mouse study in sepsis showed a marked reduction in sepsis related mortality after selective blockage of TLR2 after inoculation with Gram-

positive bacteria [46]. Furthermore, studies in animal models have demonstrated the utility of anti-CD14 monoclonal antibody therapy in septic shock and these agents are currently being evaluated in clinical phase-2 trials [47, 48]. Another strategy could involve TLR9. TLR9 recognizes CpG DNA, a specific pattern of nucleotides that is common in bacteria and viruses, but uncommon in humans. By using synthetic CpG sequences an innate and adaptive immune response could be generated involving cytotoxic T cells and disease-specific antibodies [49]. In a mouse model of severe Gram-negative sepsis, CpG treatment one hour before inoculation with *B. pseudomallei* offered protection due to the rapid induction of pro-inflammatory cytokines [50].

The treatment goal of TLR agents in sepsis should be to normalize and not to completely abolish the dysregulated and harmful inflammatory response. Maintaining a balance between host-defense functions and potentially harmful effects (e.g., tissue destruction and the induction of autoimmune disease) will be of vital importance in the development of TLR therapeutics [45].

Conclusion

The discovery of TLRs has been of enormous importance in both the field of microbiology and immunology and has shown that the innate immune system is not non-specific. TLRs form the crucial link between the innate and adaptive immune response. Surprisingly, these very complex immune responses are initiated by this family of only 11 different receptors. The first human studies on TLR expression in sepsis and experiments with mice lacking *TLR* genes have provided new insights into the pathogenesis of sepsis and have underlined the importance of TLRs as the crucial first line of defense against microorganisms. Taken together, severe sepsis can probably be seen as the clinical manifestation of a TLR mediated dysregulation of the immune response to invasive pathogens [47]. Despite this significant progress in our understanding of the sepsis enigma many of the complex immune reactions during sepsis are still a mystery [1]. These outstanding questions on pathogenesis can be summarized by the fact that we need to know precisely how dysregulation of the TLR system results in clinical syndromes such as sepsis. In the end, it comes down to the question whether all this newly gained knowledge will help to improve the care of septic patients. Hopefully, in the not too distant future, TLR genotypic profiling of patients will help clinicians to make better treatment decisions. Most importantly, unraveling the role of TLRs in sepsis will provide new, highly selective treatment targets in sepsis.

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The Emerging Role of RAGE in Sepsis

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Introduction

Sepsis and septic shock are the leading causes of death in intensive care units (ICUs) in developed countries despite recent advances in critical care medicine. Sepsis is the systemic inflammatory response to infection frequently associated with hypoperfusion followed by tissue injury and organ failure. Activation of monocytes/macrophages and neutrophils with consecutive release of proinflammatory mediators and activation of the coagulation cascade seem to play key roles in the pathogenesis of sepsis. This process is characterized by the massive release of proinflammatory mediators, such as tumor necrosis factor (TNF)-α, interleukin (IL)-1β, macrophage migration inhibitory factor (MIF), and high mobility group box-1 (HMGB-1) protein. In addition, neutrophil apoptopsis is significantly delayed by these inflammatory mediators.

Promising new experimental treatment options are interference with MIF, HMGB-1, C5a or triggering receptor expressed on myeloid cells (TREM)-1 signal transduction pathways, and inhibition of apoptosis, which may improve prognosis of septic patients in the future. In addition, recent data suggest that the inflammation perpetuating receptor, RAGE (receptor for advanced glycation end-products) is critically involved in the immune response in sepsis. Furthermore, targeting RAGE signaling pathways is a potential new target for sepsis treatment [1, 2].

Receptor for Advanced Glycation End-products

RAGE is – like TREM-1 – a member of the immunoglobulin superfamily and was first identified in lung tissue, where it is located on the basolateral membranes of alveolar epithelial cells [3, 4]. The molecule is named RAGE because it was originally described as a receptor for advanced glycation endproducts (AGEs) [5]. AGEs are products of nonenzymatic glycation and oxidation of proteins, lipids and other macromolecules that appear especially in conditions with increased availability of reducing sugars and/or enhanced oxidative stress, particularly when molecules turnover slowly and aldoses are elevated [6].

RAGE expression is both constitutive and inducible depending on cell type and developmental stage. While RAGE is constitutively expressed during embryonal development, its expression is downregulated in adult life. Known exceptions are skin and lung, which constitutively express RAGE throughout life. Most of the other cells studied so far including monocytes/macrophages, endothelial cells, smooth muscle cells, fibroblasts, and neuronal cells do not express significant amounts of RAGE under

physiological conditions, but these cells can be induced to express RAGE in situations where inflammatory mediators and ligands accumulate [7, 8]. The activation of RAGE leads to the initiation of nuclear factor-kappa B (NF-κB) [9] and mitogen-activated protein kinase (MAPK) pathways [10]. In contrast to other receptors, RAGE-mediated cellular stimulation includes an increased expression of the receptor itself. This positive feedback loop, characterized by a ligand-receptor interaction followed by increased expression of the receptor itself, suggested the role of RAGE as a propagation and perpetuation factor and leads to the two-hit model of RAGE engagement [6].

Localization and Structure of RAGE

The gene for RAGE is located on chromosome 6 near the major histocompatibility complex (MHC) III in humans and mice, in the proximity of genes encoding TNF, lymphotoxin and the homebox gene, HOX12 [11]. The extracellular domain of RAGE consists of one V-Type immunglobulin domain followed by two C-type immunglobulin domains. The V-Type domain, in particular, interacts with the potential extracellular ligands [12]. The C- and C'-domains probably have important roles in stabilizing the V-domain while docking with its ligands. The rest of the molecule is a single transmembrane spanning domain completed by a 43 amino acid highly charged cytosolic tail. This cytosolic tail lacks known signaling motifs such as phosphorylation sites, kinase domains, etc. Hofmann et al. [13] showed that the cytosolic tail is essential for signal transduction of RAGE, because a truncated form of RAGE with a deleted cytosolic tail is able to bind ligands as well as the wild-type receptor but does not mediate any cellular activation. In the rat lung, extracellular signal-regulated protein kinase-1 and -2 (ERK-1/2) were identified to bind directly to RAGE suggesting that ERK may play a role in RAGE signaling through interaction with RAGE [14]. The existence of truncated RAGE isoforms from the same gene implies that the pre-mRNA of RAGE in humans can be subjected to alternative splicing. In contrast, in mice these truncated isoforms seem to be produced by carboxyl-terminal truncation [6].

RAGE is expressed in normal tissues at low levels, aside from the lung and the skin, where RAGE is constitutively expressed. Although little is known about the physiological role of RAGE, it is possible that RAGE may fit the concept of pleiotropic antagonism [15]. This concept of an evolutionary basis for the development of age-related diseases postulates that genes that are beneficial during the reproductive phase of life may become deleterious. Interest initially focused mainly on the role of RAGE in chronic diseases. In particular, RAGE is upregulated in several chronic inflammatory settings, like rheumatoid arthritis, inflammatory kidney disease, arteriosclerosis, inflammatory bowel disease, and others [6, 13].

RAGE Interactions with its Ligands in Acute Inflammation and Sepsis

RAGE is a multi-ligand receptor that interacts with different structures to path a signal into the cell and recognizes three-dimensional structures rather than specific amino acid sequences. Therefore, RAGE fulfills the requirements of a pattern recognition receptor (PRR). As a member of the Ig superfamily it interacts with a diverse class of ligands, including AGEs, S100/calgranulins, HMGB-1, amyloid β-peptide, amyloid A, leukocytes, prions, *Escherichia coli* curli operons, and β-sheet fibrils [6, 16].

The AGE-RAGE interaction

AGEs are a heterogeneous group of compounds produced by non-enzymatic glycation and oxidation of proteins and lipids that exhibit characteristic absorbance and fluorescence properties, with N^{ε}-(carboxymethyl)lysine being a highly reactive AGE. They are protease resistant and are able to cause irreversible damage to tissues and to activate different cell types, such as macrophages/monocytes, cardiac fibroblasts, and vascular smooth muscle cells. AGEs can bind to various cellular surface receptors and thereby induce post-receptor signaling, activation of transcription factors, and gene expression *in vitro* and *in vivo*. Several receptors have so far been identified that bind AGEs, including AGE-R1, AGE-R2, AGE-R3, the scavenger receptor II and RAGE [17]. One of the receptors for which postreceptor signaling has been investigated in detail is RAGE. Binding of AGEs (and other ligands) to RAGE results in generation of intracellular oxygen free radicals and the parallel depletion of antioxidant defense mechanisms [15]. AGE-RAGE interaction transduces the activation of intracellular signal transduction pathways, such as the ERK1/2 kinases, the p38MAPK, the SAPK/JNK kinases, rho-GTPases, PI3kinases, JAK/STAT pathway and the NF-κB pathway (Fig. 1). In addition to NF-κB activation, RAGE triggering also induces *de novo* p65 mRNA synthesis resulting in a growing pool of transcriptionally active NF-κBp65, which appears to overwhelm endogeneous autoregulatory feedback inhibitory loops.

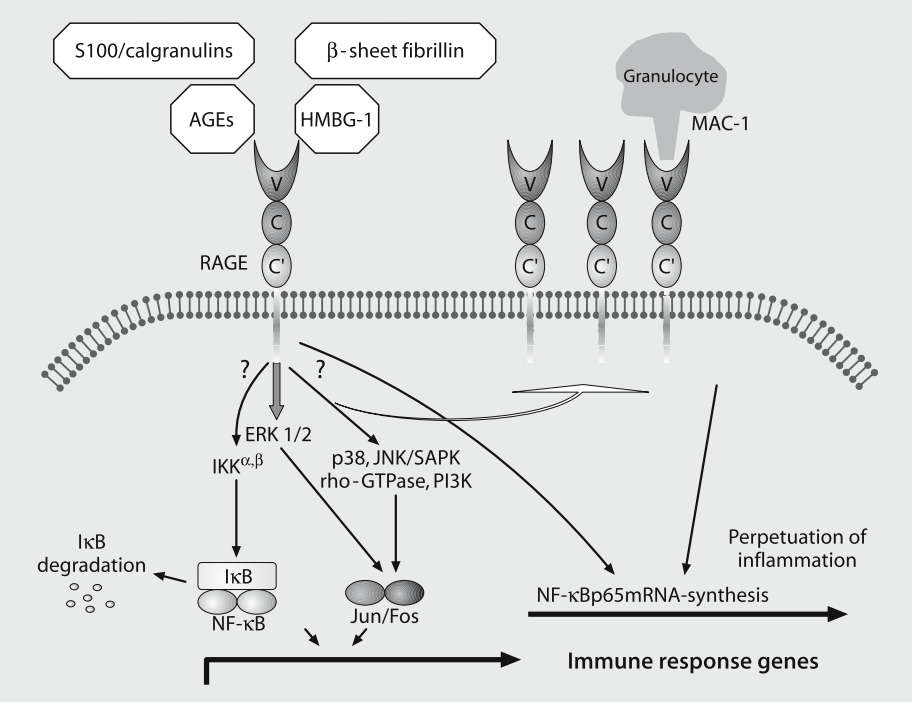

Fig. 1. Signal transduction of RAGE. AGE: advanced glycation end products; HMGB-1: high mobility group box-1 protein

In sepsis, hyperglycemia and oxidative stress are frequently present. As already described, these conditions favor formation of AGEs, which can trigger RAGE and consecutively lead to sustained inflammation. Interference with this pathway by intensive insulin therapy may be one reason why this treatment modality works.

Relevance of RAGE-S100/calgranulin Interaction

Additionally to AGEs, RAGE is a receptor for the S100/calgranulinA12, also called extracellular newly identified RAGE binding protein (EN-RAGE), and S100B [13]. S100/calgranulins, most of them being encoded on human chromosome 1q21, represent a family of multiple members, which have important intracellular properties linked to homeostatic properties, such as calcium binding. S100/calgranulin members like S100A12 and S100B activate endothelial cells, macrophages, smooth muscle cells and peripheral blood mononuclear cells including T cells via RAGE, thus triggering activation of signaling cascades and generation of cytokines and proinflammatory adhesion molecules [13, 18]. In addition, S100P stimulates cell proliferation and survival via RAGE [19]. AGEs and S100B, both augment inflammatory responses by upregulating cylooxygenase-2 (COX-2) in human monocytes via RAGE [20].

RAGE-S100 interactions have further been implicated in inflammation, since binding of S100A12 of the S100/calgranulin family to RAGE in murine macrophages resulted in the elaboration of IL-1β, TNF-α and IL-2 [13]. Furthermore, EN-RAGE induced intercellular adhesion molecule (ICAM)-1 and vascular cell adhesion molecule (VCAM)-1 expression on endothelial cells. Blockage of the engagement of RAGE by EN-RAGE decreased NF-κB activation and expression of proinflammatory cytokines. Intravenous infusion of EN-RAGE into mice led to an enhanced VCAM-1 expression in lungs, which was abrogated by soluble RAGE (sRAGE), neutralizing anti-EN-RAGE or anti-RAGE F(ab')$_2$ supporting the *in vitro* findings. In addition, treatment with sRAGE in murine models *in vivo* strongly diminished delayed type hypersensitivity and inflammatory colitis [13].

Although the precise mechanism of regulating the transcription and translation of S100/calgranulins is still poorly understood, there is evidence, that these molecules are released by activated cells, such as monocytes [21], leading to S100/calgranulin being present at sites of inflammation. Interaction of these polypeptides and RAGE might, therefore, represent a proximal step in the cascade of events perpetuating inflammation. Weigand et al. [22] demonstrated that S100 species are increased in septic patients, however, no significant difference was observed between survivors and non-survivors.

Amphoterin/HMGB-1 as RAGE ligand

Amphoterin, a member of the HMGB-DNA binding proteins (amphoterin = HMGB-1), acts also as a signal transducing ligand of RAGE. HMGB-1, encoded on human chromosome 13q12-13, 1 is a nuclear protein that is present in almost all eukaryotic cells, stabilizes nucleosome function, and acts as a transcription-factor-like protein, which regulates the expression of several genes [19]. The nonhistone chromosomal protein HMGB-1 not only has intracellular functions, it may also ex-

ist extracellulary and on the surface of cells, especially on migrating cells in neuronal development and tumors [16, 23]. It is secreted by activated macrophages, mature dendritic cells and natural killer cells. Together with S100 and heat shock proteins (HSPs), HMGB-1 is one of the main prototypes of the group of the so-called damage associated molecular pattern molecules (DAMPs), because all these molecules are released in response to tissue damage, e.g., by necrotic cells. The engagement of RAGE in tumor cells by HMGB-1 leads to an enhanced cellular migration, invasion and proliferation [23]. In addition, neurite outgrowth was found to depend on RAGE-HMGB-1 interaction through increased expression of the antiapoptotic Bcl-2. However, while nanomolar concentrations of S100B induced trophic effects in RAGE-expressing cells, micromolar concentrations promote apoptosis, likely through oxidant stress [6]. Taguchi et al. [23] demonstrated that engagement of tumor cell RAGE by HMGB-1 enhanced cellular migration, proliferation, and generation of matrix metalloproteinases. Blockage of RAGE-/HMGB-1 signaling suppressed the activation of p44/p42, p38 and SAP/JNK MAPK and tumor growth and metastasis in mice.

In addition to its amplifying role in tumor growth, HMGB-1 has a propagating role in the inflammatory responses and seems to be an important RAGE-ligand during sepsis or acute inflammation [19, 24–26]. Recent studies have shown that the monocyte-derived HMGB-1 is a late acting cytokine mediator of endotoxin lethality. HMGB-1 release by macrophage cultures is detectable in a time-dependent manner. Animal experiments demonstrate that the time-dependent inducible release of HMGB-1 by macrophage cultures is verifiable 8 hours after lipopolysaccharide (LPS) stimulation. Furthermore endotoxemia leads to a systemic increase in HMGB-1 in mice [25]. Systemic HMGB-1 levels were also measured in the serum of mice after LPS injection. Serum HMGB-1 was first detectable after 8 hours and increased to a plateau from 16 to 32 hours after LPS stimulation. Interestingly, this delayed occurrence is one of the typical observations in patients with sepsis when clinical signs appear several hours after the first detectable infection-associated cytokines in the bloodstream, and opens a therapeutic window. Examination in healthy volunteers and septic patients shows (i) no HMGB-1 in the serum of healthy humans, (ii) dramatically increased levels in septic patients; and (iii) markedly higher HMGB-1 levels in non-survivors of septic shock compared to surviving patients.

HMGB-1 itself amplifies the cytokine cascade during systemic inflammation, e.g., by an increased release of TNF and other inflammatory mediators. In addition, HMGB-1 seems to be an autocrine/paracrine regulator of monocyte invasion involving RAGE through the endothelium [27]. The pro-inflammatory activity of HMGB-1 is exerted by the B-box of the protein. Addition of this HMGB-1 B-box to enterocytic monolayers increased intenstinal permeability [28]. These effects were strongly diminished in the presence of an anti-RAGE antibody, suggesting a significant role of RAGE in HMGB-1 initiated pathogenic events. Using bone marrow macrophages from RAGE knock-out mice, Kokkola et al. [29] very recently provided the formal proof that a major component of HMGB-1's action on cells is mediated via RAGE. As a response to HMGB-1 stimulation, macrophages from RAGE knock-out mice produced significantly lower amounts of TNF, IL-1β, and IL-6. However, although there was a significant difference to wild type macrophages, cytokine production was not totally abrogated in $RAGE^{-/-}$ macrophages. In addition, phosphorylation of p38, p44/42 or SAPK/JNK kinases were similar to wild type macrophages and macrophages from IL-1 receptor I knock-out mice. These

data clearly indicate that RAGE is a major receptor for HMGB-1 but HMGB-1 also exerts important effects via different receptors.

In vivo, administration of HMGB-1 blocking antibodies resulted in an improved survival in rodents subjected to high dose LPS [25]. In an animal model of LPS-induced acute lung injury (ALI) the administration of anti-HMGB-1, notably before and after endotoxin-application reduced the typical signs of lung damage in acute inflammation like neutrophil accumulation and lung edema [30]. Recent data show that anti-HMGB-1 antibodies protect against sepsis in an animal model of cecal ligation and puncture (CLP), even when antibody administration is delayed by 24 hours [26]. In addition, studies with anti-HMGB-1 antiserum open a new potential therapeutic target by markedly improving survival in LPS- and CLP-treated mice [25, 26]. The observation that administration of HMGB-1 blocking antibodies protected mice from lethal septicemia strongly suggests that the engagement of cell surface receptors such as RAGE by HMGB-1 might mediate importantly the pathogenic effects of HMGB-1 [25].

An interesting therapeutic option targeting HMGB-1 may be ethyl pyruvate. Ulloa and colleagues reported that the administration of ethyl pyruvate 24 hours after CLP not only reduced circulating levels of HMGB-1 but the treatment also inhibited the p38 MAPK and NF-κB pathway and, finally, improved survival in mice from 30 to 88% [31].

Nevertheless, the contribution of the direct interaction of HMGB-1-RAGE to sepsis lethality has not been formally proven so far.

Potential Clinical Perspectives

Engagement of RAGE by its ligands results in sustained activation of NF-κB in all cell types studied so far, particularly mononuclear phagocytes and vascular endothlium. Sustained cellular activation leads to cellular dysfunction and tissue destruction. Using sRAGE as a decoy, RAGE neutralizing antibodies and a dominant-negative receptor, the involvement of RAGE in different chronic disease models has been shown. Studies with RAGE knock-out mice confirm that RAGE contributes, at least partially, to the development of late diabetic complications such as neuropathy and nephropathy, macrovascular disease and chronic inflammation. In contrast, RAGE deletion has no effect on the host response in delayed type hypersensitivity [6, 32].

RAGE has also a critical role in acute inflammation. A resulting deleterious inflammatory response after ischemia/reperfusion of the liver is associated with RAGE engagement in mice. The problem of ischemia/reperfusion is clinically relevant in cases of liver transplantion or resection. In an animal model of total hepatic ischemia, the blockage of RAGE by application of sRAGE led to increased survival and to fewer histological alterations in treated animals in line with a decrease in RAGE-induced signaling and activation of transcription factors [33]. Furthermore, blockage of RAGE significantly increased survival after massive liver resection [34].

We clarified the role of RAGE in sepsis, delayed type hypersensitivity, and autoimmune encephalomyelitis [32]. Several former studies investigating the role of RAGE in inflammatory diseases have used sRAGE to bind extracellulary potential RAGE-ligands [13, 23]. The problem of this approach is that sRAGE scavenges not only the ligands and prevents them interacting with RAGE itself, but, in addition, these ligands may be able to engage other receptor types and transduce completely

different signaling pathways. To overcome this problem, $RAGE^{-/-}$ mice were studied. In a setting of autoimmune encephalomyelitis, serving as a model to test the role of RAGE in the adaptive immune response, no differences between wildtype and RAGE-knock-out mice could be detected [32]. Interestingly, RAGE transgenic mice showed significantly enhanced clinical autoimmune encephalomyelitis scores. In addition, in a second model (delayed-type hypersensitivity), $RAGE^{-/-}$ mice and wildtype species developed the same inflammatory response. These results were, at first glance, in contrast to previous data from delayed-type hypersensitivity experiments with sRAGE, where a reduction of the inflammatory response in mice pretreated with sRAGE was found [13]. The application of sRAGE in wildtype and $RAGE^{-/-}$ mice, however, leads to a reduced inflammatory response in mice in the delayed-type hypersensitivity model [32]. These findings support the concept that the effects of sRAGE in delayed-type hypersensitivity were not caused mainly by prevention of ligand engagement of the cell-bound RAGE.

In contrast to the minor role of RAGE in adaptive immunity, the most interesting finding was that $RAGE^{-/-}$ mice were protected from lethal septic shock compared to wildtype mice. In a CLP model, largely dependent on the innate immune response, 80% of the $RAGE^{-/-}$ mice survived compared to 20% of the wildtype mice.

To confirm the critical role of RAGE in sepsis and to exclude artifacts by gene deletion, $RAGE^{-/-}$ mice were crossed into tie2-RAGE mice overexpressing RAGE in the vasculature. These mice displayed a similar mortality compared to wildtype mice.

In order to test whether blocking RAGE signaling pathways by sRAGE may be a therapeutic option, we injected sRAGE into wildtype mice resulting in an improved survival (40% vs. 17%) compared to untreated control animals. NF-κB activation was more strongly induced in lungs of wildtype mice compared to $RAGE^{-/-}$ mice suggesting a main contributing role in reducing mortality in $RAGE^{-/-}$ and fitting the high RAGE expression in the lung. In conclusion, these findings show that $RAGE^{-/-}$ mice have, at least in part, a normal adaptive immune system. In contrast, the pattern recognition receptor RAGE displays a central role in the innate immune system with an impact on perpetuation of the immune response.

In addition, Chavakis et al. [35] found that RAGE serves as a novel counter-receptor for the leukocyte β2 integrin Mac-1 (CD11b/CD18) and to a lesser extent p150,95 (CD11c/CD18) being directly involved in leukocyte recruitment *in vitro* and *in vivo*. This leukocyte recruitment via RAGE is enhanced in the presence of S100 proteins. Thus, RAGE acts as an endothelial adhesion receptor promoting leukocyte recruitment and subsequent inflammation. These findings are consistent with the fact that histological examination showed a reduced number of inflammatory cells adherent to the peritoneum of $RAGE^{-/-}$ mice after CLP compared with that of wildtype mice [32]. Remarkably, $RAGE^{-/-}$ seems to have a moderate proinflammatory phenotype, because C-reactive protein, basal NF-κB activation and cytokine levels were slightly increased. In conclusion, the crucial role of RAGE in experimental sepsis is not only strong evidence for its perpetuating role in the innate immune response, it may open an opportunity to develop RAGE inhibitors for treating septicemia.

A number of recent studies, including clinical investigations, have shown that genetic variants of RAGE may be of further interest [6]. These variants are seated in coding/translational as well as in the transcriptional regulatory elements. Hofmann et al. [12] found that the variant from of RAGE enhances binding and cytokine

production compared to wild-types. What kind of cellular consequences these genetic polymorphisms have and, in particular, what clinical relevance of these polymorphisms will be found in further studies is not yet predictable.

Conclusion

In conclusion, sepsis is still an important clinical challenge for ICUs with few therapeutic options. This chapter has summarized the current knowledge on RAGE, an inflammation perpetuating receptor, which plays a pivotal role in sepsis. RAGE is involved in signal transduction from pathogen substrates to cell activation during the onset of inflammation and perpetuates the immune response. Targeting this receptor might attenuate hyperinflammation. Essentially, understanding of the basic signal transduction of these receptors may offer new diagnostic and therapeutic options in septic patients.

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Oxidative Stress in Sepsis: Implications on Liver Protein Patterns and Analysis via Modified Proteomics Technology

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Introduction

Sepsis is the systemic response of the host organism to the invasion of microbial species and/or their toxins. The incidence of sepsis is steadily increasing, and, despite recent progress in intensive care research, mortality is still high, in particular when septic shock and multiple organ failure (MOF) develop [1, 2]. Diverse molecular mechanisms of inflammation and cellular damage have been implicated in the pathogenesis of septic shock and MOF [3, 4], including the excessive production of reactive oxygen species (ROS) and reactive nitrogen species (RNS).

Many cellular processes, such as inflammatory host defense and energy metabolism involve redox processes, which take place all over the cell comprising simple electron transfer reactions, radical processes as well as thiol/disulfide exchanges. To ensure proper function, the living cell has to monitor, control and maintain the intracellular redox balance. However, in septic shock an imbalance between ROS and antioxidant defense mechanisms occurs, resulting in oxidative stress [5, 6]. The reason for this imbalance is an overwhelming production of ROS and/or a deficit in antioxidant systems. The most important ROS/RNS are represented by the following candidates: superoxide anion ($O_2^{\bullet -}$), nitric oxide ($NO^{\bullet}$), hydroxyl radical ($HO^{\bullet -}$), hydrogen peroxide (H_2O_2) and peroxynitrite ($ONOO^-$) (see Table 1). Among the various ROS, $O_2^{\bullet -}$ plays a key role in the pathogenesis of hemodynamic instability and organ dysfunction during septic shock. $O_2^{-\bullet}$ is primarily produced by activated neutrophils and macrophages as part of the innate immune system [7, 8] and has been associated with the inflammatory response that accompanies tissue damage in septic shock [9]. Beside non-enzymatic antioxidants, e.g., vitamins C and E, bilirubin, reduced glutathione and albumin, superoxide dismutase (SOD), catalase and glutathione peroxidase are referred to as major *enzymatic* antioxidant systems. Under normal conditions, the formation of $O_2^{\bullet -}$ is kept under tight control by endogenous SOD enzymes. Despite their importance in innate immunity representing one important defense mechanism against invading pathogens [6], the overwhelming production of ROS threatens the integrity of various biomolecules including proteins [10], lipids as well as lipoproteins, and DNA [11] resulting in tissue damage, by lipid peroxidation of cell membranes, protein oxidation and DNA strand breaks. These pathomechanisms contribute to MOF during sepsis resulting in myocardial depression, hepatocellular dysfunction, endothelial dysfunction, and vascular catecholamine hyporesponsiveness.

It must be underscored that, beside the negative effects associated with oxidative stress, ROS exert several important and vital beneficial physiological cellular functions which have been demonstrated in different areas including intracellular sig-

Table 1. Reactive oxygen and nitrogen species (ROS/RNS): origin and metabolism

ROS/RNS molecule	Main Source	Enzymatic Defense System	Products
Superoxide ($O_2^{\bullet -}$)	Activated phagocytes Discharge of electrons from the electron transport chain Xanthine oxidase Flavoenzymes	Superoxide dismutase (SOD)	$H_2O_2 + O_2$ H_2O_2
Hydrogen peroxide (H_2O_2)	Product of SOD NADPH-oxidase (neutrophils) Glucose oxidase Xanthine oxidase	Glutathione peroxidase Catalases Peroxiredoxins (Prx)	$H_2O + GSSG$ $H_2O + O_2$ H_2O
Hydroxyl radical ($HO^{\bullet -}$)	From $O_2^{\bullet -}$ and H_2O_2 via transition metals (Fe or Cu)		
Nitric oxide (NO)	Nitric oxide synthases	Glutathione/TrxR	GSNO
Peroxynitrite ($ONOO^-$)	Product from $NO + O_2^{\bullet -}$ triggering DNA single strand breaks	Poly-(ADP-Ribose) Polymerase (PARP)	

SOD: superoxide dismutase; GSSG: oxidized glutathione; GSNO: S-nitrosoglutathione

naling and redox regulation [12, 13]. First, ROS represent a defense mechanism against invading organisms by activated phagocytes [6]; ROS are produced by the NADPH oxidase complex in this system. Second, ROS can directly affect the conformation and/or activities of all sulfhydryl-containing molecules, such as proteins or glutathione by oxidation of their thiol moiety [13]. For example, superoxide, hydrogen peroxide, and NO are well known regulators of transcription factor activities and other determinants of gene expression [14–16]. Several cytokines, growth factors, hormones and neurotransmitters use ROS as secondary messengers in the intracellular signal transduction [17]. Well-known examples of redox-sensitive transcription factors are nuclear factor-kappa B (NF-κB) and activator protein-1 (AP-1) [15, 18], the nuclear factor-E2 related factor 2 (NrF2) pathway targeting the antioxidant element [19], or the ROS mediated sensing of hypoxia [20]. The mechanisms for altered transcription factor control could be either via decreased binding to promotor regions via oxidative damage to the DNA or more direct by redox regulation of transcription factor activation [21] and/or altered DNA-binding due to redox-induced modification of the transcription factor protein [22]. Third, in addition to their physiologic beneficial effects, ROS are, due to their high reactivity, prone to cause damage being, thereby, also potentially toxic, mutagenic or carcinogenic. Thus, the targets for ROS/RNS damage include all major groups of biomolecules as already mentioned above: proteins, lipids, DNA.

As already mentioned, there are several cellular antioxidant enzymes, such as (the well characterized) SOD, as well as complex systems such as the cysteine-based redox regulation of the glutathione and thioredoxin pathways [22]. The SOD enzymes include the copper/zinc enzymes present in the cytosol (SOD1) or extracel-

lular surfaces (SOD3), and the manganese enzyme in the mitochondria (SOD2). In disease states, the production of $O_2^{-\bullet}$ is increased at a rate that overwhelms the capacity of the endogenous SOD defense system, resulting in $O_2^{-\bullet}$-mediated damage. The proinflammatory properties of $O_2^{-\bullet}$ include endothelial cell damage and increased microvascular permeability [23, 24], formation of chemotactic factors, e.g., leukotriene B_4 [25], recruitment of neutrophils at sites of inflammation [26], lipid peroxidation and oxidation, DNA single strand damage [27], release of cytokines, e.g., tumor necrosis factor (TNF)-α and interleukin (IL)-1β [28, 29], and formation of $ONOO^-$, a potent cytotoxic and pro-inflammatory molecule triggering DNA single strand breaks [30, 31].

Regarding the glutathione- and thioredoxin-reduction pathways, it has become clear that there are two parallel, interdependent enzymatic systems. On the one hand, glutathione as a reducing substrate seems to be more effective in reducing small disulfide molecules and in reacting directly with ROS, whereas, on the other hand, thioredoxin is more effective in reducing the exposed disulfides of proteins. Thus, the thioredoxin system can also be seen as an antioxidant defense/repair system for (accidentally) oxidized cysteine proteins [22].

Reduced glutathione is among the most important intracellular antioxidant within human cells. It exists in equilibrium with its disulfide form (GSSG), and the ratio of glutathione to GSSG could be used as an indicator of the redox status of the cell. Several important human antioxidant-defense systems are based around glutathione, e.g. glutathione peroxidase as a major cellular reducer of hydrogen peroxide (together with catalase and peroxiredoxin) [22]. Another important element of the defense system is formed by chaperones or heat shock proteins (HSP) [32]. Oxidized proteins tend to change their tertiary structure and when the oxidation is reversed they have to be refolded by chaperones to gain their optimal structure. Chaperones like HSP27 and HSP70 have an anti-apoptotic signaling effect. Also, the protein refolding requires ATP, increasing the energy requirement of the defense system, which also consume energy for the regeneration of reducing equivalents like NADPH. The latter is provided by pentose phosphate shunt activity or mitochondrial oxidation. Break down of the energy supply leads to apoptosis, which is reflected in a tight coupling between these two processes [33].

Under sepsis, various processes, triggered by ROS/NOS contribute to oxidative stress. Their interrelation is outlined in Figure 1. It appears that stimulated oxidative processes form one key element in the cascade of deleterious processes. Therefore, as a causative therapy, the antioxidant system should be supported. This concept has been the subject of intensive discussion [34, 35], but the results at best have been rather equivocal, to some extent certainly due to the 'friend and foe' properties of antioxidant supplementation, which was elegantly characterized as the "antioxidant paradox" [36]. Thus, the multiple interrelated processes make it difficult to assess where, when, and in which dose the supporting agent should be delivered. What is missing so far is an integrated approach that allows characterization of the state or activity of the various processes involved in oxidative stress and its defense responses. cDNA-micro array measurements [37, 38] provide such 'holistic' information about gene activation, as reflected in the mRNA levels. Microarray data allow the definition of expression patterns for specific disease states and, hence, could be used as a tool to classify sepsis or oxidative stress. Furthermore, they may reflect the state of the signaling system, but they cannot be used to infer on the activity of the proteins or enzymes they encode, because many other steps, in addition to transcription, may affect the activity of an enzyme. Hence, complementary information would be useful

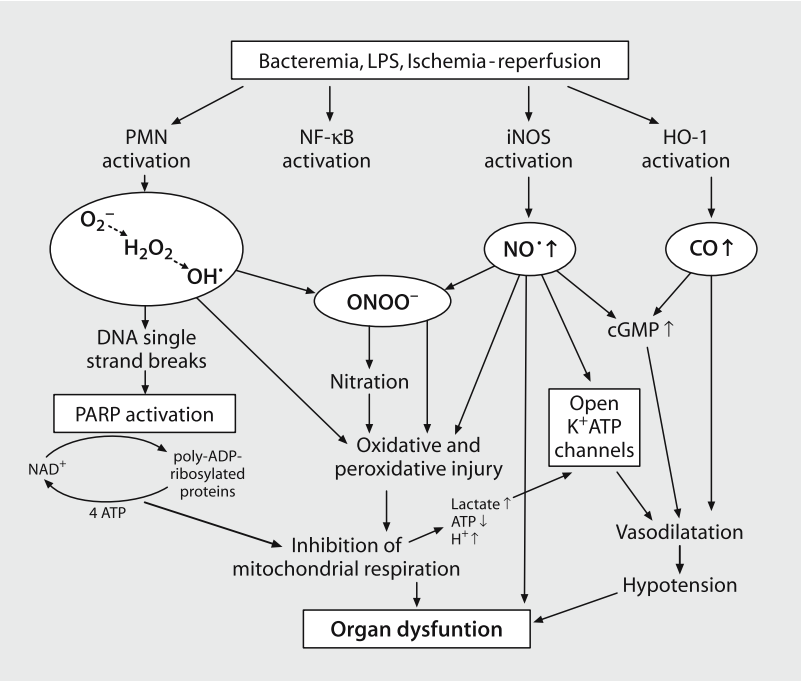

Fig. 1. Schematic representation of oxidative stress processes contributing to organ disfunction during sepsis. CO: cardiac output; NO: nitric oxide; LPS: lipopolysaccharide; iNOS: inducible NO synthase; HO: heme oxygenase. From [50] with permission

to describe the functional status of the proteins involved in the defense system, which in turn leads to the analysis of the cell proteome.

Outline of the Defense System

The major event of oxidative stress is the generation of H_2O_2, which in turn oxidizes proteins and other cellular components. As the enzymatic defense system consists of proteins, damage to the defense proteins is particularly harmful, as it may directly lead to cell death. We focus here on specific cellular strategies to repair or avoid damage and prevent oxidative inactivation of proteins, its reversibility, to remove irreversibly damaged proteins and, if necessary, to replace them by *de novo* synthesized ones. Figure 2 depicts some elements of the defense system.

The oxidation can be reversed at the expense of glutathione or NADPH consumption, which have to be regenerated via mitochondrial oxidation of glucose or the activity of the pentose phosphate shunt. The efficiency of this regeneration is monitored via the redox state of various proteins like thioredoxin or signaling proteins involved in the NF-κB or NrF2 pathway. Via this signaling the transcription of enzymes for the defence system is initiated, increasing the defence capacity. Oxidation or overoxidation of the Cys-SH groups or other amino acid residues changes the tertiary structure of the protein, which in turn evokes a misfolded protein sig-

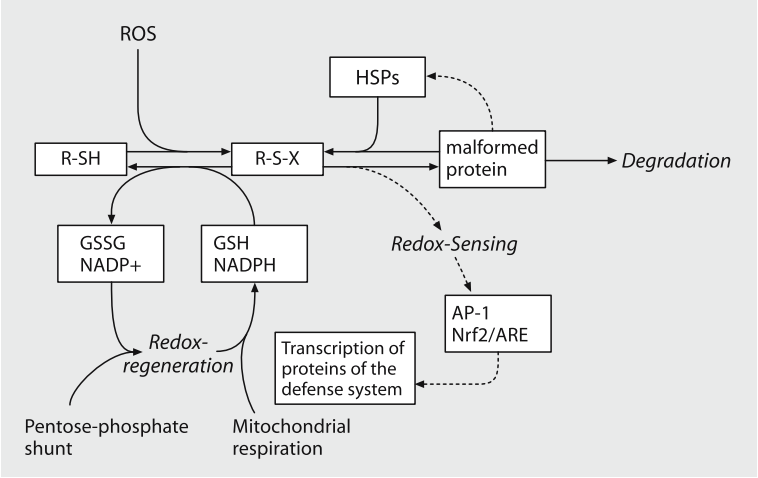

Fig. 2. Structure of a cellular defense system focusing on oxidative protein damage, protein repair, degradation and signaling pathways, regulating their interplay. Solid lines indicate flow of material or chemical reactions. Broken lines indicate stimulatory signaling effects. HSPs: Heat shock proteins; R-SH, R-SX: Proteins with free and oxidized thiol group of cysteine, respectively; ARE: anti-oxidant responsive element; NrF2: NF-E2 related factor 2; AP-1: activator protein-1

nal. This activates the chaperones and other components of the protein repair system. If the oxidation is irreversible, then the protein must be degraded.

Single elements of this defense system have been described in many cases; we are interested in the integration and coordination of the various defense processes in a clinically relevant sepsis model, and the chances to monitor them using protemic tools. The latter are geared to assess changes in the protein content of a cell or tissue.

Proteomic Tools

We are using functional and quantitative proteomic tools to monitor characteristic differences in the levels and turnover rates of selected proteins from control, sham-operated, and septic mice. A prominent tool is based on the separation of a protein mixture, derived from tissue or cell extract gel-electrophoresis, in two dimensions (2D): one for the molecular weight of the protein, and the second separating along the pKi-value of the protein. An individual protein appears as a single spot on the gel, and depending on the size of the gel and sampling conditions, more than 1000 different proteins can be separated. After 2D-separation the proteins in the acrylamide gel have to be chemically treated to become detectable. This can be done using organic dyes (coomassie brilliant blue), metal ion reduction (silver staining), fluorescence labels, or radioactive isotopes. The intensities of the detected spots reflect the protein content of the analyzed tissues or cells [39]. Changing the metabolic or stress conditions will alter the protein content. To quantify these changes, protein extracts obtained from a study and a reference sample can be tagged or labeled with different isotopes (e.g., I^{125} and I^{131}, attached to cysteines in the amino acid chain of the protein). This mixture is then separated on a single gel and the

various protein spots contain both labels (i.e., I^{125} and I^{131}). The relative proportion of these labels reflects the proportion of the specific protein in the study sample relative to the reference. The detection of the radioactive label is very sensitive and can be visualized. Changes in the protein profile can be detected including proteins that are present in low numbers of copies per tissue [40], which allows a 'differential' expression pattern to be characterized. As the protein expression is a function of signaling in the frame of regulation networks, it should provide the opportunity to assess the state of the signaling system, reflecting the activity of cytokines and other mediators, and changes thereof.

Differential expression figures, albeit very sensitive, do not provide identification of the protein spot. A common approach for identification, therefore, is based on mass spectrometry: the spot is excised from the gel and digested with a specific protease like trypsin. The protease always cuts the amino acid sequence at specific points (after Lys and Arg). With such a specific segmentation each protein yields a unique pattern of peptide fragments. The digest can be analyzed with Maldi-TOF (matrix-assisted laser desorption ionization time-of-flight) mass spectrometry, which allows detection and identification of the peptides by their molecular weight. The resulting set of observed peptides is matched to a database that contains a large set of proteins and all their peptides, predicted by theoretical tryptic fragmentation from their amino acid sequence. This protein identification is termed peptide mass finger printing (PMF). PMF is usually applied to proteins separated by 2D-gel electrophoresis. As a very sensitive detection method, silver staining is used to visualize proteins on the 2D-gels. Together with a rough quantification of the amount of protein by virtue of silver staining intensity spot, this allows to quantify and identify hundreds of proteins of a cell or tissue extract. There are, however, some limits to this concept related to the capacity and separation power of the gel and the primary detection. Only spots that can be visualized by staining can be analyzed on the gel. The dynamic range of this method is about $1:10^3$, thus only proteins with a content larger than 0.1% of the most abundant proteins can be monitored. The dynamic range in living cells from structural proteins to signaling proteins, however, is in the range of $1:10^6$. Thus, only the abundant fraction of proteins can be monitored. Moreover, general gel electrophoresis procedures are confined to proteins with a pKi value between 3 and 10 and a molecular weight larger than 10 000 Dalton.

Based on these limitations, activation of the signaling proteins depicted in Figure 1 cannot be completely visualized with the 2D-PAGE Maldi-TOF platform. Hence, the focus generally moves onto abundant proteins or enzymes, which perform the real defense task and that are regulated by the signaling structure outlined before.

What are typical defense proteins?

- Cytosolic proteins like SOD1, catalase and glutathione-peroxidase which act as first scavengers for ROS molecules.
- Glyoxalase, aldehyde reductases and enolase, which detoxify small molecules (specifically aldehydes) generated by ROS activity.
- Proteins involved in the redox-metabolism like glutathione-S-transferase, thioredoxin and peroxiredoxin.
- Proteins stimulated by the 'malformed protein signal' via the heat shock factor (HSF)-pathway [32] or from the protein repair system, like HSP27, HSP70, HSP90, protein disulfide isomerase (PDI).

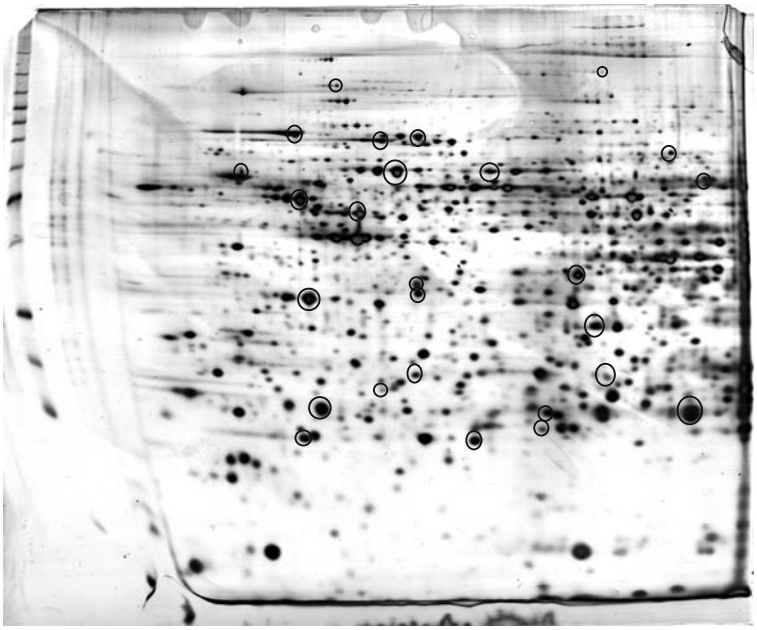

Fig. 3. SDS 2D-gel, containing proteins of a male mouse liver after surgical stress. Circled spots indicate proteins involved in the oxidative defense system, as identified by us

- Key enzymes of glycolyses and the pentose-phosphate-shunt, which provide ATP for the protein and DNA repair systems and the reducing equivalents NADH or NADPDH for the defense system.

All these proteins are part of the cellular defense system. Moreover, ROS species are generated by incomplete reduction of oxygen during cellular respiration even under resting, non-challenged conditions. They cause, to a low extent, the above-mentioned damage and, hence, a substantial constituent amount of the stress proteins is required. The same holds true for the protein repair system, which also functions as a quality control system for the protein synthesis machinery. Consequently most of these proteins are expressed at high, abundant rates even under non-stress conditions. Figure 3 shows a 2-D gel with defense proteins of the liver extract extracted from a mouse subjected to surgical stress. It turns out that defense proteins form a large, significant part of the gel-picture, and there is no need to resort to extreme stress conditions to visualize these proteins.

Dynamic Proteomics

Changing the oxidative stress level should induce changes in the expression profile of the proteins, and it is tempting to assign and trace back these expression changes to changes in cytokine activation and signaling pathways. In a model of chemically induced oxidative stress, Xiao et al. [41] exposed macrophage cells to

three different levels of diesel exhaust particles, and found a reduction in the glutathione/GSSG ratio as a function of the stress level as well as different protein expression patterns for these levels. From the gene regulation of various proteins they could assign the expression changes to changes in stimulatory pathways. For 'low' stress levels they found Nrf-2-driven changes targeting anti-oxidant response elements, for their 'medium' levels NF-κB/mitogen-activated protein kinase (MAPK)-driven changes that affect AP-1 target. The 'highest' level was associated with mitochondrial damage and apoptosis. Among the proteins identified were signaling proteins like p38, MAPK/ERK kinase kinase 1 or TNF-receptor. The latter, however, are only visible in isolated cells of the immune system and unlikely to be prominent in tissue extracts. The paper by Xiao et al. [41] clearly demonstrates both the strengths and limitations of the 2D-PAGE Maldi-TOF proteomics approach: The expression profile covering many proteins and its change, caused by altered conditions, can be monitored, and the amount of information available drastically exceeds that available using other protein-biology methods targeting specific enzymes based on antibodies or enzyme activity. The expression patterns shown, thus, would be sufficient to quantify an unknown stress level for a cell sample and to characterize the defense response. There are, however, limitations. For example, only a fraction of the enzymes involved in the antioxidant defense are listed. The above-mentioned defense and redox protein are missing despite the fact that their synthesis would be expected to be stimulated by the activation of the stress signaling pathways. These limitations arise from the detection based on spot size or intensity. First, the detection is only static, i.e., depicts protein content, but cannot separate between synthesis and breakdown, in other words, turnover rate. Second, the premise that the content of a protein is proportional to the size or staining intensity of a single spot, does not hold in each case: Defense and redox proteins tend to have a free Cys-SH group that can be reversibly oxidized or linked to glutathione, as shown for T-lymphocytes [42] and hepatocytes [43]. Free Cys-SH groups of the protein repair enzyme, PDI, oxidize or reduce the cysteine groups of other proteins, to form or break disulfide bridges that stabilize the 3D-structure of a protein, and, thereby, are susceptible to oxidation. Since sulfur can undergo different oxidation steps, irreversible overoxidation is also possible. In parallel, the residues of other amino acids can be oxidized or carbonylated [44]. All these steps shift the pKi value of the affected proteins toward more acidic values. As a consequence, some proteins appear in different spots on the gel and, in the extreme case, may depict a 'pearl chain' pattern, as seen in Figure 3. Furthermore, in some instances the protein chain is either cleaved *in vivo* by partial proteolysis to two smaller sub-chains, or the chain breaks during sample workup leading to two different protein spots with molecular weights much lower than expected. Taken together, the moiety of specific protein can be distributed over different spots. Some of these isoforms can be overoxidized and inactive, accumulating and designated for degradation [45, 46], and cannot be referred to as contributing to the content of the biologically active isoform. For example, for peroxiredoxin 2 and 3, Rabilloud et al. [45] demonstrated inactivation by irreversible oxidation of cysteine to cysteic acid. The oxidized form appears as a more acidic spot in the gel and was suggested as a marker for oxidative protein damage. Although there was a significant conversion from the active to the inactive, acidic form, the size of the spot, representing the active form of the protein, did not decrease significantly. In a subsequent study [47], it was shown, by using stable isotope labeling, that the active version of the protein was regenerated by *de novo* synthesis, whereas the oxidized

protein was not regenerated to the active form but degraded. The size or staining intensity of the active protein spot remained unchanged, as an increased synthesis was paralleled by increased irreversible conversion to an inactive state. In this specific case, spot size or measurable protein expression was not proportional to protein synthesis, violating the above-mentioned premise for profiling.

A similar situation was observed for the protein disulfide isomerase [48], a protein involved in protein folding/repair, located in the endoplasmic reticulum. Again, the loss due to protein oxidation was compensated by *de novo* synthesis. This paper also demonstrated that oxidized proteins are degraded by the proteasome system. The same group showed that oxidized proteins change their tertiary structure to unshielded hydrophobic amino acid residues [46], like large bulky aliphatic side chains or aromatic residues, and postulated that the altered hydrophobicity is the key signal for recognition by the proteasome for degradation. Oxidation-induced degradation by the proteasome has been shown for other proteins like SOD1 or mitochondrial aconitase, a key enzyme of the Krebs cycle [46].

Is oxidative protein damage a random event, affecting all proteins to a similar extent? In a study using irradiation as source for oxidative stress, Magi et al. [44] screened the cellular proteom for carbonylation and found a preferential damage for HSP next to structural proteins and some other proteins of the defense system. The preferential damage of HSP was explained with the tight contact these proteins have with other damaged proteins during protein repair, which might increase the likelihood of radical transfer from the damaged to the repair protein.

What does preferential oxidation and inactivation combined with increased degradation imply for an integrated defense system and its regulation as outlined in Figure 1? In this figure R-SH reflects any protein with a free Cys-SH group, including proteins of the defense system. Their inactivation by oxidation could lead to their degradation and trigger, via the pathways outlined there, the synthesis of new defense proteins. For PDI and peroxiredoxin, it was demonstrated that such a regulatory feedback loop could compensate for oxidative loss via signaling pathways that are not yet completely uncovered. It is also conceivable that any 'cross talk' between the different signaling methods may stimulate the expression of other proteins. This interplay cannot be detected based on monitoring the spot size/intensity alone, and consequently additional measurements for protein breakdown or synthesis are required. Using such a combined approach a detailed characterization of the defense state is possible. For example, under overwhelming toxic oxidative stress, we expect that the compensatory *de novo* synthesis will not compensate for the oxidative loss, which should be reflected in a reduced size of the spot for the active protein, increased turnover, and eventually increased size of the oxidative spot. Therefore, we recently developed an approach [49] to measure the fraction of a protein pool that is derived by *de novo* synthesis during the labeling phase (fractional synthesis rate), the proteins of which were separated and identified by 2D-gel electrophoresis and Maldi-TOF mass spectrometry. This approach is based on metabolic labeling with ^{13}C during constant infusion of uniformly labeled glucose and is sensitive enough to detect small fraction synthesis rates as low as 2% and changes thereof in the range of 0.5%. Table 2 lists the results for some proteins, which are related to the above mentioned defense system.

For a proof of principle, in a pilot study we compared the fractional synthesis rates of HSP between a septic and a sham operated condition. During sepsis we found a significant reduction in the synthesis of HSC70, a constitutive chaperone, virtually no change for HSP60 and HSP70, and a significant increase for PDI. This

Table 2. Fractional synthesis of individual proteins, participating in the antioxidant defense system, obtained from an unchallenged mouse liver

Protein	Fractional synthesis rate (± SEM)
Glutathione-S-transferase	2.8 (0.6)
Cellular glutathione peroxidase	0.7 –
Peroxiredoxin 2 (thioredoxin peroxidase 1)	5.2 (2)
Cu/Zn superoxide dismutase (SOD1)	0.9
Heat shock protein 60 (HSP60)	1.5 (0.5)
Heat shock cognate protein 70, heat shock 70 kD protein 8	15.1 (0.8)
Protein disulfide protein	8.6 (0.6)
Glucose regulated protein	9.5 (0.5)

encouraged an, at present still ongoing, detailed analysis of sepsis-induced changes in turnover and content for different defense proteins.

Conclusion

Taken together, proteins of the antioxidant defense system can be damaged by oxidative stress, and, in fact, there is evidence that they are even specifically susceptible. The oxidative loss of protein moiety is partially compensated by *de novo* synthesis. This compensatory mechanism complicates any attempt to relate mRNA profiles assessed by cDNA technology or protein expression profiles assessed by 2D-gel electrophoresis to the functionally active protein content. The dynamic cellular response with sepsis can only be revealed by disentangling the enormously complex response at the protein level. The only method able to deliver appropriate information is a proteomic platform based on differential and quantitative approaches, which is extended by synthesis or turnover measurements.

Our ultimate aim is to use this dynamic approach:

- to understand the complex interaction between the various elements of the defense system;
- to define a set of measurements necessary to characterize the various conditions of the system; and
- to develop a tool box to evaluate the efficacy of therapeutic measures intended to support the defense system.

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HSP90: The Unsung Villain of Sepsis?

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Introduction

Heat shock protein 90 (HSP90) is a molecular chaperone that ensures the correct folding and conformational maturation of specific proteins involved in a wide variety of cellular processes [1, 2]. Large multidomain proteins are prone to aggregation or to becoming involved in kinetically trapped intermediates; HSP90 is required for a specific set of difficult to fold proteins. When cells are stressed (e.g., because of growth at an unfavorable temperature, osmotic pressure, oxygen tension, pH, or in the presence of noxious chemicals or antibiotics), they upregulate HSP90 production in order to help combat the effects of protein degradation. HSP90 also aids protein stabilization and facilitates activation of many regulated proteins. HSPs have also been shown to be immunodominant and are major targets for the immune system in many infections [3, 4]. Inhibitors of HSP90 have been shown to deplete multiple proteins important in signal transduction, cell cycle regulation, apoptosis, invasion, angiogenesis, metastasis, immortalization [1].

HSP90 in humans was initially perceived as an intracellular molecular chaperone. This view has recently been challenged. In certain disease states, such as cancer, HSP90 appears on the cell surface, which is why HSP90 inhibitors are being investigated for their anti-cancer potential. The hypothesis is that impeding HSP90 would constitute an attack on multiple nodes of a cancer cell web of overlapping signaling pathways and thus be more likely to succeed than an attack on a single pathway [5]. HSPs have been shown to be released from necrotic cells and not during apoptosis. This was more pronounced in gradually dying cells such as was seen when cells were treated with acrylamide [6]. This would be consistent with a model where during severe illness dying cells increased their levels of HSP90 prior to death and then released considerable quantities of HSP90 which would freely circulate in the blood. The presence of receptors specific to HSPs such as CD91 on the surface of antigen presenting cells further characterizes the importance of this phenomenon [7]. This supports the idea that extracellular HSP90 is a surrogate marker of 'danger/alarm' [8] occurring only in pathological states such as cancer or when it is released as a consequence of cell necrosis.

The existence of fungal and bacterial homologs has also been described. Circulating HSP90 has been identified in the sera of patients with acute invasive candidiasis and its disappearance was associated with the production of a humoral response against it and the resolution of this infection [9, 10].

This raises the fascinating possibility that in the septic patient there is a mixture of HSP90 released by the invading microbes and further HSP90 released due to cell necrosis. This would result in particularly high levels leading, in part, to the clinical manifestations of septic shock.

An antibody response specific to HSP90 has been described in patients with cancer, auto-immune disease, serum from healthy patients, and a variety of microbial infectious diseases. This chapter will review these data and argue that in the septic patient HSP90 release leads to circulating HSP90 which acts as a 'toxin'. Circulating HSP90 would normally be neutralized by an antibody response rapidly mounted by the patient and a failure to produce such a reaction would lead to a poor outcome from the infection.

Cancer, Auto-immune Disease and Natural Antibody

Antibodies against HSP90 are frequently found in patients with breast cancer where they correlate with survival, the presence of lymph nodes, and metastasis occurrence [11]. In 20 high-grade osteosarcoma patients, the presence of anti-HSP90 antibodies correlated with a better response to neoadjuvant chemotherapy ($p < 0.01$), whereas their absence correlated with the development of metastases [12]. Antibody against HSP90 was found in the sera of 32% of patients with late stage ovarian carcinoma, 10% with early carcinoma, 3% with colorectal cancer, 8% with breast cancer, and 5% with benign gynecological disease. This response was restricted to a subset of patients with advanced disease where it was suggested as being associated with a better prognosis [13].

Raised levels of antibody have been reported in some series of patients with systemic lupus erythematosus (SLE) and rheumatoid arthritis. Minota et al. [14] demonstrated antibody in 50% of unselected patients with SLE and none in 10 normal subjects, 10 patients with rheumatoid arthritis, and 7 with scleroderma. Jarjour et al. [15] found a lack of antibodies to stress proteins in patients with SLE and rheumatoid arthritis.

Natural antibodies have been observed against self antigens including HSP90, myosin, tubulin, and aldolase. In normal IgG, this auto reactivity could be absorbed almost completely on F $(ab')_2$ fragments from the same IgG preparation, coupled to sepharose and could be inhibited by the effluent obtained after subjecting normal IgG to HSP90 affinity columns. These findings indicated that anti-HSP90 natural auto-antibodies were blocked by idiotypic interaction within the IgG repertoire. Unlike natural auto-antibodies, anti-HSP90 IgG from SLE patients' sera were only moderately absorbed on F $(ab')_2$ fragments of normal IgG. These results demonstrated that in SLE there is an altered idiotypic regulation of the anti-HSP90 IgG auto-antibodies. The natural antibodies were predominantly of the IgG2 isotype in contrast to those found in SLE which were mostly IgG1 [16].

Parasitic Infection

The antigenicity of HSP90 has been demonstrated in patients with malaria, leishmaniasis, and toxoplasmosis. Protection by anti-falciparum HSP90 antibodies was seen in monkeys infected with *Plasmodium falciparum* [17]; HSP90 function was essential for *P. falciparum* growth in human erythrocytes and the HSP90 inhibitor, geldanamycin, had antimalarial activity [18].

Fungal Infection

Early work examining the immunological reaction to candidiasis demonstrated the importance of antibody. Five independent groups reviewed by Matthews and Burnie [3], working with a variety of animal models, including mice and baboons, succeeded in conferring protection against systemic challenge with *Candida albicans* by passive immunization with *Candida*-specific immune serum. In addition, a single patient, prior to the availability of amphotericin B, was treated successfully with a rabbit polyclonal antiserum raised against *C. albicans*.

The development of the technique of immunoblotting allowed the humoral response to individual antigenic bands to be characterized. This demonstrated immunodominant antigens in the size range 45 to 52 kDa, which were associated with an antibody response in infected patients [3]. In an early study [9], there was a close association between survival and the ability to produce a sustained antibody response to an antigenic band at 47 kDa. In contrast, in fatal cases, the antibody response to this antigen was absent [10].

Homologous antigens have been identified in *Candida parapsilosis, Torulopsis glabrata, Candida tropicalis, Candida krusei, Cryptococcus neoformans,* and *Aspergillus fumigatus* [3, 19, 20]. In each case there was a trend or positive correlate between survival and the production of an antibody to an antigenic band which cross-reacted with one or more antibodies against HSP90.

The 47 kDa circulating antigen was shown in the urine of 10 patients with disseminated candidiasis [21]. A dot immunobinding assay using antibodies that bound to the 47 kDa antigen gave positive results in 76% of 87 patients with disseminated candidiasis [22]. These results demonstrated that the 47 kDa antigen circulated during infection, that the resolution of the infection correlated with its disappearance, and that the level initially rose in response to amphotericin B therapy. The antigen was identified as a homolog of HSP90 with an 83% homology to *Saccharomyces cerevisiae* HSP90 and 50% homology to human HSP90 [3].

Over expression of *Candida* HSP90 in recombinant *S. cerevisiae* produced a strain with enhanced virulence in infected mice; the parent *S. cerevisiae* is relatively non-pathogenic compared to *C. albicans* [23]. HSP90 has been implicated in the pheromone response in *S. cerevisiae* [24] and the genes associated with sexual mating have also been described in the genome of *C. albicans* [25]. HSP90 has been identified on the surface of *Candida* hyphae by immunoelectron microscopy revealing HSP90 both on and within the cell wall and its induction during hyphae formation [26]. Inhibition of this process would lead to a weakened cell wall and explain the synergy seen between antibody against HSP90 (such as Mycograb®) and antifungal drugs active against the cell wall or cell membrane (such as amphotericin B) – in much the same way as HSP90 inhibition by chemicals such as geldanamycin or radiciol accelerated yeast cell lysis following exposure to detergent or complement attack [27].

The availability of the sequence of the 47 kDa antigen led to its chemical synthesis as a set of overlapping peptides and definition of individual areas as potential epitopes by the method of 'epitope mapping' known as the Pepscan technique. When this technique was applied with 47 kDa antibody positive sera from patients with proven or superficial infections, the sera recognized the epitope KILKVIRK. This epitope was not recognized by eight sera from controls. The KILKVI region was shown to be essential and changing a single amino acid radically reduced the binding ability of the antibody positive sera [3]. The LKVIRK epitope is conserved amongst yeast HSP90 sequences (Fig. 1).

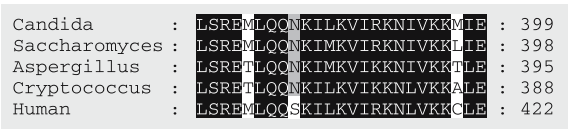

Fig. 1. Conserved amino acid sequences from *Candida, Saccharomyces, Aspergillus, Cryptococcus* and man (http://www.expasy.org). Similar amino acids are highlighted in black

A series of antibodies against this epitope was produced and their activity confirmed in murine models of infection. This included human sera from patients who had recovered from infection, polyclonal rabbit anti-sera, a mouse monoclonal antibody, and sequences derived using the FABTEC® technology where the immunoprofiles of individuals who have recovered from invasive candidiasis were characterized and the most prevalent sequences expressed in *Escherichia coli* [28]. This formed the basis for the development of the recombinant human antibody termed Mycograb® which was specific to the NKILKVIRKNIVKK epitope of fungal and human HSP90.

Recently, Yang et al. [29] expressed the epitope LKVIRK on the surface of a filamentous phage. Immunization of mice resulted in a resistance to systemic *C. albicans* infection as confirmed by fewer yeast cells in the kidneys and a longer life span after intravenous challenge. Raska et al. [30] vaccinated mice with an HSP90 expressing DNA vaccine. Following intradermal vaccination, mice challenged by live *C. albicans* survived longer (39% longer than Freund's adjuvant and 64% longer than phosphate-buffered saline [PBS]) and this survival correlated with the serum level of anti-*Candida*-HSP90 serum IgG antibody.

Bacterial HSP90 (HtpG)

The HSP90 homolog in bacteria is termed HtpG. In *Porphyromonas gingivalis,* antibody levels to an HSP90 homolog correlated with oral colonization and poor periodontal health. Heat stressing *P. gingivalis* induced a five-fold increase in expression in the 68 kDa parent molecule whilst the 44 kDa band was constitutively expressed [31]. This antibody response might explain the high levels in some patients, for example those with SLE where there is a risk of developing periodontitis.

In patients with septicemia due to *Corynebacterium jeikeium,* recovery from infection was associated with the presence of antibody, detected by immunoblotting, against bands at 50, 51 and 110 kDa. The 52 kDa band cross reacted with a monoclonal antibody raised against *Candida* HSP90, and a reverse passive latex agglutination test for detecting the *Candida* antigen in disseminated candidiasis gave false positives in patients with *C. jeikeium* infections [3].

Human HSP90

This will be subdivided into the interactions between HSP90 and interleukin (IL)-6 and HSP90 and nitric oxide (NO). Much of this information been deduced by examining the effects of the HSP90 inhibitor, geldanamycin.

Interleukin-6

IL-6 is part of the acute-phase response in infection, such that a raised level has been correlated with more severe infection and a poorer outcome. In surgical patients, a raised IL-6 was associated with the systemic inflammatory response syndrome (SIRS) [32]. At a cut-off of 310 pg/ml in patients with septic complications during their first five postoperative days the test had a sensitivity of 90% and specificity of 58% when differentiating between patients with and without postoperative septic complications [33].

In murine infection with C. *albicans,* IL-6 levels were raised and in mice which were IL-6 deficient there was a poorer prognosis [34]. The administration of recombinant IL-6 decreased the fungal load in mice with disseminated candidiasis and the level of IL-6 has been shown to be a predictor of fatal outcome in murine models of sepsis [35].

Geldanamycin potently inhibited the production of IL-6 by activated macrophages and the production of IL-6, IL-12 and NO in CpG DNA activated RAW 264.7 cells [36]. A raised IL-6 led to a raised level of C-reactive protein (CRP). This was mediated through the major signal transducer and activator of transcription (STAT) 3 which is part of a micro-domain in the plasma membrane chaperoned by HSP90. HSP90 bound to a polyclonal antibody against STAT3 confirming an interaction between STAT3 and HSP90 [37].

These observations support a model in which extracellular human or fungal HSP90 induces IL-6 and, hence, CRP release. This could be one of the causes of the pyrexia seen in infection, and may explain pyrexia in the absence of a defined pathogen where this would result from the release of human HSP90 from necrosing human cells. The temperature commonly seen after amphotericin B administration could be the result of a sudden burst of free fungal HSP90 due to acute yeast necrosis.

Nitric Oxide

NO is a gaseous free radical which is secreted by the enzyme NO synthase (NOS). In 1998, HSP90 was shown to interact with endothelial NOS (eNOS) leading to the release of NO and vasodilatation [38]. In a subsequent human volunteer study, NO mediated vasodilatation was evoked in the forearm by treating with either the endothelial agonist acetyl choline or locally heating the region to 42°C. Geldanamycin blocked this phenomenon and reversed the vasodilatation [39].

The activation of eNOS was orchestrated by an interaction between HSP90 and the protein kinase Akt, which led to phosphorylation of the serine at amino acid position 1179 in eNOS. There was preferential binding of the region of HSP90 overlapping the NKILKVIRKNIVKK epitope to eNOS when compared to constructs representing the rest of the molecule [40]. Sodium vanadate-induced NO production involved the same pathway and increased the binding of the activator protein HSP90 to eNOS [41]. Endothelial NOS activation has been shown to be critical for vascular leakage during acute inflammation *in vivo* [42].

HSP90 has been shown to activate the kinin-forming cascade leading to the production of bradykinin. This zinc dependent activation of the prekallikrein-high molecular weight kininogen complex on endothelial cells was inhibited by the addition of polyclonal antibody to HSP90 in a dose-dependent manner [43]. Bradykinin has been described as a major mediator of swelling in C1 inhibitor deficiency and responsible

for the angioedema seen with angiotensin converting enzyme (ACE) inhibitors. It is a gastrointestinal vasodilator and released from mast cells during asthma. In baboons infused with *E. coli,* activation of the plasma kallikrein-kinin system has been demonstrated, leading to hypotension and disseminated intravascular coagulation (DIC) [44]. In a septic patient, it is proposed that excessive levels of HSP90 might activate this kinin-forming cascade and mimic Gram-negative septic shock.

Mycograb® and HSP90

The discovery that an antibody response against the epitope NKILKVIRKNIVKK was associated with survival from disseminated candidiasis and that this region was key to the interaction with eNOS could best be understood by considering the molecule as three separate regions [2]. Region A of *S. cerevisiae* (1–400) contained the geldanamycin and ATP binding domains and Region B (401–620) the epitope NKILKVIRKNIVKK. Region C (621–732) was the carboxyl end containing a second ATP binding site. Exposure of this site followed a conformational change of the whole molecule after the first ATP molecule had bound to the amino end ATP binding site. This change was dependent on the sequence QQSKILKVI at the start of region B.

Mycograb® binds to this region thus preventing the conformational change. It is an active antifungal which produced synergy in combination with either amphotericin B or caspofungin in the laboratory. The results of testing a series of clinical isolates according to the methodology of Matthews et al. [28] have been summarized in Table 1. These show either synergy or an additive effect with no evidence

Table 1. Summary of results of minimum inhibitory concentration testing for clinically significant yeast isolates showing synergy, an additive effect, indifference or antagonism when Mycograb® (at 4 or 8 μg/ml) was combined with amphotericin B *in vitro*

Species	No of Isolates	Mycograb® (μg/ml)	Synergy	Additive	Indifference	Antagonism
C. albicans	31	4	12	19	0	0
		8	19	12	0	0
C. parapsilosis	20	4	9	11	0	0
		8	11	9	0	0
C. lusitaniae	12	4	10	2	0	0
		8	11	1	0	0
C. glabrata	11	4	11	0	0	0
		8	11	0	0	0
C. tropicalis	9	4	9	0	0	0
		8	9	0	0	0
C. guilliermondiae	7	4	0	7	0	0
		8	0	7	0	0
C. krusei	2	4	0	2	0	0
		8	0	2	0	0

for indifference or antagonism. This phenomenon has been confirmed in clinical studies involving 160 patients in 10 European countries and the USA, where approximately half the patients received Mycograb® plus liposomal amphotericin B (the 'test group') and half placebo plus liposomal amphotericin B (the 'placebo group') (www.neutecpharma.com).

The primary test of efficacy was based on a comparison of the frequency with which patients in the test group showed a complete clinical and mycological response by Day 10 compared to the frequency in the placebo group. This showed a highly statistically significant difference ($p < 0.001$) between the two groups; those receiving Mycograb® showing a complete overall (clinical and mycological) response in 84% of cases compared to 48% in the placebo-treated group. The frequency of deaths due to the *Candida* infection under treatment was as high as 18% in the placebo group but fell to 4% in the group receiving Mycograb®, this difference being statistically significant ($p = 0.025$).

As a further, laboratory-based test of efficacy, the speed with which culture-confirmed eradication of the fungus was achieved was compared between the two groups. The rate of culture-confirmed eradication of the infection showed a highly statistically significant difference between the two groups ($p < 0.001$), the median time to last positive culture being 3 days for the test group, compared to 23 days for the placebo group (Kaplan-Meier).

Conclusion: Is HSP90 the Unsung Villain of Sepsis?

In this chapter, we have presented data supporting the presence of circulating HSP90 in severely ill patients. This may be human in origin as the result of release from stressed and subsequently dying cells. This does not happen when cells are replaced during the normal process of apoptosis. The effect of this free HSP90 on the cardiovascular system is likely to involve the induction of eNOS leading to the production of NO resulting in vasodilatation and hypotensive shock. A separate interaction with IL-6 would produce a raised CRP. Thus free extra-cellular human HSP90 would produce a septic picture in the absence of an obvious culture positive pathogen.

In the presence of a bacterial infection, the release of HtpG would accentuate this problem and produce some of the symptoms of Gram-negative septic shock. In the presence of yeast infection, this process is highlighted as the yeast upregulates its production of extra-cellular HSP90 in response to a stress such as amphotericin B therapy and to help itself spread into the tissues. The high homology between human and yeast HSP90 results in molecular mimicry between the two molecules.

In the healthy patient, circulating HSP90 would be neutralized by part of the natural antibody pool of the patient. In sepsis, this antibody would be overwhelmed. This opens the possibility of supplementing it with an artificial antibody such as Mycograb®. Mycograb® thus becomes a logical partner in combination therapy as it would neutralize the HSP90 released by fungicidal drugs such as amphotericin B and caspofungin. HSP90 has recently been described as the 'Achilles heel' of anti-*Candida* therapy as the inhibition of this molecule has been shown to prevent the development of resistance to fluconazole and caspofungin [45]. This suggests that the co-administration of Mycograb® would both reduce the chance of resistance occurring with mono-therapy and improve outcome by neutralizing cir-

culating HSP90. This latter effect would also include human HSP90 released from necrotic cells and widen the potential clinical application of an antibody based therapy against HSP90.

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The Role of Extracellular Heat Shock Proteins in Cellular Inflammation and Injury

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Introduction

Ferruccio Ritossa first observed a novel hyperthermia-dependent puffing pattern in the giant chromosomes from the salivary glands of *Drosophila melanogaster* in 1962 [1]. By a chance occurrence, a colleague accidentally increased the temperature of one of the incubators in which he kept his specimens, and the following morning Ritossa discovered a new puffing pattern that had not been there on the previous day. Realizing the mistake, Ritossa conducted additional, properly controlled experiments and subsequently linked this new chromosomal puffing pattern with the expression of a specific group of proteins that he fittingly called heat shock proteins (HSP) [1, 2]. Since Ritossa's seminal observations in 1962, subsequent investigations into this area have continued, resulting in a growing interest in what is now commonly referred to as the heat shock response. The heat shock response is characterized by the rapid expression of a unique set of proteins collectively known as HSP [3, 4]. The structure, mode of regulation, and function of HSP are highly conserved among different species, and HSP have been identified in virtually all eukaryotic and prokaryotic species examined to date. While classically described as a response to thermal stress (hence the term heat shock response), HSP can be induced by a wide variety of non-thermal stressors and pharmacological agents (Table 1). These proteins range in molecular weight from 7 to 110 kDa and are found in virtually every cellular compartment, including the nucleus, cytoplasm, and mitochondria (Table 2). By convention, the stress proteins are classified according to their molecular weight, e.g. HSP25, HSP32, HSP47, HSP60, HSP70, HSP90, and HSP110. While new functions continue to be discovered, the stress proteins are generally thought to maintain cellular homeostasis by acting as molecular chaperones, facilitating the proper folding and assembly of nascent polypeptides, as well as assisting in the refolding and stabilization of damaged peptides. Whether induced by thermal or non-thermal stress, the stress response confers protection against subsequent and otherwise lethal hyperthermia, a phenomenon that is referred to as thermotolerance [5, 6]. Perhaps more interesting from a clinical standpoint is the phenomenon of cross-tolerance, whereby induction of the stress response confers protection against non-thermal cytotoxic stimuli.

Table 1. Inducers of the stress response

Type of stress	Agent	Comments
Environmental	Temperature	
	Heavy metals	Cadmium, zinc
	Ethanol	
	Oxygen radicals	
Metabolic	Hyperosmolality	
	Glucose starvation	
	Tunicamycin	
	Calcium ionophores	
	Amino acid analogs	
Clinical	Ischemia/Reperfusion	Reperfusion seems to be the limiting factor
	Shock	
	Anoxia	
	Endotoxin	
Pharmacologic	Sodium arsenite	Used extensively *in vitro* and *in vivo*
	Herbimycin A	Tyrosine kinase inhibitor
	Geldanamycin	Tyrosine kinase inhibitor and HSP90 inhibitor
	Prostaglandin A1	Other prostaglandins are also active
	Dexamethasone	
	Aspirin	Lowers temperature threshold for HSP induction
	Non-steroidal anti-inflammatory drugs	Lowers temperature threshold for HSP induction
	Pyyrolidine dithiocarbamate	Antioxidant; inhibitor of NF-κB
	Diethyldithiocarbamate	
	Bimoclomol	Hydroxylamine derivative, nontoxic
	Serine protease inhibitors	Concomitant inhibition of NF-κB
	Curcumin	Major constituent of tumeric; anti-inflammatory
	Geranylgeranylacetone	Antiulcerative agent

Stress Proteins and Innate Immunity

Until relatively recently, stress proteins were considered to be exclusively *intracellular* proteins, but a growing body of literature suggests that stress proteins may also exist and function outside of the cell. Several studies suggest that cellular stress results in the increased surface expression and release of stress proteins [4, 7, 8]. For example, the release of the 70 kDa stress protein, HSP70, into the extracellular environment was first reported in cultured rat embryo cells following exposure to increased temperature in the late 1980s [9]. The mechanism of release appeared to be specific, in that HSP70 release could not be reproduced by induction of cell lysis through exposure to non-ionic detergents. However, the mechanism did not appear to involve classic secretory pathways either, as it was not inhibited by either colchicine or monensin, both of which inhibit this pathway. Finally, HSP70 synthesized in the presence of a lysine amino acid analog (aminoethyl cysteine) was not released from these cells, suggesting that the altered protein structure prevented interaction with an as yet unidentified, but specific secretory mechanism [9].

Table 2.

Name	Size (kDa)	Localization	Bacterial homolog	Some known and possible functions
Ubiquitin	8	Cytosol/nucleus	–	Nonlysosomal degradation pathways
HSP27	27	Cytosol/nucleus	–	Regulator of actin cytoskeleton; molecular chaperone; cytoprotection
Heme oxygenase	32	Bound to ER, extends to cytoplasm	–	Degradation of heme to bilirubin; resistance to oxidant stress
HSP47	47	ER	–	Collagen chaperone
HSP60	60	Mitochondria	Gro EL	Molecular chaperone
HSP70	72	Cytosol/nucleus	Dna K	Highly stress inducible; involved in cytoprotection against diverse agents
	73	Cytosol/nucleus	–	Constitutively expressed chaperone
HSP90	90	Cytosol/nucleus	htpG	Regulation of steroid hormone activity
HSP110	110	Nucleolus/cytosol	Clp family	Protects nucleoli from stress

ER: endoplasmic reticulum

While some studies suggest that HSP70 is released from dying, necrotic cells [10, 11], we and others [9, 12–17] have shown that viable cells release HSP70 in a specific and inhibitable manner. For example, monensin and brefeldin A are inhibitors of the classic endoplasmic reticulum/Golgi protein transport and secretory pathways. We have shown that HSP70 release from viable THP-1 cells is not inhibited by either monensin or brefeldin A (Wheeler and Wong, unpublished data). Others have shown that HSP70 release from peripheral blood monocytes (PBMC) is inhibited by brefeldin A, but not monensin [15, 17]. In this study, HSP70 release was also inhibited by methylamine and methyl-β-cyclodextrin, both of which inhibit protein secretion via lysosomal pathways [17]. Notably, the HSP70 gene does not encode for the classic N-terminal peptide leader sequence targeting it for secretion via the endoplasmic reticulum/Golgi transport pathway [18], which is consistent with the data presented here, and recent studies suggest that HSP70 is actively released via an exosome-dependent, non-classical protein secretory pathway [19, 20]. Such a non-classical secretory mechanism may be a surprisingly common motif in the host innate immune response, as several additional examples of 'leaderless' secretory proteins exist, including interleukin (IL)-1α, IL-1β, IL-18, and high mobility group box protein (HMGB)-1 [18].

The release of stress proteins, such as HSP70, may serve to signal an impending danger to neighboring cells [21]. Several properties would suggest HSP70 to be a biologically plausible and likely candidate to serve as a host danger signal. Collectively stress proteins are the most abundant intracellular proteins, representing up to 10% of the total protein content in the cell [22]. HSP70 in particular is markedly induced in response to a diverse range of cellular insults, including increased temperature, oxidative stress, glucose deprivation, chemical exposure, ischemia-reperfusion injury, ultraviolet radiation, and infectious agents such as lipopolysaccharide (LPS). Therefore, HSP70 or additional inducible stress proteins, by virtue of their relative abundance during times of stress are reliable markers of cell stress, i.e., danger.

Stress proteins are ancient, highly conserved molecules that have been identified in virtually every organism, both prokaryotic and eukaryotic, that has been examined to date. In comparison, LPS, an important exogenous danger signal, appeared relatively late on the evolutionary time-scale and is much less ubiquitous, being unique to only Gram-negative bacteria. Similar to LPS, extracellular HSP70 elicits nuclear factor-kappa B (NF-κB)-dependent, pro-inflammatory gene expression via Toll-like receptor (TLR) 4 and TLR2 [23, 24]. Therefore, release of HSP70, whether from dying, necrotic cells or viable, but damaged cells, can activate the host innate immune response, acting as both a chaperone and a cytokine (chaperokine) [23–26]. It is perhaps no mere coincidence that HSP70-mediated and LPS-mediated activation of the host innate immune response occur via similar mechanisms (i.e., TLR-4-mediated signal transduction), and it is highly likely that the programmed response to the exogenous danger signal, LPS, is modeled on the more primitive programmed response to the endogenous danger *signal* [22]. While an attractive hypothesis, these studies linking extracellular stress proteins with inflammation should be interpreted cautiously. For example, some investigators have been unable to replicate these findings using endotoxin-free, recombinant stress proteins, suggesting that these effects were due to endotoxin or other bacterial contaminants in the recombinant protein [27]. Nevertheless, several independent lines of evidence support the concept that extracellular stress proteins play an integral role in the innate immune response [4, 8, 28].

Stress proteins, particularly HSP70, are highly immunogenic and have the capacity to mediate the induction of peptide-specific immunity. For example, as molecular chaperones, stress proteins bind to many peptides derived from the cells from which they are isolated. Stress protein-peptide complexes elicit potent T cell responses against the chaperoned peptide as well as the cell type from which the chaperoned peptide is derived, including tumors and viruses, and vaccination with stress protein-tumor peptide complexes as an immunotherapy for cancer is an active area of investigation [22, 28]. Similarly, stress protein-pathogen-derived peptide complexes have the capacity to elicit a pathogen-specific immune response [29]. Finally, as mentioned above, stress proteins themselves, especially members of the HSP60 and HSP70 families, have the capacity to activate the host innate immune response, resulting in dendritic cell activation and maturation, activation of complement, and release of pro-inflammatory cytokines [10, 23, 24, 26, 27, 30–33]. Collectively, these observations would suggest the stress proteins as likely candidates for endogenous danger signals – they are abundant, inducible, highly conserved, and appear to activate both the innate and adaptive immune responses!

Clinical Evidence of Extracellular Stress Proteins

Extracellular HSP70 (increased serum levels) is evident in a variety of clinical scenarios associated with physiologic stress. For example, increased extracellular HSP70 levels are noted following strenuous exercise [13, 14, 34–37] and following cardiopulmonary bypass in both adults [38, 39] and children (Wheeler, unpublished data). Increased extracellular HSP70 concentrations correlate with poor outcome in a variety of inflammatory disease processes, including liver disease [40], coronary artery disease [41–44], traumatic brain injury [45, 46], pre-eclampsia [47], sickle cell disease vaso-occlusive crisis [48], and septic shock [7]. Given the signaling properties recently ascribed to HSP70, these data generate a number of

functional questions. First and foremost, is extracellular HSP70 merely a marker of cellular stress, or could the release of HSP70 potentiate an already active host immune response, thereby leading to poor outcome? Second, could it be possible that extracellular HSP70 serves an as yet undefined cytoprotective function at lower levels as a normal response to infection or stress, and once a certain critical threshold is attained potentiate the dysregulated inflammatory response that subsequently results in significant auto-injury to the host? Recent experimental data [49] and the finding that extracellular HSP70 levels >15 ng/ml correlated with improved outcome following multiple trauma in adults [50] support this concept. These questions remain an active area of investigation in many laboratories, including our own.

Conclusions

The heat shock, or stress response is an ancient, highly conserved, primitive endogenous cellular defense mechanism. Traditionally, stress proteins, e.g. HSP70, have been considered to be exclusively intracellular proteins. However, increasing evidence supports a role for extracellular stress proteins, including HSP70, in the innate and acquired immune response. For example, stress proteins have been reported to stimulate the immune system via innate receptors, such as the TLR. Recent data, however, challenge this notion by claiming that it is the bacterial molecules that are trapped by the stress proteins, and not the stress proteins themselves that activate the immune system. In this brief review, we have presented evidence to suggest that stress proteins are indeed modulators of immune function. Whether activation of the immune response by extracellular stress proteins such as HSP70 serves a cytoprotective function, a pro-inflammatory function, or both, depending on context, remains to be determined.

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Quantitative *in vivo* Protein Synthesis as a Measure of Immune Function

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Introduction

Outcome in severe illness depends not only on adequate, goal-directed treatment, but also on the patient's response to the treatment. In particular, the state of the immune system is crucial in cases of severe infection. Immune suppression, regardless of the underlying mechanism, is a factor adding to a poor prognosis in patients with severe infections. Existing scoring systems, designed to reflect organ failure and to give prognosis prediction for the patient, do not include any score for the status of the immune system. The reason for that is the absence of such a measure similar to those existing for respiration, circulation, coagulation, as well as for liver, kidney and mental function.

In vivo determination of the rate of protein synthesis in immune competent cells makes it possible to measure and to quantify the ongoing metabolic activity of these cells. Such measurement may add information on the activation of various immune cells, allowing better estimates of immune competence. Application of *in vivo* protein synthesis measurements in cells of the immune system, in order to quantitatively characterize the state of their activation, is the main issue of a project within our working-group and here results are reported from a series of studies performed and published recently [1–5].

Background

Monitoring of the Immune System

Monitoring of vital organs is a necessary tool for adequate treatment of intensive care unit (ICU) patients, since insufficient function of these organs leads to multiple organ failure (MOF), with a high mortality rate despite all the advances in intensive therapy. While monitoring of organ function, such as lung, liver, kidney, etc., is well established, monitoring of immune system function is inadequate [6]. The balance of inflammatory responses can vary between pro- and anti-inflammatory phases leading to unexpected alterations in a patient's condition. In addition, despite failed clinical trials with anti-inflammatory interventions directed against tumor necrosis factor (TNF)-α and interleukin (IL)-1 [7], immunomodulatory therapies are still appealing, following a successful trial with pro-inflammatory mediators in immune-depressed sepsis patients [8]. However, the choice of such an immunomodulatory therapy has to be adjusted to the inflammatory phase, which necessitates thorough immunomonitoring.

In clinical practice, white blood cell (WBC) count and C-reactive protein (CRP) are routinely used as markers of inflammation. Both rise in response to infections, but also due to non-infectious events, such as surgery, trauma, bleeding, stroke and myocardial infarction. On the other hand, no response, or minor elevations in WBC or CRP, are observed during some viral or chronic infections. Both leukopenia and leukocytosis are components of the APACHE II scoring system and their presence in the acute phase of disease is associated with poor outcome in critically ill patients [9].

Parameters clinically widely used to describe the immunological status include absolute cell counts, as well as proportion and absolute counts of cell populations and subpopulations. A decrease in T-cell subsets reported in response to trauma, infections, sepsis or burns [10] may reflect the transient migration of circulating cells into the site of injury and not necessarily an impaired function of these cells. Furthermore, there is no correlation between the lymphocyte count and severity of illness or mortality rate. Lymphopenia and altered lymphocyte subset distribution is also observed in subjects not exposed to injury, such as patients with primary cancer or healthy volunteers in response to short-term hyperglycemia. In contrast, a rise in monocyte count is observed in patients exposed to trauma, surgery or sepsis [11].

Cell activation can also be reflected by the increase or by the *de novo* appearance of activation markers, which are surface molecules expressed only on activated or dividing cells. One of the best-characterized markers is human leukocyte antigen (HLA)-DR (MHC class II), which is upregulated on antigen presenting cells as well as expressed on a subpopulation of activated T cells. Persistent low HLA-DR expression on monocytes is proposed as a marker of compensatory anti-inflammatory response syndrome (CARS) and a predictor of poor outcome in patients with severe sepsis [6, 11]. However, some investigators have found contradictory results [12], questioning the value of HLA-DR as a single parameter to characterize the immunological status [13].

The pattern of secreted cytokines is another marker commonly used for the purpose of immunomonitoring. High concentrations of pro-inflammatory cytokines, such as TNF-*a*, IL-1, IL-6 as well as anti-inflammatory IL-10 are detected early following injury. However, interpretation of plasma cytokine levels can be difficult, because of their short half-life as well as the presence of soluble receptors or inhibitors. In addition, concentrations of cytokines may differ between the systemic circulation and the site of injury.

In vitro techniques have been widely used to measure responsiveness of the immune system. Skin tests or proliferative responses in unstimulated cells or cells stimulated with different mitogens are related to the impaired cell activity observed in patients following major surgery or in critically ill patients. However, large intraindividual variation in response to mitogenic stimulation and poor reproducibility are well-known drawbacks of these *in vitro* methods.

In vivo Protein Synthesis Determination and Immunomonitoring

An adequate function of the immune system requires rapid shifts from the 'standby' position into full activity. In health, most immune cells are quiescent. However, the constant exposure to foreign antigens implies the need for continuous scanning in order to detect harmful signals and generate immediate responses.

The defence mechanisms in the early phase of injury include activation of the innate immune system. The first phagocytic cells recruited into the site of injury are neutrophils, which produce and release reactive oxygen intermediates, a variety of proteolytic enzymes and immunoregulatory cytokines [14]. In parallel, monocytes, which upon migration into the tissue transform into macrophages, synthesize cytokines and other mediators of inflammation. As professional antigen presenting cells, they present foreign antigens on the MHC II molecules to T lymphocytes. In addition, due to the ability to produce cytotoxic agents, they are involved in phagocytosis. Adaptive immunity, which is more specific, requires some days to become effective. Upon activation by antigen presenting cells, T lymphocytes proliferate and differentiate into effector cells. Depending on the nature of the triggering signal, differentiation results in cell-mediated immunity and/or in antibody production by activated B lymphocytes.

All immunologic events, such as synthesis of cytokines or other regulatory mediators, production of enzymes, receptors and immunoglobulins, cell differentiation and proliferation are protein demanding. In metabolic terms, this means varying activity in synthesis of both structural and export proteins and can be quantitatively determined by measuring the *in vivo* rate of protein synthesis.

The *in vivo* fractional protein synthesis rate was quantitatively determined for the first time in human mononuclear cells of patients with metastasic colorectal cancer [15]. The rate of protein synthesis is lower in these patients as compared with healthy control subjects. Following a 5-day treatment of the patients with recombinant IL-2, a three-fold increase in the *in vivo* fractional protein synthesis rate is observed. Also an increased *in vivo* fractional synthesis rate in the mononuclear cells is demonstrated 24 h after cholecystectomy in otherwise healthy patients [16]. In contrast, in mononuclear cells of healthy volunteers exposed to a 6 h combined stress hormone infusion (epinephrine, cortisol and glucagon), as a model for surgical stress, a decrease is observed immediately after the end of the infusion, followed by normalization at 18 h after cessation of the infusion [17].

The possibility to perform *in vivo* measurement of protein synthesis in cells of the immune system is appealing. It provides the opportunity to assess *in vivo* metabolic activity, which may reflect immune activity and competence. In these first studies, changes in the metabolic activity of circulating mononuclear cells following immunostimulation and surgical stress were observed. This raised further questions about the activity of individual cell populations and the effects of other types of injury.

Methods

Protocols

In three studies of healthy volunteers, the effects of a combined stress hormone infusion (n = 24) (epinephrine + cortisol + glucagon), a cortisol infusion (n = 18), and an endotoxin challenge (n = 18) on *in vivo* protein synthesis in circulating immune cells was elucidated [1–3]. The protocols were chosen to represent the acute effects of trauma and sepsis in a standardized way. A parallel aim of these studies was to validate and develop the techniques used to quantify the *in vivo* protein synthesis in circulating immune cells. In the patient studies we introduced tonsil biopsies to have a measurement also in stationary immune cells. The methodology for tonsils

was developed and validated in a group of ear, nose and throat (ENT) patients (n = 11) undergoing elective surgery [4]. Finally a group of ICU patients (n = 20) on ventilators in the pro-inflammatory phase with established or a high risk of developing MOF was studied in a pilot experiment [5]. The purpose was to establish how quantitative measurements of *in vivo* protein synthesis in immune cells appear in patients with generalized inflammation and severe infections.

The *in vivo* Fractional Protein Synthesis Rate

In vivo protein synthesis rates in human tissues can be quantified by incorporation of labeled amino acids into proteins. The method is based on the assumption that free amino acids enter the intracellular amino acid pool, which is the precursor reservoir for protein synthesis. In addition, the direction of changes depends on fasting or feeding conditions [18].

Administration of labeled amino acids makes them available as precursors for protein synthesis. Measuring the fraction of labeled amino acids incorporated into the protein of interest over time in relation to the fraction of labeled amino acids in the precursor pool enables calculation of the fractional synthesis rate. Constant infusion and the flooding technique are the two available approaches to administer labeled amino acids for incorporation into proteins.

The Constant Infusion Method. The constant infusion technique has been extensively used for whole body protein turnover and for tissue protein synthesis measurements in animal and human studies. With this method, the labeled amino acid is given as a continuous intravenous infusion until a steady state is obtained in the precursor pool. In order to reduce the time to achieve an isotopic steady state, the constant infusion is often preceded by an intravenous priming dose of the tracer.

One of the main disadvantages of the constant infusion method is the difficulty to control the isotopic enrichment of the amino acids in the true precursor pool for protein synthesis. The optimal approach is to measure the isotopic enrichment in the intracellular aminoacyl-tRNA. The very low concentration of tRNA and the high rate of turnover, make this measurement technically difficult [19]. Instead other precursor pools, such as the enrichment in plasma or enrichment of transamination products of the labeled amino acids, are used as surrogate measures of the true precursor pool. However, it has been shown that enrichments of leucine and α-ketoisocaproic acid (KIC, the product of deamination of leucine) in plasma are higher than those of aminoacyl-tRNA and tissue free leucine in skeletal muscle [18]. In consequence, using plasma leucine or KIC enrichments for protein synthesis calculations falsely underestimates the protein synthesis rates. The tissue intracellular free amino acid pool enrichment is very similar to aminoacyl-tRNA enrichment and technically reasonably easy to measure. Therefore, the tissue free amino acid pool is the best substitute for the aminoacyl-tRNA when applying the constant infusion technique. A particular difficulty is that the relation between plasma and tissue enrichments is variable, due to physiological fluctuations or interventions, and not predictable.

Another drawback of the constant infusion method is that a relatively long study period is needed to allow an isotopic steady state to be reached. As the method requires not only isotopic, but also metabolic steady state during the whole study, the prolonged study time may be a limitation in studies involving critically ill patients

with unstable conditions or during surgical procedures. The prolonged study time can potentially also give a problem with recycling of the tracer. During the incorporation period, protein degradation releasing the tracer again occurs, leading to the reappearance of the labeled tracer amino acid in the precursor pool. In addition, in tissues with high protein turnover, protein synthesis rates may be underestimated because of the escape of export proteins during the labeling period.

The Flooding Method. To overcome the problems with the precursor pool enrichment and long study times, the flooding technique has been developed. In this approach, a large dose of both unlabeled and labeled amino acid is administered intravenously as a bolus over a short period of time [20]. Due to the overabundance of the given amino acid, all existing free amino acid pools are reached and equilibrated rapidly, including the true precursor pool for protein synthesis, aminoacyl-tRNA, and an isotopic equilibration is established. Indeed, the assumption on equilibration between plasma, the tissue free amino acid pool and aminoacyl-tRNA has been confirmed, which allows the plasma to be used as a valid substitute for the true precursor pool [21, 22]. Thus, using the flooding method, the problem with measuring the true precursor pool for protein synthesis is avoided. Another advantage of the flooding approach is a relatively short study time needed, making determination of protein synthesis possible within 30–90 min. Consequently, the method is more suitable for studies in unstable conditions, as well as in tissues with a high secretory activity. The short study period also minimizes the problem of recycling of the labeled amino acids.

The drawback of the flooding method is that the large dose of the amino acid gives an elevated concentration of the amino acid in plasma and tissues. The main remaining problem with the flooding technique is that the labeled and incorporated amino acids do not fulfil the criteria for being a true tracer. This may interfere with protein synthesis rates. It has been pointed out that fractional synthesis rates measured with the flooding technique were higher when compared with the constant infusion technique [23]. In particular a flood of leucine has been suggested to stimulate the protein synthesis rate in human skeletal muscle [24].

However, the discrepancies were mainly attributable to poor control of the precursor pool in the early studies employing the constant infusion technique. A comparison of the fractional synthesis rate in human skeletal muscle measured by flooding with leucine or phenylalanine, shows similar rates of protein synthesis [25]. Furthermore, comparison between the two techniques, when the precursor pool has been adequately equilibrated during a constant infusion, shows identical results [26].

Determination of Fractional Synthesis Rate by the Flooding Technique. In the studies reported here, the flooding technique was applied to determine *in vivo* fractional protein synthesis rates in immune competent cells. The main reason for choosing this approach, instead of the constant infusion, is a short study time. Cells of the immune system are capable of rapid changes in their immunological activity. By measuring protein synthesis with the flooding method and avoiding the problems with recycling of amino acids and disappearance of secreted proteins, we are more likely to capture the corresponding changes in metabolic activity of immune cells. Furthermore, labeled amino acids in plasma can be used as a reliable estimate of the true precursor pool. With the constant infusion technique, the intracellular amino acid pool would be the most correct surrogate of the aminoacyl-tRNA. How-

Fig. 1. Schematic study protocol for the flooding technique for the determination of the fractional protein synthesis rate in immune competent cells

ever, to measure the intracellular amino acid pool in immune cells is technically difficult due to the limitations in both the volume of blood samples and size of tonsil biopsies.

In vivo protein synthesis of human lymphoid tissue represented by the palatine tonsil was actually determined for the first time. Preparation of the tonsillar specimens for mass spectrometry analysis for protein synthesis determination was similar to that of muscle tissue, including freeze-drying enabling removal of connective tissue and blood [27]. The general protocol for sampling of immune cells and blood for the purpose of determination of in *vivo protein* synthesis rates is presented in Figure 1.

Cell Separation

Available methods for T lymphocyte isolation were tested and validated. The aim was to find a method that would give a high purity of T cells, without plasma protein contamination and without interference with the mass-spectrometry analysis. The magnetic cell separation methods: MACS Microbeads and Dynal® were evaluated. Unfortunately, in both cases, difficulties in removing the beads coated with antibodies resulted in the presence of magnetic particles in the samples, which disturb mass spectrometry. In addition, the presence of antibody proteins interfered with protein synthesis calculations. Besides, the cells were isolated from a relatively large blood volume, which made both methods time-consuming and unpractical. For the same reasons, the separation technique with the fluorescence-activated cell sorter was not chosen.

Isolation of T lymphocytes by rosetting with sheep erythrocytes is an old and well-established technique [28]. The method is based on the presence of receptors for sheep erythrocytes on the surface of human T lymphocytes. Lymphocytes become surrounded (rosetted) with the red cells and can then be isolated by density gradient centrifugation. In our hands, the purity of the T lymphocyte population separated with the rosette method is 90–95% as verified by flow cytometry. Mononuclear cells (MNC) were obtained by density gradient centrifugation, whereas leukocytes were isolated by lysing erythrocytes from the whole blood samples.

Tonsil Biopsies

Tonsil biopsy is a well-documented technique, which relatively easily enables access to lymphoid tissue even on an outpatient basis [29]. This technique was applied in order to compare the *in vivo* fractional synthesis rates in circulating cells of the peripheral blood with those of stationary cells of the lymphoid tissue. The biopsies were taken with a punch forceps and no complications due to bleeding were ob-

served. The procedure of tonsil biopsy in ICU patients turned out to be technically more difficult compared to that in healthy subjects. One of the explanations was the fact that ICU patients, although receiving sedatives and analgesics, were not muscle relaxed. Besides, one of the features of the early phase of critical illness is general edema, due to capillary leakage as well as fluid supply, which is a part of the intensive treatment. Owing to all these factors, visualization of the palatine tonsils was much more difficult in the ICU patients.

Phenotypic Characterization of Cells

In order to characterize the immunological status of the studied subjects, flow cytometric analysis was performed. Cell surface expression for various characteristic receptors exclusively expressed on the different immune competent cell types in peripheral blood and in palatine tonsils was determined. Expression of markers associated with differentiation or activation stage on these cells was also studied. To find out if there was a relationship between the phenotypic and metabolic manifestations of activation, the expressions of activity markers were related to the *in vivo* fractional protein synthesis rates.

Plasma Cytokines

Plasma cytokine concentrations were determined as part of the characterization of the immunological status in the investigated subjects. In the healthy volunteers subjected to endotoxin, the sandwich enzyme-linked immunosorbent assay (ELISA) was used. In the ICU patients, plasma cytokines were analyzed using the multiplex bead array assay. This relatively new method permits simultaneous flow cytometric quantitation of multiple cytokines by capturing them onto beads labeled with fluorophores and coated with antibodies, specific for the cytokines of interest [30].

Results

The *in vivo* Fractional Synthesis Rate in Circulating Cells

Determination of protein synthesis was performed in circulating peripheral blood cells, purified T lymphocytes, total mononuclear cells, and the whole population of leukocytes. The use of peripheral blood cells in studies on function and activity of the immune system has been criticized. Indeed, circulating lymphocytes represent only about 2% of the total lymphocyte pool in the normal, human body. However, in human studies the possibility to sample material from the lymphoid organs is rather limited. Thus, despite the fact that circulating cells may not reflect alterations in the whole lymphoid tissue, blood samples still remain the main source of information on the function of the human immune system.

Fractional Synthesis Rate in T Lymphocytes. The results of the *in vivo* protein synthesis determination in T lymphocytes showed that the metabolic activity of this cell population was similar in healthy volunteers and in patients. In contrast, distinct decreases in the rate of *in vivo* protein synthesis rate were observed in the subjects who received a combined stress hormone infusion and in subjects exposed to an endotoxin injection (Fig. 2).

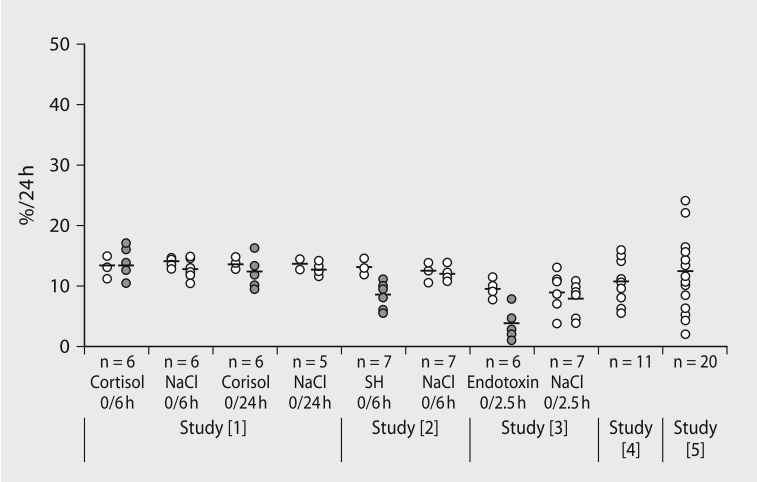

Fig. 2. The fractional protein synthesis rate in the isolated population of T lymphocytes determined in healthy volunteers [1–3], non-infected ear, nose and throat patients [4] and ICU patients [5]. Circles represent individual values, horizontal lines represent means. Open circles represent basal values, filled circles represent values following an intervention (cortisol or stress hormone (SH) infusion or endotoxin injection)

The mean value of the *in vivo* fractional synthesis rate in circulating T lymphocytes was approximately 12%/24 h. This is a relatively high rate, compared with that of human skeletal muscle having a fractional rate of protein synthesis of only 2%/24 h [31] and the human liver, producing both stationary and export proteins, which has a fractional synthesis rate of approximately 24%/24 h [32]. An *in vivo* fractional synthesis rate comparable with our results was reported in a human study where lymphocytes were separated from monocytes using iron particles [33]. T lymphocytes play an important role in maintaining the homeostasis of the immune system. Memory T cells continuously migrate via blood to lymphoid and non-lymphoid organs scanning for foreign antigens and alerted to immediate responses in case of recognition of a non-self antigen [34]. A number of mediators and chemokines are necessary to enable the different steps of this T lymphocytes traffic, such as adhesion to endothelium or transmigration into the tissues [35]. Production of these factors may be a considerable contribution to the protein synthesis rate observed in T lymphocytes under physiological conditions.

The scatter in the fractional synthesis rates in T lymphocytes was relatively low in healthy subjects, but was higher in the investigated patients. This was not surprising in the ICU group, being heterogeneous in terms of diagnosis, age, APACHE II, type of infections, etc. A possible explanation for the greater scatter in patients with minor ENT pathology might be that the age distribution was wider and that the median age was higher compared to the healthy volunteers. Known age-associated alterations include changes in immune cell composition, accompanied by varying, both diminished and enhanced, functional activity of the immune system [36]. Thus we cannot exclude that ageing is associated with alterations in the rate of *in vivo* protein synthesis in circulating T lymphocytes. Another explanation could be the difference in gender, as both male and female patients were included. Gender differences in the innate

and adaptive immune system have been reported in humans [37]. However, the *in vivo* protein synthesis rates in the T lymphocytes were not different between the men and women participating in the study.

Fractional Synthesis Rates in Mononuclear Cells. The *in vivo* fractional rate of protein synthesis in the total population of mononuclear cells was determined in a total of 45 subjects (Fig. 3). The results showed that the fractional synthesis rate was similar in volunteers and in patients undergoing elective ENT surgery. In ICU patients, the rates of protein synthesis were consistently higher, with the lowest value corresponding to the mean value in the healthy subjects. A 6-h stress hormone infusion resulted in a decrease of the mononuclear cell *in vivo* protein synthesis rate.

The population of total mononuclear cells was isolated by gradient centrifugation and in healthy subjects it consisted of approximately 60–70% T lymphocytes, 10% B lymphocytes and 20–30% monocytes [38]. The protein synthesis rate was comparable to that in the circulating T lymphocytes, suggesting that protein synthesis in both monocytes and B lymphocytes was of the same magnitude as in the T cells. There are no data available on the *in vivo* protein synthesis rate in B cells, but considering the different functions of T and B lymphocytes, it cannot be excluded that the metabolic activity of B lymphocytes in unstimulated healthy subjects is lower compared with the T cells. In contrast, monocytes, as antigen presenting cells, play an important immunoregulatory role together with T lymphocytes, which may explain a relatively high protein synthesis rate in this cell population in basic physiological conditions. The cell distribution in the ICU patients following the density gradient separation might have been different, depending on the altered composition of WBCs. The flow cytometric analysis showed a high proportion of cells expressing surface markers for monocytes and concurrently a low proportion of cells expressing surface markers for T lymphocytes. It is well known that monocytes are activated during an early phase of injury, releasing large amounts of cytokines and other proinflammatory mediators. Thus the high rate of the *in vivo* frac-

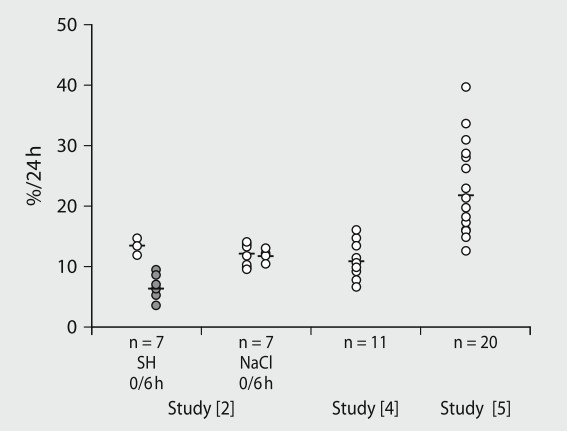

Fig. 3. The fractional protein synthesis rate in the total population of mononuclear cells determined in healthy volunteers [2], non-infected ear, nose and throat patients [4] and ICU patients [5]. Circles represent individual values, horizontal lines represent means. Open circles represent basal values, filled circles represent values following an intervention (SH, stress hormone infusion)

tional protein synthesis observed in the mononuclear cells of the ICU patients may reflect an enhanced protein synthesis rate in monocytes.

The *in vivo* rate of protein synthesis in the total mononuclear cell population has been determined previously. In surgical patients with metastatic cancer a 5-day immunostimulatory treatment with IL-2 results in an increase in the fractional synthesis rate of mononuclear cells [15]. Also, surgical trauma *per se* leads to an enhanced *in vivo* rate of protein synthesis [16]. On the other hand, a biphasic metabolic response of mononuclear cells is observed following a 6-h combined stress hormone infusion in healthy volunteers [17]. The immediate decrease of the *in vivo* fractional protein synthesis rate in the mononuclear cells at the end of the infusion is followed by return to pre-infusion levels 18 h later. The protein synthesis rates determined in the mononuclear cells in that study were lower compared with those in our study of healthy subjects. The fact that the procedure of preparing samples for the gradient centrifugation was slightly different and that samples were analyzed at another laboratory, may explain the differences between the fractional synthesis rate values. However, the magnitude of change in response to the stress hormone challenge was the same in both studies. The *in vivo* fractional protein synthesis rate has also been studied before in the total mononuclear cells of 15 ICU patients with both surgical and medical diagnoses on days 2–30 after ICU admission [39]. In that study also the results were approximately 50% lower compared with those in our present study.

The *in vivo* fractional protein synthesis rate in the total mononuclear cells of healthy volunteers has also been determined with the constant infusion technique [40], showing lower values compared with our results in study II determined with the flooding approach [2]. The discrepancy may partly depend on the problem with recycling of labeled amino acids, and partly on secretion of export proteins during the 4 h incorporation period when applying the constant infusion technique.

Fractional Synthesis Rate in Leukocytes. A striking feature of the *in vivo* fractional protein synthesis rates in the whole population of human blood leukocytes was the agreement between the volunteers and the healthy ENT patients (Fig. 4). The mean values were similar and the scatter was low. Following endotoxin administration an increase in the rate of protein synthesis was observed. The whole population of leukocytes in the ICU patients showed high protein synthesis rates, with the lowest values corresponding to the mean values measured in the healthy subjects. This was similar to the results for the mononuclear cells.

In normal, healthy subjects, whole blood leukocytes consist of 50–70% of neutrophils. In the acute phase of injury, the proportion of neutrophils in ICU patients was higher, up to 90%, as calculated from the WBC and differential counts in these patients. Neutrophils are unable to proliferate and are inactive in basic, physiological conditions [41], which fits well with their low *in vivo* metabolic activity. However, in the acute phase of injury, the neutrophils provide the first line of defense by phagocytosing the invading microorganisms. This is accomplished by the production and secretion of proteolytic enzymes, oxygen radicals, and regulatory cytokines, which may explain the high rate of protein synthesis observed in the whole population of blood leukocytes in the early phase of illness in ICU patients.

The results of the fractional synthesis rates in healthy subjects in our studies were comparable to those determined in the neutrophils of healthy volunteers when using the constant infusion technique and the intracellular free amino acids in neutrophils as the precursor pool [40].

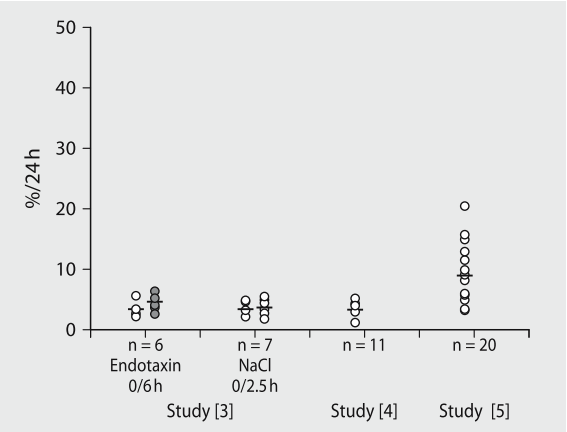

Fig. 4. The fractional protein synthesis rate in the whole population of leukocytes determined in healthy volunteers [3], non-infected ear, nose and throat patients [4] and ICU patients [5]. Circles represent individual values, horizontal lines represent means. Open circles represent basal values, filled circles represent values following an intervention (endotoxin injection)

The *in vivo* Fractional Synthesis Rate in the Palatine Tonsils

In order to compare the metabolic activity of circulating blood cells with the activity of cells in lymphoid organs, for the first time the *in vivo* protein synthesis rate was determined in the palatine tonsils of patients undergoing elective ENT surgery and of ICU patients. The palatine tonsils constitute the pharyngeal part of the mucosa-associated lymphoid tissue. Due to their location and specific functions, the palatine tonsils might not be representative for the responses during the acute phase of injury taking place in the other components of the lymphoid system, such as lymph nodes or spleen. However, they are relatively easily accessible and may add information about the functional activity of the human immune system.

The *in vivo* protein synthesis rates in the unfractioned cells of the palatine tonsils were similar in both groups of investigated subjects, and they were consistently higher compared with circulating blood cells (Fig. 5). Due to the location, healthy palatine tonsils are continuously exposed to antigens and stimulated even in a basic, physiological state, which often is considered as a permanent activation [42]. Thus, the high *in vivo* metabolic activity, corresponding to that seen in the human liver [32] is not surprising. The scatter in the ICU patients was larger, as expected. Interestingly 9 out of the 19 ICU patients studied showed a high *in vivo* protein synthesis rate, outside the range of the healthy subjects.

Discussion

The *in vivo* fractional protein synthesis rate in immune competent cells was determined as a measure of their functional activity. Furthermore, the possible application of the protein synthesis measurement as an instrument for immunomonitoring was addressed.

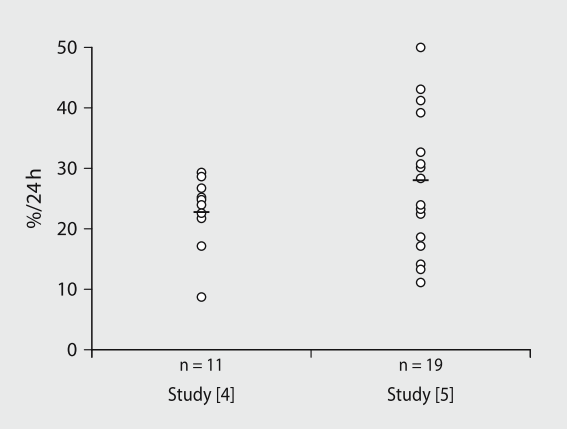

Fig. 5. The fractional protein synthesis rate in the unfractionated cells of the palatine tonsil determined in non-infected ear, nose and throat patients [4] and ICU patients [5]. Circles represent individual values, horizontal lines represent means

The importance of thorough monitoring of the immunological status, as an integral part of the surveillance in the ICU patients, has been highlighted recently [6, 7]. However, the parameters recommended as markers of different phases of the inflammatory responses have their limitations. The measurement of the *in vivo* metabolic activity in cells of the immune system may add information on their functional status.

Although components of the immune system are in a stand-by position in basic, physiological conditions, they have a varying level of basal activity. Continuous confrontation with foreign antigens implies a constant process of scanning to distinguish harmless signals from those which are dangerous and have to be disarmed. The recognition of pathogens induces rapid shifts in the activity of immune competent cells including the immediate production of multiple mediators, proteolytic substances, cell receptors, as well as cell proliferation. The metabolic reflection of these changes in immunological activity is an alteration in the ongoing protein turnover, which may be quantified by determination of the *in vivo* protein synthesis rate in immune competent cells. Quantifying the fraction of *de novo* synthesized proteins in basic, physiological conditions estimates the level of metabolic turnover, corresponding to maintenance of the basic, immunological activity. Following injury, alterations in the *in vivo* fractional protein synthesis rates manifest enhanced or diminished immunological activity.

To elucidate specific effects of different types of injury on the activity of immune competent cells, the *in vivo* fractional protein synthesis rate was studied in human models of surgical trauma and sepsis. Following the combined stress hormone infusion a decrease in the protein synthesis rate in the total population of mononuclear cells in healthy volunteers was observed, which reproduced the results reported previously [17]. In addition, the isolated population of circulating T cells showed the same type of alteration with a diminished *in vivo* metabolic activity. Immunomodulatory, mainly immunosuppressive effects of the neuroendocrine system on the immune system are well known. These effects include modulation of cytokine

expression, suppression of immune cell maturation, differentiation and proliferation, reduction of cell trafficking and diminished expression of adhesion molecules, which is in accord with a decreased *in vivo* metabolic activity. Intravenously administered endotoxin, as a well-established human model for the early course of sepsis, affected the metabolic responses of immune competent cells in several ways. Circulating T lymphocytes responded with an immediate decrease of the *in vivo* protein synthesis rate, suggesting suppression of their function. This is in line with a suppressed *in vitro* proliferative responsiveness of T lymphocytes to mitogen stimulation in healthy volunteers following *in vivo* endotoxin administration [43]. On the other hand, the whole population of leukocytes showed an enhanced *in vivo* metabolic activity, which fits well with an increased expression of leukocyte activation markers reported previously [44]. Taken together the results from our studies in human models of trauma and sepsis made it obvious that the metabolic response of immune cells to different types of injury is not uniform, but varies between individual populations of circulating peripheral blood cells.

The key question is whether the alterations in the *in vivo* protein synthesis rates of immune competent cells reflect the state of activation of the immune system in severely ill patients. In our pilot study, a group of 20 ICU patients during the initial phase of multiple organ failure (MOF) was characterized by means of clinical and immunological parameters, completed with the metabolic measurements of immune competent cells. Although heterogeneous regarding their diagnoses, the ICU patients presented a uniform, general activation of immune responses. This activation was reflected by a decrease in the number of circulating T lymphocytes and an increase in monocyte count, an enhanced activity of adhesion molecules as well as elevated levels of selected pro- and anti-inflammatory cytokines. With regard to metabolic activity, a distinct polarization of responses was observed. The *in vivo* fractional protein synthesis rates in the total circulating mononuclear cells and in the whole population of leukocytes were high, whereas the protein synthesis rates in the circulating T lymphocytes and in the tonsillar cells were not different from those observed in healthy subjects.

The metabolic activation of leukocytes in the ICU patients is in agreement with an increased *in vivo* protein synthesis rate seen in leukocytes of healthy volunteers exposed to an endotoxin injection, as a human model of sepsis. However, this enhanced metabolic reaction probably reflects an early inflammatory, non-specific immune response, as not all ICU patients had a sepsis diagnosis. Furthermore, the total mononuclear cells of the ICU patients had a high metabolic rate, which is in contrast to the results observed in the human model of surgical trauma, showing a drop in the protein synthesis rate [17]. This discrepancy may be due to the different time points of protein synthesis determination, immediately versus some days after the onset of the injury. An increase in the *in vivo* fractional protein synthesis rate in the total population of mononuclear cells 24 h after uncomplicated elective surgery [31] supports the biphasic time course of metabolic responses. The isolated population of circulating T lymphocytes in the ICU patients had an *in vivo* protein synthesis rate comparable to that observed in healthy subjects, suggesting that the elevated metabolic activity in the leukocytes and mononuclear cells was represented by cells other than T lymphocytes. It can be speculated as to whether T lymphocytes had maintained their basal metabolic activity or whether they had successively increased their activity following an initial suppression. The distinct decrease in the fractional synthesis rate in T cells in the human sepsis model supports the later explanation, indicating a dynamic time-course of the immune responses to injury.

To address the question whether determination of metabolic activity in immune cells may be useful for evaluation of the immune status of the ICU patients, the possible relationships between the *in vivo* protein synthesis rates and relevant clinical parameters were tested *post hoc*. We found a negative correlation between the *in vivo* protein synthesis rate of T lymphocytes and the plasma CRP concentration both on the first day of ICU admission ($p = 0.009$) and on the study day ($p = 0.01$), suggesting low metabolic activity of T cells in cases of pronounced inflammation. Although not statistically significant ($p = 0.056$), the negative correlation between the protein synthesis rate in T lymphocytes and ICU survival raises the question whether suppression of T cell activity is associated with poor outcome, which would be in accord with the report on T cell anergy being correlated to mortality in abdominal sepsis [45]. We also found negative correlations between the protein synthesis rate in leukocytes and the platelet count ($p=0.002$) as well as the plasma albumin concentration ($p = 0.03$). On the other hand the fractional synthesis rate of leukocytes correlated positively with the CRP level and with the sequential organ failure assessment (SOFA) score on the study day ($p = 0.01$), which together suggest a relationship between the metabolic activity of leukocytes and the severity of disease. There were also strong statistical correlations between the metabolic activity of leukocytes and IL-6 ($p = 0.000006$), IL-8 ($p = 0.000002$) and IL-10 ($p = 0.00008$) plasma concentrations, indicating that a high rate of protein synthesis in leukocytes was seen in patients with more pronounced inflammatory responses.

Conclusion

The presence of correlations between the *in vivo* rates of protein synthesis and relevant clinical parameters suggests that determination of the ongoing metabolic activity in immune competent cells reflects changes in the functional activity of the immune system, being of importance for the severity and time course of the critical illness. Our results encourage future studies, in order to characterize the alterations in the *in vivo* metabolic activity in immune competent cells in later phases of the ICU stay, characterized by a general anti-inflammatory activity and decreased resistance to opportunistic infections.

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Fluid Resuscitation and Immunomodulation in the Critically Ill

M. Raghavan, H. Murray, and J. A. Kellum

Introduction

Sepsis, a systemic inflammatory response to infection, is currently the leading cause of death among critically ill patients. In the USA alone, approximately 750,000 cases of sepsis occur each year, at least 225,000 of which are fatal [1]. The incidence of sepsis has increased dramatically in recent decades, largely due to the advancing age of the population, an increased number of invasive procedures being performed and immunosuppressive therapy. This rising sepsis mortality is similar to that observed in Europe and the rest of the world. Despite the use of antimicrobial agents and advanced life-support care, the case fatality rate for patients with sepsis has remained between 30 and 40% for the past three decades. Several billions of dollars have been spent in efforts to improve the survival of patients with sepsis. However, results have been disappointing and many observers argue that our understanding of the underlying pathophysiology of this syndrome is grossly inadequate.

Severe sepsis triggers an inflammatory response that involves a complex network of cytokines, coagulation factors and other mediators. This response not only destroys the infecting organisms but also damages the host and leaves the immune system distorted. Non-survivors of sepsis exhibit a persistent elevation of pro-inflammatory mediators. Attempts at down-regulating the inflammatory response with novel agents directed at specific pro-inflammatory mediators have uniformly met with failure [2]. Recent provocative data, however, suggest that strategies preventing excessive inflammatory response such as low tidal volume ventilation, tight glycemic control, restrictive blood transfusions, and low dose corticosteroids improve outcome in critically ill patients.

Inflammation and Mortality

During critical illness, small changes in the inflammatory response have important clinical effects in patients. Development of organ failure and mortality has been shown to correlate more with the duration of inflammation rather than the peak concentrations of mediators in patients with sepsis [3]. On the contrary, a rapid downregulation and clearance of inflammatory mediators is associated with increased survival. Therefore, interventions that reduce inflammatory response have become avenues of intense research. While resuscitation fluids have been tested extensively for their ability to restore circulating intravascular volume, the effect of resuscitation fluids on the immunologic response has not been examined as extensively. Evidence from cell biology and animal physiology suggests that fluid resusci-

tation has the potential to alter the inflammatory response and may have significant implications during critical illness [4, 5].

While fluid resuscitation is both an integral part of therapy for sepsis and other forms of shock, it is also a potential source of inflammation. Moreover, it is unknown as to what type of fluid resuscitation is best, both in terms of effectiveness as well as safety in critically ill patients. There has been increasing evidence that choice of resuscitation strategy is an important variable, and different fluids can have widely divergent impact on the immune response, neutrophil activation, and tissue injury. Emerging data suggest that a variety of resuscitation fluids upregulate cytokine expression, neutrophil activation and adhesion, thus contributing to or prolonging the pro-inflammatory state. We have previously demonstrated that inflammation induced by fluid resuscitation is associated with significant adverse physiologic effects and decreased survival in animals [4, 6].

Fluid Resuscitation and Cytokine Expression

Critically ill and injured patients frequently require large volume resuscitation. Recent studies suggest that there is no mortality difference in patients receiving small volume resuscitation with 0.9% saline versus albumin in saline [7]. Thus, for typical low-volume resuscitation, crystalloid resuscitation is likely to remain the standard of care. However, numerous studies have suggested that large-volume (e.g., > 100 ml/kg) resuscitation with crystalloids may actually exacerbate the pro-inflammatory cytokine response during shock states [4, 6]. During hemorrhagic shock, circulating levels of cytokines such as interleukin-1 (IL-1α and β), and tumor necrosis factor (TNF) have been shown to remain persistently elevated after volume resuscitation despite significant hemodilution [8]. A rapid and significant increase in plasma IL-6 and IL-8 levels within 12 hours of resuscitation has also been observed following trauma. Hemorrhage-induced elevations in TNF and IL-6 mRNA levels have been shown to increase further after saline resuscitation. Gushchin and colleagues evaluated the differential cytokine expression by isotonic and hypertonic fluids by analyzing the cytokine gene profile of human leukocytes. Increased gene transcription of pro-inflammatory cytokines (IL-1α, IL-6, IL-10, and TNF) as well as others (IL-5, IL-7, and IL-16) was found after incubation with these fluids [9].

'Ab'normal Saline and Iatrogenic Acidosis

In North America, 0.9% sodium chloride (normal saline) is currently the most commonly used resuscitation fluid. However, large volumes of saline infusion cause metabolic acidosis by increasing the plasma chloride concentration relative to the plasma sodium concentration [10, 11]. The result is a reduction in the strong ion difference (SID), the difference between positive and negative charged electrolytes, which in turn produces an increase in free hydrogen ions [12]. The resulting effect is a metabolic acidosis often named 'dilutional acidosis'. While transient acidosis in an otherwise healthy subject as a result of exercise is well tolerated without any significant long-term sequelae, metabolic acidosis in critically ill patients is a powerful marker of poor prognosis; although causality has not been established. This acidifying effect could represent a drawback for extended use of 0.9% saline in the critically ill in whom a pre-existing metabolic acidosis is frequently associated.

Metabolic Acidosis and Inflammatory Response

A potentially important consequence of acidosis is its effect on the immune response. Several studies have documented the effects of decreased extracellular pH on the synthesis and release of inflammatory mediators. Different degrees of metabolic acidosis have been shown to cause induction of inflammatory markers such as nitric oxide (NO), IL-6, and IL-10 [5]. Hyperchloremic acidosis has been shown to induce TNF synthesis and release, as well as to increase nuclear factor kappa-B (NF-κB) DNA binding, suggesting that the overall effects of hyperchloremia appear to be pro-inflammatory [5, 13] (Fig. 1). Numerous studies using hydrochloric acid (HCl) have consistently shown pro-inflammatory effects at the level of NF-κB DNA binding or TNF synthesis provided pH was not less than 6.0, although TNF secretion was reduced even at pH as high as 7.0. In addition acidosis induces an inflammatory response through its effect on catecholamine synthesis [14]. Critically ill and injured patients may be adversely affected by even short-term alterations in the immune response (augmentation and attenuation). Since some forms of metabolic acidosis are largely iatrogenic in origin [10, 15], the influence of iatrogenic acidosis on immune response and outcome in critically ill patients needs to be evaluated in larger clinical trials.

Impact of Resuscitation Fluids on Clinical Outcomes

Immune changes and acid-base changes associated with fluid resuscitation are associated with significant adverse effects many of which could be important clinically. Metabolic acidosis has been associated with decreased myocardial contractility and hemodynamic instability. Pedoto and colleagues have shown that metabolic acidosis may increase inducible NO synthase (iNOS), and this may lead to vasodilation and shock [16]. We have previously demonstrated that hyperchloremic metabolic acidosis, induced by hydrochloric acid infusion, significantly reduced systemic mean arterial pressure in normotensive, septic animals [6]. In a study using an endotoxic shock model in rats, we demonstrated that saline resuscitation was associated with a significantly shorter survival time compared to a more physiologic fluid containing starch in a balanced electrolyte solution (Hextend) [4]. Furthermore, survival time was correlated with the decrease in pH and negatively correlated with the increase in serum chloride following initial resuscitation.

Wilkes and coworkers compared starch preparations in saline solution versus balanced electrolyte solution for elderly gastrointestinal surgery patients and described worse acid-base balance and more adverse events with the saline-based fluid [17]. Similar findings have also been reported with 0.9% saline solution when compared with lactated Ringer's solution [18]. In addition, the hyperchloremia associated with saline-based solutions has been shown to decrease splanchnic mucosal perfusion, glomerular filtration, and cause coagulopathy [19–21]. Acidosis, even in the absence of sepsis or endotoxemia, has been shown to be associated with gut barrier dysfunction. Thus, while there is little evidence that treating metabolic acidosis improves clinical outcomes, there is mounting evidence that iatrogenic metabolic acidosis is harmful.

In an attempt to avoid the acidifying effects of non-metabolized anions, the use of metabolized ones such as acetate, lactate, and bicarbonate has been advocated.

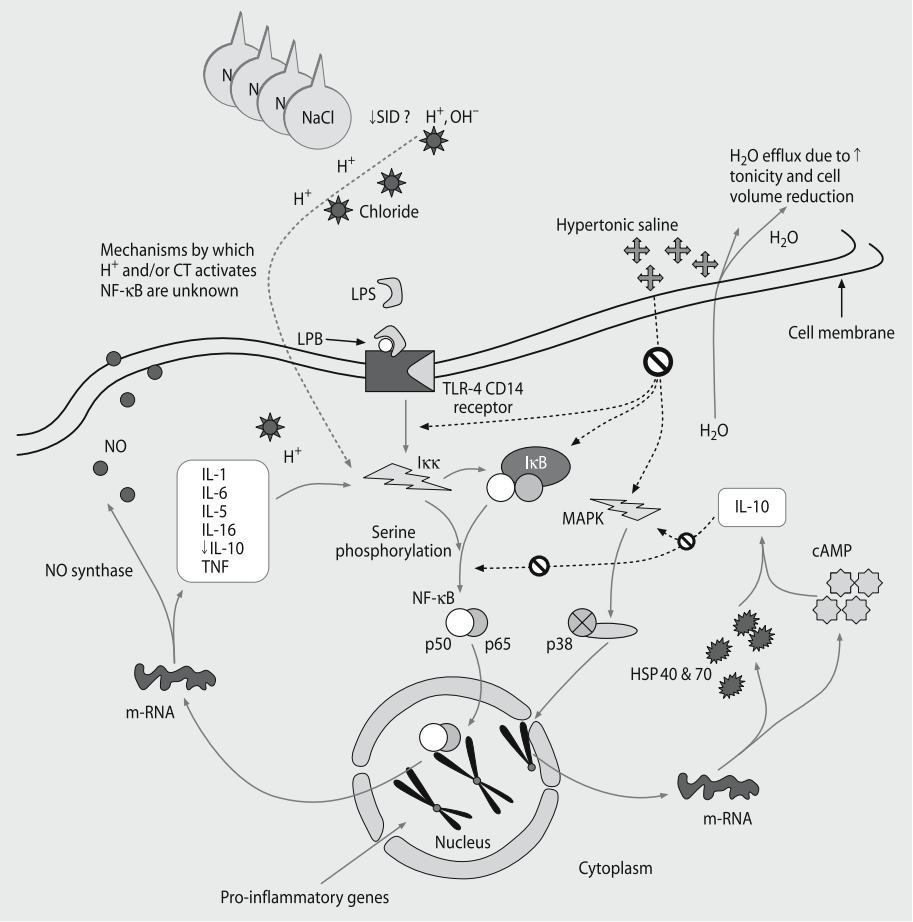

Fig. 1. Schematic illustration of fluid resuscitation induced immunomodulation. Metabolic acidosis induced by saline resuscitation results in a pro-inflammatory response. Solutions with a low strong ion difference (SID) increase water dissociation, and hydrogen (H^+) and/or chloride (Cl^-) ions activates nuclear factor kappa B (NF-κB). Although the mechanisms of H^+ and or/Cl^- induced activation of NF-κB are unclear, this results in a cascade of activation of pro-inflammatory genes and cytokines. Bacterial lipopolysaccharide (LPS) acting through Toll like receptor (TLR)-4 to induce inhibitory kappa kinase (IκK), which in turn activates NF-κB from inhibitory kappa B (IκB). DNA binding of NF-κB induces pro-inflammatory gene expression including interleukins (IL)-1 IL-6, IL-5, IL-16, nitric oxide (NO) and tumor necrosis factor (TNF). Hypertonic saline exerts anti-inflammatory effects through its actions on IκK and subsequent NF-κB activation. Hypertonic saline also inhibits mitogen activated protein kinase (MAPK), thereby inhibiting activation of p38 binding. Cyclic AMP (cAMP) and heat shock protein (HSP) mediate the synthesis of IL-10, which exerts further anti-inflammatory effects

Mustafa et al. compared the metabolic and hemodynamic effects of hypertonic sodium lactate versus hypertonic sodium chloride (7.5%) in three groups of surgical patients who underwent elective coronary artery bypass grafting [22]. Although both solutions produced significant increases in cardiac index and oxygen delivery, hypertonic saline infusion induced a modest, although significant, decrease in arte-

rial pH and bicarbonate, whereas hypertonic lactate infusion increased these two parameters.

Lactated Ringer's Solution

Lactated Ringer's is an interesting alternative to saline because lactate is well metabolized even in poor hemodynamic conditions [23]. Evidence from animal models of hemorrhagic shock suggests that survival is improved with lactated Ringer's solution compared with saline. However, this finding has not been consistent across all studies and some authors have raised concerns about the hypotonicity of lactated Ringer's solution. In recent years, there have been conflicting reports on the effects of lactate on the inflammatory response both *in vitro* and *in vivo*.

Lactated Ringer's solutions have been shown to have pro-inflammatory effects in some studies. During hemorrhagic shock, administration of lactated Ringer's solution has been shown to be associated with increased neutrophil activity compared with non-shock animals and hypertonic saline treated animals [24]. Alam et al. demonstrated that infusion of lactated Ringer's solution increased the expression of E- and P-selectin mRNA in lung and splenic tissue thereby promoting neutrophil activation [25]. Recent work has demonstrated that the pro-inflammatory effects of lactated Ringer's solution may be due to the D-lactate stereoisomer in this traditionally racemic solution. L-lactate is a normal intermediary of human metabolism and is easily metabolized by the liver. However, D-lactate is produced by microorganisms or from ketone bodies and its metabolism is significantly slower. A recent study by Koustova et al. demonstrated that racemic mixtures of lactated Ringer's solution are proinflammatory, whereas solutions containing only L-lactate lactated Ringer's were not [26]. This has prompted some but not all manufacturers to produce lactated Ringer's solutions containing only L-lactate.

Balanced Electrolyte Solutions

Hextend (6% hetastarch in a balanced electrolyte solution) has less chloride compared with 0.9% saline (124 vs. 154 mmol/l), closely resembles plasma and does not produce metabolic acidosis in humans. We compared resuscitation with Hextend against 0.9% saline and lactated Ringer's solution in rats with septic shock [4]. Animals resuscitated with Hextend survived 45% longer than saline-resuscitated animals and had significantly less metabolic acidosis. Survival time was correlated with the changes in pH and negatively correlated with the increase in serum chloride after initial resuscitation. The decrease in pH appears to have been brought on by changes in chloride, lactate, and $PaCO_2$. However, lactate values were not different between groups and changes in $PaCO_2$ were not correlated with survival time. Thus, hyperchloremia and the resulting metabolic acidosis were strongly associated with early mortality in these endotoxemic animals.

Hypertonic Saline

Although like 0.9% saline, hypertonic saline solutions (3 and 7.5%) have an SID of zero and produce hyperchloremic metabolic acidosis. Hypertonic saline has several hemodynamic and immunomodulatory properties that could be beneficial. Hyper-

tonic saline has been shown to decrease the predisposition to sepsis following traumatic shock [27], and improve survival following septic shock [28]. Hypertonic saline inhibits the activation of neutrophils and macrophages [29]. Decreased production of pro-inflammatory cytokines such as TNF and augmented synthesis of anti-inflammatory mediators such as IL-10 has been documented with hypertonic saline.

Several physical properties of hypertonic saline may contribute to this net immunomodulatory effect. Increased serum tonicity alters neutrophil adhesion properties, which decreases mediator release [30]. Hypertonicity attenuates a variety of neutrophil functions, including CD11b expression, elastase release, superoxide production, phagocytosis, and transmigration [31, 32]. Hypertonicity also has been shown to inhibit the phosphorylation of a specific mitogen-activated protein kinase, p38 MAPK, which may mediate many of the anti-inflammatory effects of hypertonic saline [33]. Hypertonic saline has also been shown to decrease NF-κB activation through upregulation of IL-10 [34] (Fig. 1).

Hypertonic treatment of neutrophils has been shown to increase cAMP, and to down-regulate the inflammatory activity of leukocytes and may be involved in the modulation of macrophage activity [35]. Recently, heat shock proteins (HSP) have been implicated in the immunomodulatory effects of hypertonic saline. Murao et al. observed reduced intestinal damage and apoptosis after hypertonic saline resuscitation of hemorrhagic shock in mice due to preservation of HSP40 and HSP70 expression [36]. Furthermore, it has been suggested that the anti-inflammatory effects of hypertonic saline are associated with cytoskeletal changes induced by changes in cell volume. Rizoli et al. showed that L-selectin shedding by neutrophils was cell volume dependent [32]. These cytoskeletal changes may be responsible for dysfunctional cell signaling proximal in the signaling cascades. Due to promising animal data and human studies, a randomized clinical trial of hypertonic saline is currently underway in patients with sepsis. However, since many of the potentially beneficial effects of hypertonic saline are likely due to its tonicity rather than its chemical makeup, other hypertonic solutions (e.g., lactate, starch) might prove even better. At this time, very little information concerning these other fluids is known.

Conclusion

Fluid resuscitation induces immunomodulation in the critically ill. Current evidence is robust enough to suggest that interventions that induce/prolong inflammatory responses are associated with adverse outcomes in critically ill patients. Therefore, an ‘ideal’ resuscitation fluid in addition to being an effective volume expander should minimize iatrogenic metabolic acidosis and pro-inflammatory mediator expression. While normal saline and perhaps even lactated Ringer’s solution appear to be associated with pro-inflammatory effects, fluids such as hypertonic saline may be associated with anti-inflammatory effects. Solutions such as Hextend may be less likely to be immunomodulating. Further large human studies are required to characterize these effects and their impact on outcomes in the critical care setting.

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Cellular Dysfunction

Mitochondrial Dysfunction and Critical Illness Myopathy

E. Kothmann and S. V. Baudouin

Introduction

Generalized muscle weakness is increasingly recognized to be a common and serious complication after prolonged intensive care treatment. This form of muscle weakness and paralysis was initially described by Bolton as a critical illness neuropathy [1]. However, research has demonstrated that critical illness polyneuropathy is frequently associated with critical illness myopathy, as well as existing as the sole pathology [2, 3]. This chapter will focus primarily on the pathophysiology of critical illness myopathy, and the possible role of mitochondrial dysfunction as a contributing factor.

Critical Illness Myopathy

Critical illness myopathy, a primary myopathy, is a term that encompasses a number of different types of myopathy. This includes a spectrum from myopathies with a pure functional impairment with normal histology, to those with atrophy and necrosis on histology [4]. There are also electrophysiological and biochemical criteria to support the diagnosis. The true occurrence of critical illness myopathy is unknown, as different incidences are reported depending on the case-mix, the diagnostic criteria used and the timing of the evaluation. However, in one multicenter study, 25% of all patients ventilated for 7 or more days had severe weakness during recovery [3]. Of those who underwent muscle biopsy, all had some element of myopathy as judged by histology.

Critical illness myopathy is probably under recognized because it has a similar clinical appearance to critical illness polyneuropathy and muscle biopsy is usually necessary to firmly establish the diagnosis [5]. Long-term follow-up of survivors with the acute respiratory distress syndrome (ARDS) suggest that critical illness myopathy may be more common than initial reports indicated. Prolonged (>1 year) muscle weakness was reported by the majority of patients at follow-up [6]. In addition, severe weakness and functional impairment were universally reported in intensive care unit (ICU) survivors in another long-term study [7]. Both of these reports suggest that critical illness myopathy may be an almost universal occurrence in the critically ill. Only the more extreme cases are currently identified clinically, but subtle weakness may be present in most survivors of severe illness.

Normal Skeletal Muscle Physiology

Skeletal muscle is composed of a large number of individual muscle fibers (Fig. 1). These cells lie parallel to one another and are separated by connective tissues, which contain blood vessels and nerves. There are two distinct types of muscle cells, type I and type II, which appear in a random mix. Each muscle cell is made up of myofibrils, which lie parallel to one another. Each myofibril contains many shorter components called sarcomeres, which are also arranged in parallel. The sarcomeres are composed of myofilaments. A myofilament consists of two parts: a thick filament composed of myosin molecules and a thin filament composed mainly of actin molecules together with troponin and tropomysin. The thick and the thin filaments interact to produce muscle contractions. Another important feature of skeletal muscle is the mitochondria [8], which lie next to the muscle filaments and produce the energy required for muscle contraction. These mitochondria are found in two distinct muscle compartments. Subsarcolemmal mitochondria are found close to the muscle periphery, under the sarcolemma. The mitochondria in the fiber centre, i.e., between the myofibrils, are called intermyofibrillar mitochondria. Different external stimuli, e.g., exercise, have selective effects on these populations.

The marked plasticity of human skeletal muscle is well documented [9]. Both atrophy and hypertrophy are under complex physiological control and a number of factors that influence muscle plasticity have clear relevance to the critically ill. These include [10]:

- Inactivity/bed rest/immobilization
- Denervation (neuromuscular blockade)
- Caloric restriction (malnutrition)
- Ageing
- Hyperglycemia
- Hormonal factors
- Pro-inflammatory mediators

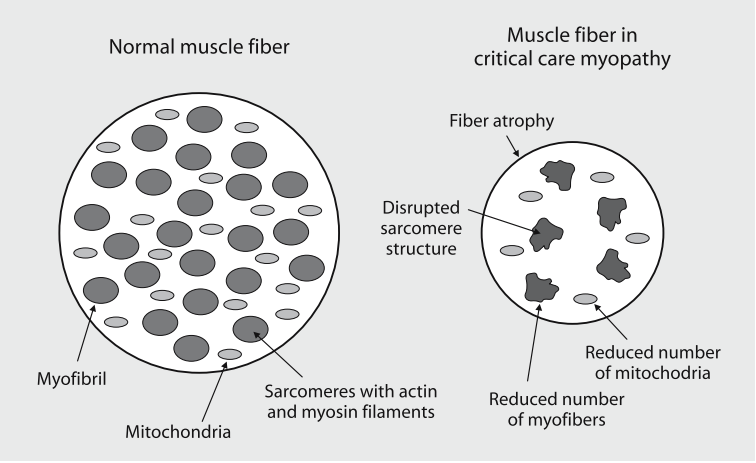

Fig. 1. Changes in skeletal muscle cell structure in critical illness myopathy. These include: 1) atrophy of myofibrils, 2) loss of sarcomere structure, 3) increase in space between myofibrils, and 4) reduction in mitochondria numbers. Selective loss of myosin filaments has also been reported

Structural Changes in the Muscle in Critical Illness Myopathy

There is a spectrum of histological changes that appear to be associated with critical illness myopathy, from normal histology to atrophy and necrosis (Fig. 1). The pathological features of myopathy are complex, but are classified into three main types:
1) atrophy of myofibers predominantly involving type 2 fibers;
2) degenerative-necrotic changes with signs of regeneration; and
3) selective loss of thick myosin myofilaments [11].

However, there is often significant overlapping of pathologies in the same patient.

Stibler and co-workers performed muscle biopsies on 11 patients with critical illness myopathy, as part of a study to develop a method to quantify myosin [12]. They found pathological changes in all muscle specimens. These were described as severe and extensive structural changes. On light microscopy, structural alterations included muscle fiber atrophy, degeneration, necrosis and regeneration. Loss of myosin ATPase activity was found to a varying degree in all the specimens, affecting both type I and II fibers. In severely affected muscles there was a loss of fiber differentiation. The most striking histological change was rounded and enlarged nuclei. On electron microscopy there was preferential loss of thick filaments, with a resulting loss of normal sarcometric structure. The loss of myosin filaments was seen even in the early stage of critical illness myopathy. In more severe disease stages there was increased space between the myofibrils, and complete loss of the sarcomeric pattern.

Bednarik and colleagues reported similar myopathic changes in muscle biopsies from patients with critical illness myopathy [11]. Ultra-structural examination showed myofibrilar disarray with loss of myosin filaments. Loss of myosin was considered to be a pathognomonic change with atrophy of myofibers being less specific.

Helliwell and co-workers examined 98 muscle biopsies from 57 critically ill patients, using histochemical staining for myosin ATPase and cytochrome c oxidase [13]. These authors found that fiber atrophy was a common observation and was related to the loss of the filamentous structure of myosin. The loss of structure occurred before there was substantial degradation of actin or cytoskeletal protein and was associated with increased expression of lysosomal enzymes and ubiquitin. Degenerative changes in the fibers were often associated with fiber atrophy, but both appeared to occur independently of muscle fiber necrosis [13].

In summary, there is only a limited amount of histological information on critical illness myopathy. Most studies have, of necessity, focused on severely weak patients. A broad range of changes are reported. While there is a consensus that severe inflammatory changes are lacking, there do not appear to be any clear defining histological features consistently present.

Causes of Critical Illness Myopathy

Critical illness myopathy was initially reported in patients following a prolonged ICU stay, with a higher incidence in patients diagnosed with sepsis, septic shock, and multiple organ failure (MOF) [14]. For example, de Letter and colleagues re-

ported that the APACHE III score, a quantitative index of disease severity based on clinical and laboratory physiologic data, was a valuable predictor of development of critical illness myopathy [15]. The association with severe sepsis led to the hypothesis that microcirculatory dysfunction or hyperinflammation may be involved in the damage of the motor neuron integrity [16]. In recent years, further research has shown that other factors contribute to critical illness myopathy. These include the use of glucocorticoids and muscle relaxants and the presence of hyperglycemia.

High-dose glucocorticoids may induce significant myopathy, with loss of the thick myofilaments. However, no simple relationship between the duration or dose and occurrence of myopathy has been observed. Short-term, high-dose steroid administration as used in acute exacerbations of asthma may lead to myopathy [17]. Steroids may cause weakness by inducing a form of functional denervation. Rich and Pinter have demonstrated a hyperpolarizing shift of voltage-dependence of sodium channel fast inactivation in rat muscle fibers exposed to steroids, which caused reduced excitability [18].

Patients receiving prolonged muscle relaxants have a higher incidence of critical illness myopathy in some studies. The mechanism is likely to be similar to that observed in denervation, both experimental and following spinal cord injury.

Hyperglycemia also appears to be a risk factor for critical illness myoneuropathy [16]. In a study of patients with ARDS, 60% had electromyographic evidence of myoneuropathy and this was associated with poor glycemic control. Insulin resistance and hyperglycemia accompany critical illness. It has recently been shown that preventing hyperglycemia with insulin improves outcomes. The Leuven study showed that the benefit of intensive insulin therapy in the ICU was particularly apparent among patients with prolonged critical illness [19]. In addition to decreasing mortality, intensive insulin therapy also prevented complications such as critical illness polyneuropathy and muscle weakness.

The Role of Mitochondria in Muscles

Mitochondria are a subcomponent of muscle cells that are bound by a double membrane [20]. They are involved in cellular homeostasis, intracellular signaling, apoptosis, intermediary metabolism, and in the metabolism of amino acids, lipids and nucleotides. They play a central role in cellular energy metabolism, including fatty acid oxidation, the urea cycle and the final pathway for ATP production, which involves the respiratory chain. The respiratory chain is a group of five enzyme complexes, which are situated on the inner mitochondrial membrane. Each complex is composed of multiple subunits. Electrons are donated to complex 1 and 2 from reduced cofactors (NADH and FADH). These electrons flow between the complexes, down an electrochemical gradient. They are transported via complexes 3 and 4 and by two-electron carriers, ubiquinone and cytochrome c. The liberated energy is ultimately used by complex 5 to synthesize adenosine triphosphate (ATP) from adenosine diphosphate and an inorganic phosphate. The overall process is called oxidative phosphorylation. ATP is the high-energy source, which is used for all active metabolic processes within the cells. It is released from the mitochondrion in exchange for cytosolic ADP. In muscle, mitochondria are strategically placed next to the muscle filaments to enable energy produced by the mitochondria to be transported easily to the site where it will be used by the muscle filaments.

Mitochondrial proteins are encoded for by two distinct genetic systems: mitochondrial DNA (mtDNA) and nuclear DNA [21].

Mitochondria and Critical Illness Myopathy

The existence of several, inherited conditions, known as mitochondrial myopathies, demonstrates that mitochondrial dysfunction can cause severe muscle weakness [20]. These illnesses are characterized by DNA mutations, either mitochondrial or nuclear, in the proteins of the mitochondrial respiratory chain. Mitochondria are very sensitive to damage by both intrinsic and extrinsic factors and it can be postulated that both short-term and longer-term damage to mitochondria could occur in the critically ill (Fig. 2).

Short-term changes could include:

- Direct damage to the mitochondria caused by both exogenous and endogenous toxins.
- A 'physiological' shutdown of mitochondrial energy generation. This postulated protective mechanism has been called the "hibernation hypothesis" of MOF [22].
- A physiological loss of mitochondrial numbers driven by disuse atrophy and prolonged bed rest.

Longer-term problems could involve illness-induced mutations in mitochondrial DNA which could impair skeletal muscle recovery and regeneration. These changes could be accentuations of those reported during normal human ageing [23].

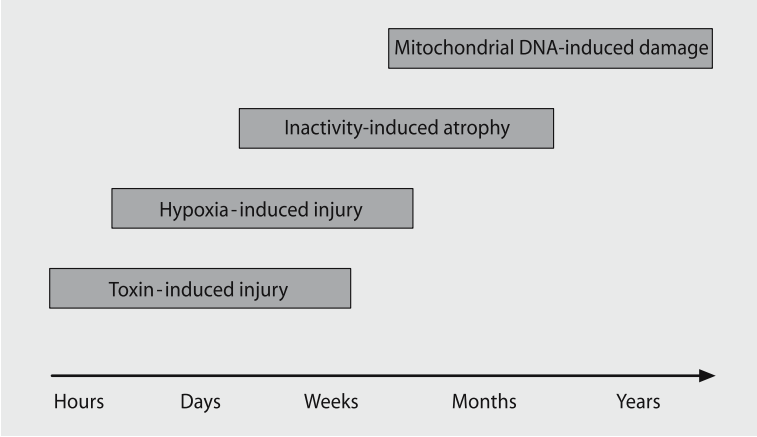

Fig. 2. Proposed time course of onset of various processes that may damage or disrupt skeletal muscle mitochondria in the critically ill

Short-term Damage to Mitochondria

Toxin-induced Injury

There is a substantial body of evidence that pro-inflammatory toxins cause mitochondrial dysfunction and damage [reviewed in [24]]. This evidence includes studies using separated mitochondria, whole cell preparations, isolated organs (including skeletal muscle), and animals exposed to experimental sepsis and endotoxins. A variety of molecular mechanisms has been proposed, including physical injury to the mitochondrial membrane and inhibition of respiratory chain proteins. In man, the data are less substantial. One of the few studies in human sepsis was conducted by Brealey and colleagues who performed skeletal muscle biopsies on 28 critically ill septic patients on the ICU [25]. The biopsy samples were analyzed for respiratory-chain activity, ATP concentration, reduced glutathione concentration, and nitrite/nitrate concentrations and were compared with control specimens. These researchers found that skeletal muscle ATP concentrations and ATP:ADP ratios were significantly lower in the septic patients who died, compared with survivors. Complex 1 activity appeared to be inversely related to illness severity. Damage or inhibition of complex 1 could decrease mitochondrial capability to generate ATP. Sepsis, therefore, appeared to cause mitochondrial dysfunction and decreased ATP concentrations that related to organ failure. Their data suggest bioenergetic failure as a pathophysiological mechanism underlying MOF [25]. Although the study did not directly investigate skeletal muscle function, it seems likely that mitochondrial energy failure in skeletal muscle would lead to significant muscle failure which would be clinically apparent as weakness.

Hypoxemia-induced Injury

Oxygen delivery is frequently impaired in the severely ill and could result in mitochondrial dysfunction. Hoppeler and co-workers reviewed the response of skeletal muscle mitochondria to hypoxia [26]. They examined the effects of short-term hypoxia (minutes to hours) and long-term hypoxia (weeks), both situations that are applicable to critically ill patients. Muscle biopsies from healthy volunteers exposed to long-term hypoxia (simulated Everest ascent) showed loss of muscle mitochondrial volume of approximately 30%, with a net loss of muscle oxidative capacity. Muscle biopsies showed considerable signs of muscle wasting and an accumulation of lipofuscin granules in muscle cells. Hypoxemia may induce mitochondrial damage by causing the release of reactive oxygen species (ROS) from mitochondria [27]. This could lead to a self-perpetuating situation where mitochondria exposed to hypoxia produce increased ROS which further damage the mitochondria.

Dietary/glucose-induced Injury

Dietary composition and caloric restriction can influence mitochondria function. It is, therefore, interesting to note the presence of ultra-structural abnormalities in mitochondria in a recent study of patients who died following a critical illness [28]. Post-mortem studies reported hypertrophic mitochondria in the liver in the majority of patients not treated with intensive insulin therapy to maintain blood glucose between 4.4 mmol/l and 6.1 mmol/l. Increased numbers of abnormal and

irregular cristae and reduced matrix density were also seen. In contrast, skeletal muscle obtained from patients in both groups had normal ultrastructure. The striated muscle cells contained mainly normal sarcomeres, and the mitochondrial arrangement appeared normal. Enzyme activities of the mitochondria in skeletal muscle were not significantly affected by intensive insulin therapy. However, skeletal muscle total protein content was higher in the intensive insulin group. In a subanalysis of septic versus non-septic patients, complex I activity was reduced in the septic group. This seems to confirm the existing evidence that sepsis is a contributing factor to mitochondrial dysfunction in skeletal muscle. The difference in the effects of insulin on the mitochondria in the liver and skeletal muscle remains unexplained but different mechanisms of glucose uptake may be important.

Inactivity-induced Injury

Critically ill patients have prolonged periods of inactivity due to the severity of the illness as well as through enforced sedation and muscle relaxation to facilitate appropriate treatment. Substantial evidence exists indicating that mitochondrial biogenesis is stimulated by aerobic exercise and reduced by inactivity [29]. Muscle mitochondrial oxidative enzymes, cytochrome-c oxidase and citrate synthase, and mRNA concentrations of several genes encoding mitochondrial proteins are enhanced by aerobic exercise. Mitochondrial biogenesis and mitochondrial DNA replication are stimulated by the expression of a constitutively active form of calcium/calmodulin-dependent protein kinase IV. Chronic electrical stimulation of skeletal muscle causes a sustained rise in intracellular calcium and activates calcium-regulated enzymes such as calcineurin and calcium/calmodulin-dependent protein kinase, both of which have been shown to activate slow and oxidative fiber gene expression in muscle cells. Muscle contractile activity though this mechanism enhances mitochondrial biogenesis and oxidative phenotype of skeletal muscle [23].

Longer-term Damage to Mitochondria

Age and Reduction in Mitochondrial Function

The population currently admitted to critical care units is increasingly elderly with median ages of over 70 years reported by some groups. Skeletal muscle function falls with age with a concomitant reduction in mitochondrial function [23]. Oxidant-induced mitochondrial damage, resulting in progressive loss of cellular energy resources is believed to play a key role in aging [30]. Mitochondria suffer more damage than other cellular components as they are the primary site of ROS formation.

The underlying cause of the reduction in mitochondrial biogenesis and ATP production seem to be decreases in mitochondrial DNA and messenger RNA content. Increased mitochondrial DNA oxidative damage with aging and cumulative DNA damage could explain the overall reduction in mitochondrial DNA in skeletal muscle. This reduction may contribute to reduced mRNA which results in reduced mitochondrial protein synthesis and enzyme activity. The overall effect is a reduced capacity for oxidative phosphorylation.

Mitochondrial alteration in ageing myocytes has been described extensively in skeletal muscles [31]. Aged myocyte mitochondria are more variable in size; some mitochondria enlarge enormously. Swelling, loss of cristae, and even complete destruction of inner membranes result in the formation of electron dense material. There is a drop in the inner membrane potential and a decrease in energy production.

A small number of experimental studies suggest that sepsis could produce persistent structural damage to mitochondrial DNA. In one study, liver mitochondrial DNA was examined following intra-peritoneal injection of lipopolysaccharide (LPS) in rats [32]. An oxidant-dependent mitochondrial DNA deletion was reported in the region encoding NADH dehydrogenase sub-units 1 and 2 and cytochrome oxidase sub-unit 1. The total liver mitochondrial DNA copy number also fell. The study did not examine changes in skeletal muscle. Similar, short-term falls in mitochondrial DNA copy number were reported in hearts from rats exposed to LPS [33]. This was reflected in a substantial fall in many respiratory chain proteins coded by mitochondrial DNA. In both studies, mitochondria recovery occurred by biogenesis. Whether similar damage to mitochondrial DNA occurs in human sepsis is unknown but the similarity of sepsis-induced mitochondrial DNA damage to that observed in normal human ageing is interesting.

Conclusion

Muscle weakness following critical illness is a significant clinical problem. It is likely to contribute to the continuing morbidity and mortality of prolonged ICU stay. However, its pathophysiology remains poorly understood. Several major issues remain unresolved. These include the question of whether critical illness myopathy is a distinct illness or represents a continuum of skeletal MOF.

Also unresolved is the differentiation of a postulated specific, sepsis-related muscle failure from other causes of muscle dysfunction in the critically ill. In particular, differences between diffuse atrophy, the normal ageing process, and sepsis-induced injury need to be explored. Finally, the question of possible mitochondrial DNA damage in human sepsis should be addressed. Normal human ageing is characterized by gradual damage to mitochondrial DNA. Could the long-term adverse consequences of prolonged critical care be partially a result of an increase in this process – a sort of 'ageing in the fast lane'?

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Lactic Acidosis and Hyperlactatemia

B. Levy

Introduction

Traditionally, hyperlactatemia in critically ill patients and particularly those in shock was interpreted as a marker of secondary anaerobic metabolism due to inadequate oxygen supply inducing cellular distress [1]. Many arguments have since refuted this view [2]. With lactate metabolism being extensively described in classical biochemistry manuals, this chapter will focus only on those aspects as they relate to critically ill patients. Distinction between lactic acidosis, metabolic acidosis with hyperlactatemia, and isolated hyperlactatemia will also be addressed.

Lactate Metabolism [3]

Arterial lactate concentration is dependent on the balance between its production and consumption. In general, it is less than 2 mmol/l, although daily production of lactate is actually 1500 mmol/l. In physiological conditions, lactate is produced by muscles (25%), skin (25%), brain (20%), intestine (10%) and red blood cells (20%) which are devoid of mitochondria. Lactate is essentially metabolized by liver and kidney.

Lactate is produced in the cytoplasm according to the following reaction (Fig. 1):

$$\text{Pyruvate} + \text{NADH} + \text{H}^+ \Leftrightarrow \text{lactate} + \text{NAD}^+$$

This reaction favors lactate formation yielding a 10-fold lactate/pyruvate ratio. Lactate therefore increases when production of pyruvate exceeds its utilization by the mitochondria. Pyruvate is essentially produced via glycolysis; hence any increase in glycolysis, regardless of its origin, can increase lactatemia. Pyruvate is essentially metabolized by the mitochondrial aerobic oxidation pathway via the Krebs cycle.

$$\text{Pyruvate} + \text{coenzyme A} + \text{NAD} \Rightarrow \text{acetyl CoA} + \text{NADH} + \text{H}^+ + \text{CO}_2$$

This reaction leads to the production of large quantities of ATP (36).

Generated lactate can be transformed into oxaloacetate or alanine via the pyruvate pathway or can be utilized directly by periportal hepatocytes (60%) to produce glycogen and glucose (neoglycogenesis and neoglucogenesis) (Cori cycle). The kidney also participates in the metabolism (30%) of lactate with the cortex classically

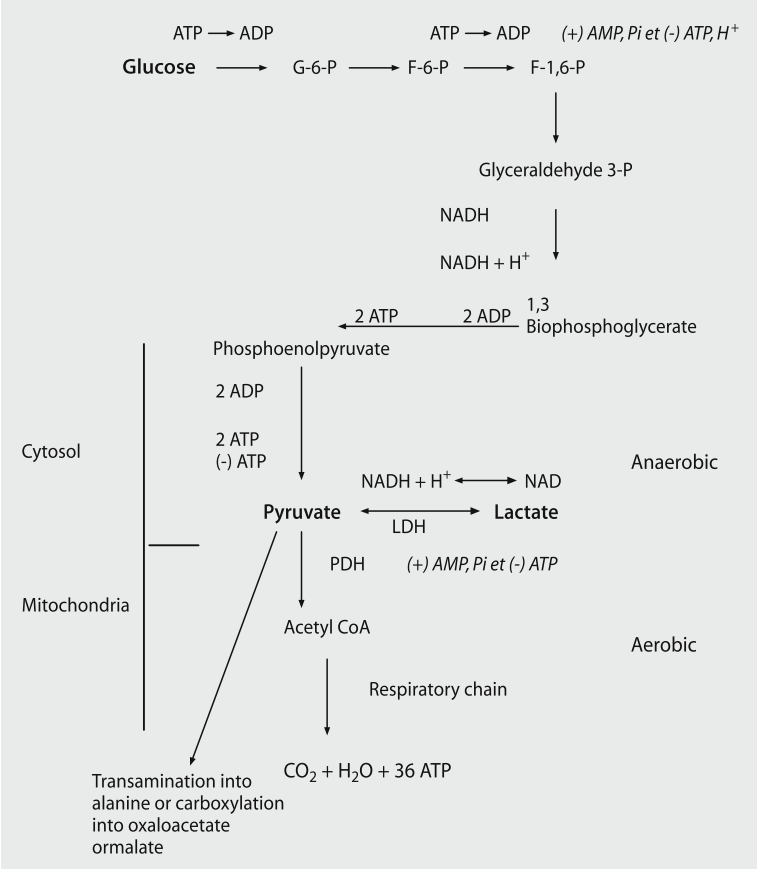

Fig. 1. Overview of carbohydrate metabolism

acting as the 'metabolizer' by neoglucogenesis and the medulla as a producer of lactate. The threshold of renal excretion is 5–6 mmol/l meaning that, physiologically-speaking, lactate is not excreted in urine.

Hence, lactatemia reflects a balance between production and utilization of lactate. Consequently, for the same etiological mechanism producing an increase in lactate, one can either observe a hyperlactatemia (if its metabolism decreases) or a normolactatemia. Understanding this concept is vital, notably to avoid treating solely a numerical value of lactate.

Formation of Lactate in Cases of Tissue Hypoxia

By definition, hypoxia blocks mitochondrial oxidative phosphorylation [4], thereby inhibiting ATP synthesis and reoxidation of NADH. This leads to a decrease in ATP/ADP ratio and an increase of NADH/NAD ratio. A decrease in the ATP/ADP ratio induces both an accumulation of pyruvate which cannot be utilized by way of

phosphofructokinase (PFK) stimulation and a decrease in pyruvate utilization by inhibiting pyruvate carboxylase, which converts pyruvate into oxaloacetate. An increased NADH/NAD ratio also increases pyruvate by inhibiting pyruvate dehydrogenase (PDH) and hence its conversion into acetyl coenzyme A.

Consequently, the increase in lactate production in an anaerobic setting is the result of an accumulation of pyruvate, which is converted into lactate stemming from alterations in the redox potential. This conversion allows for the regeneration of some NAD^+, enabling the production of ATP by anaerobic glycolysis, although clearly less efficient from an energy standpoint (2 ATP produced versus 36). It is important to consider that the modification of the redox potential induced by an increase in NADH/NAD ratio activates the transformation of pyruvate into lactate and consequently increases the lactate/pyruvate ratio.

All in all, anaerobic energy metabolism is characterized by hyperlactatemia associated with an elevated lactate/pyruvate ratio, greater glucose utilization and low energy production.

Lactate/Pyruvate Ratio

Lactate/pyruvate interconversion can be described by the following equation:

$$\text{Pyruvate} + \text{NADH} + \text{H}^+ \Leftrightarrow \text{lactate} + \text{NAD}$$

and, at equilibrium

$$\text{Lactate/pyruvate} = \text{K} \cdot \text{NADH/NAD} \cdot \text{H}^+$$

where K represents the dissociation constant.

Therefore, an increase in the NADH/NAD ratio or a drop in cytosolic pH triggers an increase in the lactate/pyruvate ratio. The use of this ratio has been advocated for differentiating hypoxia-related hyperlactatemia from hyperlactatemia resulting from an increase in glycolytic flux without hypoxic stress.

However, the above equation clearly demonstrates that this NADH/NAD ratio can be altered by factors other than the inability to transfer electrons to oxygen. Furthermore, in order for the plasma lactate/pyruvate ratio to properly reflect the redox potential, one would need to demonstrate that this ratio is identical to both cytosolic and mitochondrial ratios and that the rate of cell efflux of pyruvate and lactate are also identical [5]. Lastly, use of a pyruvate assay is precarious since the latter is quickly degraded and can therefore lead to falsely elevated lactate/pyruvate levels.

We have nonetheless demonstrated [6] that this ratio is very high (40 ± 6) in cardiogenic shock patients with low cardiac output compared to controls (8 ± 2), these patients representing a clinical model of tissue hypoxia (we will see further that this notion can in fact be debated). We also found a definite increase of this ratio in patients with refractory septic shock characterized by elevated catecholamine dosages, low blood-pressure, metabolic acidosis and normokinetic state. On the other hand, in stabilized septic shock patients, this ratio was slightly increased (14 ± 1) or otherwise normal when corrected for pH. Interestingly, for equal concentrations of lactate, septic patients, with the exception of refractory septic shock patients, have

higher pyruvate levels, thus implying a mechanism other than hypoxia. In the end, the prognostic value of the lactate/pyruvate ratio was no better than that of lactate and failed to provide any additional information.

Classification of Hyperlactatemia

For instructional purposes, the classification of Cohen and Woods remains the tool of reference (Table 1). It differentiates hyperlactatemia associated with signs of tissue hypoperfusion (type A) from hyperlactatemia without tissue hypoperfusion (type B).

Lactate and Shock States

Classically, hyperlactatemia in shock is considered as secondary to tissue hypoxia induced by a decrease in tissue perfusion. This notion is potentially true in certain clinical situations.

Situations where Hyperlactatemia is Predominantly a Reflection of Tissue Hypoperfusion

Shock states induced by low cardiac output should theoretically be accompanied by a hypoxic hyperlactatemia. Cardiogenic shock, as demonstrated previously, is associated with hyperlactatemia with a very high lactate/pyruvate ratio. In theory, hemorrhagic shock should behave in an identical fashion.

The problem encountered with sepsis is more complex; although at least two situations are usually accompanied by hypoxia-associated hyperlactatemia. The first is

Table 1. Causes of hyperlactatemia adapted from Cohen and Woods.

Type A: Imbalance between oxygen demand and supply	▮ Shock ▮ Severe hypoxemia, carbon monoxide poisoning ▮ Severe anemia, excessive increase in oxygen demand (seizure, hyperpyrexia, shivering, strenuous exercise)
Type B: Metabolic derangements	▮ Cancer (tumor production or liver metastasis) ▮ Liver failure ▮ Cyanide poisoning ▮ Alkalosis ▮ Sepsis ▮ Beta-2 agonist ▮ Ketoacidosis ▮ Vitamin deficiency: thiamine, biotine ▮ Ethanol intoxication (increases in hepatic NADH and decreases in the conversion of lactate to pyruvate) ▮ Metformin ▮ Inborn error of metabolism

septic shock with catecholamine-resistant cardio-circulatory failure, especially in situations of low cardiac output. The second circumstance is septic shock pre-emptively observed prior to volumetric expansion as illustrated in the study by Rivers et al. [7] in which hyperlactatemia was associated with signs of poor oxygen delivery. These two situations are nonetheless close to low output states.

Situations where Hyperlactatemia Reflects a Metabolic Adjustment, such as Sepsis

Indeed, many arguments argue against tissue hypoxia as the major cause of hyperlactatemia in septic shock. Theoretically, if septic shock hyperlactatemia was indeed induced by tissue hypoxia caused by hypoperfusion, then:

- hyperlactatemic septic patients should display collapsed oxygen delivery, which should be corrected with increased oxygen transport, which is not the case [8]
- tissue PO_2 should be low; however, and in contrast to cardiogenic shock, muscle PO_2 measured in septic shock patients is actually elevated [9]
- ATP levels should be decreased. Yet these levels were found to be normal when measured in human muscle, as in many animal models [10]
- dichloroacetate, a PDH activator, should not lower lactatemia in septic patients or animals since it increases the conversion of pyruvate into acetyl CoA used in the respiratory chain. However, numerous animal models and several human studies have shown that dichloroacetate significantly decreases lactatemia in septic states [11]
- Finally, it has been postulated that lactate may originate from a regional source. Splanchnic circulation was initially targeted but De Backer et al. [12] demonstrated that the splanchnic area in general consumed lactate and that splanchnic production was uncommon and in any case not quantitatively sufficient to explain systemic hyperlactatemia. The lungs can also produce lactate, essentially in acute respiratory distress syndrome (ARDS), although this production is mostly explained by the presence of infiltrating inflammatory cells [13] and not by hypoxia.

Aerobic Production of Lactate

Aerobic is defined as any situation involving oxygen. Lactate formation occurring during the first part of glycolysis is termed anaerobic, as it does not require the presence of oxygen. A sepsis-associated inflammatory state induces an increase in pyruvate production combined with accrued synthesis of glucose transporter-1 (GLUT-1) mRNA [14]. This state, called accelerated aerobic glycolysis, occurs when the rate of carbohydrate metabolism exceeds the oxidative capacity of the mitochondria. Pyruvate is produced by an increased influx of glucose [15] but also via muscle protein catabolism, releasing amino acids subsequently transformed into pyruvate and, thereafter, lactate. Moreover, PDH dysfunction has been described in sepsis and thus may participate in the accumulation of pyruvate [16].

Compartmentalization of Glycolysis, Epinephrine, Muscle and Na^+K^+ATPase Pump. Cytosolic glycolytic flux is functionally divided into two distinct compartments. There are two distinctive glycolytic pathways utilizing separate glycolytic enzyme pools. The first pathway participates in oxidative metabolism via the Krebs cycle. The sec-

ond pathway is linked to activity of the Na^+K^+ATPase pump (Fig. 2). Indeed, ATP produced by this pathway is used to fuel this membrane pump [17, 18].

Numerous studies [19, 20] have demonstrated that epinephrine, via β_2-adrenoceptor stimulation, increases cAMP production inducing the stimulation of glycogenolysis and glycolysis (ATP production) as well as activation of the Na^+K^+ATPase pump, which in turn will consume this ATP, thereby producing ADP. This generated ADP via PFK stimulation, will re-activate glycolysis and hence generate more pyruvate and, thereafter, lactate. Muscle tissue, which represents approximately 40% of total cell mass in the body is particularly implicated in this mechanism, not to mention that over 99% of muscle adrenergic receptors are β_2 receptors [21].

In order to confirm this hypothesis, we utilized muscle microdialysis in hyperlactatemic septic shock patients under catecholamine treatment. This technique consists of inserting into the quadriceps muscle a very fine catheter perfused with a liquid similar to the extracellular medium but lactate-free. The catheter is comprised of a membrane similar to a dialysis membrane, therefore enabling the retrieval, following an equilibrium period, of a fluid whose composition is in equilibrium with the interstitial fluid. When the liquid is perfused very slowly (0.3 µl/min), the composition of the collected fluid is equal to the composition of the interstitial fluid. Furthermore, it is possible to add a biologically active substance to the perfusate whose effect will be strictly limited to cells surrounding the catheter. Finally, by measuring the arterial concentration of the compound of interest, one can establish an interstitial muscular-arterial gradient which, if positive, indicates muscle production.

Our working hypothesis stipulated that epinephrine, secreted in response to a shock state, boosted production of muscle lactate by activating the Na^+K^+ATPase pump. We, therefore, introduced two microdialysis catheters; the first perfused with lactate-free Ringer's and the second perfused with the same solution in combination with ouabain, a selective inhibitor of the Na^+K^+ATPase pump. A key finding

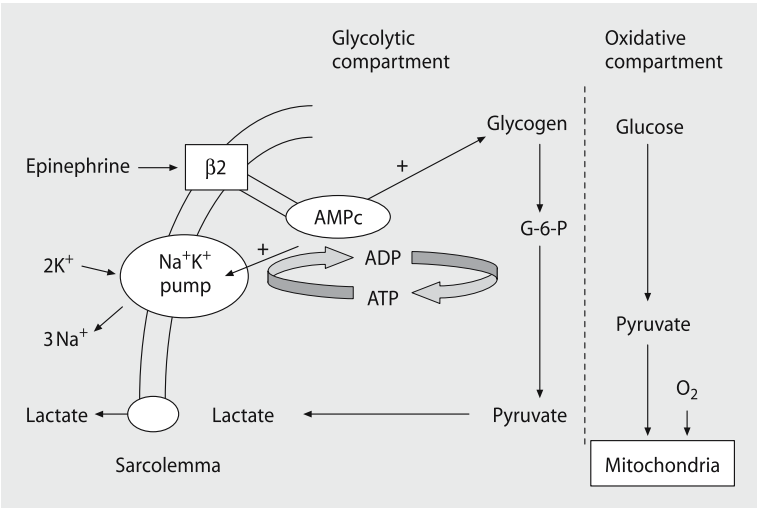

Fig. 2. Epinephrine-increased glycolysis is coupled to Na^+K^+ATPase activity. The ATP furnished by the glycolysis under epinephrine stimulation is used to fuel Na^+K^+ATPase activity

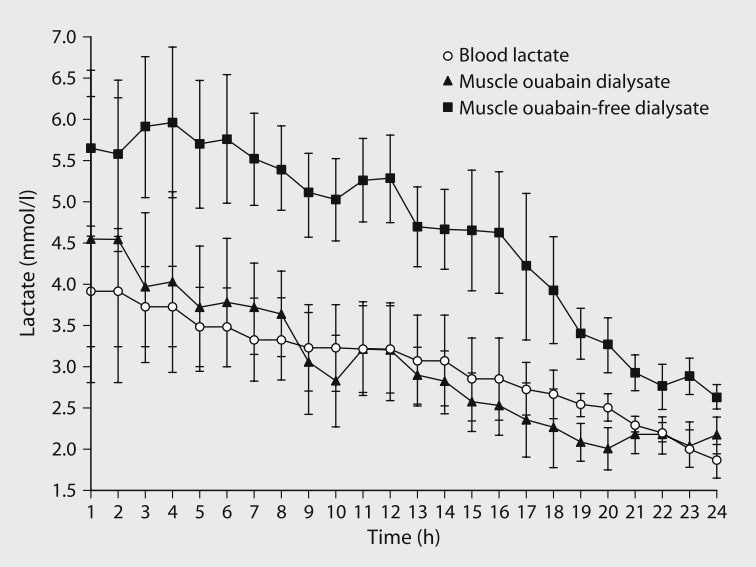

Fig. 3. Lactate concentrations in 14 patients with septic shock in 24 h of study. From [22] with permission

revealed that muscle lactate was consistently greater than arterial lactate thus indicating muscle production (Fig. 3) and that this production was totally inhibited by ouabain confirming a Na^+K^+ATPase–dependent mechanism, but independent of tissue hypoxia [22].

Significance. Muscle lactate, produced under the effect of epinephrine and released into the bloodstream is utilized by the liver to produce glucose through neoglucogenesis (Cori cycle) (Fig. 4) or by other cells for oxidative purposes. Neoglucogenesis is associated with a lower energetic efficiency since 2 ATP are produced per molecule of glucose to generate lactate, while 6 ATP are consumed for every molecule of glucose generated from lactate. This process nonetheless allows the liver to use the ATP generated by fatty acid β oxidation to produce glucose. Hence, fatty acids which supply large quantities of available energy, albeit in a slow process, are used to produce limited stocks of glucose. This mechanism underscores the pivotal role of lactate during aerobic energy metabolism. The 'lactate shuttle' theory suggests that aerobic production of lactate represents an important mechanism by which various tissues share a common source of carbons for oxidation and other biochemical processes such as neoglucogenesis. Hyperlactatemia in shock states may, therefore, constitute an adaptive protective mechanism by favoring the oxidation of lactate rather than that of glucose in tissues where oxygen is available, thus preserving glucose in tissues where oxygen content is rare. Thus, an elevated lactate/pyruvate ratio is an indicator of a cytoplasmic accumulation of reduced equivalents (NADH) from which NADH can be used to regenerate ATP ($ADP + NADH + H^+ \Rightarrow ATP + NAD$). Henceforth, the combination of lactate/pyruvate could be considered as an adaptive energetic substrate, able to navigate from cell to cell or from organ to organ [23].

This hypothesis is largely supported by several experimental studies demonstrating, for example, that the brain [24] or heart can utilize lactate as a preferred

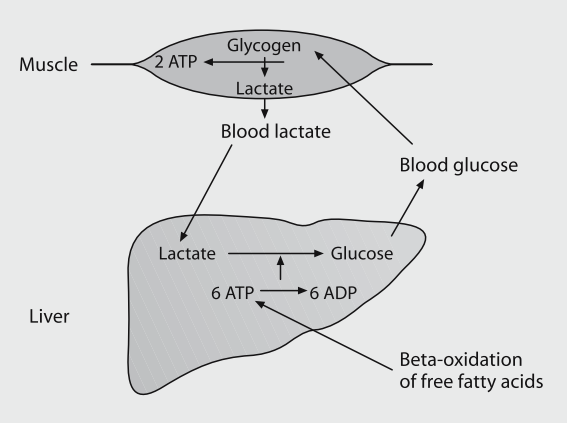

Fig. 4. Cori cycle: Lactate is produced by muscle and released in the blood. It reaches the liver where it enters the Cori cycle and becomes glucose. The energy for such gluconeogenesis is supplied by beta-oxidation of fatty acids. The glucose is either used locally or recycled to supply fast energy to cells. These pathways ensure conversion of slow energy stored as fat into fast energy that is readily available as glucose. The energy cost is –4 moles of ATP [32]

source of energy in certain situations of stress. It was also demonstrated that lactate depletion in the myocardium resulting from hemorrhagic shock reduced myocardial performance [25].

Other Etiologies of Non-hypoxic Hyperlactatemia

Reduction in lactate clearance: Levraut et al. [26] have elegantly demonstrated through the use of labeled lactate that persistent hyperlactatemia in hemodynamically-stable septic shock patients was due to a reduction in lactate clearance and not an increase in lactate production.

Pyruvate Dehydrogenase dysfunction: PDH converts pyruvate into acetyl CoA allowing pyruvate to enter the mitochondria. PDH activity was found to be lower in septic muscle and restored by dichloroacetate. Dichloroacetate lowers lactatemia in septic patients. It is, therefore, likely that there is a certain degree of dysfunction or saturation of PDH activity in septic states [27], although this phenomenon remains secondary.

Protein degradation: Protein catabolism generates the release of amino-acids which are converted into pyruvate and thereafter into lactate.

Accelerated Glycolysis is a Universal Mechanism in Shock States

Indeed, the initial reaction to a state of shock or to aggression, regardless of etiology, is catecholamine secretion. It, therefore, appears logical that at least a portion of the hyperlactatemia observed in low cardiac output states, such as that observed during hypovolemia, hemorrhagic or cardiogenic shock, would be due to a Na^+ K^+ATPase pump-dependent mechanism. McCarter et al. [28] demonstrated in a rat

model of hemorrhagic shock that muscle lactate was produced under the stimulatory effect of the epinephrine-induced Na^+K^+ATPase pump. Using muscle microdialysis, we have demonstrated that non-selective beta blockers, ouabain as well as a selective β_2 blocker, considerably decreased muscle lactate production in hemorrhagic shock, although not suppressing it completely (unpublished data); the conclusion of this study being that hyperlactatemia is in large part secondary to the stimulation of muscle β_2 receptors, including low output shock states.

Lactate and Metabolic Acidosis

Lactic acidosis is generally defined by a build-up of lactic acid (lactatemia >5 mmol/l) and metabolic acidosis (pH <7.25). Classically, it is considered that during tissue hypoxia, acidosis is induced by the hydrolysis of ATP, from which the resulting release of H^+ ion is accumulated in the cytoplasm. On the other hand, in the absence of hypoxia, ATP hydrolysis also leads to the release of H^+ ions which will ultimately be recycled during glucose metabolism, thereby explaining the absence of acidosis.

This concept has been questioned by Stewart [29], who demonstrated that the acid-base balance is dependent on PCO_2, the presence of weak acids (phosphates and proteins) and a 'strong ion gap [SIG]' defined by $(Na^+ + K^+ + Ca^{++} + Mg^{++}) - (Cl^- + \text{lactate})$. Lactate, as does chloride or citrate for example, reduces the gap between positive ions and negative ions. This reduction in [SIG] alters the dissociation of plasmatic water. Water, which is normally partially dissociated into H^+ and OH^-, becomes more dissociated, thus generating more H^+ ions measured by a reduction in pH. While it appears evident that lactate is a significant component of [SID] by increasing the concentration of H^+ ions, it is not solely responsible for the variations in pH. This explains why a certain number of hyperlactatemias are not accompanied by acidosis or exhibit lesser acidosis relative to the actual concentration of lactate.

Prognostic Value of Lactate

Regardless of the mechanism of production, hyperlactatemia and especially the persistence of hyperlactatemia, remains a major prognostic factor in diseases with etiologies as varied as polytrauma or shock, whether it be septic, hemorrhagic or cardiogenic [30, 31]. Persistence of an elevated lactate level can be due to an incessant overproduction related to a persistence of the initiator mechanism but also to a lowering of lactate clearance notably due to hepatic dysfunction.

Line of Conduct when Facing Hyperlactatemia

Lactate must be assayed in all situations predisposing to its formation and particularly in the diagnosis and follow-up of shock states, including all cases of severe sepsis. Rivers et al. [7] demonstrated, for example, that a large proportion of patients with severe sepsis without hypotension exhibited hyperlactatemia and low central venous oxygen saturation ($ScvO_2$), and that this hyperlactatemia was corrected during ensuing management.

Initiated treatment should be based on alleged mechanisms of formation but mostly on observed physiopathological disorders as they relate to objective parameters warranted by the situation: cardiac output, blood-pressure, echocardiography, mixed venous oxygen saturation (SvO_2), abdominal pressure. Lactate can be used to monitor the efficiency of initiated therapy insofar as confounding factors such as catecholamines, particularly epinephrine, and hepatic function are taken into account. The major concern of the treating critical care specialist when facing hyperlactatemia and, even more so, when it is accompanied by metabolic acidosis, is cardiovascular dysfunction, regardless of its origin. Once this diagnosis is eliminated, when warranted, by treatment aimed at increasing oxygen delivery the etiological diagnosis will rest on a knowledge of the various etiologies involved (Table 1). To date, there is no specific treatment available; furthermore, several pathophysiological components cited above actually suggest that hyperlactatemia could even be beneficial.

Conclusion

Measurement of plasma lactate remains a primordial component for a sound diagnostic and therapeutic line of conduct in critical care. The concept of lactate as merely a metabolic waste product (bad lactate) has now evolved towards lactate being viewed as an energy shuttle (good lactate). In most clinical critical care situations, hyperlactatemia must be perceived as an adaptive response to an aggressive state and not as a marker of tissue hypoxia. Nevertheless, irrespective of its mechanism of formation, hyperlactatemia remains an excellent prognostic marker.

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Cardiovascular Dysfunction

Physiology and Pathophysiology of the Natriuretic Peptide System

J. Boldt and S. W. Suttner

Introduction

The natriuretic peptide family consists of four structurally similar but genetically distinct peptides with unique biochemical and physiologic properties (Table 1). In 1981, de Bold and colleagues infused atrial homogenate extracts into rats and noted massive diuresis and natriuresis [1]. The structure of atrial natriuretic peptide (ANP), the peptide hormone responsible for these actions, was identified by Kangawa and Matsuo in 1984 [2]. Four years later, another peptide with natriuretic and diuretic properties similar to ANP was identified in extracts of porcine brain [3]. Although this 32-amino acid polypeptide was called brain natriuretic peptide (BNP), it was soon determined that that the primary site of BNP synthesis was in the ventricular myocardium [4]. Since then, additional members of the natriuretic peptide family of extracardiac origin have been identified:

- C-type natriuretic peptide (CNP) is a 22-amino acid peptide mainly present in the central nervous system (CNS) and vascular endothelial cells with very low concentrations in human plasma [5].
- The fourth member of the natriuretic peptide family is dendroaspis natriuretic peptide (DNP), isolated from the venom of the green mamba snake with structural similarity to ANP, BNP, and CNP [6]. It has also been reported that DNP-like immunoreactivity is present in human plasma and in the atrial myocardium [7].

While understanding of the actions of CNP and DNP is incomplete, the cardiac natriuretic peptides have a fundamental role in cardiovascular remodeling and in regulating the body's volume homeostasis. Both cardiac natriuretic peptides act as counter-regulatory hormones to the increased sympathoadrenal and neurohormonal activation in response to ischemic myocardial injury and heart failure [8]. Nu-

Table 1. The natriuretic peptide family

Peptide	Primary origin	Stimulus for release
ANP	Atrial myocardium	Atrial myocyte stretch
BNP	Ventricular myocardium	Ventricular myocyte stretch
CNP	Endothelial cells/central nervous system	Shear stress
DNP	Atrial myocardium/snake venom	Atrial myocyte stretch

merous experimental and clinical investigations have focused on their diagnostic usefulness for cardiovascular conditions ranging from asymptomatic myocardial ischemia without ST-segment elevation to transmural myocardial infarction and acute heart failure. The natriuretic peptides have also emerged as important therapeutic agents in patients with congestive heart failure that may offer an interesting approach for managing the critically ill patient.

Structure, Metabolism, and Biological Function

The four molecules of the natriuretic peptide family are synthesized as high molecular weight precursor proteins that undergo intracellular processing to the active forms. Although the precursor prohormone for each natriuretic peptide is encoded by a separate gene, they share significant amino acid sequence homologic characteristics with a common 17 amino acid ring structure and a cysteine-cysteine cross-link, which is present in all natriuretic peptides [9]. The structure of these peptides is shown in Figure 1.

ANP is produced primarily in the atrial myocardium, with lower concentrations found in the ventricles and kidney. Pro-ANP (126 amino acids) is the major form of ANP that is stored in atrial granules. Cleavage of human pro-ANP in the endoplasmic reticulum by endoprotease releases a 98-amino acid amino-terminal fragment (NT-ANP) and the 28-amino acid active hormone into the circulation [9]. The biologically active ANP has a molecular weight of approximately 3000 Dalton and the biological half-life of ANP in humans is 1 to 5 min. The half-life of NT-ANP is about 8- to 10-fold longer than that of ANP.

BNP is synthesized and secreted mainly by the ventricular myocardium. Within the myocyte, BNP is derived from the precursor preproBNP, a 134 amino acid polypeptide, which is cleaved to the prohormone proBNP (108 amino acids) and a signal peptide (26 amino acids). Unlike ANP, which is packaged into secretory granules, proBNP gene expression must be upregulated before it can be released into the blood. Thus, the concentration of BNP does not fluctuate as quickly as does ANP. Under conditions of sustained ventricular expansion and pressure overload, proBNP is released into blood where it is cleaved into BNP, the physiologically active hormone, and N-terminal BNP (NT-BNP), an inactive metabolite [9]. BNP has a biological half-life of approximately 20 minutes.

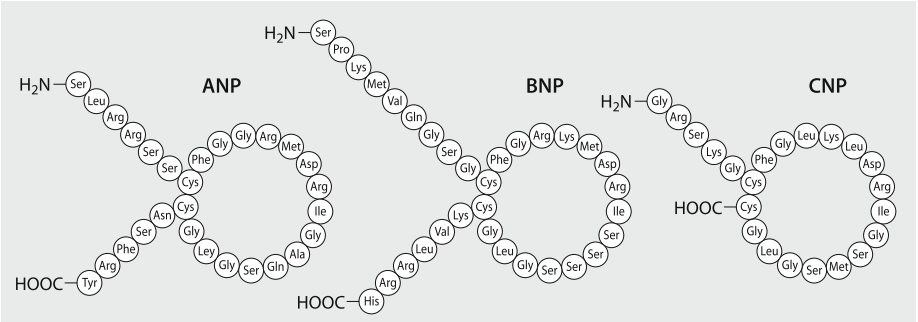

Fig. 1. Structure of the natriuretic peptides

Table 2. Factors associated with natriuretic peptide release

Cardiac disease	Congestive heart failure, myocardial ischemia, left ventricular hypertrophy
Other disease	Septic shock, acute or chronic renal failure, pulmonary hypertension
Neurohumoral and other endocrine factors	Epinephrine, norepinephrine, acetylcholine, vasopressin, glucocorticoids, thyroid hormone, endothelin-1, angiotensin II
Inflamatory cytokines	Tumor necrosis factor-α, interleukin 6
Other factors	Age, gender, circadian variation, heart rate, exercise, heat exposure, cold

ANP and BNP are released continuously from the heart, but the rate of release increases in response to appropriate stimuli. Myocyte stretch is regarded as the central regulator of ANP and BNP release. Several endogenous vasoactive factors, neurotransmitter, pro-inflammatory cytokines, and hormones directly stimulate ANP and BNP secretion. In addition, there are many conditions that are associated with an increase in natriuretic peptide release (Table 2).

The natriuretic peptides are ligands for three different high affinity natriuretic peptide receptors – somewhat unfortunately labeled A-type, B-type, and C-type. These labels do not reflect an association with the similarly labeled natriuretic peptides, leading to a confusing nomenclature. Two of these receptors (A-type and B-type) are generally considered to mediate all the known biological actions of these hormones, while it has been suggested that the C-type receptor mainly has a clearance function [10]. ANP and BNP bind preferentially to the A-type receptor, CNP binds to the B-type receptor. Specific natriuretic peptide binding sites have been reported in various organs such as vascular smooth muscle cells, endothelial cells, inner medullary collecting duct cells, and in organs such as the lung, kidney, adrenal gland, liver, and intestine [9, 10]. Most of the biological functions of natriuretic peptides are mediated by intracellular accumulation of guanosine 3′,5′-monophosphate (cGMP) through the activation of a particulate guanylyl cyclase [9, 10].

Receptor-mediated binding, uptake, and metabolism in target tissues, degradation by enzymes and excretion, for example, into urine, are involved in the clearance of natriuretic peptides from the circulation [9, 10]. The major mechanisms for ANP and BNP clearance, however, are uptake by the clearance receptor (C-type receptor) and enzymatic degradation by neutral endopeptidase. These zinc metallopeptidase enzymes are present within vascular cells and tubular cells.

The natriuretic peptides exhibit a wide range of potent biological effects at multiple sites in the cardiovascular system. The physiology of ANP is very similar to that of BNP (Fig. 2). Both peptides result in increased venous capacitance, decreased vascular tone, and inhibition of the activities of several hormone systems, including the renin-angiotensin-aldosterone system, endothelins, cytokines, and vasopressin [8-10]. One of the unique properties of ANP and BNP is their ability to decrease cardiac preload without subsequently inducing reflex tachycardia. This reduction is probably caused by stimulation of vagal afferents, suppression of sympathetic outflow from the CNS, and a reduced release of catecholamines from autonomic nerve endings [11]. In addition to these vascular properties, the natriuretic

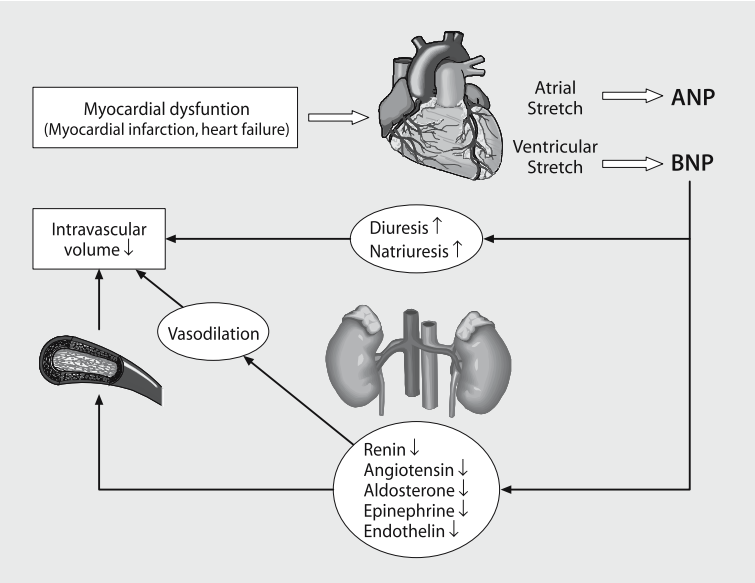

Fig. 2. Physiologic effects of the cardiac natriuretic peptides, atrial natriuretic peptide (ANP) and brain natriuretic peptide (BNP)

peptides have a direct effect on renal hemodynamics and function. Increased pressure within the glomerular capillaries and, thereby, increased glomerular filtration is the result of vasodilatation of the afferent renal arterioles and vasoconstriction of the efferent arterioles [12]. There also appears to be a direct tubular effect on sodium and water handling, resulting in natriuresis and diuresis. The net effect of these properties is balanced vasodilatation in the arterial and venous beds as well as natriuresis and diuresis. Additional evidence suggests a direct vasodilatory effect on the coronary arteries. The natriuretic peptides also appear to exhibit an antimitogenic effect on the heart and other organ systems, suggesting a potential role in the modulation of cell growth.

The physiologic effects of CNP are different from those of ANP and BNP. CNP has only limited diuretic or natriuretic effects and does not function as a circulating hormone. It is more likely to act in an autocrine or paracrine fashion in the vasculature as a vasodilator and inhibitor of vascular cell proliferation [5, 13].

Clinical Utility of Cardiac Natriuretic Peptides

Diagnostic Markers

Because of their unique characteristics and their role in cardiovascular physiology, the cardiac natriuretic peptides are currently being used as diagnostic and prognostic markers in a variety of cardiovascular conditions such as hypertension, congestive heart failure, and acute coronary syndromes [14]. The final common pathway in the majority of these diseases is left ventricular dysfunction resulting in a

process of neurohumoral activation that can ultimately lead to myocardial apoptosis, fibrosis, and remodeling. Of the natriuretic peptides, BNP seems to be the most sensitive and specific indicator of cardiac dysfunction [14]. Results from experimental and clinical studies strongly confirm the close correlation of increased blood BNP concentrations and the severity of left ventricular dysfunction [15–19]. Accordingly, increasing BNP concentrations correspond to New York Heart Association (NYHA) functional classes and closely reflect alterations in hemodynamic indices including right atrial pressure, pulmonary capillary wedge pressure (PCWP), and left-ventricular end-diastolic pressure [15, 16]. Quantitative measurement of BNP provides an indicator for assessing the degree of heart failure. Within a NYHA class, mean and median blood BNP concentrations progressively increased from class I to IV [15]. Commercially available point-of-care BNP testing allows monitoring of BNP in the emergency department or intensive care unit (ICU), so that treatment can be initiated immediately [15, 17, 18]. In the Breathing Not Properly Multinational Study, Maisel et al. studied BNP levels of 1,586 patients presenting to the emergency department with acute dyspnea [15]. In this study, concentrations of BNP were highest in patients with acute decompensated heart failure (675±450 pg/ml), intermediate in patients with ventricular dysfunction without exacerbation (346±390 pg/ml), and lowest in those without heart failure or ventricular dysfunction (110±225 pg/ml) [15]. A BNP cut-off value of 100 pg/ml had a sensitivity of 90% and a specificity of 76% in distinguishing congestive heart failure from other causes of dyspnea. For ruling out heart failure, the authors found that a BNP cut-off level of 50 pg/ml had a negative predictive value of 96%. Very importantly, a single point-of-care test of BNP performed immediately upon arrival in the emergency department was more accurate than any historical or physical findings or laboratory values in identifying heart failure as the cause of dyspnea [15].

In the single center B-Type Natriuretic Peptide for Acute Shortness of Breath Evaluation Study, 452 patients who presented to the emergency department with acute dyspnea were randomly assigned to a diagnostic strategy involving point-of-care BNP testing or not [17]. A BNP level of 100 pg/ml was used to distinguish dyspnea caused by heart failure from other causes of dyspnea. In patients with a BNP level below 100 pg/ml, the diagnosis of heart failure was considered unlikely, and other causes of dyspnea had to be investigated. In patients with a BNP level more than 500 pg/ml, heart failure was considered the most likely diagnosis, and therapy with angiotensin-converting-enzyme (ACE) inhibitors, morphine, and diuretics was recommended [17]. Rapid measurement of BNP in the emergency department reduced the need for hospitalization and intensive care by 10 percentage points. The median length of hospital stay was reduced by three days, and the mean total cost of treatment by about $1,854, with no adverse effects on mortality or the rate of subsequent hospitalization [14, 17]. Accordingly, a single US Food and Drug Administration (FDA) approved cut-off value of 100 pg/ml has been established for the diagnosis of congestive heart failure. This cut-off level, however, is still under debate, because BNP levels ranging from 100 to 500 pg/ml may be attributable to causes other than congestive heart failure [19]. There is some evidence that age, gender, and renal impairment might affect plasma BNP concentrations, especially in critically ill patients [19, 20]. Chronic kidney disease may be an important confounding factor that influences the cut-off point for BNP in the diagnosis of heart failure. Thus, McCullough et al. suggested a higher cut-off value of 200 pg/ml for patients with markedly reduced glomerular filtration rate and those undergoing hemodialysis [19]. In a prospective cross-sectional study of 121 patients

admitted to a general ICU, McLean and colleagues found that elderly individuals and women have BNP levels that are 2 to 3 times higher than those seen in younger individuals and men [20]. These findings suggest that the application of a single cut-off level for BNP may not be adequate to diagnose cardiac dysfunction in critically ill patients. Hence, BNP must be viewed in conjunction with the clinical scenario and patient characteristics, including age, gender, and the presence or absence of renal disease.

BNP as a Prognostic Tool

A number of studies have demonstrated the prognostic value of BNP testing and it is now well established that in patients with heart failure and acute coronary syndromes higher concentrations of BNP are associated with increased cardiovascular and all-cause mortality, independent of age, NYHA class, previous myocardial infarction, and left-ventricular ejection fraction [16, 21–24]. In a study of 452 patients with a left-ventricular ejection fraction < 35%, Berger et al. found by univariate analysis that BNP levels were strong and independent predictors of sudden death [21]. A cut-off BNP level of 130 pg/ml differentiated patients with high and low survival rates. Using this cut-off point, only 1 of 110 patients (1%) below the cut-off point, but 43 of 227 patients (19%) above the cut-off point, died suddenly. Thus, measurement of plasma BNP allows classification of patients with higher risk of sudden death. To determine the prognostic value of BNP in acute coronary syndrome, de Lemos et al studied 2,525 patients presenting with a broad range of ischemic symptoms, including myocardial ischemia without ST-segment elevation and transmural myocardial infarction [24]. A single measurement of BNP obtained in the first days (40±20 hours) after the onset of symptoms correlated with the long-term risk of death and non-fatal cardiac events, including a second myocardial infarction or the need for cardiac catheterization or coronary artery bypass surgery. At 30 days and 10 months, the odds ratio of an adverse outcome ranged from 3.8 to 5.8 fold and was proportional to the concentration of BNP. Moreover, this study demonstrated for the first time that BNP was predictive of recurrent ischemic events when the BNP level was greater than 80 pg/ml [24].

Other Roles for Cardiac Natriuretic Peptides

Monitoring of Drug Therapy and Therapeutic Efficacy

Having identified BNP as an important marker for diagnosis and risk stratification, it remains to be elucidated whether this marker can be also used to guide appropriate drug therapy in patients with heart failure or acute coronary syndromes. In a study by Troughton et al., 69 patients with symptomatic heart failure were allocated at random to receive treatment with ACE inhibitors, vasodilators, and diuretics guided by plasma NT-BNP concentrations or by standardized clinical assessment [25]. The treatment target in the clinical group was clinically compensated heart failure according to an objective score, and in the BNP group, NT-BNP concentrations below 200 pmol/l. Whenever these targets were not achieved, drug treatment was intensified according to a strict and predetermined stepwise protocol comprising maximization of ACE inhibitors, increase in loop diuretics, and addition of digoxin, spironolactone, and isosorbide mononitrate. Using death, hospital admission,

or heart failure decompensation as clinical endpoints at nine month, patients monitored by NT-BNP had a reduction in total cardiovascular events and delay to the presence of the first event [25]. In another study, in 30 patients with idiopathic dilated cardiomyopathy, Kawai et al. analyzed the relationship between changes in plasma BNP levels and left-ventricular function during beta-blocker therapy [26]. They found that beta-blocker therapy improved left-ventricular function and significantly decreased BNP concentrations during 6 months of treatment. Thus, these studies suggest that BNP might be useful for monitoring the success of drug therapy. However, large prospective clinical trials are needed to fully determine the potentials of BNP-guided drug therapy.

Therapeutic Uses

Currently patients with heart failure are treated with inhibitors of the renin-angiotensin-aldosterone system, e.g., ACE inhibitors, angiotensin-II-receptor antagonists, aldosterone antagonists, which produce vasodilation, induce natriuresis and diuresis, and medications that block beta-adrenergic receptors and suppress neurohumoral overactivity. Given the favorable biological effects of ANP and BNP, it is not surprising that a synthetic recombinant human BNP ("nesiritide") was recently approved by the FDA for short-term infusion in patients with decompensated heart failure, to improve signs and symptoms of volume overload and cardiac decompensation. The results of two important clinical trials have been reported demonstrating the utility of nesiritide in patients who required hospitalization and intravenous therapy for decompensated congestive heart failure [27]. The first was a double-blind, placebo-controlled efficacy trial designed to assess short term hemodynamic responses to nesiritide. Patients in this trial underwent pulmonary artery catheter monitoring and a PCWP >18 mmHg was required for entry. After oral vasoactive medications and intravenous diuretics had been withheld for at least 4 hours, 127 patients were randomized to receive either placebo or nesiritide at a dose of 0.015 or 0.03 μg/kg/min. The infusion was continued for 6 hours with a 50% dose reduction allowed for hypotension. The primary endpoint in this study was a decrease in PCWP at 6 hours with several secondary endpoints, including other hemodynamic measures and patient global clinical status and symptoms. At 6 hours, the two doses of nesiritide provided a significant dose-dependent decrease in PCWP by 6 and 9.6 mmHg versus a 2 mmHg rise in the placebo arm. Statistically significant decreases in right atrial pressure, systemic vascular resistance, and a moderate, but statistically significant increase in cardiac index were observed with the infusion of nesiritide. Nesiritide also provided global improvement in clinical status with both doses compared to placebo. In the second trial, patients were randomized to either nesiritide in the two doses used in the previous trial or standard therapy with a conventional intravenous vasoactive agent chosen at the discretion of the investigator [27]. Patients generally received treatment with vasoactive drugs for one to two days but a small percentage of all treatment groups (9–14%) were treated for more than 5 days. In this trial, although there were no significant differences in global status, dyspnea, or fatigue among the three groups, there were less intravenous diuretics given to those patients on nesiritide despite a similar loss of weight. Asymptomatic or mild dose-related hypotension was the most common adverse effect in both trials. Overall, nesiritide functions as both a potent venous and arterial vasodilator and has been shown to improve cardiac hemodynamics more rapidly

and to a greater extent than conventional vasodilator treatment, as well as having fewer side effects. Therefore, recombinant human BNP may offer a new and useful form of therapy for the treatment of decompensated heart failure.

Conclusions

ANP and BNP are secreted from cardiac myocytes in response to atrial or ventricular wall stretch. The physiologic effects of both cardiac natriuretic peptides include natriuresis, diuresis, and inhibition of the activities of several neuroendocrine systems, including the renin-angiotensin-aldosterone system, endothelins, cytokines, and the sympathetic nervous system. Single and serial plasma measurement of BNP is a promising tool for diagnosis and risk stratification of patients with heart failure and acute coronary syndromes. Levels of BNP could also be used to guide drug therapy in these patients. Finally, the administration of nesiritide, a synthetic recombinant human BNP, appears to offer a novel approach in the management of acute heart failure.

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Heart Failure as a Co-Morbidity in the ICU

M. R. Pinsky

Introduction

We often treat patients with heart failure in the intensive care unit (ICU) setting, and clearly, severe heart failure carries a very high mortality rate. However, non-fatal heart failure commonly accompanies processes that cause patients to become critically ill. In these cases, heart failure becomes a co-morbidity. Although intuitively obvious that one needs forward blood flow to sustain life, it is not clear to what extent decreased cardiac reserve impairs outcome from acute illness other than acute coronary syndrome. It is important, therefore, to consider the impact that heart failure may have on outcome from critical illness.

Heart failure, defined as a reduced ability to sustain forward blood flow, can affect mortality from critical illness in several ways (Table 1). If severe enough, heart failure leads to a low output state associated with organ failure and pulmonary edema. Clearly, if the heart is unable to pump blood, life cannot be sustained. Hypoperfusion causes profound microcirculatory disturbances [1]. However, such events are exceedingly rare, except in the conditions of acute heart failure following myocardial ischemia or cardiac reconstructive surgery. Still, profound cardiovascular collapse is the hallmark of the terminal phase of most critical illness. Where heart failure primarily expresses increased morbidity and mortality is at the start of critical illness and at its resolution. In the early stages of critical illness, being able to sustain an appropriately elevated cardiac output to maintain sufficient tissue oxygen delivery (DO_2) is a major factor determining outcome, whereas increased DO_2 later in the course of critical illness is not associated with improved outcome. Thus, when cardiac reserve limits the initial maximal cardiac response to increasing metabolic demands in patients with severe sepsis and related critical illness, mortality

Table 1. Impact of heart failure on outcome from critical illness

Severe heart failure
▮ Primary organ hypoperfusion
– Gut hypoperfusion
– Pre-renal azotemia
▮ Acute respiratory failure
Mild to moderate heart failure
▮ Failure to attain 'survivor levels' of DO_2
▮ Prolonged mechanical ventilation

increases. Second, following critical illness or insults, impaired cardiac reserve limits maximum exercise tolerance. Cardiovascular impairment is the most common cause of failure to wean from mechanical ventilatory support, prolonging morbidity. Let us review these two distinct processes separately.

Achieving Maximal Oxygen Delivery

All studies of septic shock and high-risk surgical patients have documented that mortality is inversely proportional to the maximal level of DO_2 that can be spontaneously generated by the patient [2]. Although originally considered 'survival levels' of DO_2, these data primarily defend that sustained cardiovascular fitness is associated with improved survival. In support of this concept, the more profound the circulatory shock the greater the degree of anaerobic metabolism and the higher the circulating lactate levels [3]. Thus, the impact of heart failure on outcome from critical illness is complex. It appears to be related more to maximal sustainable levels of cardiac output, than to ventricular pump function *per se*. The case for this argument follows.

Clearly, resuscitation from circulatory and respiratory failure represents the mainstay of emergency and critical care management. Most of the time, resuscitation is done from a position of relative hypovolemia [4]. However, clinical studies have demonstrated that restoration of total blood flow, arterial oxygenation and even arterial pressure to otherwise normal levels by the use of vasoactive agents is not universally good for either organ function or host outcome, if delivered late in the course of critical illness [5]. Exogenous vasopressor therapy impairs normal autoregulation of blood flow among organs and may induce occult tissue ischemia in vital but silent vascular beds, such as the gut mucosa and renal subcortex. Furthermore, microcirculatory oxygen utilization is more a function of local metabolic demands and capillary flow than global blood flow or arterial oxygen content. Regrettably, significant regional ischemia or rescue can occur without perceptible changes in global oxygen uptake (VO_2) [6]. Although fluid and vasopressor therapies may normalize organ perfusion pressure they may not induce normal organ perfusion nor prevent organ dysfunction [7].

Still, it is clear from numerous clinical studies that tissue hypoperfusion is bad and that avoidance of ischemia improves outcome from stress states. Early aggressive resuscitation, referred to as early goal-directed therapy, was shown in one study to improve outcome from severe sepsis if delivered in the Emergency Department of a single medical center [8]. Thus, the rapid restoration of normal hemodynamics by conventional means, including fluid resuscitation and surgical repair, results in a superior outcome than inadequate or delayed resuscitative efforts.

Since critically ill patients often have abnormal blood flow regulation, increasing DO_2 to what would otherwise be considered supranormal levels theoretically may treat the lethal occult tissue hypoxia that is a hallmark of many forms of circulatory shock. Accordingly, interest centered on 'hyper-resuscitation' such that DO_2 is exogenously increased to supranormal levels, levels often seen in subjects who spontaneously survive acute circulatory insults, the so-called 'survivor levels' of DO_2. Most studies that have aimed at augmenting DO_2 or VO_2 to 'survivor levels' have documented that if DO_2 can increase, subjects do better [9]. However, this improvement in survival appears to be independent of whether the subject was part of the group with intentional augmented DO_2.

Only two prospective clinical trials of aggressive resuscitation have data that would allow one to address the issue of the impact of cardiac reserve of resuscitation outcome. The first study was of septic shock patients from a single center. Tuchschmidt et al. [10] studied 51 critically ill patients, 25 of whom were given supranormal DO_2 targets. Just like all other studies that addressed this issue previously, they found that mortality was similar in both groups. However, those patients who spontaneously reached the higher 'survival levels' of DO_2, independent of treatment group, had a markedly improved survival. Though not specifically addressed in this study, these data are consistent with the assumption that baseline cardiac reserve plays a major role in determining outcome from critical illness. The second study was a multicenter study of patients with diverse causes of critical illness. Gattinoni et al. [11] studied 762 critically ill patients from 56 centers. These patients' diagnoses included high-risk surgery, massive blood loss, sepsis, respiratory failure, and trauma. Goals of therapy were separated into three treatment groups: I: CI 2.5–3.5 l/min/m^2; II: CI >4.5 l/min/m^2; and III: SvO2 >70%. Importantly, therapeutic goals were achieved in less than half of group II and group III patients, demonstrating that primary cardiac depression was a central part of the failure to achieve these target resuscitation goals. By *post hoc* analysis those subjects that did not reach these target DO_2 related goals did not have an increased mortality (personal communication). As in the study by Tuchschmidt et al. [10], although these data do not specifically address the issue of cardiac reserve, they are consistent with the assumption that baseline cardiac reserve plays a major role in determining outcome from critical illness.

One can take this concept further in patients with established sepsis. Aggressive therapies aimed at augmenting DO_2 may actually increase mortality because the artificial increase in cardiac output induced by therapy once organ injury has occurred should not improve organ function but will still be associated with the complications of that therapy. In support of that concern, Hayes et al. [5] studied 100 critically ill patients with severe circulatory shock, stratifying them to either aggressive supranormal DO_2 levels or normal DO_2 levels. These investigators found a markedly increased mortality in the treatment group compared to the control (54 v. 34%, $p<0.05$). Thus, the use of aggressive hyper-resuscitation therapies and supranormal levels of DO_2 in patients with established sepsis and organ injury is dangerous and should not be done.

Hence, a low DO_2 in a critically ill patient is probably a marker of critical illness, rather than a parameter of effective resuscitative therapy. Interestingly, the most impressive beneficial outcomes from clinical trials have all included prevention of hypoperfusion rather than resuscitation from shock [8]. This form of preemptive resuscitation, treatment before the insult has even occurred, is referred to as 'pre-optimization'. An impressive number of studies have documented that attaining high levels of DO_2 prior to high-risk surgery [12] or during the initial hour of presentation with severe sepsis [8] improves outcome, even if survivor levels of DO_2 are not achieved [13].

Based on the above evidence, aggressive hemodynamic therapies in patients in septic shock or at risk for development of multiple organ dysfunction and death improves survival if given before or during the onset of tissue injury. Often these benefits are seen without measurable differences in DO_2 or VO_2 during therapy [12, 13]. The cumulative clinical data to date suggest that a major benefit of aggressive resuscitation therapy is realized only if efforts are started very early and primarily when the host can manifest an increased cardiac output response. However,

once circulatory shock and/or organ dysfunction has occurred there appears to be little additional benefit and real risk of harm from aggressive resuscitation therapies that increase DO_2 or VO_2 to levels above what would otherwise be considered normal. Thus, the contribution that ventricular pump function plays once initial resuscitation has finished is unclear, but probably of lesser relevance.

Is Heart Failure a Result of Critical Illness?

Up until this point, we have assumed that the patient presents with critical illness and preexistent heart failure. However, cardiac injury commonly occurs with critical illness due to associated hypotension and decreased coronary perfusion or from direct myocardial injury, as is the case with anterior chest trauma causing myocardial contusions. Still, many critically ill patients, especially those with hypotensive septic shock, often have an increased cardiac output following fluid resuscitation. How then is it possible that they have impaired cardiovascular reserve? This may not be an entirely academic question, because treatments aimed at restoring vasomotor tone increase arterial pressure. Animal studies have long shown that vasopressors induce profound hypoperfusion in septic shock [14]. Similar findings were seen with inhibitors of nitric oxide synthase (NOS) [15]. Several recent clinical trials have documented that patients with severe sepsis have impaired cardiac reserve. However, this impaired reserve is masked by the pathological vasodilation that sustains a decreased left ventricular afterload. Two recent clinical trials of the inducible NOS inhibitor, L-NMMA, in human sepsis underscore this point. Kilbourn et al. [16] showed that NOS inhibition reduced blood flow while restoring blood pressure in cancer patients being treated with interleukin (IL)-2 chemotherapy. Furthermore, the multicenter clinical trial of L-NMMA in the treatment of sepsis was stopped because of increased mortality in the treatment group, which to my thinking was because of the decreased cardiac output and regional blood flow it induced. Thus, cardiac performance is not a major issue in determining outcome from critical illness once stabilized, unless the system is altered artificially to increase its workload. Then, occult heart failure will emerge.

Heart Failure in Severity Scoring Systems

Another way to examine the impact of heart failure on outcome from critical illness is to look at large retrospective data sets used to predict risk. Systems, such as APACHE II, simplified acute physiology system (SAPS) II, and sequential organ failure assessment (SOFA) scores, all attempt to estimate risk of a bad outcome from assessing measures of pre-morbid functional status, physiological state and intensity of interventions. Interestingly, the role played by heart failure, *per se*, figures minimally in these scoring systems. Even if one focuses on the EuroSCORE analysis of post-cardiac surgery mortality risk, the impact of isolated reductions in left ventricular ejection fraction are small [17]. For example, for a 65-year old male without other co-morbidities, having the ejection fraction decrease from $>50\%$ to 30–50% to $<30\%$ increases perioperative mortality risk from 1.3 to 1.9 to 3.8%, respectively, whereas the presence of an acute myocardial infarction in an otherwise healthy (left ventricular ejection fraction $>50\%$) 65 year old male would increase

mortality to 2.2%, or critical perioperative status to 3.2%. Importantly, if one has both depressed ventricular function (left ventricular ejection fraction < 30%) and a critical perioperative state, the risk of death increases to 8.9%. Thus, heart failure and critical illness are coupled in their impact on outcome, not merely additive. However, based on all the data summarized above, the role of heart failure outside of its ability to limit the initial hyperemic response to acute stress is probably minimal.

Preventing Liberation from Mechanical Ventilation

Having said that cardiac reserve is of minimal importance during the recovery phase of critical illness, does heart failure play a role further on in the course of critical illness? The answer is most definitely yes, but in the specific conditions in which sustained increases in DO_2 are needed to support independent function. Here, the most widely studied phenomenon is the role that heart failure plays on the ability to wean from mechanical ventilatory support. Most studies of weaning from mechanical ventilation demonstrate that subjects do not 'wean' *per se*, they merely stop needing ventilatory support. Mechanical ventilation, within the context of respiratory failure, then relates to the work cost of breathing by reversing respiratory muscle fatigue and decreasing the stress on the cardiovascular system, allowing it to deliver blood to other organs. The act of slowly decreasing ventilatory support is done primarily to allow the cardiovascular system to adjust to increased demands and to allow the physician to identify impending cardiovascular collapse prior to it becoming a crisis. The primary presenting sign of cardiogenic shock is acute respiratory failure [18].

The transition from positive-pressure to spontaneous ventilation (weaning) can profoundly alter cardiovascular function via complex, conflicting, and often opposite processes. These processes reflect the interaction between myocardial reserve, ventricular pump function, circulating blood volume, blood flow distribution, autonomic tone, lung volume, intrathoracic pressure, and the surrounding pressures for the remainder of the circulation. Clearly, the final response to ventilatory stress is dependent on the baseline cardiovascular state of the subject.

Lung volume increases in a tidal fashion during both spontaneous and positive-pressure inspiration. However, intrathoracic pressure increases during positive-pressure inspiration due to passive lung expansion to increasing airway pressure, whereas intrathoracic pressure decreases during spontaneous inspiration owing to the contraction of the respiratory muscles. Thus, changes in intrathoracic pressure and the metabolic demand needed to create these changes represent the primary determinants of the hemodynamic differences between positive-pressure and spontaneous ventilation [19].

Spontaneous Ventilation is Exercise

Although ventilation requires normally less than 5% of total DO_2 [20], in lung disease states the work of breathing is increased, such that its metabolic demand for oxygen may reach 25% of total DO_2. If cardiac output reserve is also limited, then blood flow to other organs can be compromised, causing tissue hypoperfusion, ischemic dysfunction, and lactic acidosis [21]. When subjects are weaned from mechanical venti-

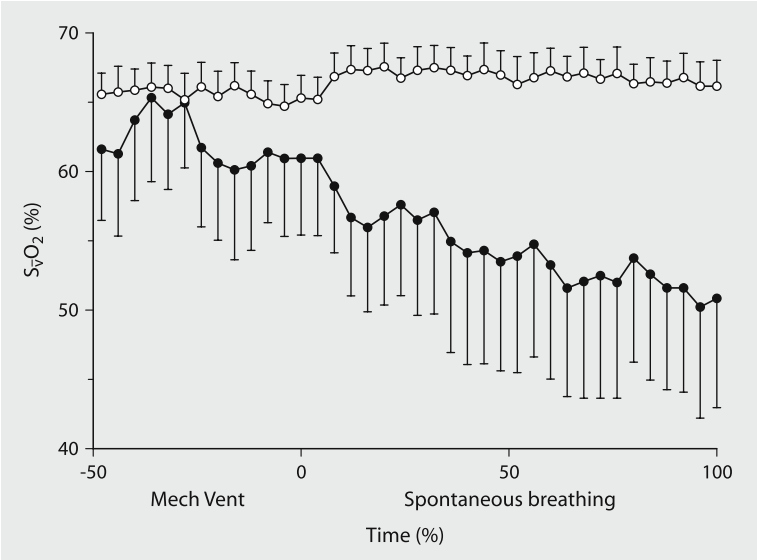

Fig. 1. Dynamic changes in SvO_2 in patients who successfully weaned (open circles) and did not wean (closed circles) from mechanical ventilatory support. From [28] with permission

lation they experience an obligatory increase in the work of breathing, which can be minimized by mask continuous positive airway pressure (CPAP) [22]. The resultant cardiovascular effects of this spontaneous ventilation exercise will include a decreased DO_2 to other organs, increased serum lactate levels, and decreased mixed venous oxygen saturation (SvO_2). The obligatory decrease in SvO_2 may result in a decreased PaO_2 if fixed right-to-left shunts exist, even if cardiac output and gas exchange are maintained at their baseline levels. Finally, if cardiac output is severely limited, respiratory muscle failure develops despite high central neuronal drive, such that many heart failure patients die a respiratory death prior to cardiovascular standstill [23].

Ventilator-dependent patients who fail to wean from mechanical ventilation may display impaired baseline cardiovascular performance [24], but routinely develop signs of heart failure only during weaning. The transition from positive-pressure to spontaneous ventilation can be associated with pulmonary edema [24], myocardial ischemia [25, 26], tachycardia, and gut ischemia [27]. Jubran et al. [28] demonstrated that although all subjects increase their cardiac outputs in response to a weaning trial, consistent with the increased metabolic demand, those who subsequently fail to wean also display a decrease in SvO_2 saturation (Fig. 1). Since weaning from mechanical ventilatory support is a cardiovascular stress, it is not surprising that weaning-associated electrocardiographic (EKG) and thallium cardiac blood flow scan–related signs of ischemia have been reported in both subjects with known coronary artery disease [25] and in otherwise normal patients [26]. Similarly, initiating mechanical ventilation in patients with severe heart failure and/or ischemia can reverse myocardial ischemia [29].

Hemodynamic Effects of Ventilation Depend on Cardiopulmonary Status

In patients who are otherwise normal, their cardiovascular state is characterized by preload-dependency. Thus, in normal subjects or in patients with hypovolemia (e.g., hemorrhagic shock, severe vomiting, diarrhea, loss of vasomotor tone, spinal cord shock) cardiac output and organ perfusion are often increased during the transition to spontaneous ventilation from positive-pressure ventilation. Withdrawal of ventilatory support in patients with limited cardiovascular reserve should be done slowly, because the increased load on the heart can precipitate heart failure and pulmonary edema [24].

Patients with chronic obstructive pulmonary disease (COPD) are at an increased risk of hyperinflation, either due to bronchospasm, loss of lung parenchyma or dynamic hyperinflation (inadequate expiratory time). Hyperinflation will compress the heart, increase pulmonary vascular resistance, and impede right ventricular filling. Intrinsic positive end-expiratory pressure (PEEP, hyperinflation) alters hemodynamic function similar to extrinsic PEEP but carries with it the added burden of increased work of breathing. Importantly, during spontaneous ventilation trials, the degree of hyperinflation determines the decrease in cardiac output [30]. Most of the decrease in cardiac output can be reversed by fluid resuscitation [31, 32]. If cardiac output does not increase with fluid resuscitation, then other processes, such as cor pulmonale, increased pulmonary vascular resistance, or cardiac compression, may also be inducing this cardiovascular depression [30].

The cardiovascular benefits of positive airway pressure can be seen in the extubated spontaneously breathing patient by withdrawing negative swings in intrathoracic pressure. Increasing levels of CPAP improve cardiac function in patients with heart failure, but only once the negative swings in intrathoracic pressure are abolished [33]. Nasal CPAP can also accomplish the same results in patients with obstructive sleep apnea and heart failure [34], although the benefits do not appear to be related to changes in obstructive breathing pattern [35]. Prolonged nighttime nasal CPAP can selectively improve respiratory muscle strength, as well as left ventricular contractile function if the patient has preexisting heart failure [36].

Conclusion

Heart failure is a co-morbidity for critical illness at both the beginning and end of the critically ill process. At the beginning it limits the ability of the host to sustain an adequate DO_2 necessary to prevent the initial ischemia-induced organ injury, while at the end, it limits the host's ability to wean successfully from mechanical ventilatory support. When treating critically ill patients, one must remember that failure to achieve an adequate DO_2 may reflect occult heart failure. Since the treatment for heart failure is often the opposite to the treatment of hypovolemia, which itself is the other major cause of cardiovascular insufficiency, this consideration is of profound practical importance in the management of the critically ill.

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The Vascular Bed during Critical Illness: Evaluation in Animal Models

J. Gorrasi, J. Takala, and S.M. Jakob

Introduction

Vascular reactivity has a fundamental role in regulating blood flow and tissue oxygen consumption. Vascular tone is regulated by receptors in endothelial and smooth muscle cells which can be stimulated by biochemical signals or a physical stimulus [1]. Receptor abundance and their response to stimuli is different among the different vascular beds, which enables fine tuning between organ perfusion and oxygen consumption according to different metabolic needs [1]. Vascular reactivity contributes to maintain the adequacy of tissue perfusion in response to acute injury such as sepsis and trauma [2]. This compensatory response can redirect regional blood flow towards organs where a decrease in oxygen consumption would have detrimental consequences for the organism such as the brain and the coronary arteries [3].

Definition of Vascular Reactivity

Vascular smooth muscle is functionally different from striated cardiac or skeletal muscle. Vascular smooth muscle undergoes slow tonic contractions to maintain pressure and to reduce vessel diameter [1]. Actin, myosin, and troponin-like regulatory proteins are used for regulation of vascular smooth muscle contraction [4, 5]. Vascular smooth muscle contraction can be induced with electrical stimuli, stretching, and with chemical stimuli such as norepinephrine, angiotensin II, vasopressin, endothelin-1 and thromboxane A2 [1]. Each of these mediators binds to receptors that trigger metabolic pathways to increase the intracellular calcium concentration [6]. The intracellular calcium concentration is regulated by several mechanisms: Phosphoinositolphosphate and diacylglycerol increase calcium concentration in response to stimulation by norepinephrine, angiotensin II and endothelin-1; nitric oxide (NO) and cyclic guanosine monophosphate (cGMP) inhibit calcium entry from the extracellular space and, hence, contraction; β-agonists, by acting via G protein-coupled pathway increase cyclic adenosine monophosphate (cAMP) which in turn inhibits myosin light chain kinase.

Vascular reactivity can be defined as the normal response of a vessel after a chemical, physical or electrical stimulus under controlled conditions hence vascular reactivity dysfunction is characterized by a vessel response that is significantly different from a 'normal' response during controlled conditions.

Methods for the Evaluation of Vascular Reactivity

In vivo vascular reactivity can be assessed by measuring the regional blood flow response to a physiological or pharmacological challenge [7]. *In vitro* vascular reactivity can be evaluated by using wire myography [8]: Isolated strips from arteries are fixed in an organ chamber, and the effects of stimulation (contraction or relaxation) are measured (Fig. 1). In the organ chamber, the biological preparation is immersed in physiological buffer and rinsed several times to wash out mediators and pharmacological agents that may have been tested before. Alternatively, vessels can be cannulated, mounted on glass pipettes and perfused at fixed flow rates or at constant pressures. Pressure changes across the cannulated vessels in response to a stimulus can then be measured [8]. Vascular reactivity of small arteries can also be evaluated by intravital (and *ex vivo*) microscopy [9]. Some of the key findings from experimental models investigating vascular reactivity are summarized in Figure 2.

Methods for the Evaluation of Endothelial Function

The endothelium can be evaluated by the acetylcholine response test: Acetylcholine releases NO which induces vasodilatation by stimulation of smooth muscle guanylate cyclase and consequent production of cGMP [10]. Acetylcholine acts on endothelial cells by activation of muscarinic receptors and NO release from the constitutive NO synthase (cNOS). Hence, vasodilatation after acetylcholine stimulation demonstrates intact endothelial cell-smooth muscle cell coupling and endothelial function [11]. On the other hand, N^G-methyl-L-arginine causes endothelium-dependent contraction and inhibition of cyclic GMP formation [12].

Mediators of Vascular Reactivity

Effects of important mediators are summarized in Table 1.

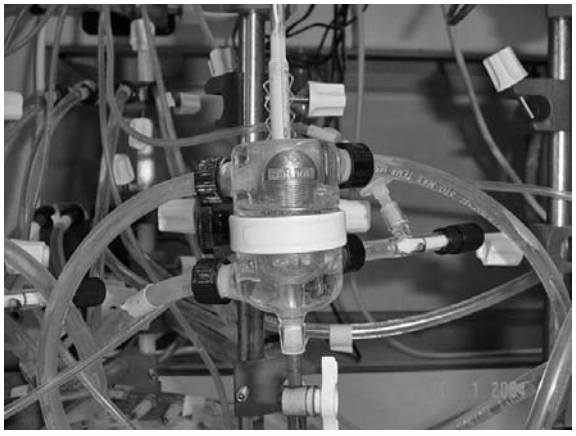

Fig. 1. Single organ chamber. Vascular rings are mounted in the chamber and connected to a pressure transducer. Each chamber has controlled conditions for temperature, oxygen tension and pH

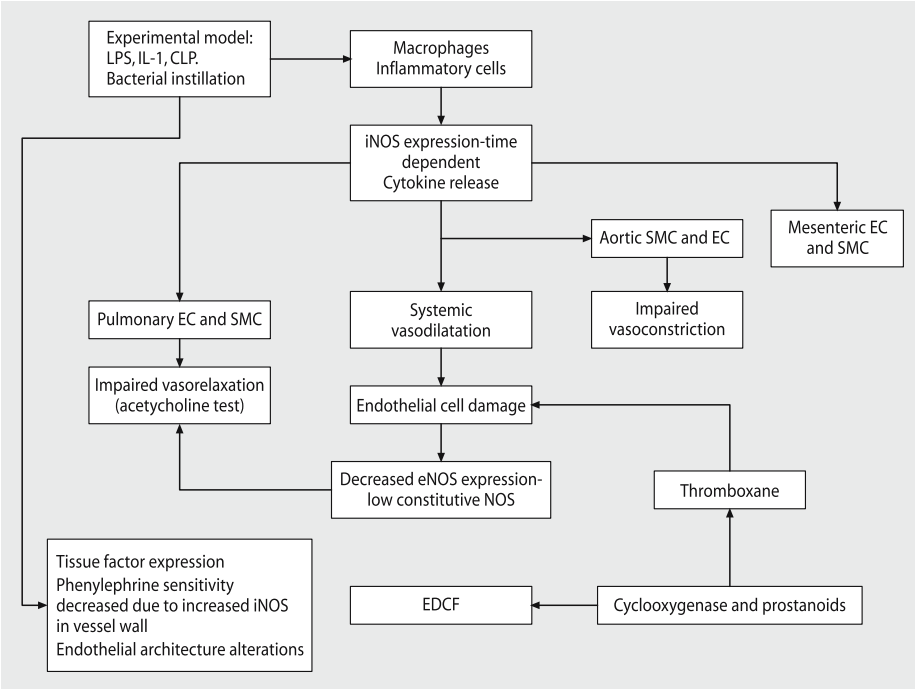

Fig. 2. Findings from experimental models investigating vascular reactivity. Inflammatory stimuli can induce inflammatory gene expression in macrophages and inflammatory cells with high expression of inducible nitric oxide synthase (iNOS) and cytokine proteins. These inflammatory proteins induce the release of vasoactive substances into the circulation. As a result, systemic vasodilatation (*in vivo*) and impaired vasoconstriction in response to adrenergic compounds (*in vitro*) occur. At the same time, these inflammatory mediators induce endothelial dysfunction and cell damage. LPS: lipopolysaccharide; IL-1: interleukin-1; CLP: cecal ligation and puncture; SMC: smooth muscle cells; EC: endothelial cell; eNOS: endothelial nitric oxide synthase; EDCF: endothelial derived contracting factor

Adenosine

The effect of adenosine on arteries has been assessed in an isolated dual-perfused liver model [13]. ATP administration elicited hepatic arterial vasodilatation. Furthermore, the administration of a purino-receptor antagonist inhibited the response to injected ATP. In ischemic conditions, ATP is metabolized to adenine nucleotides, such as adenosine in order to provide energy. Adenosine mediates increases in local blood flow at different vascular beds: It has been reported that adenosine dilates coronary arteries to allow matching between oxygen supply and consumption [14].

Prostanoids

It has been postulated that attenuated pulmonary and systemic vascular contractility is – at least in part – mediated by prostanoids and prostaglandins. This was tested in a rat model of hyperdynamic sepsis induced by cecal ligation and perfora-

Table 1. Effects of drugs commonly used to test vascular reactivity

Substance	Smooth vascular cell	Endothelial cell
Norepinephrine	Contraction	
Phenylephrine	Contraction	
KCl	Contraction	
Acetylcholine	Relaxation, at high doses contraction	Activation of muscarinic receptors
ACE inhibitors		NADPH oxidase modulation
Sodium nitroprusside	Relaxation	
Adenosine	Relaxation	
Interleukin-1	Relaxation	
Thromboxane	Contraction	
Nitric oxide	Relaxation	
Oxidation-derived products	Contraction	
Low oxygen tension	Contraction	
Angiotensin II	Contraction	
Ascorbate		Restores vasodilatation by acetylcholine

tion, and with the use of the cyclo-oxygenase inhibitor meclofenamate [15]. In septic animals, both hypoxic pulmonary vasoconstriction and the effect of phenylephrine on systemic and pulmonary arteries was impaired. When meclofenamate was administered, hypoxic pulmonary vasoconstriction improved while vasoconstriction in response to phenylephrine was not changed. These data suggest that vasodilator prostaglandins may contribute to the attenuated pulmonary pressor response in sepsis.

Endothelins

Endothelin-1 is a potent vasoconstrictor peptide [16]. During hypoxia, endothelin receptor antagonists block the hypoxic pressor response in pulmonary arteries [17]. When the endothelium is removed, the pulmonary vasoconstrictor response to hypoxia is abolished. In sepsis, blockade of endothelin-1 receptors abolishes the lipopolysaccharide (LPS)-induced pulmonary artery hypertension and worsens systemic hypotension [18].

Angiotensin Converting Enzyme (ACE) Inhibitors

In endotoxic shock in rabbits, impaired vascular relaxation was improved by adding the ACE inhibitor perindopril [19]. The effect of perindopril depended on the availability of arginine, a substrate for NOS, and was abolished by inhibition of NOS. Hence, endothelial release of NO by perindopril seems to play an important role for re-establishment of vascular relaxation properties in resolving endotoxic shock in rabbits.

Coagulation and Vascular Reactivity Dysfunction

This has been reviewed elsewhere [20]. Briefly, NO prevents platelet aggregation and clot formation [21]. Consequently, inhibitors of NOS have the potential to promote or enhance endotoxin-induced disseminated intravascular coagulation (DIC). In a porcine model of endotoxic shock, the administration of an NOS inhibitor resulted in coagulation abnormalities and histological changes consistent with increased activation of intravascular coagulation [22]. Alterations in coagulation during sepsis are associated with organ dysfunction and increased mortality [23].

Vascular Reactivity during Anesthesia and Surgery

Little is known on vascular reactivity during anesthesia and surgery in humans. In pigs, the maximal *in vitro* contraction in response to norepinephrine after surgery and prolonged anesthesia is lower in hepatic as compared to superior mesenteric arteries [24]. In rats, anesthesia and surgery had profound effects on the mesenteric pressure profile; immediately after surgery, the large arteries dissipated 4% of the total pressure drop across the hepatosplanchnic circulation, arcade small arteries 16%, the intramural circulation 67%, arcade veins 9% and the remaining veins plus the hepatic circulation 7% [25]. In conscious animals, the corresponding values were 5%, 31%, 51%, and 6%. Hence, anesthesia and surgery can induce a low mesenteric flow state with the potential to induce organ damage, especially when resting perfusion is already critical or evolving hypovolemia is not appropriately treated.

Vascular Reactivity in Sepsis, Trauma and Heart Failure

During shock, tissue hypoperfusion occurs, stimulating compensatory mechanisms in an attempt to maintain arterial pressure and organ perfusion. In this setting, vascular reactivity is modulated by physiological and inflammatory mediators, intracellular release of calcium and uptake by G protein activation. Later, the physiological vascular response (i.e., vasoconstriction) shifts towards a hypo-responsive state mediated by NO and activation of ATP-sensitive potassium channels [26]. During sepsis and severe inflammation, *in vivo* vascular reactivity is characterized by a decreased capacity to both vasodilate in response to acetycholine, and to contract in response to phenylephrine and norepinephrine (see below).

Sepsis and related syndromes release inflammatory mediators that induce vascular reactivity dysfunction, which can be associated with heterogeneous tissue perfusion and dysoxia [27–29]. Experimental data support that interleukin-1 (IL-1) plays a role in vasodilation in sepsis and during non-septic inflammation [30]. The effect of IL-1 on vascular contraction was tested using phenylephrine and potassium chloride (KCl), both with and without intact endothelium. IL-1 inhibited vascular smooth muscle contraction to both phenylephrine and KCl. This effect was not endothelium-dependent since de-endothelization did not alter the response. However, it has been shown that the effect of IL-1 on vascular reactivity is mediated by guanylate cyclase activation in response to modification of protein synthesis [31]. In vascular smooth muscle cell cultures, cGMP and oxidative products of NO such as

nitrite increased after 6 hours, with a maximum at 36 hours, when stimulated by IL-1 [32]. These effects were absent when either protein synthesis was blocked or L-arginine subtracted from the culture medium. In summary, these results suggest that during sepsis, inflammatory substances such as interleukins induce NO production in vascular smooth muscle and endothelial cells, which can mediate vascular tone and initiate tissue blood flow redistribution. Hence, the refractory vasodilation that characterizes hyperdynamic septic shock is mediated by enhanced release of NO.

Sepsis also induces endothelial-cell injury and pulmonary hypertension that promote modifications in vascular reactivity [33]. In a swine model of *Pseudomonas* sepsis, a decreased response of pulmonary vessels to KCl and impaired acetylcholine-induced relaxation was found [34]. In contrast, the response to sodium nitroprusside was not altered. However, contractility and endothelial functions decreased after five hours exposure to *Pseudomonas*. These results suggest a sepsis-induced alteration in pulmonary artery endothelial cell receptor sensitivity to acetylcholine, and a sepsis-induced alteration of muscle contraction pathways.

Regional differences of vascular reactivity have been evaluated in a rat model of sepsis [35]. In this model, the *in vitro* full contraction properties of arteries, norepinephrine response and vasorelaxation properties were tested after endotoxin infusion. The effects of endotoxemia (attenuated vasoconstriction to depolarizing KCl and the decrease of maximum contractile force with norepinephrine) were more pronounced in renal and coronary arteries than in superior mesenteric and hepatic arteries. Renal arteries showed an improvement after aminoguanidine administration and in the absence of L-arginine (a substrate for NOS). In contrast, the response of the hepatic artery was not influenced by aminoguanide or L-arginine. After 20 hours of endotoxemia, the regional variability of vascular reactivity dysfunction in response to alpha agonists increased. It has been suggested that this could be due to a regionally heterogeneous decrease in calcium sensitivity [35].

During sepsis, inflammatory mediators promote free radical production and oxidative stress [36]. It has been reported that oxidative stress plays a role in vascular dysfunction during sepsis [37]. In a mice model of cecal ligation and puncture, intravenous administration of ascorbate restored arterial responsiveness and normalized NO metabolites and inducible NOS (iNOS) expression [38]. Restoration of vascular reactivity dysfunction by ascorbate can also be explained by prevention of excessive NO production [39].

However, when endothelial NOS is blunted during sepsis, endothelium-dependent relaxation is altered. This has been demonstrated in the microcirculation and in large arteries in a rat model of polymicrobial sepsis [40]. In this model, the endothelium was selectively affected because both the contractile force after norepinephrine stimulation and relaxation in response to sodium nitroprusside were preserved. Since vasodilatation improves microcirculatory blood flow [41], inhibition of NOS does not seem to be a useful clinical option. In fact, NOS inhibition by NG-methyl-L-arginine hydrochloride has been associated with increased mortality in a clinical trial [42].

Vascular Reactivity during Hypoxia and Low Cardiac Output States

Regional ischemia and acidosis during cardiopulmonary bypass (CPB) and in low flow states such as cardiac tamponade or cardiogenic shock may induce blood flow redistribution and/or alterations in regional oxygen extraction [43–45]. Oxygen supply and consumption are tightly regulated by vascular tone [46]. Endothelial cells react to hypoxia through a complex mechanism of depolarization and hyperpolarization that is transmitted along the vascular bed through vascular gap junctions. It has been demonstrated that hypoxia induced relaxation is mediated by NO, and that NO production is regulated by tissue PO_2 [47, 48].

During cardiac surgery and in low flow states, especially when vasopressor agents are used, vascular reactivity dysfunction is likely to contribute to the increased risk of mesenteric ischemia [49]. In a rat model of CPB, vascular reactivity dysfunction was associated with increased plasma levels of tumor necrosis factor (TNF)-α [50], suggesting an association between inflammation and vascular dysfunction even when infection is absent. In another model of hemorrhagic trauma, shock and resuscitation, mean arterial pressure was lowered to 40 mmHg, and endothelial functions were evaluated after hemorrhage had been completed and 1.5 and 4 hours following resuscitation [51]. The responses to norepinephrine and acetylcholine were both decreased at maximal bleeding, and 1.5 and 4 hours after resuscitation. In contrast, the response to nitroglycerine did not change. These results could explain the persistent and refractory hypotension despite adequate volume resuscitation after traumatic injury [51].

Vascular Reactivity and Organ Dysfunction

Hepatic dysfunction occurs early in the course of sepsis and multiorgan dysfunction and is associated with increased morbidity and mortality [52]. Release of enzymes, accumulation of metabolic products, and coagulation disorders are late biomarkers of liver dysfunction. Early induction of hepatic dysfunction is associated with vascular reactivity dysfunction with an abnormal response to vasoactive mediators such as endothelin-1 [53]. In a model of cecal ligation and puncture, endothelin-1 infusion was associated with a distinct decrease in sinusoidal diameter and volumetric flow [53]. In addition, portal pressure increased, and high plasma alanine transferase release demonstrated hepatocellular injury. These findings suggest that sepsis enhances the effects of endothelin on vascular smooth muscles in the liver.

The effect of bacteremia with and without prior hemorrhage and resuscitation was tested in a rat model using intravital microscopy [54]. Acute bacteremia, with or without prior hemorrhage, caused significant vasoconstriction in large-caliber arterioles with concomitantly decreased blood flow. This constriction was blunted at 24 hours after hemorrhage but was completely restored by 72 hours. In pre-mucosal vessels, a marked dilation was observed both at 24 and 72 hours. Hemorrhage and bacteremia resulted in a progressive enhanced reactivity to the endothelial-dependent stimulus of acetycholine in the pre-mucosal vessels at 24 and 72 hours. Reactivity to endothelial-independent smooth muscle relaxation and subsequent vessel dilation was similar for animals with and without hemorrhage prior to bacteremia. These findings indicate that there is altered endothelial control of the intestinal mi-

crovasculature after hemorrhage in favor of enhanced dilator mechanisms in premucosal vessels with enhanced constrictor forces in inflow vessels. The data also indicate that microvascular blood flow responses to systemic inflammation can be modified by prior pathophysiological events.

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Hemodynamic Monitoring

Intensive Care Echocardiography

A. S. McLean and S. J. Huang

Introduction

Echocardiography continues to exert an increasing influence in the practice of intensive care medicine. Rapidly and non-invasively obtaining diagnostically accurate pictures of the heart can lead to major management changes when treating critically ill patients. Most intensivists regularly encounter its use, either in receiving a report after the study is performed by someone else, or by personally engaging in the acquisition of the required hemodynamic information. A number of hemodynamic and cardiac function parameters can be measured including cardiac output, pulmonary artery pressure, left atrial pressure, transvalvular pressures, in addition to evaluating the presence of underlying myocardial ischemia. At the very least, an intensivist training today should, from viewing a study, be comfortable in differentiating a well volumed, strongly contracting left ventricle from an underfilled, functionally impaired heart. This chapter is a brief outline of the many applications of echocardiography that are helpful in the intensive care unit.

Comparing the relative value of either the transthoracic (TTE) or transesophageal (TEE) approach is only of use when focusing on the specific patient. Skills in both modalities are essential for the intensivist. Most information is available from the TTE, which fortunately is applied more rapidly. Yet when searching out a left atrial appendage thrombus or infected valve for example, the TEE technique is *par excellence*. Whether a TTE or a TEE is performed, the examination attempted must be a rigorous, full and systematic study. A quick glimpse only of the heart will often turn out to be inadequate and leads to sloppy technique. The TTE study outline should follow the classical 'parasternal-apical-subcostal views' approach (Fig. 1). Gastric, lower/mid/high esophageal views should be performed when performing a TEE.

Common Hemodynamic Equations in Echocardiography

Theory underlying the application of echo and Doppler needs to be encountered in that certain equations need to be appreciated. One of these equations is the Bernoulli Equation, which states that the pressure drop across any section of a conduit, such as valves or orifices, depends on the convective acceleration, flow acceleration and the viscous friction. However, the flow acceleration and viscous friction can be ignored in most clinical situations. Assuming the proximal velocity is low compared to the distal (peak) velocity, the Bernoulli Equation can be modified to:

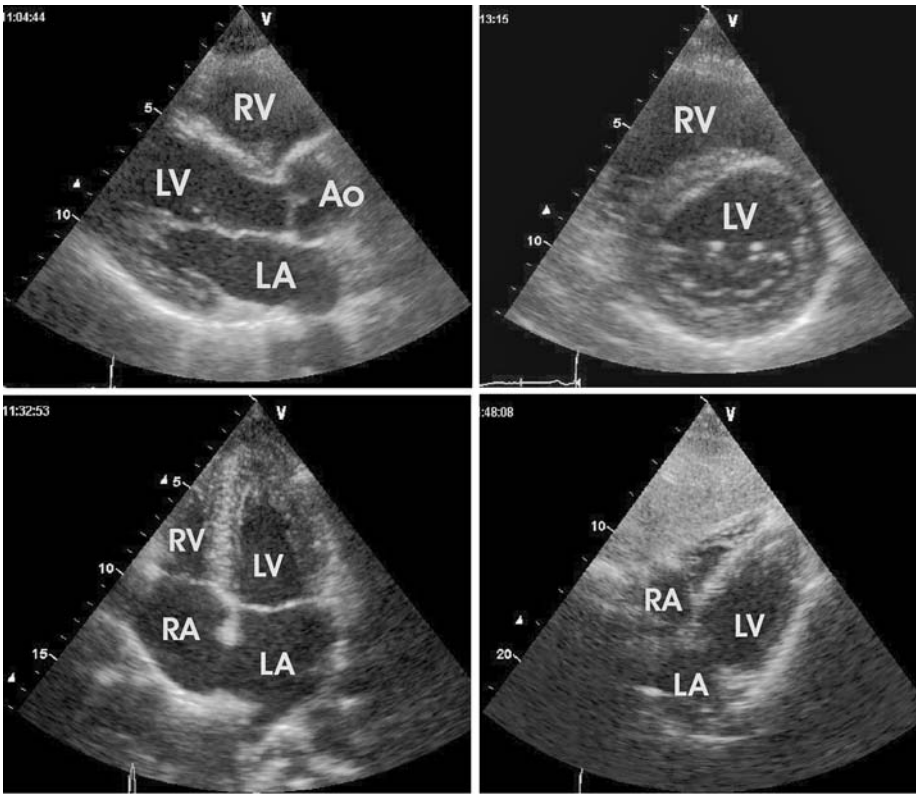

Fig. 1. Standard transthoracic echocardiographic views. Upper left, parasternal long axis; upper right, parasternal short axis; lower left, apical four chamber; lower right, subcostal. Ao, aorta; LA, left atrium, LV, left ventricle, RA, right atrium, RV, right ventricle.

$$\Delta P = 4V^2$$

where ΔP is the pressure gradient and V is the transvalvular (peak) flow velocity. This modified Bernoulli Equation is very versatile in echocardiography. It can be used to estimate chamber pressures in intracardiac shunts, to determine severity of valvular stenosis from the mean or peak pressure gradients, and to estimate pulmonary artery pressures and left atrial pressure [1].

The continuity equation is another important equation in echocardiography and can be used to calculate the area of a stenotic or regurgitant valve [2]. The concept of conservation of flow dictates that the volume of blood entering a valve must be the same as that exiting (i.e., 'what goes in must come out'). Hence, for a stenotic valve

$$A_1 \times VTI_1 = A_2 \times VTI_2$$

where A_1 is the cross-sectional area of the location proximal to the stenotic valve, A_2 is the valvular area, VTI_1 and VTI_2 are the velocity time integrals obtained at these two locations, respectively. If A_1 and both VTIs are known, the stenotic valve

area can be worked out. The application of the continuity equation is not limited to the stenotic or regurgitant valve, it can also be used to estimate the ratio of pulmonary flow to systemic flow (Qp:Qs) in intracardiac shunt.

Assessment of Left Ventricular Function

Left Ventricular Systolic Function

The most common use of echocardiography in the critically ill patient is the assessment of left ventricular (LV) contraction. Even though the study may be suboptimal, such as in the person undergoing cardiopulmonary resuscitation, useful information is often obtained [3]. In less critically unstable patients a more objective assessment of LV ejection fraction (LVEF) is possible, with some methods being more useful in the critically ill population. A well-established method using two-dimensional (2D) echocardiography is the biplane method of discs or modified Simpson's rule, which is based on apical 4 and 2 chamber views. Calculation of volume results from the summation of multiple discs or cylinders. Most machines now contain built in quantitative programs to perform the calculations [4, 5]. Combining Doppler measurements with 2D diameter measurements allows volumetric flow to be calculated by LV outflow tract (LVOT) diameter and estimation of flow through the LVOT by pulse wave Doppler. From these data, both stroke volume and cardiac output can be calculated [6]. Although originally practiced with TTE, these same methods have been validated with TEE [7]. Other methods are available in certain patients, such as measuring dP/dt from the mitral regurgitant signal [8]. Although most studies have been performed on a stable population, there have been comparisons of some of the different methods in the critically ill population [9]. Visual estimations of the LVEF are the most frequently used, evidence indicating that in experienced hands this equates with more formal methods of assessment [10, 11]. The presence of underlying ischemic heart disease may be detected such as when segmental wall defects or aneurysm are seen (Fig. 2).

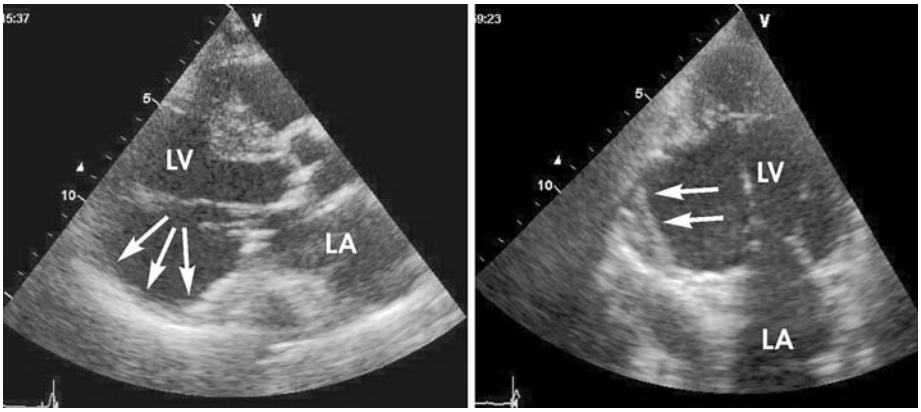

Fig. 2. Left ventricular inferior wall aneurysm in a patient with cardiogenic shock. Left: long axis view; right: apical 2 chamber view. Note the large aneurysm at the basal inferior wall (arrows). LA, left atrium, LV, left ventricle

Left Ventricular Diastolic Function

Diastolic dysfunction of the left ventricle is an area of intrigue where as clinicians we are aware of its importance, yet measuring it has proved difficult. Its importance in the critically ill is unknown but must be significant when it is estimated that 30–40% of ambulant patients with symptoms and signs of congestive cardiac have normal LV systolic function and their condition is considered to be a result of diastolic dysfunction [12, 13]. The ratio of early to late diastolic velocity (E/A), deceleration time of mitral inflow, isovolumic relaxation time (IVRT) and pulmonary wave form analysis have been useful, but far from adequate, tools for evaluating diastolic function for many years [14]. Fortunately the advent of Doppler tissue imaging (DTI) has provided more accurate and easily applied tools to assess diastolic dysfunction, as can be seen with measuring mitral valve annulus motion [15, 16].

Assessment of Right Ventricular Function

The crescentic-shaped right ventricle and its position in the chest (beneath the sternum) render the assessment of right ventricular (RV) function a challenge in echocardiography. Many of the quantitative evaluations for the left ventricle are not directly applicable to the right ventricle. For example, the estimation of RV ejection fraction (RVEF) is virtually impossible, and segmental wall motion analysis has no direct bearing on the RV assessment. To date, the function of the right ventricle is most commonly inferred from RV size and thickness – a qualitative approach. RV dilation is normally associated with volume or pressure overloading (Fig. 3). RV hypertrophy (wall thickness >0.5 cm) is regarded as abnormal and is suggestive of long-standing pressure overload (e.g., cor pulmonale), although it may also be associated with infiltrative or hypertrophic cardiomyopathies.

Quantitative evaluations of RV function are limited. The lateral tricuspid annular displacement (TAD) appears to be the easiest and most consistent method to quantify RV systolic function [17]. Briefly, the lateral peak-to-peak TAD is obtained

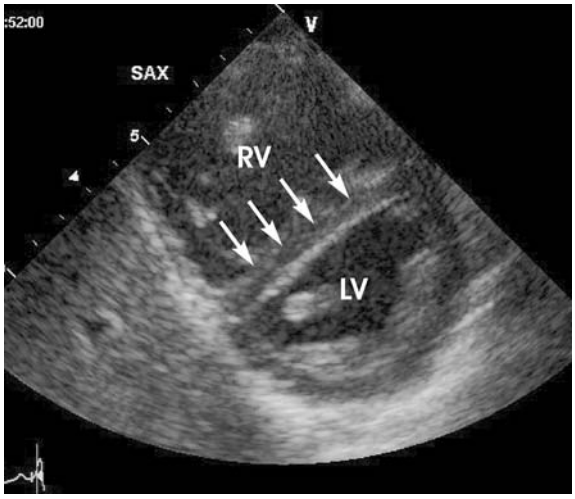

Fig. 3. Parasternal short axis view demonstrating volume overloading in an ICU patient. Note the dilated right ventricle (RV) and flattening of septum towards the left ventricle (D-shaped) during diastole.

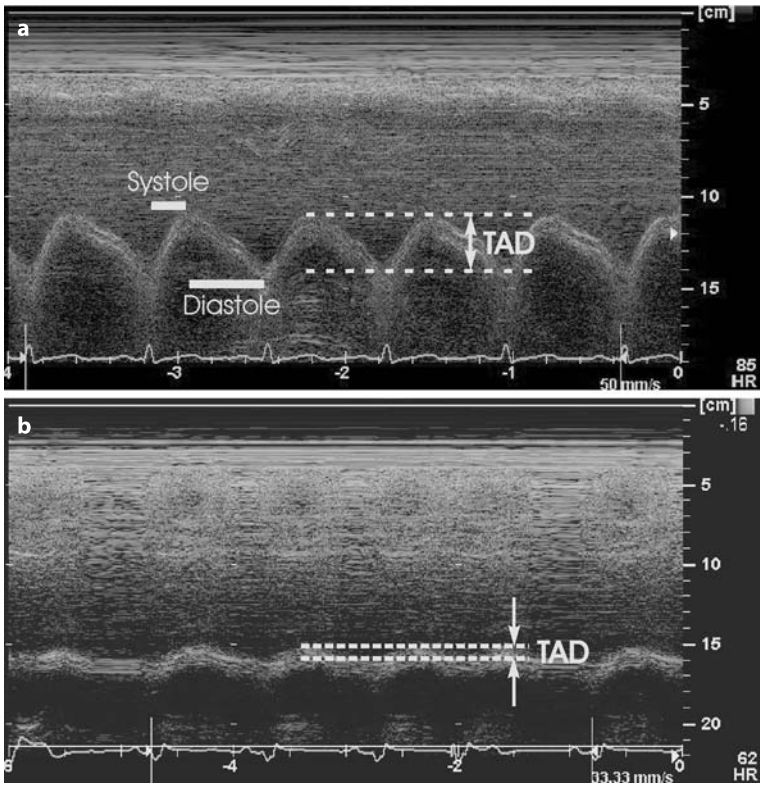

Fig. 4. Lateral tricuspid annular displacement (TAD) in a normal patient (a) and in a patient with right heart failure (b).

from the M-mode in the apical view (Fig. 4). A value of < 2.0 cm suggests impairment in systolic function. The tricuspid annular tissue Doppler velocity has also been demonstrated to correlate with RVEF [18]. Global myocardial function can be assessed by the myocardial performance (or Tei) index – calculated from the measurement of Doppler-derived time intervals (Fig. 5) [19]. The index is a measure of the ratio of isovolumic time intervals and ejection time. While the isovolumic contraction time increases and ejection time decreases in systolic dysfunction, the IVRT is prolonged in diastolic dysfunction. Prolongation of the Tei index is, therefore, associated with RV dysfunction. It should be noted, however, that although this index has been well validated in some categories of right heart dysfunction, the validity is nullified by the presence of heart block and arrhythmias.

Preload Assessment

In clinical practice the term 'preload' is used to represent the filling volume and pressures of both the right and left ventricles. A number of tools are utilized for this purpose in the ICU, including the invasive indices of central venous pressure (CVP), pulmonary artery occlusion pressure (PAOP), intrathoracic blood volume

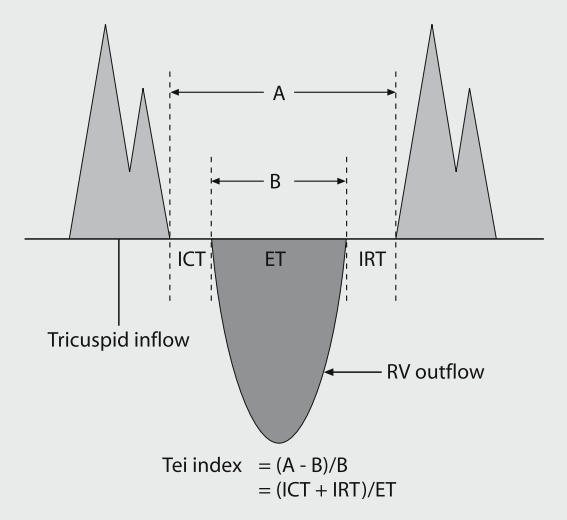

Fig. 5. Schematic diagram demonstrating the calculation of the Tei index for the right ventricle. The Tei index is calculated by measuring the time intervals A and B, and is a measure of the ratio of isovolumic time intervals and ejection time. ICT: isovolumic contraction time; IRT: isovolumic relaxation time; ET: ejection time.

(ITBV) as determined by PiCCO – all requiring invasive monitoring. Echocardiography is very useful in the critically ill patient because it is readily applied, non-invasive, and often provides additional information relevant to improving organ perfusion. If, and when, selected parameters need to be continuously monitored, the decision to proceed with invasive monitoring can be made. The fact that numerous echocardiographic techniques are available, attests either to the imprecision of some of the techniques in certain scenarios (i.e., in the patient with atrial fibrillation) or the obvious level of skill required.

Subjective review of chamber sizes, although a crude guide to preload status, can be helpful especially where marked hypovolemia is suspected [20]. Decreased size of the cardiac chambers can be a quick guide to inadequate filling, especially when resuscitating the patient. Fixed bowing of the interatrial septum, from left to right, during the cardiac cycle, indicates a PAOP greater than 18 mmHg [21]. In the operating room, the use of LV end-diastolic area (LVEDA) as a guide to volume status is well established. In the critical care setting, this parameter is not always so helpful in that it is not influenced by fluid challenge, nor predictive of increasing stroke volume with a fluid challenge [22, 23]. Pulsed wave Doppler echocardiography has been validated in non-critically ill subjects [24]. Analysis by Doppler of the mitral inflow and pulmonary vein waveforms in addition to utilizing DTI have demonstrated good correlation to invasive measurements and are helpful in the clinical setting where experienced echocardiographers perform the procedure.

DTI of the mitral valve annulus velocities can also assist in assessing left atrial pressure. Combining mitral inflow E velocity measurements with the mitral annulus Em DTI measurement, Ommen and colleagues described an accurate method of measuring LV filling pressures in 100 consecutive patients undergoing cardiac catheterization [25]. An E/Em < 8 indicated normal mean LV diastolic pressures and an E/Em > 15 indicated an elevated mean LV diastolic pressure. Although useful in the normal and very high ranges, additional Doppler data was required to determine those in the range between 8–15 mmHg. This method has been validated in critically ill ventilated patients [26].

For many years now right atrial pressure has been accurately estimated using inferior vena cava (IVC) and hepatic vein diameters and their alterations with inspiration [27]. The collapse of the IVC by >50% during inspiration indicates a right atrial pressure of <10 mmHg. A useful extension in measurements of the major venous veins has come from work by Vieillard-Baron and colleagues [28]. The variation of the superior vena cava (SVC) diameter in mechanically ventilated patients, the SVC being extrathoracic in location, serves as a guide to preload dependence (as opposed to measuring preload itself) in that it predicts the hemodynamic response to intravascular filling. The same authors have demonstrated that respiratory variation in the aortic or pulmonary outflow Doppler signal also serves as a guide to preload dependence, but when combined with the IVC variation with mechanical ventilation, is a very useful predictor of fluid loading response [29].

Miscellaneous Diagnoses and Applications of Echocardiography

Some cardiovascular pathologies are only readily obtainable by echocardiography, particularly when speed to diagnosis is important. Infectious endocarditis, thoracic aorta dissection, pericardial tamponade, left atrial appendage thrombi, intracardiac shunts, severe valvular dysfunction – if identified can lead to a dramatic change in management (Fig. 6). Alternatively ruling these pathologies out can reduce the number of investigations that require transfer of the patient outside the ICU or emergency department. Tables 1 to 3 provide quick guides to echocardiography in selected clinical scenarios.

Although echocardiography cannot fulfill a minute-to-minute monitoring purpose, it can nevertheless be used as a guide for treatment monitoring over a period of hours or days such as in fluid resuscitation, reversible myocardial depression and acute heart failure [30].

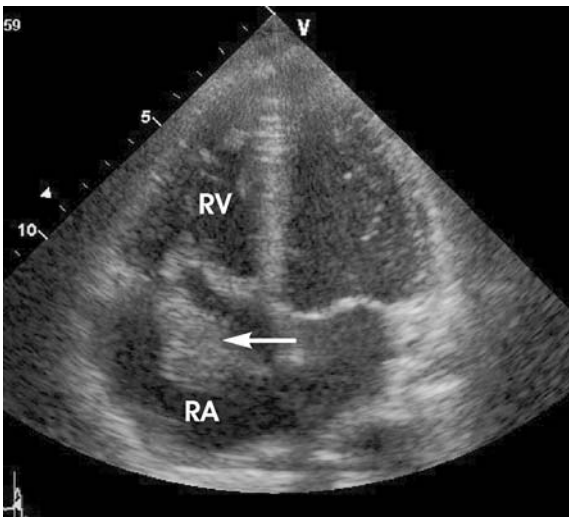

Fig. 6. Detection of endocarditis in an ICU patient. Apical four chamber view showing vegetation on the tricuspid valve leaflet (arrow).

Table 1. Guide to echocardiography: Hypotensive Patient

Consider	Review
1. Hypovolemia	▮ chamber sizes – end-systolic ventricular cavity – left atrial size – hyperdynamic small ventricles ▮ E/Em ▮ SVC variation
2. Pump failure	▮ left ventricular contraction – ? Pre-existing reduced LV contraction – ? RWMD – ischemic/non-ischemic – ? Acute myocardial infarction ▮ right ventricular contraction
3. Valvular	▮ visualize valve opening: – ? stenosis – ? subvalvular obstruction ▮ color Doppler to identify significant regurgitation ▮ presence of vegetations
4. Obstructive	▮ pericardial tamponade ▮ valvular stenosis ▮ pulmonary embolus – RA/RV size/contraction – PAP by TR method
5. Miscellaneous	▮ consider less common pathology – intracardiac shunts – LV diastolic dysfunction

E/Em: mitral inflow/mitral annulus Doppler tissue velocity; PAP: pulmonary artery pressures; RA: right atrial; RV: right ventricular; RWMD: regional wall motion dysfunction; SVC: superior vena cava; TR: tricuspid regurgitation.

▮ Training and Accreditation

Who performs echocardiography in the ICU is dictated by local institutional factors. If the ICU is within an institution with large and active cardiology or radiology departments which provide a quick efficient echocardiographic service 24 hours a day/7 days a week, then intensivists do not need to replicate the service. In smaller hospitals where limited number of intensivists work this is also desirable. Many hospitals are now in the position of having large and busy cardiology departments where immediate echocardiography availability is compromised because of competing work interests. Even where an urgent study can be performed, experienced specialist interpretation is often delayed. Yet the presence of a trained and experienced doctor during performance of the study greatly assists decision making, especially when speed is of the essence. It is on this background that many intensivists have personally taken up the challenge of performing echocardiography. The challenge for the intensivist echo trainee is twofold. The first is defining what is an acceptable level of training (and how to gain and grant accreditation). The second is how to obtain this training. The objective should be full and comprehen-

Table 2. Guide to echocardiography: Septic patient

Consider	Review
1. Intravascular volume status	▮ chamber sizes ▮ hepatic vein diameter ▮ left atrial pressure ▮ E/Em ▮ SVC variation
2. Myocardial contractility	▮ LV contraction overall ▮ LV segmental wall defects ▮ RV contraction
3. Endocarditis	▮ valvular: native/prosthetic ▮ rarely: pacing wire, Eustachian valve
4. Cardiac output	▮ Simpson's method ▮ LVOT continuity equation

E/Em: mitral inflow/mitral annulus Doppler tissue velocity; SVC: superior vena cava; RV: right ventricular; LVOT: left ventricular outflow tract

Table 3. Guide to echocardiography: Dyspneic patient

Consider	Review
1. Left ventricular failure	▮ contractility ▮ diastolic function
2. Left heart valve disease	▮ mitral ▮ aortic
3. Right heart failure	▮ contractility ▮ ventricular wall thickness ▮ chamber sizes
4. Pulmonary hypertension	▮ right heart chamber sizes ▮ PAP by TR method
5. Pericardial effusion	▮ impairment of chamber filling ▮ RA diastolic indentation
6. Pleural effusions	

PAP: pulmonary artery pressure; RA: right atrial

sive training. Echocardiographic studies (apart from TEE) are usually more difficult in the critically ill subject and hence prior adequate experience with the ambulant, relative agile subject (for better positioning during the study) is very important. Also, unlike the elective study, many critically ill patients are 'blind studies' in that the underlying diagnosis is not known prior to the commencement of the procedure. The existence of structured training courses for intensivists varies from country to country, but there are few worldwide. Critical care echocardiography is

different to that necessary for the cardiac patient and as such specific training requirements are necessary, yet paradoxically less training is available. In our institution in Australia the standards match those of cardiology training (300 TTE and 100 TEE routine studies) enhanced by a one year Fellowship based in Critical Care Echocardiography.

Perhaps intensivists should be creating an international diploma in critical care echocardiography. This would surmount many of the national politics encountered by many of our colleagues facing other craft groups whose actions are dictated by a perceived 'loss of turf'. This attractive proposition, however, faces the obvious challenges of which body takes on this responsibility, what is an acceptable training program, and how accreditation should be undertaken.

Conclusion

The interplay between cardiac function and general systemic disturbances is at the core of intensive care practice. Echocardiography is becoming 'mainstream', in that many ICUs now have their own machine and the objective is to utilize it during, as well as outside, regular working hours. Clinical urgency demands application at any time. Echocardiography frequently yields important diagnostic information, non-invasively, usually rapidly, and it can be readily reapplied as the situation demands. It has evolved from being a useful adjunct in the past to what is now an indispensable tool in the management of the critically ill patient.

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Intrathoracic Blood Volume: Clinical Applications

G. Della Rocca and M.G. Costa

Introduction

Optimization of volume status to improve cardiac performance in critically ill and in high risk patients requires adequate preload monitoring. The pulmonary artery catheter (PAC) has been a milestone in the management of the hemodynamically unstable patient in the intensive care unit (ICU) for the last 30 years. Recently, the therapeutic utility of the PAC has been challenged based on studies (whose primary objective was 'presumed' to be patient outcome) suggesting an unfavorable balance of risk and benefits [1, 2]. Kern and Shoemaker in a meta-analysis reviewed 21 randomized controlled trials with various approaches to treatment and revealed statistically significant mortality reductions with hemodynamic optimization when patients with acute critical illness were treated early to achieve optimal goals before the development of organ failure, when there were control group mortalities of more than 20%, and when therapy produced differences in oxygen delivery between the control and protocol group [3]. However, that paper stressed the timing of treatment and not the technique used for monitoring. Squara and colleagues performed a study to look at the variability of treatment with the PAC and concluded that the problem lies in the users [4]. Kumar and colleagues in a prospective, nonrandomized, non blinded interventional study demonstrated that neither central venous pressure (CVP) nor pulmonary artery occlusion pressure (PAOP) appeared to be useful predictors of ventricular preload with respect to optimizing cardiac performance [5].

Today, transesophageal echocardiography (TEE) is used in critically ill patients. The TEE is a seminvasive and safe technique that provides additional information regarding preload and contractility and can help to guide fluid replacement and catecholamine therapy [6]. Measuring left ventricular (LV) end-diastolic area (LVEDA) by TEE, although considered to be the clinical 'gold standard' for the estimation of preload, is not yet routinely performed in most operating rooms because of its high costs and dependency on physician ability. In contrast to the widely used cardiac filling pressures, end-diastolic volume estimates of the left ventricle are better indicators of end-diastolic LV fiber length, (i.e., the real preload according to the Frank-Starling law). In recent years, new devices have allowed the estimation of 'volumes', in particular the intrathoracic blood volume (ITBV) derived from the transpulmonary thermodilution technique.

Intrathoracic Blood Volume: The Theory

The transpulmonary artery thermodilution indicator technique with the injection done through a central venous line and the change in temperature sensed in a thermistor that is embedded in an arterial (femoral or axillary) catheter is now available (PiCCO System, Pulsion Medical System AG, Munich, Germany) [7–12].

The transpulmonary artery thermodilution indicator technique without the use of indocyanine green (ICG) enables quantification of the intrathoracic thermal volume (ITTV) by using the mean transit time (MTt) approach:

$$\mathrm{ITTV} = \mathrm{CO} \times \mathrm{MTt}_{\mathrm{therm}} \text{ or } \mathrm{ITTV} = \mathrm{GEDV} + \mathrm{PBV} + \mathrm{EVLW}$$

where $\mathrm{MTt}_{\mathrm{therm}}$ is the mean thermal transit time and PBV the pulmonary blood volume. According to the decay time or exponential downslope time (DSt) of indicator dilution, the DSt of the indicator allows the measurement of the largest individual mixing chamber in a series of differently sized chambers with identical flow [13]. The pulmonary thermal volume (PTV), which represents the largest mixing chambers in the system consisting of the right heart, lung, and left heart, can be quantified by:

$$\mathrm{PTV} = \mathrm{CO} \times \mathrm{DSt}_{\mathrm{therm}} \text{ or } \mathrm{PTV} = \mathrm{PBV} + \mathrm{EVLW}$$

where $\mathrm{DSt}_{\mathrm{therm}}$ is the thermal decay time and EVLW the extravascular lung water. The global end diastolic volume (GEDV), which represents the end-diastolic volume of the right and left heart, can be calculated by:

$$\mathrm{GEDV} = \mathrm{ITTV} - \mathrm{PTV}$$

The linear relationship between ITBV and GEDV described earlier now enables estimation of the ITBV by thermodilution alone. Using the model of structural regression analysis, the following equation calculates ITBV index (ITBVI):

$$\mathrm{ITBVI} = 1.16 \times \mathrm{GEDV} + 97\,(\mathrm{ml})$$

For comparison, the authors calculate in parallel the thermodilution ITBV according to the equation:

$$\mathrm{ITBVI} = 1.25 \times \mathrm{GEDV}\,(\mathrm{ml})$$

This equation is used in the manufacturer's software (PiCCO V4.1, Pulsion Medical System AG, Munich, Germany) for calculation of thermodilution ITBV [14]. It is based on the findings in a large population of non-cardiac surgery patients.

The ITBV is composed of GEDV (between 2/3 and 3/4) and to a minor degree by PBV. Total vascular volume is formed by arterial volume (AV) and by the low pressure capacitance system containing; the first represents 15% of total blood volume (TBV), while the second is 85% of TBV [14]. The application of the regression analysis concept to the ITBV/TBV ratio produces the following equation, valid in normal subjects:

$$\mathrm{TBV} = 3.2 \times \mathrm{ITBV} + \mathrm{AV}$$

where 3.2 ITBV = 85% TBV and, therefore, the ITBV is about 26% of TBV.

Recent experimental and clinical data demonstrate that a single arterial thermodilution-derived ITBV correlates well with the respective values measured by the double indicator technique [14–16]. Neumann, in an experimental model of lung injury performed in 13 mechanically ventilated pigs, showed that single thermodi-

lution ITBV and EVLW were reasonably accurate compared to the same data obtained with the double indicator technique [15]. Buhre et al. [16] comparing ITBV derived by the double indicator versus ITBV derived by the single indicator in 10 patients undergoing neurosurgical procedures, observed that during surgery in the supine and sitting positions the relative changes in ITBV values by the single indicator were similar to those assessed by double indicator dilution. The authors confirmed that the assessment of changes in ITBV by single thermodilution is valid for clinical purposes and relatively less expensive when compared with the double indicator technique. Sakka et al. [14] in a recent study, compared the simpler approach using single arterial thermodilution-derived measurements of ITBV and EVLW with the double-indicator technique. Structural regression analysis of the first two thermodilution measurements in a derivation population of 57 critically ill patients revealed ITBV = (1.25 × GEDV–28.4 ml). This equation was then applied by the authors to all first measurements in a validation population of 209 critically ill patients and single-thermodilution ITBV and EVLW were calculated and compared to thermo-dye dilution derived values. Linear regression analysis yielded a strong correlation for ITBV ($r = 0.97$, $p < 0.0001$; bias 7.6 ml/m^2 SD 57.4 ml/m^2), and EVLW index (EVLWI) ($r = 0.96$, $p < 0.0001$; bias –0.2 ml/Kg, SD 1.4 ml/Kg) confirming the accuracy of single thermodilution [14]. Reuter et al. revealed that measurement of ITBVI by single thermodilution agreed closely with those obtained by transpulmonary arterial dye dilution and enhancement in cardiac preload was adequately detected by single thermodilution ITBVI in cardiac surgical patients [12].

'Volume' Monitoring

Good correlation has been observed between the absolute values of and changes in cardiac index (CI) and/or stroke volume index (SVI) and ITBVI (Table 1). Since calculation of ITBVI mathematically relies on cardiac output, concerns have been raised as to the validity of these measurements based on mathematical coupling of data. McLuckie and Bihari showed, by increasing CI with dobutamine (in 12 euvolemic patients) and observing that ITBVI did not change, that ITBVI and CI can change independently. As CI increased, the MTt of the dye fell to a similar extent, leaving the product of the two unchanged [17]. Caution should be exercised, however, in extrapolating the findings of this study, which used the double indicator dilution COLD system (PiCCO System. Pulsion Medical System AG. Munich, Germany), to ITBVI measurements made with the single indicator dilution PiCCO system, since the method of calculating ITBVI is different with the two systems. The authors excluded mathematical coupling as the explanation for the close relationship observed between double indicator dilution measurements of ITBVI and CI and supported the continued use of ITBVI in the assessment of myocardial preload. No evidence of such coupling was found in a recent study performed by Buhre and colleagues in which cardiac output was decreased by esmolol [18].

Faybik and colleagues showed that the transpulmonary arterial dye dilution technique performed with a single indicator can be performed using room temperature instead of iced temperature injectate [19]. If room temperature injectate is used, it must be remembered that there may be slightly higher values of cardiac output and EVLWI compared to iced temperature injectates. Based on these results, the transpulmonary arterial dye dilution technique should be performed with room temperature injectates that are easy to administer and more convenient for both the

Table 1. Clinical validation of intrathoracic blood volume index (ITBVI) and global end diastolic volume index (GEDVI) as a cardiac preload index compared with cardiac index (CI), stroke volume index (SVI), or end-diastolic area (EDAI)

Authors	Year	Ref	Clinical setting	Patients	Data analyzed	r
Hinder et al.	1998	28	Cardiac surgery+ICU	15	ΔITBVI/ΔEDAI	0.87
Gödje et al.	1998	22	Cardiac ICU	30	ΔITBVI/ΔSVIart	0.76
					ΔGEDVI/ΔSVIart	0.82
Sakka et al.	1999	11	ICU-septic shock	57	ITBVI/SVI(1^{st}data)	0.66
					ITBVI/SVI(2^{nd}data)	0.67
Buhre et al.	2000	28	Neurosurgery	10	ITBV/SVI	0.78
Gödje et al.	2000	27	Heart transplantation	40	ΔITBVI/ΔSVI	0.734
					ΔGEDVI/ΔSVI	0.646
Holm et al.	2000	33	Burn shock patients	24	ΔITBVI/ΔCI	0.45 [a]
Della Rocca et al.	2002	29	Lung transplantation	45	ITBVI/SVI	0.41 [a]
Della Rocca et al.	2002	32	Liver transplantation	60	ITBVI/SVI	0.55 [a]
Hofer et al.	2002	41	Pneumoperitoneum	30	ITBVI/SVI	0.79
Schiffmann et al.	2002	39	Critically ill pediatric patients	10	GEDVI/SVI	0.76
					ITBVI/SVI	0.56
Reuter et al.	2002	12	Cardiac surgical patients	19	ΔITBVI/ΔCI	0.85
					ΔITBVI [b]/ΔCI	0.76
Michard et al.	2005	23	ICU-septic shock	36	ΔGEDVI/ΔSVI	0.72
Hofer et al.	2005	26	Cardiac surgical patients	20	ΔGEDVI/ΔSVIpa	0.267 [a]
					ΔGEDVI/ΔSVIart	0.576 [a]

[a]: $r^{2:}$ CIart: transpulmonary indicator dilution cardiac index recorded in femoral artery; SVIpa: stroke volume index (from PAC); SVIart: stroke volume index (from transpulmonary indicator dilution technique);
[b]: changes in ITBVI obtained with the single indicator technique.

patients and the medical staff. More recently, Wolf and co-workers retrospectively examined data obtained in a neurosurgical population using the transpulmonary arterial dye dilution single indicator technique to evaluate whether a single injection is sufficient to adequately measure cardiac output and derived thermodilution parameters [20]. The authors misadvise the use of a single injection for the measurement of transpulmonary thermodilution especially for preload parameters, the mean of at least three repeated measurements better reflects patient status.

Intrathoracic Blood Volume in Critically Ill Patients

ITBV has been shown to be better correlated with SVI and CI than filling pressures (CVP, PAOP) in different clinical scenarios [6–12, 21–33]. Sakka et al. [14] compared each preload variable in the early phase of hemodynamic stabilization in 57 critically ill patients with sepsis or septic shock in whom intrathoracic pressure and myocardial compliance were changing due to the course of the disease, changes

in respiratory adjustments, and alterations in catecholamine treatment. In the analysis of the first hemodynamic profiles after admission to the ICU, linear regression analysis revealed that changes in ITBV correlated better with variations in stroke index when compared with PAOP or CVP. Changes in dosages of vasoactive drugs and/or respiratory adjustment may be, in part, responsible for this finding, especially since correct determination of transmural cardiac filling pressures by PAOP may be difficult in ventilated patients with large respiratory changes in intrathoracic pressures. These data confirm the controlled studies by Lichtwarck-Aschoff et al. [21] and Gödje et al. [22], which demonstrated that ITBV is a more reliable indicator of preload than the cardiac filling pressures. Michard and colleagues demonstrated that in septic shock patients, in contrast to CVP, the transpulmonary thermodilution GEDV index behaves as an indicator of cardiac preload (ΔSVI vs ΔGEDVI $r = 0.72$, $p < 0.001$) [23]. They showed that GEDV increased with volume loading but not with dobutamine, and the lower the pre-infusion GEDV, the more marked the hemodynamic effects of volume loading. Bindels and co-workers in a prospective study performed in a medical ICU evaluated the relationships between the changes in SVI, measured in both the aorta and the pulmonary artery, and the changes in ITBVI, as well as the relationship between changes in aortic SVI and changes in PAOP. ITBVI was determined with thermal-dye dilution. The authors found a good correlation between changes in ITBVI and changes in aortic SVI while no correlation was found between changes in PAOP and changes in aortic SVI. The authors concluded that ITBVI seemed to be a more reliable predictor of cardiac filling, because changes in ITBVI closely correlated with changes in aortic SVI [24].

To assess the impact of high intrathoracic pressures on LV volume and function and to test the hypothesis that right ventricular (RV) end-diastolic volume (RVEDV) and ITBV represent cardiac preload and are superior to CVP or PAOP, Luecke and colleagues performed a prospective study in fifteen adult sheep. All animals were studied before and after saline washout-induced lung injury, undergoing volume-controlled ventilation with increasing levels of positive end-expiratory pressure (PEEP, 0, 7, 14 and 21 cmH_2O) [25]. The overall correlation of RVEDV and ITBV with LVEDV was satisfactory ($r = 0.56$ and $r = 0.62$ respectively) and clearly superior to cardiac filling pressures. The authors concluded that ventilation with increasing levels of PEEP did not alter RV function, while LV function was impaired at the highest level of PEEP. Unlike cardiac filling pressures, ITBV and RVEDV both provide valid estimates of cardiac preload even at high intrathoracic pressures.

Intrathoracic Blood Volume in Cardiac Surgical Patients

Several studies have been performed in the cardiac surgical field to test the efficacy of ITBV as a preload index and to find an alternative device to the PAC in this population. Conventional CVP and PAOP were compared with ITBV and GEDV in thirty patients after coronary artery bypass grafting (CABG). No correlation was found between filling pressures and SVI/CI, while ITBVI correlated well with SVI and CI (coefficients were 0.76 and 0.83, respectively). The correlation coefficients of GEDVI versus SVI/CI were 0.82 and 0.87. ITBV and GEDV were, therefore, confirmed as better cardiac preload indexes, compared with CVP and PAOP in cardiac surgical patients [22].

Using two different thermodilution techniques, volume preload monitoring was compared with LV preload assessment by TEE. Twenty patients undergoing elective cardiac surgery with preserved left-right ventricular function were studied after induction of anesthesia. The authors found that GEDVI, continuous end-diastolic volume (CEDVI), LVEDA index (LVEDAI) and $SVI_{PiCCO/PAC}$ increased significantly after fluid load. They also found a correlation coefficient (r^2) for ΔGEDVI vs ΔLVEDAI of 0.658 and for ΔCEDVI vs ΔLVEDAI of 0.161. The relationship between ΔGEDVI and ΔSVI_{PiCCO} was stronger ($r^2 = 0.567$) than that between ΔCEDVI and ΔSVI_{PAC} ($r^2 = 0.267$). They concluded that GEDVI assessed by the transpulmonary arterial dye dilution technique gives a better reflection of echocardiographic changes in LV preload, in response to fluid replacement therapy, than CEDVI measured by a modified PAC [26].

The validity of ITBV and GEDV as a preload index was confirmed also in 40 heart transplanted patients although with a lesser degree of correlation probably due to the denervation of transplanted hearts that cannot change with heart rate or contractility [27]. Hinder et al. showed a high correlation between changes in ITBVI and EDAI measured using TEE during anesthesia and perioperative ICU stay in patients undergoing cardiac surgery [28].

Intrathoracic Blood Volume in Solid Organ Transplantations

Lung Transplantation

End stage lung disease necessitating lung transplantation is frequently associated with abnormal intrathoracic and pulmonary pressures, impaired efficacy of gas exchange, and different degrees of RV dysfunction. PAC monitoring represents the current clinical standard during lung transplantation because pulmonary artery monitoring is needed particularly during cross clamping of the pulmonary artery and after graft reperfusion phases. We evaluated the relationship between pressures (PAOP) derived from PAC, and the volume (ITBVI) preload variable derived from the PiCCO System, with respect to SVIpa (obtained from the PAC) during lung transplantation [29]. We concluded that the assessment of ITBVI by the transpulmonary arterial dye dilution technique is a useful tool in lung transplant patients providing a valid index of cardiac preload that may be superior to PAOP. These results agree with the results of a previous study in which we described the usefulness of volumetric monitoring during anesthesia and postoperative care also in patients undergoing double lung transplantation for cystic fibrosis [30].

Liver Transplantation

Inadequate cardiac filling leading to sub-optimal tissue perfusion and multiple organ failure (MOF), or excessive filling resulting in pulmonary edema and worsening respiratory function may contribute to both perioperative morbidity and mortality in liver transplanted patients. Krenn et al., monitoring patients undergoing liver transplantation with the transpulmonary arterial dye dilution technique performed with double indicator, observed that a post-reperfusion increase in ITBVI influenced pulmonary function, as demonstrated by the increase in Q_S/Q_T without impairment in EVLWI or oxygenation [31]. We studied the relationships between PAOP and ITBVI with respect to CI and SVI in 60 patients undergoing liver transplantation [32]. To

measure ITBVI, we used the single indicator thermodilution technique. In terms of preload variables, the main findings of this study were a good correlation between ITBVI and SVI and CI while PAOP failed to correlate. Statistically significant correlations were obtained when analyzing the predefined steps (after anesthesia induction, during the anhepatic phase, and at the end of surgery), confirming the validity of ITBVI as a preload index also during phases characterized by major hemodynamic changes due to the clamping of the inferior vena cava, unclamping of the anastomoses and graft reperfusion, bleeding and surgical manipulations.

Intrathoracic Blood Volume in Burn Patients

Adequate resuscitation from burn shock has long been recognized as the single most important therapeutic intervention in burn treatment and it is one of the most difficult tasks in the treatment of the severely burned. These patients, in the past treated with liters of fresh frozen plasma and human albumin, require large volumes of isotonic crystalloid solution to restore and maintain overall cardiovascular and vital organ perfusion during the first hours after burn. To this end, empirical formulas, basing fluid infusion on burn size and body weight, have traditionally been used. The formula predominantly used for burn resuscitation over the past 25 years has been the Parkland formula. The transpulmonary arterial dye dilution technique was applied in burns patients to guide fluid challenge based not only on old formulas but on hemodynamic-volumetric data. This area requires further investigation in the burn population as different results have been reported with the double and single transpulmonary arterial dye dilution techniques [33–36].

Holm and colleagues have recently introduced the ITBVI as a possible end-point to guide major burn fluid resuscitation [33]. Kuntscher and co-workers showed in recent studies that the transpulmonary arterial dye dilution technique performed with the single indicator is not suitable to assess ITBV and EVLW in burn shock, while the method is suitable to assess cardiac output and its derived parameters in burn resuscitation [34]. Moreover, Holm and colleagues showed that hemodynamic monitoring based on the transpulmonary arterial dye dilution double indicator technique resulted in more aggressive therapeutic strategies and was associated with a significant increase in fluid administration when compared to fluid resuscitation based on the Baxter formula [35]. A recent study showed that burn shock resuscitation using the Baxter formula led to significant hypovolemia during the first 48 h following burn. Hemodynamic monitoring resulted in more aggressive therapeutic strategies and was associated with a significant increase in fluid administration [36].

Intrathoracic Blood Volume in Pediatric Patients

Measurement of cardiac output is rarely performed in seriously ill children. The transpulmonary thermodilution cardiac output has shown to be as accurate as the Fick technique in pediatric patients [37]. Shiffmann and co-workers demonstrated that the transpulmonary arterial dye dilution technique enabled measurement of cardiac output and intravascular volume status in critically ill neonates and infants at the bedside whereas the CVP was not indicative of changes in intravascular volume status [38–39]. They found that GEDV and ITBV better reflected cardiac preload while CVP failed to predict cardiac preload changes after a fluid challenge.

Intrathoracic Blood Volume during Pneumoperitoneum and Positioning

Interesting studies have been performed using the transpulmonary arterial dye dilution technique in different clinical conditions such as laparoscopic procedures with pneumoperitoneum or during neurosurgical procedures performed in the sitting position [40–44]. Changes in body position caused significant decreases in ITBV and were accompanied by a significant decrease in CI, SVI, and mean arterial pressure (MAP). Changes in ITBV correlated ($r = 0.78$) with changes in SVI [40]. Thus, a change in blood volume distribution between the intra and extrathoracic compartment occurred after a change from the supine to the sitting position. The authors concluded that indicator dilution enables quantification of this shift and may be helpful in guiding fluid therapy in selected patients.

A significant increase in ITBV was observed after pneumoperitoneum induction, persisting both in the supine, head-up, and head-down positions [41]. The authors concluded that the onset of pneumoperitoneum, even with moderate intra-abdominal pressures, is associated with an increased ITBV in ASA I-II patients. However, Hachenberg et al. and Andersson et al. found no increase in ITBV in response to induction of pneumoperitoneum [42, 43]. The key difference between these studies is the fluid challenge performed by Hofer (crystalloid 10 ml/kg plus 2 ml/kg/h) during the induction while the others administered only crystalloids at 2 ml/kg/h without initial fluid loading. Unchanged ITBV after elevation of intra-abdominal pressure in these studies may reflect relative hypovolemia. These studies are difficult to compare also because of the different anesthesia techniques and ventilator strategies applied.

Valenza and colleagues applied the transpulmonary arterial dye dilution single indicator technique in the experimental setting to investigate whether negative extra-abdominal pressure improves respiratory function and induces a blood shift from the intrathoracic compartment and to assess whether these effects are influenced by abdominal pressure [44]. They concluded that negative extra-abdominal pressure increases lung volume and cause a shift of blood from the intrathoracic compartment.

Limitations

In a case report, an aortic aneurysm and huge cardiac chambers were responsible for high ITBV values [45]. Volumes will be overestimated in the presence of large aortic aneurysms or catheters placed too far peripherally, i.e., in a radial artery, due to a prolonged mean transit time. Furthermore intracardiac shunts may limit the usefulness of this technique. The transpulmonary arterial dye dilution method is not suitable for patients with severe peripheral vascular disease, those undergoing vascular surgery, or those that have other contraindications for femoral artery cannulation.

In another case report a pathological low ITBV value, despite a high CVP, was detected and these data were firstly erroneously interpreted as hypovolemia. Unfortunately in this case the low ITBV value was due to a pulmonary embolism [46]. Another condition to take into account is a severe reduction in pulmonary vascular bed which correlates with substantial errors in PBV quantification. When the PBV reduction is more than 50% (i.e., after a pneumonectomy) the PBV will be only

10% of ITBV and not the 20%; therefore, ITBV can be underestimated by 10% if detected with the single indicator technique [47].

Conclusion

The state of the art of the preload condition, volemia and cardiac performance in critically ill patients is still under investigation. After several years of 'filling pressures', PAOP and CVP values should be limited only in well known clinical conditions. The ITBV, correlates well with the cardiac output and with the stroke volume, and provides the opportunity to evaluate the central blood volume. The integration of ITBV together with other hemodynamic data and organ perfusion parameters today offers a chance to better understand the volemic condition. This has been studied and demonstrated in cardiac surgical patients, in sepsis, in solid organ transplantation, in positioning changes, and in children. In all other fields more investigations should be done.

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Hemodynamic Management Guided by Esophageal Doppler

X. Monnet and J.L. Teboul

Introduction: Principle of Esophageal Doppler Measurements

The use of esophageal Doppler is aimed at monitoring cardiac output by continuously measuring the blood flow in the descending thoracic aorta. Initially, the Doppler technique attempted to measure the blood flow in the *ascending* part of the vessel. A Doppler probe was positioned on the skin at the level of the supra-sternal notch and manually moved until a correct positive signal was obtained [1]. Although it provided a reliable estimation of cardiac output [2, 3], this technique was never widely adopted because it did not allow continuous monitoring and because the position of the supra-sternal probe was unstable [4].

Using the anatomical proximity of the thoracic descending aorta and the esophagus, the principle of monitoring the aortic blood flow by the Doppler effect then moved to esophageal Doppler [5]. With this technique, a flexible probe of small diameter is introduced into the esophagus. The tip of the probe is equipped with a Doppler transducer (4-MHz continuous wave or 5-MHz pulsed wave, according to the manufacturers) that records the velocity of red blood cells circulating into the descending thoracic aorta. For estimating the velocity of blood flow from a single Doppler line through the esophageal and the aortic walls, one must accept the three following hypotheses:

- the blood flow in the thoracic aorta is axial with a minimal radial component, which is true during the high speed systolic phase of flow,
- the velocity profile into the aorta is flat, which is not absolutely true [5] and which might result in an overestimation of flow by esophageal Doppler and
- the aorta is a cylindrical vessel with a circular section.

For determining the cross-sectional area of the aorta at the level of measurement, two different methods are used. The CardioQ^{TM} device (Deltex Medical^{TM}) – previously ODM^{TM} device (Abbott^{TM}) – does not measure the aortic diameter but estimates it from a nomogram based on age, height and weight [6]. The Hemosonic^{TM} device (Arrow Intl^{TM}) – previously Dynemo 3000^{TM} (Sometec^{TM}) – measures the aortic diameter by means of a second echo beam that is oriented at a right angle with the aorta and that emerges from a second 10-MHz transducer placed on the probe [7]. This diameter assessment has been demonstrated to be reliable when compared to measurements made with transesophageal echocardiography (TEE) [8].

Practical Use of Esophageal Doppler

After its oral introduction, the probe is advanced into the esophagus and adjusted to obtain the highest Doppler velocity signal from the descending aorta. For the Hemosonic^{TM} device, the positioning adjustment also takes into account the quality of the diameter signal that is simultaneously displayed.

The diameter of the esophageal probe is similar to that of a nasogastric tube such that the technique is considered as minimally invasive. Esophageal disease must be considered as an obvious contraindication. Usually a correct Doppler signal can be displayed within a few minutes after inserting the probe [9]. Furthermore, the technique requires minimal skill and the operator's performance can be improved after only a short period of training [10, 11]. The quality of the signals may be poor in obese patients. The insertion of the probe is usually not hampered by the presence of a nasogastric tube and the probe can be easily left in place for hours. However, the discomfort associated with the insertion of the probe requires mild sedation such that the technique is reserved for intubated patients receiving mechanical ventilation.

Hemodynamic Information Provided by Esophageal Doppler Monitoring

Aortic Blood Flow

Basically, the esophageal Doppler device continuously calculates the aortic blood flow value from the velocity and the diameter values. Although the diameter value is continuously measured by the Hemosonic^{TM} device, it is considered as constant if the CardioQ^{TM} device is used. All esophageal Doppler devices also provide a value of cardiac output that is based on the hypothesis that there is a constant distribution of the systemic blood flow between the upper territories and the descending aorta. It is generally considered that blood flow in the descending thoracic aorta represents around 70% of the systemic blood flow [7].

Numerous clinical studies have demonstrated that esophageal Doppler aortic blood flow measurements are reliable. Most of these validation studies compared the value of cardiac output estimated by esophageal Doppler with that directly measured by the thermodilution method, considered as a gold standard. Dark and Singer recently reviewed the most pertinent of these validation publications [12]. Taking into account eleven validation studies that simultaneously assessed cardiac output by thermodilution and by esophageal Doppler [6, 11, 13–21], they demonstrated that the validity of the method was high, with minimal bias but limited clinical agreement.

Importantly, Cariou and coworkers demonstrated that the dobutamine-induced changes of aortic blood flow measured by the Hemosonic^{TM} device correctly tracked the changes in cardiac output measured by thermodilution [8]. Other authors showed that esophageal Doppler monitoring devices were able to reliably track the cardiac output changes under various hemodynamic interventions [18, 20, 22, 23]. Taken together, these studies clearly confirm the reliability of cardiac output estimation by esophageal Doppler.

It could be questioned whether it is preferable to actually measure the aortic diameter rather than to estimate it. On the one hand, one may argue that the measurement of aortic diameter by the technique could introduce an additional source of error that may be magnified to the second power since the area of the aorta is a quadratic function of its radius. In this regard, it would be better to consider the theoretical value of aortic diameter rather than a false measured one [4]. On the other hand, the measurement of aortic diameter by esophageal Doppler was shown to be in good agreement with that obtained from TEE [8]. Moreover, no study has compared estimated and measured values of the aortic cross sectional area to a gold standard. More importantly, one must keep in mind that the aortic diameter varies physiologically with arterial pressure [24]. In this respect, a change up to 20% of aortic diameter has been observed in patients during liver transplantation [25]. Thus, measuring aortic diameter rather than estimating it could be important for assessing the whole amplitude of hemodynamic changes, although this point remains to be demonstrated.

Another issue rises from the estimation of cardiac output from the descending aortic blood flow assuming that a constant fraction of the cardiac output passes through the descending aorta. Indeed, the distribution of cardiac output between the upper and the lower parts of the arterial tree may change with spontaneous or therapeutic changes in hemodynamic status especially when they are associated with changes in the sympathetic tone. Nevertheless, one can consider that measuring blood flow at the level of the descending aorta makes sense from a physiological point of view since the perfusion of the most important vascular areas hampered by circulatory failure (i.e., the splanchnic and the renal vascular areas) depends upon the *descending* aortic blood flow.

Duration of the Systolic Aortic Flow

The time elapsed between the beginning and the end of the aortic velocity signal, i.e., the duration of the systolic flow through the aorta (so called 'flow time' in the CardioQ^{TM} system and 'left ventricular ejection time' in the Hemosonic^{TM} system)

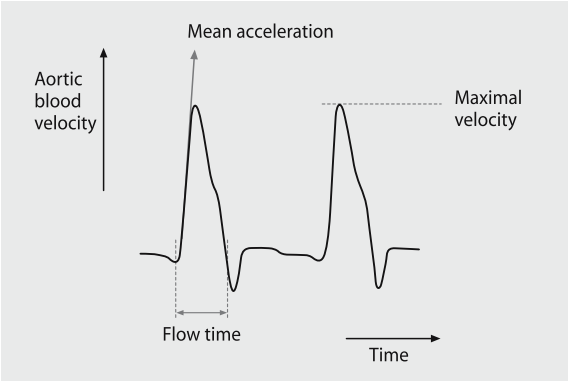

Fig. 1. Velocity waveform obtained with esophageal Doppler. Mean acceleration and peak velocity are considered as reflecting the left ventricular inotropic state. The flow time – corrected for heart rate – is inversely related to vascular resistance; it is particularly narrowed when cardiac preload is low

is usually considered as a reflection of cardiac loading conditions (Fig. 1). It must be corrected for heart rate by dividing its value by the square root of the cycle time [16]. This ejection time decreases when cardiac preload decreases and when afterload increases: in low filling states, the time devoted to ejection is low (1) because the volume to be ejected by the left ventricle is low and (2) presumably because afterload is increased by the sympathetic-dependent vasoconstriction. Similarly, when the left ventricular afterload is primarily increased, the aortic systolic time of flow is decreased. This variable is not altered by inotropic alterations [16].

Acceleration and Maximal Velocity of the Aortic Flow

The mean acceleration of the aortic flow and the maximal value of the aortic velocity have been postulated to reflect left ventricular contractility (Fig. 1) [16]. In this regard, these indices were shown to increase with dobutamine and decrease with esmolol administration in healthy subjects [16]. However, the clinical evaluation of these indices is scarce and it must be remembered that these parameters depend not only on ventricular contractility but also on ventricular loading conditions. For example, the peak velocity was shown to follow the directional changes in ventricular afterload [6, 16]. To summarize, the analysis of the shape of the aortic velocity waveform may help to recognize hemodynamic alterations in shocked patients [4].

Hemodynamic Management by Esophageal Doppler

Esophageal Doppler Driven Protocols in the Operating Room

Because it is relatively non-invasive and easy-to-perform and since it does not require any repositioning – provided that the patient is not moving – esophageal Doppler is becoming more and more popular for intra-operative cardiovascular monitoring. Furthermore, numerous studies have demonstrated outcome benefits in patients undergoing esophageal Doppler monitoring in the operating room.

Mythen and Webb first demonstrated, in cardiac surgical patients, that a perioperative fluid therapy with a colloid challenge based on the value of stroke volume provided by esophageal Doppler reduced the incidence of gut mucosal hypoperfusion, the rate of major complications, and the in-hospital and in-intensive care unit (ICU) length of stay [26].

In a randomized study in patients undergoing repair of hip fracture, Sinclair and coworkers [27] compared conventional intraoperative fluid management with repeated colloid fluid challenges monitored by esophageal Doppler ultrasonography to maintain maximal stroke volume throughout the operative period. The esophageal Doppler driven protocol was based on a simple algorithm that took into account the stroke volume as well as the flow time corrected for heart rate as a marker of preload. Patients of the protocol group exhibited higher intraoperative values of cardiac output. Finally, they had a significantly faster postoperative recovery as well as a shorter hospital length of stay than conventionally managed patients. Similar results were obtained by Venn and coworkers [28] and by Gan and coworkers [29].

Clinical Utility of Esophageal Doppler in Critically Ill Patients

While numerous clinical studies have showed that esophageal Doppler can be useful in the operative room, the utility of such a monitoring technique has never been demonstrated in critically ill patients. Obviously, this ultrasonographic method does not provide the critical care giver with some important hemodynamic parameters like mixed venous oxygen saturation (SvO_2), cardiac filling pressures, pulmonary artery pressures or extravascular lung water (EVLW) that are available with other monitoring devices such as the pulmonary artery catheter (PAC) or PiCCO^{TM} system. In this regard, it seems difficult to recommend that esophageal Doppler alone should be used in patients with complex forms of circulatory shock, in particular if they have concomitant respiratory failure. However, in patients with less severe circulatory shock or in those without lung injury/prior cardiac failure, esophageal Doppler monitoring can be used as the first line monitoring technique since it allows the monitoring of cardiac output and the assessment of preload and preload responsiveness.

Monitoring of Cardiac Output

Esophageal Doppler monitoring enables a continuous measurement of aortic blood flow. One advantage of the technique is that it provides a real-time, beat-to-beat estimation of stroke volume [9]. In this regard and in contrary to 'continuous cardiac output' PACs, esophageal Doppler monitoring is able to detect instantaneous changes in hemodynamic status. This advantage makes the esophageal Doppler method particularly attractive to follow the short term effects of a therapeutic challenge like passive leg raising (see below). However, one of the major inconveniences of this method is clearly the need to reposition the probe each time it has moved away from its correct position facing the aorta. This problem might be minor in the operating room where the patient is motionless. In the ICU, this inconvenience does not hamper monitoring of hemodynamic interventions since their effect is usually expected to occur over a short period during which the probe can be maintained in its optimal position.

Assessment of Preload and Preload Responsiveness

Since it provides a continuous and reliable estimation of cardiac output, esophageal Doppler can be used for monitoring the hemodynamic changes induced by a fluid challenge [29]. The esophageal Doppler technique may also help physicians to assess adequate cardiac preload conditions in critically ill patients.

The duration of the aortic velocity signal is usually considered as an index of cardiac preload as it increases after volume expansion [9, 16, 30]. Recently, McKendry and coworkers have shown that managing fluid therapy in the postoperative period with a protocol using esophageal Doppler data and delivered by nurses, significantly shortened the hospital stay of patients who underwent cardiac surgery [31]. However, the aortic flow time suffers from the limitations of static markers of cardiac preload for predicting volume responsiveness [32]. Indeed, a static value of flow time corrected for heart rate is unlikely to predict whether the heart is on the preload-dependent or preload-independent part of the Frank-Starling relationship. We recently reported that the corrected flow time poorly predicts fluid responsiveness [9].

Nonetheless, esophageal Doppler can provide the physician with dynamic markers of fluid responsiveness. Parameters like pulse pressure variation or 'pulse contour' stroke volume variation were shown to be more valuable than static markers of preload to assess volume responsiveness [32]. Because it measures aortic blood flow on a beat-to-beat basis, the esophageal Doppler technique is able to assess its respiratory variation. Slama and coworkers showed that the respiratory variation of aortic blood velocity progressively increased during incremental blood withdrawal in anesthetized rabbits and returned to normal values after blood restitution [33]. In patients with acute circulatory failure, we demonstrated that the magnitude of the respiratory variation of aortic blood flow was higher in responders to volume expansion than in non responders. Importantly, we observed that an aortic blood flow respiratory variation greater than 18% predicted volume responsiveness with a sensitivity of 90% and a specificity of 94% [9].

There are some limitations of using heart-lung interaction indices to predict volume responsiveness in critically ill patients [34]. In particular, these indices cannot be used in patients ventilated with a low tidal volume, although this is still a matter of debate [33], or in patients with spontaneous breathing activity or with arrhythmias. In such cases, performing passive leg raising has been proposed to distinguish patients who will respond to fluid from patients who will not [35]. Indeed, raising the legs passively to 45° induces a transfer of blood from the legs toward the intrathoracic vascular compartment and hence increases cardiac preload. Once the legs are returned to the horizontal position, the blood shift vanishes: passive leg raising acts like a reversible fluid challenge for predicting preload reserve. Accordingly, the changes in cardiac index induced by fluid loading correlated nicely with the changes induced by passive leg raising on pulse pressure [36]. More im-

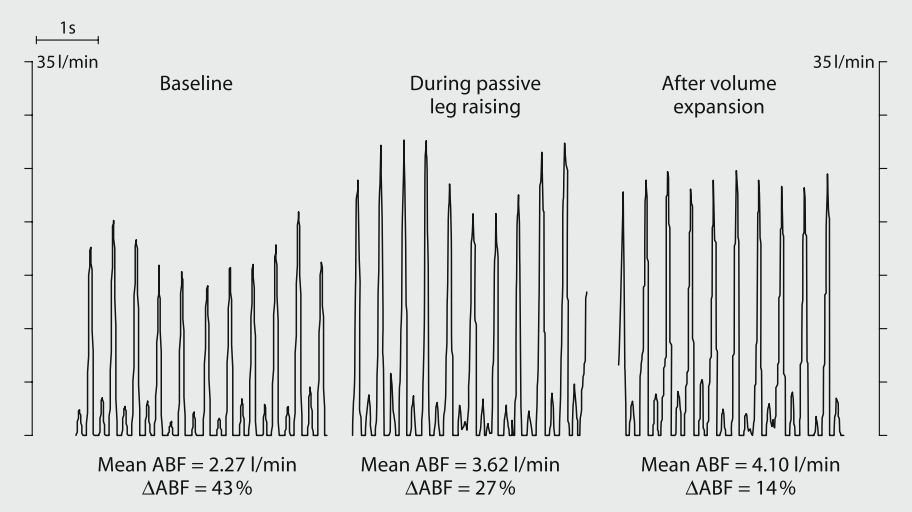

Fig. 2. Typical waveform of aortic blood flow (ABF) in a patient with hemodynamic failure and preload responsiveness. At baseline, the aortic blood flow is low and its variation during the mechanical respiratory cycle (ΔABF) is high. Passive leg raising induces a significant increase in the mean value of aortic blood flow. These two parameters predict the increase in mean aortic blood flow induced by volume expansion

portantly, we have recently demonstrated that the changes in aortic blood flow observed by esophageal Doppler during passive leg raising were highly predictive of a positive hemodynamic response to a subsequent fluid expansion (Fig. 2) [37]. Importantly, this prediction was fair not only in patients with perfect adaptation to the mechanical ventilator and with sinus cardiac rhythm, but also in those with spontaneous breathing activity and arrhythmias, i.e., a subgroup in when the respiratory variation of hemodynamic signal could not be used for predicting fluid responsiveness [37]. Such results further emphasize the ability of the esophageal Doppler technique to track immediate changes in aortic blood flow during hemodynamic interventions. These findings also suggest that esophageal Doppler monitoring can be positively included in the management of hemodynamically unstable patients.

Conclusion

The esophageal Doppler technique has progressively emerged as a minimally invasive and reliable tool for measuring aortic blood flow and estimating cardiac output. Numerous studies have emphasized its clinical utility by showing improved outcome in surgical patients when hemodynamic therapy was driven by algorithms based on esophageal Doppler data. In the setting of the ICU, the ability of esophageal Doppler to track the changes in aortic blood flow in a beat-to-beat manner makes it particularly suitable for predicting fluid responsiveness, either by the measurement of the aortic blood flow respiratory variation or by testing the effects of passive leg raising.

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Less Invasive Cardiac Output Monitoring: Characteristics and Limitations

C. K. Hofer and A. Zollinger

Introduction

Cardiac output is monitored in critically ill patients in the intensive care unit (ICU) and during anesthesia to maintain and improve cardiac function with the primary goal of adequate tissue perfusion. Since the introduction of the pulmonary artery catheter (PAC) into clinical practice in 1970, this invasive technique has been considered to be the gold standard for cardiac output measurement. However, its risk-to-benefit ratio has been increasingly questioned in recent years based on conflicting study results. Connors et al. [1] showed that the use of the PAC was associated with a higher mortality in a large series of critically ill medical patients with organ failure. Comparable results were found in patients with myocardial infarction [2] and in surgical patients with organ failure [3]. By contrast, some studies reported no difference in relation to mortality [4] or even an improved outcome when a PAC was used [5]. Apart from the inherent potential risk of morbidity in association with the PAC [6] differences in outcome observed in these studies may be related to differences in hemodynamic changes between medical and surgical patient groups and the presence or lack of defined treatment concepts based on hemodynamic findings. Several less invasive cardiac output monitoring techniques have been advanced in recent years with the aim of avoiding the risk associated with PAC use. These less-invasive techniques have the potential to replace the current gold standard for a variety of established indications. Furthermore, they may be applied earlier in situations at risk for hemodynamic instability to a larger patient population than the PAC. However, before these alternative techniques can be generally applied, their safe and easy handling, as well as their accuracy in providing hemodynamic data, needs to be demonstrated compared with the PAC.

This chapter gives a synopsis of the major, currently available less invasive cardiac output measurement methods, i.e., echocardiography and Doppler measurements, pulse contour analysis, partial carbon dioxide rebreathing, pulsed dye dilution technique, and electrical bioimpedance. The technical fundamentals of these methods are summarized and their validation, as well as their advantages and limitations compared to the PAC, are outlined. As a result of an increasing number of publications in this field only studies performed in adult critically ill or severely compromised patients presenting Bland and Altman analysis [7] published in major journals in the last five years have been listed (Table 1). References have been limited to five publications for each technique.

Table 1. Recent study results comparing less invasive cardiac output (CO) measurements with the intermittent thermodilution technique by the pulmonary artery catheter (PAC)

	Setting	Pat Non	Bias ± 2SD l/min (l/min/m^2) #	Correlation r^2	
TEE					
Bettex et al. [9]	CS ICU	30	−0.21 ± 2.26	–	on-line CW-Doppler: aortic valve
			1.12 ± 2.68	–	on-line PW-Doppler: aortic valve
			1.55 ± 2.92	–	on-line PW-Doppler: left ventricular outflow tract
			−0.41 ± 2.30	–	on-line Simpson
Zhao et al. [10]	CS OR	26	0.07 ± 0.86	0.70	off-line PW-Doppler: aortic valve
			0.12 ± 0.98	0.76	off-line PW-Doppler: left ventricular outflow tract
			0.17 ± 0.82	0.77	off-line PW-Doppler: right ventricular outflow tract
Akamatsu et al. [12]	CS ICU	30	0.01 ± 1.16	0.85	automated CO assessment: mitral valve
			−1.47 ± 2.3	0.41	automated CO assessment: left ventricular outflow tract
Transesophageal Doppler					
Leather et al. [19]	UR OR	14	−0.89 ± 1.78/0.55 ± 3.75	–	ODM II: before/after lumbar sympathetic blockade
Hullett et al. [16]	CS	20	−0.56 ± 1.28	0.38	CardioQ
Kim et al. [18]	BU ICU	20	0.77 ± 2.74	0.64	CardioQ
Jaeggi et al. [17]	CS	22	0.23 ± 1.60 #	0.09	HemoSonic 100
Moxon et al. [21]	CS	13	−0.23 ± 2.12	0.66	HemoSonic 100
Pulse contour analysis					
Rodig et al. [27]	CS OR	26	−0.04 ± 2.02/1.20 ± 3.75	–	PiCCO: old algorithm before/after phenylephrine
Goedje et al. [24]	CS ICU	24	0.08 ± 2.62/-0.20 ± 2.40	0.53/0.77	PiCCO: old/new algorithm
Della Rocca et al. [26]	LI OR	62	−0.11 ± 1.30/0.18 ± 1.55	0.86 §	PiCCO: CO <8 l/min/8 l/min, •total
Felbiger et al. [28]	CS ICU	17	−0.05 ± 0.24 #	0.98	PiCCO: new algorithm during rapid preload changes
Yamashita et al. [30]	CS OR	23	0.71 ± 2.65/0.30 ± 1.97	0.49/0.52	PulseCO: before/after sternotomy
			0.76 ± 3.86	0.55	PulseCO: end of surgery

Table 1 (continued)

	Setting	Pat Non	Bias ± 2SD l/min (l/min/m^2) #	Correlation r^2	
Pulsed dye dilution					
Bremer et al. [35]	CS ICU	58	−0.39 ± 1.00	0.93	nose sensor
Hofer et al. [36]	CS ICU	31	−0.53 ± 1.28 #	0.04	finger sensor
			−0.08 ± 1.56 #	0.20	nose sensor
Sakka et al. [37]	ICU	16	−0.80 ± 3.40	0.41	nose sensor
Partial carbon dioxide re-breathing					
Odenstedt et al. [40]	ICU	15	−0.05 ± 1.92	0.81	
Kotake et al. [41]	CS OR	28	−0.58 ± 1.90	0.64	PAC intermittent vs. continuous: bias ± 2SD = 0.38 ± 2.34
Mielck et al. [42]	CS ICU	22	−0.64 ± 2.78	–-	
Tachibana et al. [44]	CS ICU	25	0.09 ± 2.00	0.20	tidal volume: 12ml/kg, RR 10/min
			−0.67 ± 1.46/−1.73 ± 2.54	0.62/0.32	tidal volume: 6ml/kg, RR 20/min/RR10/min
			0.18 ± 2.82	0.05	SIMV-PSV
Rocco et al. [43]	ICU	12	−0.01 ± 0.80/−2.30 ± 2.4	0.90/0.38	low shunt (Qs/Qt < 20%)/ high shunt (Qs/Qt > 35%)
Bioimpedance					
Sageman et al. [46]	CS ICU	20	−0.07 ± 0.80 #	0.86	TEB: BioZ
Spiess et al. [47]	CS ICU	47	−0.28 ± 1.34 #	0.49	TEB: BioZ
Hirschl et al. [48]	ICU	29	0.61 ± 1.49 #	—	TEB: Cardioscreen
Cotter et al. [49]	mixed	122	0.001 ± 1.36 #	0.77	WHEB: NiCaS
Imhoff et al. [50]	ICU	22	1.62 ± 4.64	0.08	WHEB: NiCaS

BU: burn; CS: cardiac surgery; CW: continuous wave; ICU: intensive care unit; LI: liver transplantation; OR: operating room; PSV = pressure support ventilation; PW: pulsed wave; RR: respiratory rate; SIMV: synchronized intermittent mandatory ventilation; UR: Urologic.

Transesophageal Echocardiography (TEE)

Two echocardiographic approaches to cardiac output determination by a standard 5 MHz multiplane probe are used in clinical practice: the biplane application of the Simpson formula and Doppler flow measurements. When using the Simpson formula, the longitudinal axis and different cross-sectional areas of the left ventricle have to be determined in systole and diastole and left ventricular (LV) volumes are then calculated on the basis of a cone shaped geometric model of the left ventricle. Cardiac output is obtained by the difference of the two volumes times the heart rate. The second, more popular approach to cardiac output assessment is to measure flow primarily by pulsed-wave Doppler beam across a cardiac valve but also in the left or right ventricular outflow tract and to assess the cross-sectional area at the site of the flow quantification. From these two measurements and heart rate, cardiac output is calculated. Prerequisites for optimal measurements are a Doppler beam orientation parallel to the blood flow and an unchanged cross-sectional area during the blood flow. These needs are considered to be best met by a Doppler assessment at the LV outflow tract or the aortic valve with the echo probe in the trans-gastric position (Fig. 1). The measurements are time consuming and require

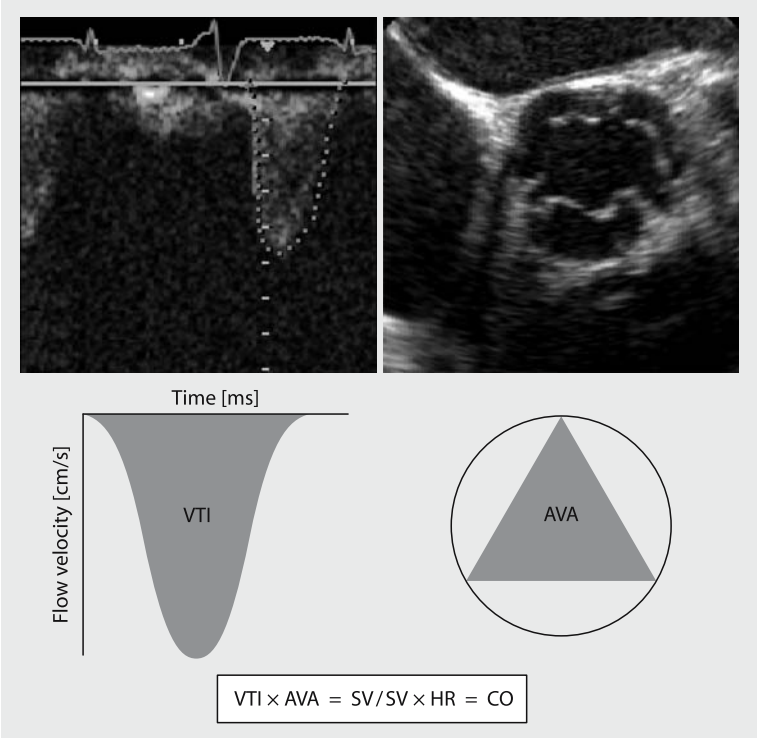

Fig. 1. Determination of cardiac output (CO) by transesophageal echocardiography (TEE). Echocardiographic recordings of the Doppler assessment with the probe in the transgastric position and the basal shot-axis view of the aortic valve and the corresponding diagrams of the measurements required for cardiac output calculation are shown. AVA: aortic valve area; SV: stoke volume; HR: heart rate; VTI: velocity time integral

a certain level of operator skill and knowledge. Although ultrasonic measurements are possible by the transthoracic or transesophageal access, the latter is preferred because of enhanced image quality. However, the placement of the TEE probe is not free of complications [8].

Numerous investigations comparing cardiac output measurement by TEE and PAC have been performed; the results of the latest studies are summarized in Table 1. Bettex et al. [9] determined cardiac output by TEE on- and off-line at different sites using pulsed-wave and continuous-wave Doppler as well as the manual Simpson approach. Best results were obtained at the aortic position with the continuous-wave Doppler, but all approaches revealed large limits of agreement and a signal failure rate up to 16.7%. By contrast, Zhao at al. [10] observed good agreement between pulsed-wave Doppler and thermodilution measurements in patients before cardiopulmonary bypass, with a 20% failure rate to obtain an adequate signal. Immediately after bypass, the agreement decreased and improved again over time potentially because of the temperature-sensitive cardiac output determination by the PAC. However, variations of accuracy and agreement between TEE and PAC may be mainly related to the inherent technical problems of TEE measurements (image quality, variations in the determination of the orifice area, excessive angle of the Doppler beam) and the numerous assumptions that have to be made for cardiac output determination by this technique (constant cross-sectional area over time, flat flow velocity profile, ideal geometry of anatomic structures [11]). The automated cardiac output measurement technique (ACOM) may have the potential to partly overcome these problems. Multiple color flow Doppler measurements by spatial and temporal integration in a predefined area of interest are used to measure cardiac output without the determination of an orifice area and the time consuming placement of the echo probe in different positions. However, as a result of individual gain adjustment for optimal signal quality, the inherent TEE problem of inter- and intraobserver variability may not be reduced. Initial results by Akamatsu et al. [12] for measurements in the mitral position were promising, but showed less reliable results for the left outflow tract analysis with a signal failure rate of 56%.

Although TEE may not be an alternative to the PAC for long-term cardiac output measurement, this visual technique provides essential hemodynamic information of left and right ventricular global function, wall motion abnormalities or cardiac filling with considerable therapeutic impact in many acute, critical hemodynamic situations [13].

Transesophageal Aortic Doppler Ultrasound

Another method of less invasive cardiac output assessment by TEE is the measurement of Doppler flow velocity in the descending aorta [14], where adequate characteristic flow signals can be obtained much easier than in the above mentioned areas of the heart as a result of the close anatomic proximity of the esophagus and the aorta. The esophageal probe in only one position with minor adjustments may allow fast, reliable, and continuous measurements. These advantages may minimize interobserver variability and have induced the development of dedicated cardiac output Doppler probes and devices (e.g., ODM II, Abbott, Maidenhead, UK; CardioQ, Deltex Medical Ltd, Chichester, UK; HemoSonic 100, Arrow, Reading, PA, USA). Cardiac output is derived with these devices from measured aortic blood flow and aortic cross-sectional area (Fig. 2) obtained either from nomograms (ODM II, CardioQ) or by M-mode ultrasound determination (HemoSonic). Because

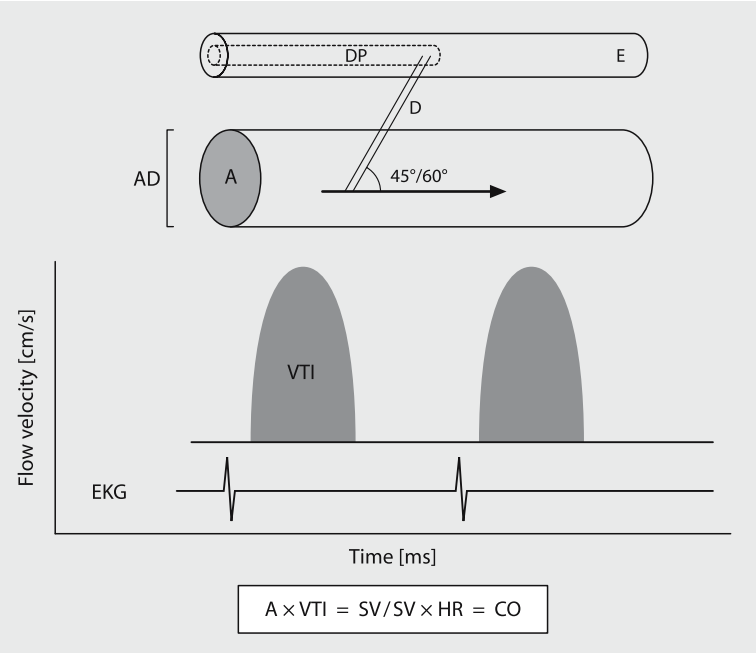

Fig. 2. Cardiac output (CO) assessment using an aortic transesophageal Doppler probe. The Doppler transducer is located at the tip of the probe and the beam is directed at the descending thoracic aorta (angle between Doppler beam and blood flow typically 65° or 45°). Cardiac output is determined by Doppler flow measurements and the aortic area calculated from the diameter of the descending aorta either obtained from nomograms or assessed by M-mode echocardiography (HemoSonic 100). A: aortic area (aortic diameter × π/4); AD: aortic diameter; D: Doppler beam; DP: Doppler probe; E: esophagus, EKG: electrocardiogram; HR: heart rate; SV: stroke volume; VTI: velocity time integral

coronary and brachiocephalic flow are not measured, cardiac output is calculated assuming a constant partition between caudal (70%) and cephalic (30%) blood supply areas. Handling, especially the insertion of the probes in the sedated, intubated patient, is facilitated as the probes are smaller than conventional TEE probes and steep learning curves for probe positioning to obtain highest possible peak velocity have been reported [15].

Clinical trials over the last 20 years have demonstrated inconsistent results in terms of correlation, accuracy, and repeatability of cardiac output measurement using this method compared to the intermittent thermodilution technique using the PAC (Table 1) [16–21]. Problems in flow signal detection have to be considered as a cause for these findings because two of the recently published studies [16, 17] reported a Doppler signal failure in 2 out of 22 patients investigated in each trial (9%). On the other hand, the assumption of fixed regional blood flow, that has to be made for cardiac output determination, may be a major source of error. In fact, Leather and Wouters [19] clearly demonstrated that flow redistribution induced by epidural anesthesia increased bias between Doppler and thermodilution measurements. Moreover, determination of aortic cross-sectional area by nomograms used with some devices can introduce a systematic bias. The HemoSonic 100 device was

developed to eliminate this problem by the echocardiographic assessment of the aortic diameter, but optimal adjustment of both the Doppler and the ultrasonic signal may be challenging [17].

Based on these findings, the esophageal Doppler technique may not be recommended for monitoring absolute cardiac output values. However, a considerable number of studies have showed that Doppler devices are able to reliably track cardiac output changes over time. This feature has been used in combination with the heart rate-adjusted Doppler flow time as preload indicator to optimize perioperative fluid therapy and different studies have demonstrated an improved outcome using this concept [22]. Recent advances in suprasternal Doppler technique with favorable initial clinical results [23] may expand the use of the Doppler method for this goal directed approach to non-sedated patients.

Pulse Contour Analysis

Arterial pulse contour analysis as a continuous measurement of cardiac output has recently been introduced into clinical practice as an alternative to the PAC. It is based on the principle of predicting vascular flow by means of the arterial pressure wave form. The arterial pressure wave form is the result of an interaction between stroke volume and mechanical characteristics of the systemic vascular system. Thus, in order to assess stroke volume and cardiac output by this technique, resistance (vascular tone, which determines mean arterial pressure for a given flow), compliance (pressure change per unit volume change), and characteristic impedance at the site of the signal detection have to be considered. Different models are used to address these issues in the currently available pulse contour analysis devices (PiCCO plus, Pulsion Medical Systems, Munich, Germany; PulseCO, LiDCO Ltd, London, UK; FloTrac/Vigileo, Edwards LifeSience, Irvine, CA, USA). Using the PiCCOplus system, aortic pressure wave forms are recorded typically via femoral, but also via brachial or radial access by a thermistor tipped arterial catheter. Cardiac output is then calculated on a beat-to-beat basis using an algorithm measuring the area under the systolic part of the pressure wave form after calibration by transpulmonary thermodilution. The original algorithm has recently been modified to better address the individual patient's aortic compliance by shape analysis of the systolic pressure wave form and the calibration data [24] (Fig. 3). The PulseCO and the Vigileo devices obtain their signal by a standard peripheral arterial line. PulseCO measurements are based on harmonic waveform analysis (Fourier transformation) and integrate beat duration, ejection duration and mean arterial pressure. Lithium indicator dilution is used for calibration. Compared to thermodilution, this technique is not temperature sensitive, but requires accurate measurement of serum sodium and hematocrit concentrations and allows only limited injections per time unit [25]. In contrast to the other systems, the Vigileo monitoring system does not require external calibration. For cardiac output assessment, the standard deviation of pulse pressure measured during a period of 20 seconds is empirically correlated to the stroke volume based on underlying patient data (age, gender, body length, and weight). Aortic compliance is also estimated using these data, whereas resistance is derived by analyzing the actual pressure wave form characteristics. As a result of the primary principles used, all pulse contour devices share the inherent need of an optimal arterial signal quality for valid cardiac output assessment and arrhythmias preclude reliable measurements.

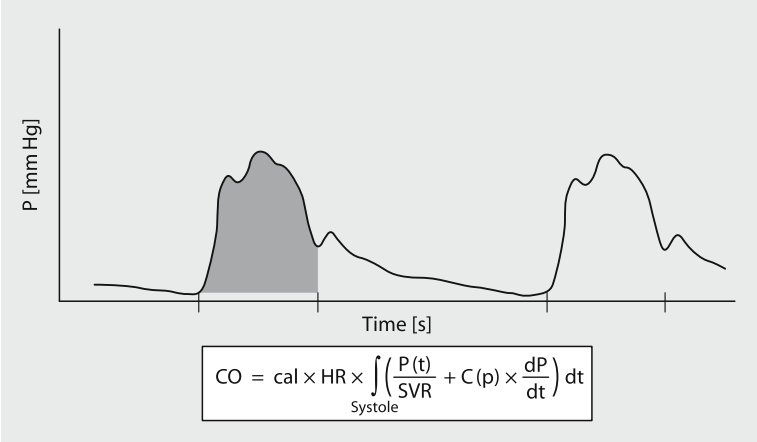

Fig. 3. Principle of cardiac output (CO) assessment by pulse contour analysis using the PiCCOplus system. Cal: specific calibration factor determined by transpulmonary thermodilution; C(p): aortic compliance; P(t)/SVR: area under the pressure wave curve; dP/dt: shape of the pressure wave curve; SVR: systemic vascular resistance

Pulse contour analysis, especially the PiCCO system, has been validated extensively against intermittent thermodilution by the PAC in the last decade (Table 1). Most PiCCO studies found a good agreement between values obtained using the two methods [24, 26]. Inaccurate measurements as a result of variations in systemic vascular resistance were observed in a study performed by Rodig et al. [27] when the initial algorithm for cardiac output calculation was used. However, the modified algorithm appears to be more robust in situations of rapid hemodynamic change [28]. Good results in terms of bias, limits of agreement and correlation were observed by Hamilton et al. [29] evaluating the PulseCO device. However, Yamashita et al. [30] showed that agreement can vary considerable during different stages of cardiac surgery, which may be mainly related to alterations in vascular compliance and resistance. Therefore, recalibration at intervals of 4 to 6 hours, which is also used to determine actual vascular status (i.e., compliance and resistance) may improve and maintain accuracy of cardiac output readings by these devices in critically ill patients, when changes in vascular resistance are likely to occur. Published validation studies for the Vigileo device are still missing, but preliminary results indicate that valid cardiac output monitoring using a pulse contour analysis system without calibration may be possible [31].

In addition to cardiac output, all the above mentioned devices allow the assessment of stroke volume (and pulse pressure) variation by pulse contour analysis which has been shown to predict LV fluid responsiveness in mechanically ventilated patients [32]. Moreover, global end-diastolic volume (GEDV) as a volumetric preload parameter and extravascular lung water (EVLW) can be determined by transpulmonary thermodilution integrated in the PiCCOplus system. GEDV has been shown to be a more reliable cardiac preload parameter than the conventional filling pressures assessed by the PAC [33], whereas EVLW may be an important prognostic factor in patients with sepsis and severe acute respiratory distress syndrome (ARDS) [34]. Thus, these devices offer new parameters with the potential to improve hemodynamic management.

Pulsed Dye Densitometry

Pulsed dye densitometry by the DDG 2001 analyzer (Nihon Kohden, Tokyo, Japan) allows intermittent cardiac output measurement based on transpulmonary dye dilution with transcutaneous signal detection adapted from pulse oximetry. After venous injection of the dye, i.e., indocyanine green (ICG), as indicator, its concentration is estimated in the arterial blood flow by means of optical absorbance measurements at two wave lengths in consideration of an overlap between the ICG and hemoglobin absorption spectra. From the observed dye dilution curve, cardiac output is calculated according to the thermodilution method using the Stewart-Hamilton formula.

Validation studies have been performed in patients after cardiac surgery [35, 36] but also in other typical intensive care settings (Table 1) [37]. The best agreement as compared with the reference method was found by Bremer et al. [35]. However, they excluded 10% of their patients because of low peripheral signal quality. Other investigators [36, 37] observed only weak to moderate agreement and a signal detection failure in up to 33% of all measurement attempts [36]. Poor peripheral circulation, vasoconstriction, and tissue factors like interstitial edema, but also movement artifacts or ambient light influences have been suggested to limit appropriate signal detection. Improved sensor technology with local tissue warming in combination with another methodological approach may be more suitable for cardiac output measurements [38]. However, to date pulsed dye densitometry cannot be recommended for cardiac output assessment in daily clinical practice.

Despite the limitations for cardiac output measurement, bed-side assessment of the ICG elimination rate by this technique has been shown to reliably provide information on liver function and may serve as a prognostic factor in critically ill patients [39].

Partial Carbon Dioxide Rebreathing

One of the first methods to estimate cardiac output was described by Fick in 1870. Based on the postulation that complete arterial oxygen uptake takes place in the lungs, cardiac output can be calculated as the ratio between oxygen consumption and arteriovenous oxygen difference. This principle can be applied to carbon dioxide (CO_2) for cardiac output measurement and it is used in the CO_2 rebreathing technique by the NICO system (Novametrix Medical Systems, Wallingford, CT, USA). The system consists of a disposable rebreathing loop, which is connected to the breathing circuit and contains a pneumatically controlled valve allowing an intermittent partial rebreathing state in cycles of 3 minutes (induction of an additional dead space of 50–80% tidal volume). For CO_2 analysis a mainstream infrared and airflow sensor is used. CO_2 production (VCO_2) is calculated as the product of CO_2 concentration and air flow during a breathing cycle, and arterial CO_2 content ($CaCO_2$) is derived from end-tidal CO_2 ($etCO_2$) and the CO_2 dissociation curve. The rebreathing cycle induces an increase of $etCO_2$ and a drop in VCO_2. The obtained differences of these values, ΔVCO_2 and $\Delta etCO_2$, are used to calculate cardiac output on the basis of a differential Fick's formula (Fig. 4). To adjust for pulmonary blood flow to non-ventilated lung areas, shunt fraction is estimated from the inspired O_2 fraction and O_2 saturation, measured by a pulse oximeter attached to the

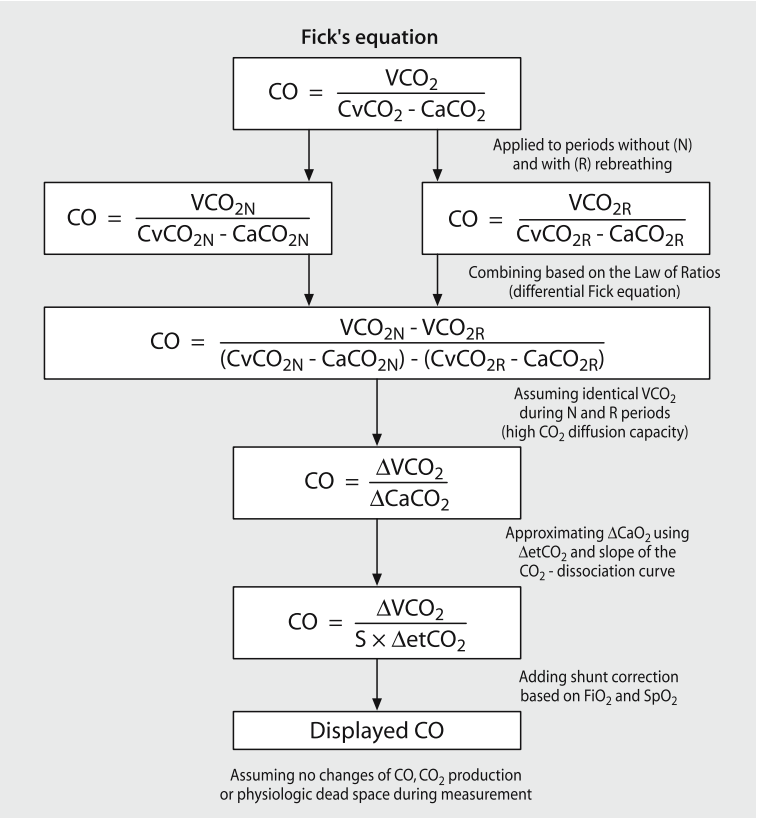

Fig. 4. Application of the differential Fick formula for cardiac output (CO) determination by the partial CO_2 rebreathing technique. $CaCO_2$: arterial CO_2 content (ml/100 ml blood); $CvCO_2$: mixed venous CO_2 content (ml/100 ml blood); $etCO_2$: end-tidal CO_2 concentration (mmHg); FiO_2: fraction of inspired oxygen; N: normal ventilation; R: rebreathing; S: CO_2 dissociation curve; SpO_2: oxygen saturation obtained by pulse oximetry (%)

NICO monitor. When arterial O_2 tensions measured by blood gas analysis are used for shunt calculation, improved accuracy can be achieved.

Different validation studies with conflicting results have been published in the last few years (Table 1). For instance, Odenstedt et al. [40] as well as Kotake et al. [41] found a fair agreement between the rebreathing technique and intermittent thermodilution using a PAC. Furthermore, comparable findings for intermittent and continuous cardiac output measurements by the gold standard were observed [41] and cardiac output changes during the study period could be reliably followed [40]. Therefore, the authors concluded that the device may be a valuable alternative for cardiac output measurement. By contrast, studies like the one by Mielck et al. [42] revealing only loose agreement raised concerns about the utility of this technology using parameters for cardiac output determination inherently influenced by ventilation and pulmonary function. Indeed, studies addressing this issue [43, 44] showed that different ventilatory settings in mechanically ventilated patients re-

sulted in differences in agreement between cardiac output measured by the NICO system and thermodilution. Moreover, spontaneous, mechanically assisted breathing [44] and the application of this technique to patients with lung pathologies [43], i.e., patients with increased shunt fraction, decreased the accuracy of cardiac output readings. Thus, cardiac output may only be reliably assessed by the partial CO_2 rebreathing method, when fixed ventilatory settings can be applied to mechanically ventilated patients without serious or alternating pulmonary shunt fractions.

Apart from cardiac output, the NICO system displays different ventilatory data and allows the determination of the individual shunt fraction. However, the NICO shunt calculation underestimated shunt fraction by 10.8% (±2SD = 17.8%) when compared with values calculated by a standard formula [40] and further refinements of the NICO technology seems to be warranted for use in clinical practice.

Electrical Bioimpedance

Electrical bioimpedance uses constant electrical current stimulation for identification of electrical impedance variations induced by vascular blood flow. The electrical current is applied and the impedance measured by electrodes either attached to the neck root and the thorax at the xyphoid level (referred to as thoracic electrical bioimpedance = TEB) or by electrodes at one wrist and the collateral ankle (termed as whole body electrical bioimpedance = WBEB). From the measured signal variations, cardiac output values are estimated on a continuous basis according to the formula $\Delta V = \Delta R/R \times$ body weight (g), where ΔV equals the aortic systolic volume change, i.e., stroke volume, ΔR is the systolic resistance change (= impedance variation), and R the baseline impedance of the electrical field involved. The basic formula has been adjusted for the distance between the sensing electrodes, patients' body length, blood resistivity and left ventricular ejection time to better reflect individual vascular flow. However, clinical experiences with bioimpedance devices using these adjusted formulas were often disappointing and advanced the development of more sophisticated algorithms considering gender, hematocrit, cardiac cycle length and other factors [45]. These are used in the currently available second-generation TEB (e.g., BioZ, Cardiodynamics International, San Diego, CA, USA; Cardioscreen, Messtechnik, Illmenau, Germany) and new WBEB (e.g., NiCaS, NI Medical, Hod-Hasharon, Israel) devices.

Despite technological advances, the recently published clinical trials using TEB still report conflicting results (Table 1). Sageman et al. [46] and Spiess et al. [47] showed a fair to moderate agreement between TEB and the reference method, whereas other investigators [48] found a weak agreement with a bias >0.5 l/min/m in 56% of paired measurements. Inconsistent results were also found in clinical studies using a new WBEB system [49, 50], which may be explained by the considerable limitations for the use of TEB and WBEB. Pronounced aortic disease (aortic dilatation and aneurysm, coarctation, aortic valve insufficiency), intra- and extracardiac shunt, tachyarrhythmia, significant edema, pleural effusion and interventions resulting in a large fluid shift, increased positive end-expiratory pressure (PEEP), but also movement artifacts and electrical interference may render cardiac output readings by TEB and WBEB unreliable. Therefore, further development of the bioimpedance technology may have to address some of these limitations in order to be suitable to a larger patient population.

Conclusion

At present, evidence suggests that none of the available less invasive techniques for cardiac output measurement may fully replace the PAC in daily clinical practice. Based on probe placement and frequent re-positioning for optimal signal quality, TEE and Doppler measurements are highly operator dependent. The underlying assumptions render these techniques often unreliable. Pulse contour analysis devices calibrated by indicator dilution techniques may be reliably used for cardiac output assessment in different clinical settings. Rapid changes of systemic vascular resistance during hemodynamic instability may require repeated calibration for accurate measurements. The recently introduced pulse contour analysis without calibration still has to prove its use in these situations. The partial CO_2 rebreathing technique may be applied for accurate cardiac output measurements in a precisely defined clinical setting to mechanically ventilated patients only. Both pulse contour analysis and partial CO_2 rebreathing have the potential to be used in situations where hemodynamic monitoring is desirable, but the PAC may not be used. Pulsed dye densitometry in its current form has to be considered as experimental and bioimpedance, influenced by a variety of clinical conditions, is limited to an investigational setting.

Despite their potential limitations for cardiac output assessment many of these methods allow useful additional information on actual patient status to be obtained: TEE is an important diagnostic tool, esophageal Doppler may be used to follow aortic flow trends, pulse contour analysis devices enable enhanced preload assessment, and the determination of the ICG elimination rate may be helpful in the bed-side assessment of liver function.

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Pulse Pressure Analysis

M. Cecconi, J. Wilson, and A. Rhodes

Introduction

Cardiac output monitoring is part of routine practice in the critically ill patient. Recently, there has been increasing interest in continuous cardiac output monitoring, which has seen the development of new devices less invasive than the pulmonary artery catheter (PAC). The insertion of a PAC allows semi-continuous monitoring of cardiac output using the thermodilution technique but these new devices allow continuous monitoring by analyzing the arterial pressure wave. This analysis is known as pulse pressure analysis. This chapter explores the issues associated with pulse pressure analysis and presents the mathematical basis for the devices available.

From Arterial Pressure to Cardiac Output

Arterial pressure is one of the most commonly monitored variables in critical care medicine; however, arterial pressure is not in itself enough to assess cardiac output. In fact, arterial pressure is the result of a combination of cardiac output and the resistance of the vasculature:

$$P = CO \times R$$

Where P = arterial pressure, CO = cardiac output, and R = resistance of the vasculature.

It is apparent that the resistance of the vasculature must be known to calculate arterial pressure. Unfortunately, resistance is not constant, but it is possible to relate variations in pressure to variations in stroke volume due to a feature known as compliance. Compliance is the relationship between volume change and pressure change:

$$C = \Delta V / \Delta P$$

Where C = compliance, ΔV = volume change, and ΔP = pressure change.

Therefore, if we know the value of compliance, we can measure the pressure change (ΔP) to calculate the volume change (ΔV).

$$\Delta V = C \times \Delta P$$

Compliance in the arterial tree is not a linear function (Fig. 1). Moreover, compliance is not constant for one blood vessel. For instance, vasoconstriction causes arteries to become stiffer and this leads to a decrease in compliance. Vasodilation leads to an increase in compliance (Fig. 2).

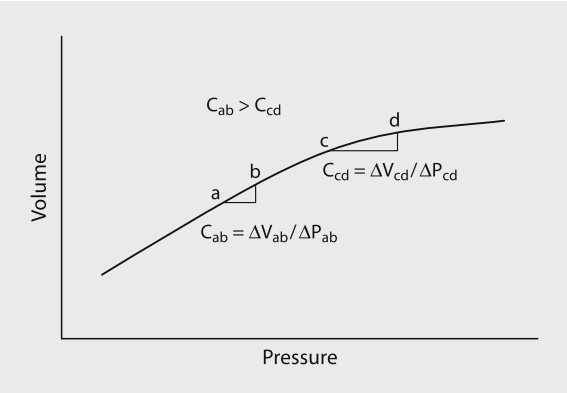

Fig. 1. ΔV_{ab} and ΔV_{cd} have the same value. ΔP_{ab} is lower than ΔP_{cd}. C_{ab} ist then higher than C_{cd}. ΔV = change in volume, ΔP = change in pressure. C = compliance

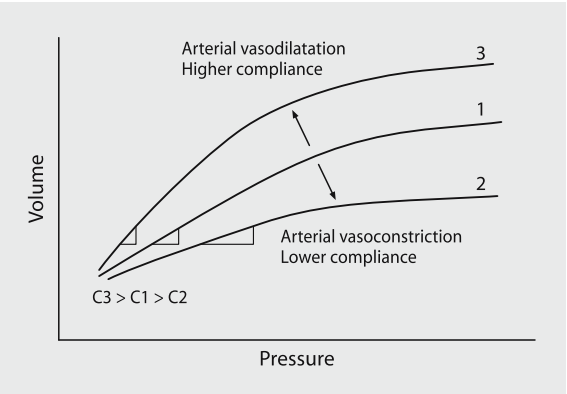

Fig. 2. Compliance curve can shift to different states and the same change in volume can determine very different changes in pressure. C3, C1, C2 = compliance respectively for curve 3, 1, 2

The proximal aorta carries the blood initially pumped from the heart. The aorta is filled according to three forces:

- The force of injection of the blood by the pumping of the heart
- An opposing force dependent on pulsatile inflow (impedance)
- An opposing force depending on the change in volume (compliance).

The peripheral resistance is the force that opposes the blood flow to the periphery. Ideally, these forces should be measured at a site as proximal as possible to the aorta. However, as pulse pressure devices have a low level of invasiveness, they will, by their very nature, record the arterial wave peripherally.

Arterial pressure measured in the periphery is affected by at least three effects:

- The generation of reflection waves in the periphery
- Damping of the arterial pressure measurement system
- Differences in the flow-pressure relationship centrally and peripherally.

Damping is a common problem in clinical practice. The fluid filled tubes used to measure intravascular pressure form a resonant system that can oscillate. The performance of such a resonant system is determined by the frequency of oscillation and by the damping coefficient.

If a signal being analyzed has a similar frequency to the resonant frequency of the measuring system, the two oscillations can combine, leading to the phenomenon called dampening. If this combination amplifies the signal, it is known as underdamping, if the combination fades the signal, it is known as overdamping. Both underdamping and overdamping lead to incorrect measurements of arterial pressure.

The relationship between flow and pressure depends on the intrinsic characteristics of the cardiocirculatory system. Systole generates two types of wave, pressure waves and flow waves. An arterial transducer will measure pressure waves. In the proximal aorta, the pressure wave and the flow wave will occur at almost the same moment; however, the pressure wave is transmitted to the periphery about twenty times faster than the flow wave. Also, the proximal aorta is filled during systole, resulting in an almost pulsatile flow whereas flow in the periphery is more constant, flowing in both systole and diastole [1, 2]. Therefore, pulse pressure analysis algorithms have been, and are being, developed to relate arterial pressure to cardiac output. Given an initial stroke volume value obtained by calibration, these algorithms use correction factors for the above issues to generate a volume vs. time curve from a pressure vs. time curve.

In summary, an ideal algorithm should:

- Work independently of the arterial site where pressure is being monitored, given the changes in waveform shape and pressure through the arterial tree from the core to the periphery.
- Correct for the non-linear nature of compliance and individual variations in aortic characteristics to give an absolute cardiac output.
- Not be affected by changes in vascular resistance caused by increases of arterial pressure due to reflected waves.
- Not be reliant on identifying the details of wave morphology.
- Be minimally affected by damping effects seen in arterial lines [3].

History: From Frank to Wesseling

The history of pulse pressure dates back more than 100 years. In 1899, Otto Frank developed the Windkessel (air chamber) model to simulate the heart-vessel interaction [4]. This model comprises a circuit in which fluid is pumped in tubes through chambers. The tubes are completely fluid-filled but the chambers contain some air. As the fluid is not compressible but the air is, the behavior of the air mimics aortic distension in blood vessels (compliance). Frank also deduced that by knowing the compliance, the stroke volume could be calculated from the change in pressure.

In 1904, Erlanger proposed a correlation between stroke volume and change in arterial pressure and suggested there would be correlation between cardiac output and the arterial pulse contour [5]. This eventually led to the development of algorithms relating the pulse pressure analysis 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.

The first algorithm to be used in clinical practice was the Wesseling algorithm in 1983 [6]. This algorithm is based on the hypothesis that the contour of the arterial pressure wave is dependent on stroke volume and this can be estimated from the integral of the change in pressure over the interval from the end of diastole to the end of systole (Asys) (Fig. 3).

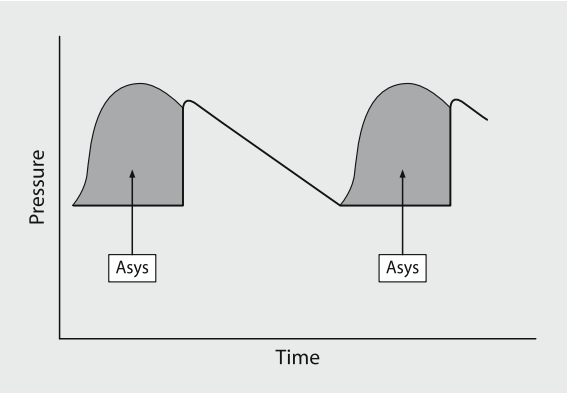

Fig. 3. Stroke volume is derived by determining the area under the curve of the systolic part of the arterial pressure (Asys)

The integral calculation gives the value of the systolic area from the beginning of systole to the end of systole (dicrotic notch). The stroke volume (Vz) is the value of Asys divided by the value of aortic impedance (Zao):

$$Vz = Asys/Zao$$

The stroke volume is adjusted (Vcz) to take account of heart rate (HR, to adjust for the effect of reflection waves in the periphery), mean arterial pressure (MAP) and age:

$$Vcz = Vz[0.66 + 0.005\,HR - 0.01\,Age(0.014\,MAP - 0.8)]$$

Vcz is multiplied by heart rate to give the cardiac output. The system is then calibrated with thermodilution to equalize the pulse contour cardiac output to the measured one:

$$COcz = cal \times HR \times Vcz$$

Where COcz is the continuous cardiac output, and cal is the calibration factor derived by the comparison of COcz with cardiac output measured by thermodilution [7].

This was the first algorithm of pulse pressure analysis used to determine cardiac output and from this starting point, new algorithms have been developed. In the Modeflow algorithm, Wesseling et al. refined their original algorithm to take account of compliance and the flow-pressure relationship. Both the Wesseling and Modeflow algorithms have shown good correlation with the gold standard pulmonary thermodilution [8–11] and led to the development of new devices. 'Pulse pressure analysis' continues to be an evolving field in hemodynamic monitoring.

Available Devices

There are presently four suppliers of devices able to calculate cardiac output from analysis of intravascular arterial pressure:

- Pulsion with PiCCO monitor
- LidCO with LiDCO^{TM}plus System
- FIAB SpA with pressure recording analytical method (PRAM) system
- Edwards Lifesciences with FloTrac technology and Vigileo Monitor.

These devices and their calculation of continuous cardiac output is briefly described below.

PiCCO

Line requirements:
- Central line for transpulmonary thermodilution
- Femoral arterial line (specialized catheter) or radial arterial line (radial arterial long catheter) for transpulmonary thermodilution and pulse contour analysis.

The PiCCO (Pulsion, Munich, Germany) monitors cardiac output and several volumes using transpulmonary thermodilution (e.g., intrathoracic blood volume (ITBV), extravascular lung water (EVLW)). Early versions of the device were the first to implement a modified Wesseling algorithm. Later versions use a more robust algorithm that includes analysis of arterial pressure during the diastolic phase to address issues around non-linear compliance and flow-pressure relationships. Both algorithms use transpulmonary thermodilution to calibrate the continuous cardiac output derived from the algorithm to the measured output.

After calibration, the continuous cardiac output stroke volume is:

$$\text{First algorithm: } SV = \text{cal}\,(163 + 0.48\,HR^{*}MAP)\,Asys$$

$$\text{Second algorithm: } SV = \text{cal}\,(Asys + C_{(p)}dP/dt)dt$$

where SV = stroke volume, cal = calibration factor, HR = heart rate, MAP = mean arterial pressure, $C_{(p)}$ = compliance corrected for arterial pressure, P = pressure, and t = time [6, 12].

The first algorithm uses a very similar approach to the Wesseling algorithm but excludes age. For a long time, continuous cardiac output using the PiCCO was the only pulse pressure analysis device available for use in intensive care and anesthesia. PiCCO continuous cardiac output has been validated against the PAC in several conditions [13–18] and has proven to be reliable, only needing regular recalibration in the event of major hemodynamic changes [19].

LiDCO

Line requirement:
- Central line or peripheral line for lithium bolus for transpulmonary dilution
- Arterial line for lithium transpulmonary dilution and continous cardiac output.

The LiDCO system is a new cardiac output monitor that measures cardiac output using lithium transpulmonary thermodilution. The LiDCO^{TM}plus system implements an algorithm for continuous cardiac output monitoring derived from the arterial pressure wave. The LiDCO system is not truly a pulse contour monitor, rather it uses pulse power analysis based on the hypothesis that the change in power in the system (arterial tree) during systole is the difference between the amount of blood entering the system (stroke volume) and the amount of blood flowing out peripherally. It is based on the principle of conservation of mass/power and an assumption that following correction for compliance and calibration there is a linear relationship between net power and net flow [3]. The algorithm overcomes the problem of reflected waves by taking account of the entire beat and uses an 'auto-

correlation' to determine what proportion of the change in power is determined by the stroke volume. Once this is determined, cardiac output is easily calculated, multiplying stroke volume by heart rate. The first stage of the algorithm transforms the arterial pressure wave into a standardized volume waveform (arbitrary units) using this formula:

$$\Delta V/\Delta P = \text{calibration} \times 250 \times e^{-k.p}$$

Where V = volume, P = blood pressure, k = curve coefficient, and 250 is the saturation value in mls, i.e., the maximum above the starting volume at which atmospheric pressure that the aorta/arterial tree can fill [3].

Autocorrelation uses the volume waveform and derives the period of the beat plus a net effective beat factor, proportional to the nominal stroke volume pumped into the aorta. This nominal stroke volume is then calibrated to be equalized to a measured stroke volume. The calibration is obtained using the lithium dilution technique. The continuous cardiac output of LiDCO has been validated in a number of studies [20–23] and proved to be reliable in surgical and intensive care patients. Studies of the device in different situations are continuing and will likely further refine this method.

PRAM

Line requirement:
- an arterial line.

PRAM (Mostcare FIAB SpA) is a new continuous cardiac output monitor. The most innovative feature of PRAM is the lack of a requirement for calibration. The algorithm is based on the principle of perturbations, analyzing the arterial wave using a collecting signal of 1000 Hz. The most important points on the arterial wave for the calculation are the initial point of the pulse wave (diastolic pressure), the highest point (systolic pressure), and the point of closure of the aortic valve, represented by the dichotic notch or incisura dicrota. The PRAM algorithm uses these and other points of perturbance to take into account the interaction of heart contraction, aortic impedance and compliance and peripheral resistance. For each cardiac cycle, the whole area under the curve is measured and analysis of the perturbations gives a factor Z that correlates changes in volume to changes in pressure. The algorithm does not use recorded curves or the age or sex of the patient to calculate compliance but calculates it individually cycle by cycle.

PRAM gives the formula for stroke volume as:

$$SV = A/(P/t \times K)$$

where A = whole area under the systolic portion of the pressure curve, P = description of the pressure wave profile expressed as the variation in pressure (P) over time (t) during the entire cardiac cycle, and K = factor inversely related to the instantaneous acceleration of the vessel's cross-sectional area, obtained from the ratio between expected and measured mean blood pressure.

The most novel feature of this device is that calibration does not need to be performed to give an absolute cardiac output and a patient can be connected to PRAM as soon as an arterial catheterization is performed. PRAM is still under validation. So far it has been validated against pulmonary thermodilution in animals and in cardiac patients. In the study in cardiac surgery patients, the Bland Altmann plot

showed a good agreement between PRAM and standard thermodilution (mean difference 0.027 l, standard deviation 0.43 l, with limits of agreement –0.83 and +0.89 l) [24–26].

FloTrac and Vigileo

Line requirement:
- an arterial line.

Flotrac (Edwards Lifescience) is the name of the transducer incorporated in the Vigileo monitor. As with PRAM, this device does not require calibration and only requires an arterial line. The algorithm is primarily based on the standard deviation of the pulse pressure waveform:

$$CO = f\,(\text{compliance, resistance}) \times \sigma_p\,HR$$

where σ_p is the standard deviation of the arterial pressure, HR is the heart rate, and f (compliance, resistance) is a scale factor proportional to vascular compliance and peripheral resistance.

The standard deviation of the arterial pressure waveform is computed on a beat-to-beat basis using the following equation:

$$\sigma_p = \sqrt{\frac{1}{N-1}\sum_{k=0}^{N-1}\left[P(k) - P_{avg}\right]^2}$$

where P(k) is k^{th} pulse pressure sample in the current beat, N is the total number of samples, and P_{avg} is the mean arterial pressure.

Compliance and resistance are derived from the analysis of the arterial wave. The hypothesis is that the shape of the arterial pressure wave can be used to calculate the effects of compliance and peripheral resistance on flow. Additional parameters, such as the pressure dependent Windkessel compliance, C_w, based on Langwouters' study [27], heart rate and the patient body surface area (BSA) are also included to take other patient specific characteristics into account. This algorithm is now under validation. The two publications available [28, 29] show reasonable bias and precision in comparison with pulmonary thermodilution. Further studies will probably be available shortly.

Conclusions

Pulse pressure analysis has an important role in the management of critically ill patients. There are currently several devices available that are less invasive and serve to provide continuous and accurate measurements of cardiac output. PiCCO is the oldest device and has been validated in several clinical situations. LiDCO is more recent and has also been validated, with more studies to follow. PRAM and FloTrac are very new devices that have the advantage of not requiring calibration and being quick and easy to use. There is evidence validating PRAM and results on the use of FloTrac are expected in the near future. There has been much criticism about the use of the PAC recently. It is our vision that pulse pressure analysis will be implemented in clinical protocols to change management and outcome in critically ill patients.

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Pulse Pressure Variations in Managing Fluid Requirement: Beware the Pitfalls!

A. Vieillard-Baron

Introduction

Mechanical ventilation in controlled mode has long been known to induce cyclic changes in systolic blood pressure. This occurrence was first described by Massumi et al. as an increase in systolic blood pressure related to lung inflation, followed by a decrease during expiration, and called "reverse pulsus paradoxus" [1] (Fig. 1). These changes stem from cyclic modifications in systemic venous return [2] and in right ventricular (RV) afterload [3] related to alterations in intrathoracic pressure and in transpulmonary pressure, respectively. Indirectly, this reflects cyclic changes in left ventricular (LV) stroke volume induced by positive-pressure ventilation. Such a phenomenon is always present but is limited in a patient with a normal hemodynamic status. Coyle et al. were probably the first to use these variations to detect hypovolemia [4]. Using the baseline value of systolic blood pressure observed at end-expiration, they also separated systolic blood pressure variations into two components, delta Up (dUp) and delta Down (dDown) (4) (Fig. 1), the latter being closely correlated with the level of induced hemorrhage in dogs [5] and with fluid responsiveness in septic patients [6].

However, systolic blood pressure changes not only reflect changes in LV stroke volume but are also related to the transmission of the intrathoracic pressure to the aorta [7]. For this reason, Michard et al. recently suggested not using systolic blood pressure variations in clinical practice but rather pulse pressure variations (ΔPP) [8]. Pulse pressure is calculated as the systolic minus the diastolic blood pressure. It is also higher during inspiration and lower during expiration [9] (Fig. 1). Assum-

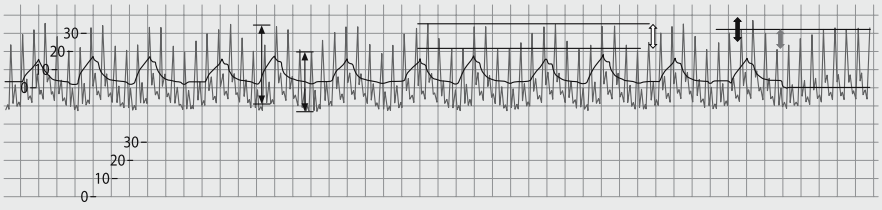

Fig. 1. Variations in blood pressure in a mechanically ventilated patient. Use of an end-expiratory pause allows separation of the dUp from the dDown effect. dUp is defined as an absolute increase in systolic blood pressure during inspiration whereas dDown is defined as an absolute decrease during expiration. ⇳ Systolic blood pressure variations; ⇕ dDown; ↕ dUp; ↕ Pulse pressure

ing that the intrathoracic pressure is transmitted similarly to the systolic and diastolic pressures, pulse pressure more accurately reflects LV stroke volume. Michard et al. nicely demonstrated that, in certain conditions, ΔPP could be a strong predictor of fluid responsiveness: the higher the ΔPP, the greater the increase in LV stroke volume induced by volume expansion [8].

There is now an enthusiasm for less invasive monitoring of fluid requirement. However, many potential pitfalls must be avoided if this index is to be used reliably. False-positives (significant ΔPP not related to fluid responsiveness) and false-negatives (low ΔPP despite fluid responsiveness) have been reported [8, 10], leading to inappropriate and deleterious fluid infusion or inadequacy of plasma volume. There is a strong physiological rationale underlying these limitations, as described below.

The False-positives

False-Positives Related to Right Ventricular Dysfunction

As briefly recalled above, ΔPP reflects changes in LV stroke volume related to tidal ventilation. Because the left ventricle is directly filled from the pulmonary circulation, which is therefore its filling reserve [11], respiratory changes in LV stroke volume, and thus ΔPP, are directly related to changes in the amount of blood in the pulmonary reservoir: during lung inflation, the pulmonary circulation is emptied because the blood is boosted toward the left cardiac chambers [12], whereas, at the same time, a decrease in RV ejection precludes its immediate refilling [9]. Two conditions have been described that prevent any immediate refilling of the pulmonary circulation by the right ventricle, thereby inducing significant ΔPP: 1) hypovolemia leading to a marked inspiratory decrease in RV ejection related to a decrease in systemic venous return, but also 2) severe RV dysfunction also leading to an inspiratory decrease in RV ejection but related to an increase in RV afterload [13]. In the first case, the fluid infused reaches the pulmonary circulation and thus corrects ΔPP and significantly increases LV stroke volume. In the second situation, the fluid does not reach the pulmonary circulation because of the RV dam and thus does not correct ΔPP and does not increase LV stroke volume.

The specificity of ΔPP in the study of Michard et al. was 96%, in a very selected population of 40 septic patients with circulatory failure [8]. Interestingly, these authors excluded patients with severe acute respiratory distress syndrome (ARDS) [8]. We have previously demonstrated that ARDS patients may exhibit acute cor pulmonale [14], a type of RV dysfunction related to an excessive and acute increase in RV afterload [15]. In another recent study in 66 septic patients, in which patients with ARDS were included, we reported a specificity of ΔPP of only 87% with six false-positives [10]. We also reported that most of our false-positives exhibited acute cor pulmonale, demonstrating the truthfulness of this limitation, which was also recently suggested by Magder [16] and by De Backer et al. [17]. Two examples are given in Figure 2.

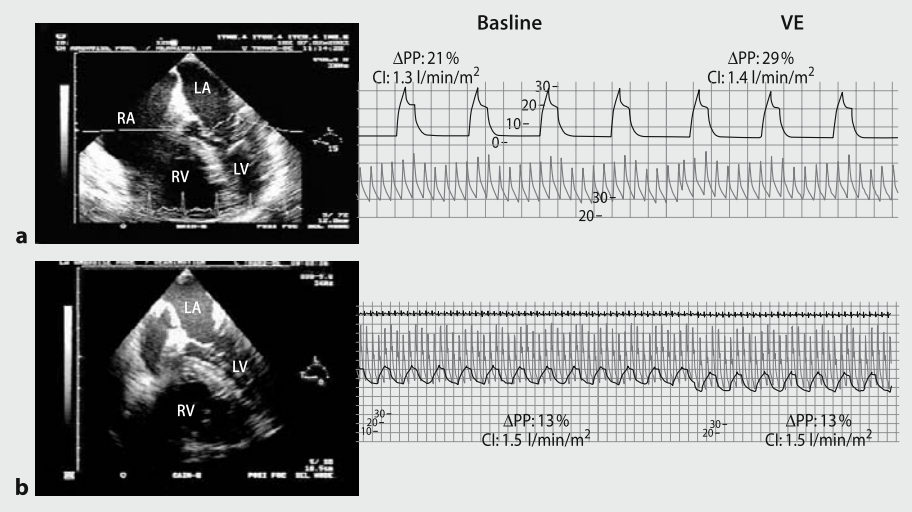

Fig. 2. Significant pulse pressure variations (ΔPP) related to acute cor pulmonale in two patients (**a**, **b**) ventilated for severe acute respiratory distress syndrome. Echocardiography demonstrated marked right ventricular dilatation leading to marked restriction of the left ventricle. After volume expansion (VE), ΔPP was not corrected and cardiac index (CI) was not increased. Note that patient **b** was receiving a norepinephrine infusion. RA: right atrium; LA: left atrium; RV: right ventricle; LV: left ventricle

False-positives Related to a 'dUp Effect'

As proposed by Michard et al. [8], ΔPP is very simple to calculate just by using the maximal pulse pressure during inspiration and the minimal during expiration. In most cases this is a good idea in clinical practice and is probably one of the main reasons why this parameter has been very successful. However, the absence of an end-expiratory pause makes it difficult to bring ΔPP to a 'dUp effect' (absolute increase in pulse pressure during inspiration) or to a 'dDown effect' (absolute decrease in pulse pressure during expiration). In the latter situation, we have seen above that this frequently leads to volume expansion, except in the case of acute cor pulmonale. On the other hand, in the first situation, this could lead to an inappropriate and potentially deleterious volume expansion.

We have previously shown that most patients exhibit a dDown and also a dUp (Fig. 3) [12]. We have also demonstrated that dUp is related to the blood in the pulmonary capillaries which is boosted toward the left atrium and the left ventricle when the lung is inflated [12]. This improvement in LV preload is immediately responsible for a significant increase in LV stroke volume and thus for a dUp [12]. dUp increases with the amount of blood in the pulmonary circulation. Therefore, this effect is more frequently observed in congestive heart failure, a situation where the 'ad hoc' therapeutic arm is fluid removal rather than volume expansion (Fig. 4). Once again, it is difficult to evaluate the incidence of this limitation of ΔPP in the study by Michard et al. because patients with severe hypoxemia or with a pulmonary artery occlusion pressure (PAOP) ≥ 18 mmHg were excluded [8].

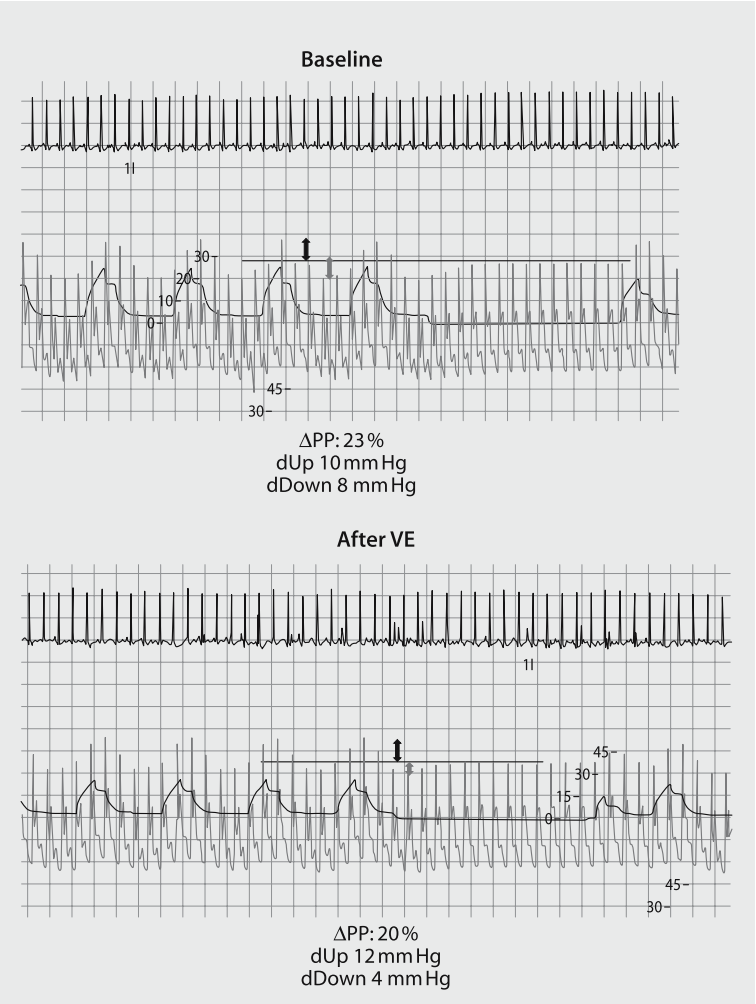

Fig. 3. ΔPP related to a dDown (⇕) and a dUp (⇕) in a patient with peripheral vascular disease. At baseline, a significant ΔPP of 23% was related to a significant dDown associated with a significant dUp. By increasing the amount of blood in the pulmonary circulation, volume expansion (VE) led to a decrease in dDown and an increase in dUp, without significant change in ΔPP.

The False-negatives

False-negatives Related to an 'inadequate' Tidal Volume

Much debate surrounds the impact of a low tidal volume on the accuracy of pulse pressure variations in patients ventilated for ARDS [18]. The rationale is that too low a tidal volume could be insufficient to generate significant changes in intrathoracic and transpulmonary pressures and so ΔPP could appear non-significant even if patients require volume expansion. This discussion was largely boosted by Reuter

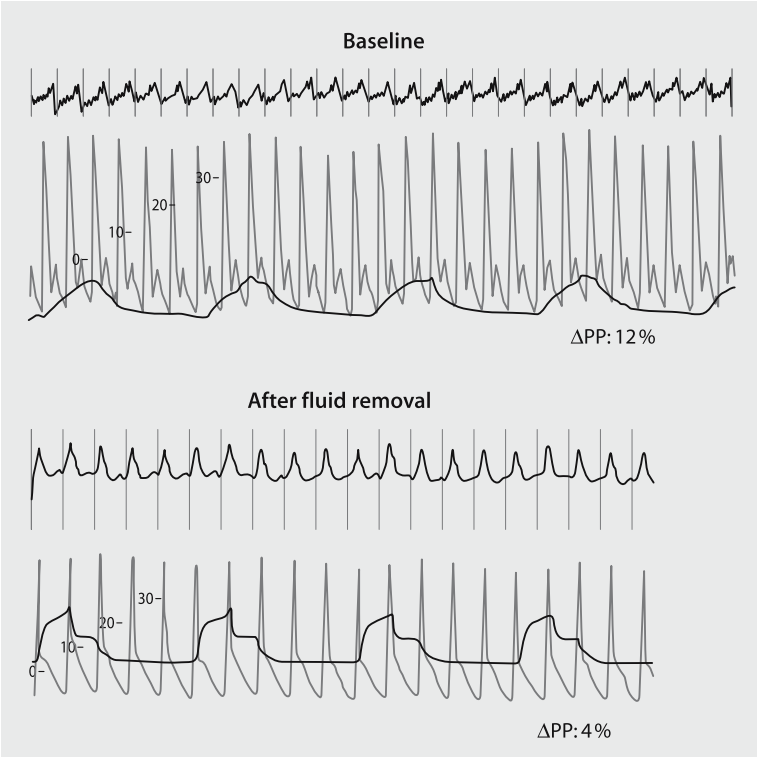

Fig. 4. ΔPP induced by fluid overload. In this patient with marked fluid overload, ΔPP was corrected by fluid removal using continuous veno-venous hemodiafiltration

et al. who demonstrated that the level of tidal volume significantly influences LV stroke volume variations [19]. However, this finding was perfectly expected: in a given patient, the higher the tidal volume, the greater the cyclic changes in airway pressures and so the greater the LV stroke volume variations. In ARDS, the debate is a little different. Tidal volume is significantly reduced because lung and often chest wall compliances are significantly impaired [20]. So, significant airway pressures are still present despite major reduction in tidal volume.

Among studies reporting the accuracy of pulse pressure variations or systolic blood pressure variations in predicting fluid responsiveness, those of Tavernier et al. [6] and of Michard et al. [8] were performed in patients ventilated with a tidal volume above 8 ml/kg, i.e., between 8 and 12 ml/kg, leading to a large range of plateau pressure (11 to 34 cm H_2O in the study by Tavernier et al. [6]). In our recent study [10], ΔPP was still accurate although all patients were ventilated with tidal volumes that were relatively low (8 ml/kg) but still greater than the recommendations of the ARDS Net study [21]. Finally, De Backer et al. recently tried to resolve this issue in 60 critically ill patients separated according to their tidal volume [17]. Although they suggested that ΔPP is inaccurate for a tidal volume below 8 ml/kg, this study suffers from a few limitations [18] and no final conclusion can be drawn.

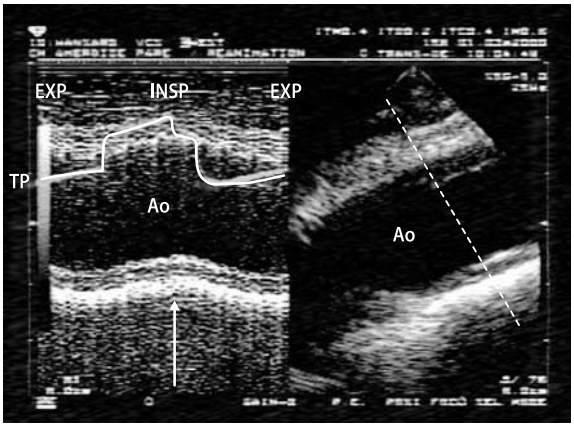

Fig. 5. Effect of intrathoracic pressure on the aorta. In this mechanically ventilated patient who exhibited marked impairment of chest wall compliance, transesophageal echocardiography, using the two-dimensional mode coupled with motion mode (dotted line), demonstrated a compression of the aorta (Ao) (arrow) during lung inflation. EXP: expiration; INSP: inspiration; TP: tracheal pressure

Potential Limitation Related to Arterial Compliance

By hypothesizing that arterial compliance remains constant through the respiratory cycle, which is not completely true because of the external constraint exerted by the intrathoracic pressure on the aortic wall (Fig. 5), pulse pressure is a reliable reflection of LV stroke volume. But pulse pressure also depends on arterial compliance: the more compliant the artery, the lower the pulse pressure for a given LV stroke volume. One can, therefore, hypothesize that small respiratory changes in LV stroke volume in a preload-unresponsive patient could induce significant ΔPP in the case of stiff arteries (this probably in part explains the ΔPP observed in Figure 3). On the other hand, large changes in LV stroke volume could only induce small ΔPP in a preload-responsive patient with very compliant arteries. Artery compliance may differ from one patient to another, or in the same patient depending on the time of measurement, the disease, and whether or not there is infusion of vasoactive drugs. This could impose a significant limitation on the accuracy of ΔPP in a large and unselected population. However, unlike the other limitations described above, which can be avoided when forewarned, it is difficult to predict such a limitation.

Conclusion

Variation in pulse pressure is probably a good parameter for management of fluid requirement in patients with circulatory failure. Its measurement is less invasive than that of other parameters, and several studies have reported its value in clinical practice. However, reliable use of this parameter is dependent on awareness that there are pitfalls with a strong underlying physiological rationale, especially in an unselected population.

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Minimally Invasive Hemodynamic Monitoring Using the Pressure Recording Analytical Method

S. Scolletta, S.M. Romano, and P. Giomarelli

Introduction

The commonly employed reference techniques for assessment of cardiac output are represented by the direct Fick method in physiology, and by intermittent thermodilution in clinical practice. According to the Fick principle, cardiac output is determined by the ratio of oxygen uptake to the difference in oxygen content between arterial and mixed venous blood [1]. The validity of the method depends on the assumption that pulmonary blood flow closely approximates systemic blood flow and that lungs themselves do not extract oxygen. The major limitations of the method are the need for right heart catheterization to obtain truly mixed venous blood, the availability of techniques for measuring oxygen uptake and content, and the attainment of a steady state in which oxygen consumption matches tissue oxygen utilization [1, 2]. Likewise, the intermittent thermodilution method requires fulfillment of several conditions, such as complete mixing of the thermal indicator with blood, no loss of indicator within the dilution volume, and constant blood flow during the dilution time [3]. Inconsistency of these assumptions may occur in many clinical conditions, leading to inaccuracy in cardiac output measurements. In particular, variability of blood flow may result from hemodynamic instability related to changes in heart rate, cardiac arrhythmias, valvular or congenital heart disease, and application of mechanical ventilation [4–6].

Despite some disadvantages of bolus intermittent thermodilution, this is the most commonly used clinical method of cardiac output determination and continues to be considered as the established standard reference method for validation of the other methodologies. Nevertheless, the bolus intermittent thermodilution technique only measures cardiac output over a variable time period of 15–45 s, and it may not be so appropriate as a gold standard technique for evaluation of a continuous method of measuring systemic blood flow. Therefore, the thermal filament-wrapped pulmonary artery catheter (PAC) employing the thermodilution principle to measure cardiac output continuously might be more appropriate for comparison of beat by beat techniques [7–9]. However, the cardiac output displayed is updated every 30 seconds and reflects the average flow from the previous 3–4 minutes. As a consequence, this could generate errors in comparing hemodynamic values without an exact event marker to synchronize continuous thermodilution cardiac output with beat-to-beat techniques. These concerns are present and must be acknowledged.

According to the hypothesis that continuous monitoring of cardiac output could allow the detection of sudden hemodynamic changes which may influence patient management and outcome [10–12], different approaches, such as continuous ther-

Table 1. Characteristics of the ideal monitoring system

Accuracy
Reproducibility or precision
Fast response time (real time, beat-to-beat)
Operator independency
Easy to use
Continuous use
Cost effectiveness
Minimally invasive
Clear data display and interpretation
Real time afterload, preload, and oxygen delivery
Neonates to adults

modilution [13, 14], pulse contour method [15], bioimpedance [16], and Doppler ultrasonographic techniques [17] have been utilized more recently.

The pulse contour methods, unlike bolus thermodilution, which measures cardiac output over a limited time span, operate on a beat-to-beat basis and, for this reason, could be suitable for the continuous monitoring of cardiac output. Such a device would have significant advantages over the use of intermittent thermodilution and may provide the concept of an ideal monitor (Table 1). Pulse contour methods are based on the main assumption, proposed by Wesseling et al. [18], that the pressure rise during systole is related to the systolic filling of the aorta and proximal large arteries [19–21]. Various models of the arterial system have been devised to approximate the relationship between arterial pressure and flow [18, 22–25]. The most used pulse contour approach [25] relies on the aortic pressure-cross-sectional area relationship that is modeled from unrelated *in vitro* measurements on segments of human thoracic aorta [26]. Pulse contour methods can monitor changes in cardiac output over prolonged periods of time either from the pressure signal recorded in a systemic artery [25, 27, 28] or from that detected non-invasively at the finger [29–32]. To obtain absolute values of cardiac output, it is, however, necessary to determine, at least once for each patient, a calibrating factor for the model parameters by comparing the pulse contour method result with an absolute cardiac output value. This greatly limits the usefulness of pulse contour methods since the calibrating technique is invasive (e.g., thermodilution) and must be repeatedly applied when changes in the experimental procedure, that may alter the physical properties of the arteries, are induced [32].

Recently, a minimally invasive method has been developed: beat-to-beat values of cardiac output can be obtained by a pressure recording analytical method (PRAM). This new method is based on the mathematical analysis of the arterial pressure profile changes. It allows beat-to-beat stroke volume assessment from the pressure signal recorded invasively in the aorta or non-invasively at the finger [33], or from that recorded invasively in radial and femoral arteries [34, 35].

PRAM: Scientific and Physical Principles

Changes in volume which occur in all arterial vessels are mostly due to wall radial expansion in response to blood pressure changes. This depends on various physical factors:

- the force of blood ejection generated by the left ventricle
- the arterial impedance counteracting the pulsatile blood inflow
- the arterial compliance that permits to elastically store a portion of the kinetic energy of the cardiac upstroke, and
- the peripheral vessels' resistance generating backward reflections of the pressure wave depending on heart rate and relative tightening, bifurcations, and stiffness of downstream arterial vessels.

These variables are closely interdependent and need to be evaluated simultaneously. To this end, a variable called Z, representing the relationship between changes in pressure and changes in volume with time, is taken into account for the evaluation of stroke volume in the various approaches to determine cardiac output by pulse contour methods. Pulse pressure is converted to stroke volume by calculating the area under the pulsatile portion of the pressure wave, and Z ($mmHg \times s\ cm^{-3}$) is calculated as a factor retrospectively approximated from the results of *in vitro* experiments or by calibration with an independent measure of stroke volume (i.e., thermodilution bolus). Such retrospective adjustment represents the principal limitation of pulse contour methods.

At variance with other pulse contour methods, PRAM is the practical application of a model developed completely *a priori*. The model does not require adjustments based on experimental data [33]. The concept behind PRAM is based on the physics theory of perturbations [36], by which each physical system under the effects of a perturbing term tends to react to reacquire its own condition of stability (i.e., the situation of minimal energy required). With PRAM, the whole, instead of the pulsatile, systolic area under the pressure curve is measured in each cardiac cycle (Fig. 1). Simultaneously, Z is obtained directly from the morphologic analysis of both the pulsatile and the continuous components of the pressure waveform, with no need for calibrating factors. The derivation of Z requires no predicted data apart from the expected mean arterial pressure (MAP).

Briefly, according to PRAM [34], Z is equal to $(P/t) \times K$, and stroke volume (SV) is calculated as follows (cm^3):

$$SV = \frac{A}{P/t \times K}$$

where A ($mmHg \times s$) is the whole area under the systolic portion of the pressure curve; P/t ($mmHg \times s^{-1}$) is a description of the pressure wave profile expressed as the variations in pressure (P) over time (t) during the entire cardiac cycle (systolic and diastolic portion); and K is a factor inversely related to the instantaneous acceleration of the vessel cross sectional area ($s^2 \times cm^{-1}) \times (1 \times cm^{-2}$).

The A, P/t and K variables are closely interdependent in each cardiac cycle. To compute P/t, PRAM assumes that peak systolic pressure and the pressure at the dicrotic notch represent points of dynamic equilibrium among the various forces which concur to the blood flow along the arterial system. The value of K is ob-

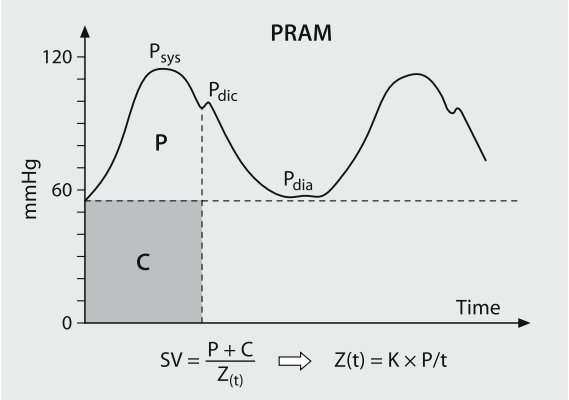

Fig. 1. Analysis of the arterial waveform by PRAM and stroke volume computation. PRAM measures the whole systolic area (A = P + C, pulsatile + continuous) under the pressure curve. Stroke volume (SV) is calculated by P + C divided by Z(t). Z(t) represents the relationship between changes in pressure and changes in volume with time. Z is equal to (P/t) × K, and P/t is a description of the pressure wave profile expressed as the variations in pressure (P) over time (t) during the entire cardiac cycle (systolic and diastolic portion). K is a factor inversely related to the instantaneous acceleration of the vessel cross sectional area. For computing P/t PRAM assumes that peak systolic pressure (P sys), the pressure at the dicrotic notch (P dic), and the diastolic pressure (P dia) represent points of dynamic equilibrium among the various forces which concur to the blood flow along the arterial system (see text for details).

tained from the ratio between expected and measured mean blood pressures. The numerator of the relationship is constant (theoretical MAP), and the denominator is measured. As a consequence, K may change from cardiac cycle to cardiac cycle, and the constant value at the numerator is taken as a reference to gauge the deviation from normality of MAP. Because MAP is lower peripherally with respect to central arteries [37], PRAM applies two different values of expected mean pressure for the computation of K at the central (aorta) and the peripheral levels (e.g., radial or femoral), namely the values originally indicated by Burton [37] and Guyton [38] (i.e., 100 mmHg at the central and 90 mmHg at the peripheral level). Thus, PRAM allows the use of two proper algorithms for central or peripheral arteries to obtain stroke volume for each cardiac cycle. The value of K will differ from unity in the presence of physical phenomena that may affect the pressure wave transmission (e.g., low stroke output from the left ventricle or backward wave reflections from the peripheral vasculature). Because perturbations of the pressure wave are reflected in the instantaneous acceleration of the arterial vessel cross-sectional area, the correction of P/t by a value of K above or below unity yields a corrected value of Z that takes into account the effect of the wave reflection [33].

PRAM: Cardiac Output Measurements

PRAM needs to be connected to a standard arterial catheter (or to the analogic-output of the monitoring system) for computing cardiac output and other hemodynamic parameters from the radial or femoral pressure waves. The pressure signals are acquired at 1000 Hz by means of an analogic-digital multifunction card (DAQ Card-700, Na-

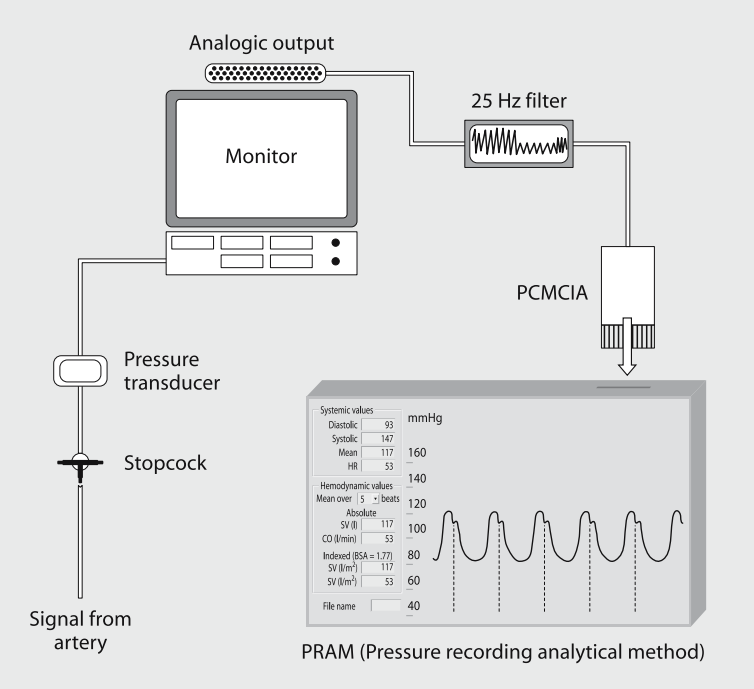

Fig. 2. Schematic representation of PRAM. PRAM can be connected to the analogic-output of the monitoring system or to the pressure transducer for the continuous recording of the arterial pressure waves and the subsequent computation of cardiac output (CO). The signals are acquired at 1000 Hz by means of an analogic-digital multifunction card (PCMCIA) and filtered at 25 Hz to avoid the resonance effects caused by the catheter-transducer system without degrading the pressure wave amplitude. On a personal computer, PRAM cardiac output values and arterial pressure waves are displayed. Dotted lines under the pressure curves represent the identification of the dicrotic notches.

tional Instruments Corp., Austin, TX) working on the tension signals with 12 bit from –2.5 to 2.5 volts. All the signals are recorded on a personal computer. The pressure signals are filtered at 25 Hz to avoid resonance effects caused by the catheter-transducer system without degrading the pressure wave amplitude. PRAM analyzes the shape of systolic and diastolic phases, identifies the dicrotic notch, and calculates the whole area under the systolic portion of the arterial pressure wave (Fig. 2). Blood pressure, heart rate, cardiac output, systemic vascular resistance (SVR), stroke volume, stroke volume variation (SVV), cardiac cycle efficiency (CCE), and dP/dT ratio values are displayed on a dedicated personal computer (Fig. 3).

PRAM Versus other Pulse Contour Methods: Advantages and Disadvantages

PRAM is based on the principle that in any given vessel volume changes occur mainly because of radial expansion in response to variations in pressure [33]. Similar approaches have been studied by several authors in the course of the last three

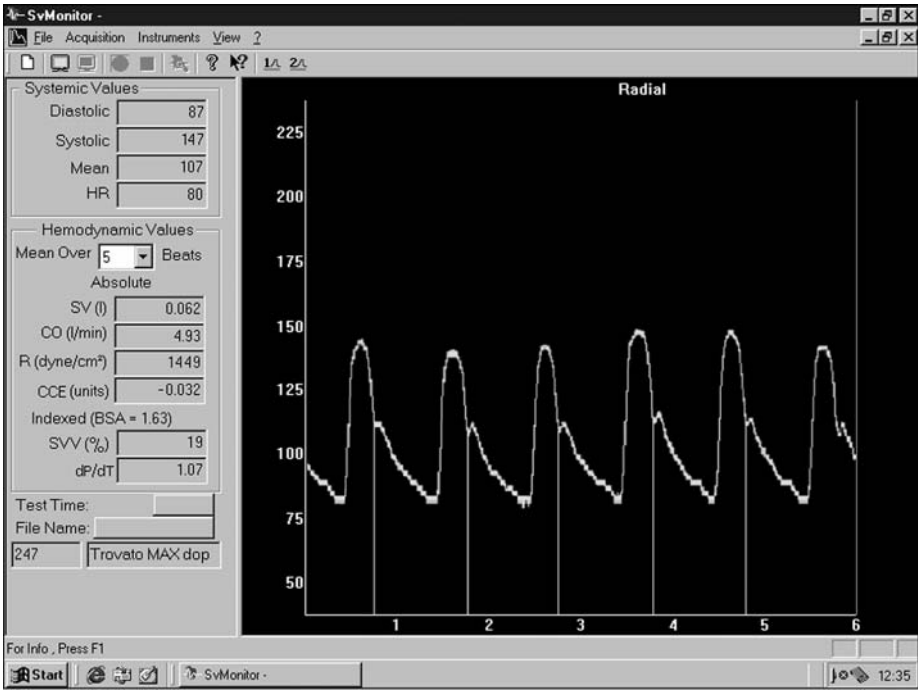

Fig. 3. Beat-to-beat recording of the arterial pressure waveform by PRAM. The figure shows the screen of PRAM and a real time analysis of the arterial waveforms. On the left side of the monitor the hemodynamic parameters are shown. From top to bottom are blood pressure values (diastolic, systolic, mean), heart rate (HR), stroke volume (SV), cardiac output (CO), systemic vascular resistance (R), cardiac cycle efficiency (CCE), stroke volume variation (SVV), and dP/dT ratio. On the right side of the monitor are shown 5–6 pressure waveforms from the radial artery. The vertical lines under the pressure curves identify the dicrotic notches at each cardiac cycle.

decades, and have led to the development of important clinical applications (e.g., PiCCO system, LiDCO system) [39–41].

The PiCCO device also allows the measurement of global end-diastolic volume (GEDV) and intrathoracic blood volume (ITBV) as surrogate preload markers together with extravascular lung water (EVLW) and pulmonary vascular permeability index (PVPI) [42], and recent studies have suggested that ITBV or GEDV are better indicators of cardiac preload than parameters obtained with a PAC [43]. However, the PiCCO system does not start without a central thermodilution cardiac output calibration, which is performed at a given time point with a given aortic impedance at that time. Therefore, there is a need for recalibration in case of vasomotor tone and resistance changes [42]. Finally, the PiCCO system recommends arterial catheter placement in the femoral site. This may be a limitation because many intensivists choose to avoid the femoral site for intravascular access due to concerns about increased infection rates. Conversely, the LiDCO system is less invasive, uses an existing arterial access, and does not require central circulation catheterization for calibration (lithium can be injected via the peripheral venous route). However, little is known about possible toxic effects or accumulation with long-term use of

lithium, especially in patients with organ failure. Finally, even if the LiDCO system starts pulse contour cardiac output computations directly after connection to a radial artery pressure signal with a trending of cardiac output, it has a higher accuracy only after considering external calibration data [39, 41]. On the contrary, PRAM can measure absolute values of stroke volume, independently from calibration, by determining parameters able to characterize the elastic properties of the arteries from the objective analysis of the pressure wave profile [33–35].

Our research group investigated PRAM in low-risk and hemodynamically stable patients undergoing coronary artery bypass surgery, and concluded that PRAM was accurate for real-time monitoring of cardiac output during cardiac surgery and the post-operative period [34]. However, we did not obtain good agreement between PRAM cardiac output and thermodilution cardiac output measurements after the end of extracorporeal circulation, and hypothesized that our results may be explained by the loss of reliability of PRAM due to vascular tone changes and to the increased forearm blood flow observed during the rewarming after the end of extracorporeal circulation [44]. On the other hand, thermodilution may also not provide truthful cardiac output values after the end of extracorporeal circulation [45, 46]. Notoriously, variability of the thermodilution method occurs because of differences in injection technique, fluctuations in blood temperature, electrical interference, and cyclical variations in cardiac output with ventilation [45–47]. Variations in pulmonary artery blood temperature transiently increase after extracorporeal circulation, and the increased thermal noise may cause significant errors in the results of thermodilution techniques [45, 46]. Unfortunately, this interpretation cannot be demonstrated in the absence of a third method or a gold standard preferable to thermodilution, and it is not possible to offer a definitive explanation of the disagreement between PRAM and thermodilution after the end of extracorporeal circulation. Nevertheless, PRAM was able to identify two patterns of beat by beat cardiac response during the weaning phase from extracorporeal circulation: whereas most patients showed stable cardiac output values following the reduction of the blood flow generated by the heart-lung machine, a group of patients showed a decrease in cardiac output values and required a longer weaning time and inotropic drug support [48]. Thus, beat-to-beat cardiac output monitoring by PRAM can prove valuable in identifying patients at risk of developing a low flow state during the weaning from extracorporeal circulation.

Over recent years, only two major studies have provided an indication of the intrinsic accuracy of PRAM [33, 34], but they did not give a direct answer to the questions arising from the accuracy of the method in measuring cardiac output in various hemodynamic states. To this purpose, we studied PRAM under various hemodynamic conditions in animals, since it enabled us to evaluate the performance of the system over a wide range of cardiac outputs that could not be tested ethically in humans. Our research group demonstrated that PRAM gave comparable results to cardiac output determined by electromagnetic flowmetry, and by conventional thermodilution during various hemodynamic states in a swine model [35].

Generally, PRAM and other pulse contour methods may have advantages over PAC-derived thermodilution measurements. They provide a fast response time (beat-to-beat readout), and abrupt changes in cardiac output resulting from blood loss, tamponade, off-pump cardiac surgery, or changes in arterial resistance may be detected more quickly than with thermodilution. In addition, in mechanically ventilated patients, blood pressure can develop changes from interaction between the heart and lungs during positive pressure ventilation, and the changes in arterial

pressure during the respiratory cycle can match different fluid status predictors: systolic pressure variation (SPV), pulse pressure variation (PPV), and SVV. For example, a SVV value under 10% implies that the patient probably does not need volume administration, and a value over 15% implies that the patient probably does need volume expansion [43]. These dynamic markers of fluid responsiveness, combined with more traditional parameters, may permit more appropriate fluid management in the ventilated patient. However, variations in stroke volume or pulse pressure may not be as readily attributed to hypovolemia in the spontaneously breathing patient or in the presence of an irregular cardiac rhythm. As a result, these parameters may not be reliable in a large proportion of critical care patients. Finally, the use of these variables requires more research as the clinical consensus is not universal and there is some controversy over the interpretation of the arterial pressure variation analysis [49].

PRAM also provides a new parameter, the CCE, which could represent the performance of the left ventricle. The CCE ranges from –1 to +1, with –1 being the worst and +1 the best possible cardiac cycle performance. Recently, in 70 patients undergoing coronary surgery, the CCE by PRAM was compared with the left ventricular ejection fraction (LVEF) by echocardiography before and after cardiopulmonary bypass. Overall, the correlation coefficient between LVEF and CCE values was 0.82 ($r^2 = 0.91$, $p < 0.001$), and the correlation coefficients at each time of the study ranged from 0.80 to 0.84, $p < 0.001$ [50]. Thus, PRAM seemed a reliable tool to detect changes in cardiac cycle performance and to monitor myocardial function and recovery during cardiac surgery.

Some disadvantages of this new method still need to be addressed. Several factors could affect the accuracy of cardiac output measurements based on the analysis of arterial waveforms, such as stenosis of the arterial tree and arterial pathology in the proximal segments. Damped waveforms and inadequate pulse detection (e.g., severe arrhythmias, catheter dislodgement) may influence the precision of the pressure wave analysis [42]. Because the radial artery is the commonly employed site for assessment of cardiac output by PRAM, we would have to consider the following: the more peripheral the arterial access point, the greater the potential for error in cardiac output determinations. Finally, intrathoracic volume quantification is not provided with PRAM.

Conclusion

Clinicians today have an outfit of several new techniques that provide for minimally invasive hemodynamic assessment. Currently no method fulfils the criteria of the ideal monitor. However, these monitoring systems do not exclude each other, as they have different advantages and limitations and each has something to offer a given patient population, health care institutional budget, and clinical user [42].

PRAM seems to be easy to use and does not require external calibration by thermodilution, or any additional invasive procedure. Since it does not require injection of a thermal solution, a central line is not required, avoiding both time-consuming and potential complications due to the insertion of a central catheter. Moreover, the positioning of a PAC for thermodilution measurements may be contraindicated in minor surgical procedures and in low-risk patients [47]. Therefore, PRAM might be considered a practical alternative to the traditional intermittent thermodilution method when siting a PAC is deemed harmful or not essential for clinical

management. Finally, further studies should concentrate on the impact of using PRAM monitored variables on the length of intensive care unit patient stay, morbidity, and survival.

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Coagulopathies

Thrombotic Microangiopathy Syndrome in the ICU

S. Samy Modeliar, M. Monge, and M. Slama

Introduction

Since the first descriptions of haemolytic-uremic syndrome (HUS) by Moschowitz and thrombotic thrombocytopenic purpura (TTP) by Gasser, our knowledge about thrombotic microangiopathy (TMA) has grown considerably [1]. TMA now refers to a group of diseases comprising mechanical hemolytic anemia, peripheral thrombocytopenia, and varying degrees of organ failure. The incidence of TMA is increasing in the USA. Considerable progress has recently been made in the understanding of the pathophysiological mechanisms of TMA. These rare diseases, characterized by platelet thrombi in the microcirculation, are responsible for often serious organ dysfunction leading to the admission of these patients to intensive care units (ICUs). The prognosis of TMA was extremely poor prior to plasma therapy and especially plasma exchange. TMA is a serious, life-threatening disease that requires early diagnosis and urgent specialized therapeutic management.

Definition

TMA syndrome comprises a group of diseases characterized by a combination of:
- mechanical hemolytic anemia
- peripheral (consumption) thrombocytopenia
- varying degrees of organ failure.

Histologically, TMA is defined by the presence of thrombi in terminal arterioles and capillaries, responsible for varying degrees of organ failure.

The two main types of TMA are:
- TTP, characterized by frequently multiple organ involvement,
- HUS, in which renal involvement is predominant.

HUS and TTP share similar clinical features. Other forms of TMA are associated with multiple factors: cancers, certain chemotherapeutic agents, stem cell transplantation, human immunodeficiency virus (HIV) infection, malignant hypertension, pregnancy, post-partum, disseminated intravascular coagulation (DIC), HELLP syndrome (hemolysis, elevated liver enzymes and low platelets), giant hemangiomas and hemangio-endotheliomas.

Pathophysiology

General Description

The early phenomenon common to all forms of TMA is damage to, or activation of, the vascular endothelium, responsible for local platelet aggregation, promoting the formation of platelet thrombi in the microcirculation. Various factors are responsible for this endothelial cell activation: infections, drugs, cancers, stem cell transplantation, etc. [2].

Thrombotic Thrombocytopenic Purpura

Role of von Willebrand factor (vWF) and ADAMTS-13 protein (A Disintegrin And Metalloprotease with ThromboSpondin type 1 motif). Physiologically, vWF is a multimeric glycoprotein that triggers the formation of platelet clot, and transport of clotting factor VIII. It is synthesized by megakaryocytes and endothelial cells, and then stored in endothelial and platelet Weibel-Palade bodies. Ultra-large vWF multimers (ULvWF) are composed of several vWF monomers linked by disulfide bonds. vWF multimers have more intense hemostatic properties than monomers [2].

The ADAMTS-13 protein is a metalloprotease that cleaves vWF multimers into monomers [3, 4]. TTP is associated with very low levels of ADAMTS-13 protein activity[1], resulting in elevated plasma levels of ULvWF [5].

In this context of metalloprotease deficiency, ULvWF are released in response to endothelial cell damage, and then accumulate and adopt an optimal procoagulant configuration, resistant to the shearing forces of the microcirculation. ULvWF bind to platelet GPIIb and GPIIb/IIIa receptors, inducing excessive platelet aggregation. This mechanism is responsible for consumption thrombocytopenia and the formation of microthrombi decreasing the caliber of capillaries of the microcirculation, leading to tissue ischemia and erythrocyte fragmentation on thrombi (schistocytes) [2].

Deficiency of the protease cleaving ULvWF in TTP is attributed either to acquisition of an IgG inhibitory auto-antibody or to a mutation of the gene coding for the protease (familial cases) [6–10].

Role of infections in the pathogenesis of TTP. TMA is frequently associated with infection, whether or not there is another underlying disease [11]. In a retrospective intensive care study, 53% of cases of TMA were associated with infection [1]. These infections have a major impact on the patient's outcome [11]. Various microorganisms have been isolated during the acute phase of TTP, or during the days preceding TTP (Table 1 [12–20]). An underlying infection must be systematically sought in all cases of TMA.

The infectious agent involved in the pathogenesis of TTP induces a direct or indirect endothelial lesion (mediated by sepsis), as various mediators (interleukin [IL]-1 and IL-6, interferon gamma [IFN-γ, tumor necrosis factor [TNF]-α) are released during the inflammatory reaction of sepsis and generate endothelial lesions.

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[1] NB: ADAMTS-13 protein activity can be decreased in other pathological situations: DIC, ITP-idiopathic thrombocytopenic purpura, systemic lupus erythematosus, cirrhosis, pregnancy, postoperatively, but it remains detectable in these situations, while it is virtually undetectable in TTP (activity $< 5\%$).

Table 1. Non-exhaustive list of the main microorganisms isolated during TTP

Bacteria	Viruses	Yeasts
▮ Pneumococcus	▮ HIV	▮ Cryptococcus
▮ Tuberculosis	▮ Herpes	
▮ Borrelia	▮ CMV	
▮ Legionella		
▮ Bacteroides		
▮ Rickettsia		

HIV: human immunodeficiency virus; CMV: cytomegalovirus

Damaged endothelial cells degranulate, releasing procoagulant substances (ULvWF, platelet-activating factor [PAF]) into the plasma, express adhesion molecules and produce chemokines such as IL-8. These phenomena promote adhesion and activation of neutrophils, which participate in endothelial lesions. Activated endothelial cells also show decreased synthesis of prostaglandin I2 (PGI2), the most potent platelet aggregation inhibitor in the body. These mechanisms lead to a hypercoagulability state, which persists in individuals presenting predisposing factors.

Hemolytic-uremic Syndrome

The pathophysiology of HUS is characterized by intravascular coagulation specifically involving the renal microcirculation. Two very different forms are distinguished:

- post-diarrheal HUS, usually endemic and mainly affecting young children (1–5 years),
- HUS occurring in the absence of diarrhea or atypical HUS, observed in older children and adults.

Post-diarrheal HUS [21]**.** This is the most frequent form of HUS (90% of cases). The majority of cases are due to toxin-producing *Escherichia coli* gastrointestinal infection. The serotype most frequently isolated is *E. coli* 0157:H7, but many other serotypes as well as many other bacteria have been incriminated (Table 2). The *E. coli* toxin is called Shiga-like-toxin (SLT) due to its structural analogy with *Shigella dysenteriae* toxin type 1.

The pathophysiology of post-diarrheal HUS starts with ingestion of food usually contaminated by a strain of toxin-producing *E. coli*. Intestinal colonization by *E. coli* is responsible for liquid diarrhea. This colonization phase is accompanied by massive toxin production. This toxin is released into the intestine and is responsi-

Table 2. Main bacteria involved in the pathogenesis of post-diarrheal HUS

▮ Escherichia coli
▮ Shigella dysenteriae
▮ Salmonella typhi
▮ Campylobacter jejuni
▮ Yersinia pseudotuberculosis

ble for direct microvascular and mucosal lesions, causing another episode of potentially hemorrhagic diarrhea. The toxin binds to specific receptors, is internalized and then enters the systemic circulation. *E. coli* bacteremia is not usually observed in post-diarrheal HUS. The toxin is transported by neutrophils to target tissues, i.e., the kidney, where it binds to specific receptors situated on endothelial cells of the renal cortex and medulla and tubular epithelial cells. It is internalized in renal cells and induces inhibition of protein synthesis via ribosomal inactivation (depurinization), leading to apoptotic cell death. In addition to these lesions, the toxin also stimulates cytokine production.

Endothelial cell damage has several consequences: expression of tissue factor on the cell surface, activation of coagulation, release of PAF into the circulation, and induction of overexpression of plasminogen activator inhibitor (PAI)-1 on the endothelial cell surface. Damaged endothelial cells also show decreased PGI2 production, and activate platelets and neutrophils predisposing to platelet aggregation on the endothelial cell surface. These phenomena lead to the formation of fibrin-rich microthrombi in renal capillaries, resulting in renal failure, formation of schistocytes and consumption thrombocytopenia.

Atypical HUS [2, 22]**.** Atypical HUS is much rarer (7 to 10% of cases), occurring sporadically in adults and older children. It is characterized by the absence of gastrointestinal infection. Its pathophysiology is poorly elucidated. Various hypotheses have been proposed in adults (viral infections, bacterial infections, certain drugs, etc.) to be responsible for endothelial lesions and features of HUS. A circulating factor present in the kidney has also been proposed.

Atypical HUS in children has been reported to be associated with persistent consumption of the C3 fraction of complement via the alternative pathway [23]. Decreased complement levels can be associated with factor H deficiency (factor H inhibits the alternative complement pathway). Atypical HUS with factor H deficiency usually corresponds to sporadic cases rather than familial cases and is rare in adults [24, 25]. There is no clearly established correlation between factor H deficiency and the development of atypical HUS (factor H deficiency induces excessive C3 consumption, causing increased activation of neutrophils and excessive platelet aggregation).

HUS and pneumococcal infection (in children). This is a particular pathophysiological entity related to expression of the Thomson Friedenrich antigen on the surface of erythrocytes, endothelial cells, and glomeruli. This antigen, normally masked by sialic acid, is revealed by neuraminidase secreted by *Pneumococcus*. This antigen is then recognized by circulating IgM, leading to platelet aggregation and endothelial and glomerular lesions.

Diagnosis

TMA disorders are rare in intensive care, with a prevalence of 0.35% of admissions according to a retrospective study conducted in adult intensive care units between 1998 and 2001 (64 patients) [11]. They represent 1 in 300 admissions, including cases of pre-eclampsia, stem cell transplantation, HELLP syndrome and terminal cancer.

Table 3. Complementary investigations in TMA (non-exhaustive list, to be completed as a function of clinical data)

- Complete blood count, reticulocytes, PT, APTT
- Fibrinogen, D-dimers (ELISA)
- Test for schistocytes (to be repeated)
- Proteins C, S, antithrombin
- Blood and urinary electrolytes, serum creatinine, plasma urea
- LDH, total and free bilirubin
- Protein profile
- Protein electrophoresis: plasma protein immunoelectrophoresis
- Antinuclear, anti-native DNA, antiphospholipid antibodies
- Complement C3, C4, CH50, assay of factor H
- Tissue plasminogen activator and its inhibitor
- Activity of ADAMTS 13 protein (repeated on D3 of plasma therapy) and its inhibitor
- Inhibitory protein of the alternative complement pathway
- Urinary urea and creatinine with analysis of urine sediment
- Urinary protein electrophoresis with immunoelectrophoresis
- Bacteriological work-up guided by clinical findings (blood culture, urine culture, etc.)
- Systematically:
  - hepatitis B and C, HIV, HTLV1 and 2, HHV6 serologies, CMV antigenaemia and CMV viruria
  - stool examination looking for *E. coli* (with serotyping), PCR stool verotoxin
  - serodiagnosis of enteropathogenic bacteria
- Renal ultrasound in the presence of renal failure
- Brain CT if neurological signs, EEG if convulsions, etc.

CT: computed tomography; EEG: electroencephalogram; HIV: human immunodeficiency virus; CMV: cytomegalovirus; PCR: polymerase chain reaction; HTLV: human T-cell leukemia virus; HHV: human herpesvirus

Early diagnosis is important (the prognosis is better when appropriate treatment is rapidly initiated). A diagnosis of TMA must be considered in any case of sudden onset of poorly defined neurological symptoms and hematological abnormalities (especially thrombocytopenia and anemia). TMA is observed in severely ill patients, often requiring admission to the ICU. In a retrospective study of TMA in 63 adult ICU patients conducted in 14 French teaching hospitals, the mean simplified acute physiology score (SAPS) II on admission was 45 ± 26 [11]. In another retrospective study, the mean SAPS II score on admission was 37 ± 18 (30 patients) [1].

The criteria for admission to the ICU are mainly life-threatening disease due to renal failure and/or neurological signs (convulsions, ischemia, coma). The other reasons for admission are hemorrhage, metabolic disorders (hepatocellular insufficiency), arrhythmias, myocardial infarction, etc [26].

Clinical features of TTP [2, 27]

The clinical presentation of TTP is marked by a sudden onset in an adult in previously good health. It has a female predominance (M/F ratio: 3/2) and generally occurs in the 4th decade. However, it can also occur in older or younger people.

The estimated incidence is 4 per million inhabitants per year and has been constantly increasing over recent years. In 40% of cases, there is a prodromal phase resembling viral infection for several days before the acute episode (asthenia, arthralgia, myalgia, low back and abdominal pain). In 80% of cases, the triad suggestive of the diagnosis is observed immediately (mechanical hemolytic anemia, peripheral thrombocytopenia and neurological signs). In 40% of cases, two other cardinal signs are present (hyperthermia and renal failure). The diagnosis of TTP must always be considered in a patient with anemia and thrombocytopenia associated with organ failure.

Anemia is the most constant and earliest feature and consists of severe, regenerating (reticulocytes > 120,000 mm^3), normochromic, normocytic, hemolytic (elevated lactate dehydrogenase [LDH] and bilirubin, and very low haptoglobin), microangiopathic anemia.

The blood smear reveals schistocytes, confirming the mechanical hemolysis (negative direct Coombs test). The presence of schistocytes may be delayed and should be looked for on subsequent smears [28]. Peripheral thrombocytopenia is marked (often less than 20,000/mm^3) and constant and reflects disseminated intravascular platelet hyperaggregability. The hemorrhagic manifestations related to this thrombocytopenia are varied in terms of site and severity. Clotting tests are normal. Fibrin and fibrinogen degradation products (FDP) may be observed in rare cases.

Neurological signs are present in only 60% of cases at the initial phase of the disease (and globally in 84 to 92% of cases during the course of the disease). They are characterized by their sudden onset and transient nature, and intermittent involvement of various territories over an interval of several hours. Neurological signs comprise: obtundation, confusion, hemiparesis, dysarthria, aphasia, coma, disorders of consciousness, sensory deficit. Deep tendon reflexes are often brisk. Generalized convulsions are observed in 20% of cases and can evolve to status epilepticus.

Hyperthermia is present in 20% of cases at the initial phase, and then in 59 to 89% of cases during the course of the disease. It is usually only low-grade and reflects underlying hemolysis and cellular ischemia.

Renal failure occurs in one half of cases. It is usually moderate and, in the majority of cases, consists of macroscopic hematuria or slight proteinuria (less than 3 g/24 h). Oligo-anuric renal failure is rare.

Other organ lesions may be observed, particularly myocardial infarction or myocarditis that may lead to acute cardiogenic shock and chronic heart failure. Acute respiratory distress syndrome (ARDS) requiring mechanical ventilation, colonic ischemic, acute pancreatitis and sometimes ocular lesions may also be observed.

Sporadic TTP that resolves definitively is distinguished from recurrent forms with regular and frequent relapses, and intermittent TTP associated with irregular relapses [29].

Clinical Features of HUS

HUS comprises a combination of mechanical hemolytic anemia, peripheral thrombocytopenia and acute renal failure. The renal lesion is predominant in all forms and is classically associated with severe hypertension at the time of diagnosis. This hypertension is more severe and more frequent than during TTP, while neurological signs are less frequent than during TTP.

Anemia and thrombocytopenia have similar characteristics to those of TTP (see above). Thrombocytopenia can be more severe than during TTP. Clotting abnormalities may be observed: slight reduction of fibrinogen and clotting factors V and VII. Elevation of fibrin degradation products, tissue plasminogen activator and PAI reflect activation of homeostasis by tissue factor in the kidney.

Kidney needle biopsy is only performed when there is a doubt about the diagnosis or in the case of persistent renal failure [30].

Epidemic (post-diarrheal) HUS. Post-diarrheal HUS has a sudden onset in 90% of cases, presenting with diarrhea (bloody in 70% of cases and febrile in 50% of cases). It is essentially observed in children, but also in adults. The renal lesion appears one week after onset of the diarrhea (which has resolved at the time of diagnosis in 50% of cases). Clinical manifestations are dominated by renal failure with severe proteinuria and hematuria (microscopic or macroscopic). Fifty percent of children are anuric at the time of diagnosis. One half of patients with renal failure will require dialysis. Severe hypertension is often associated with marked hyponatremia. Central nervous system signs are present in 10 to 30% of cases, often accentuated by hyponatremia, consisting of irritability, drowsiness, convulsions or even coma. The microorganism responsible is often no longer present in the stools at the time of the diagnosis of HUS, but the toxin can be isolated in two thirds of cases. The clinical and laboratory diagnosis of HUS is usually easy to establish and kidney needle biopsy is rarely necessary.

Atypical HUS. The clinical features are often less typical, and kidney needle biopsy is more often required to establish the diagnosis. Acute renal failure is often anuric with moderate proteinuria (1 to 2 g/24 h), but proteinuria may also exceed 3 g/24h. Microscopic hematuria is frequent and hypertension is present in 50% of cases at diagnosis. Thrombocytopenia is present in only 50% of cases at diagnosis. There are few or no extra-renal signs (fever, neurological signs). A history of infection is frequently reported. Adults sometimes present a context of auto-immune disease, drugs or pregnancy.

The assessment of atypical HUS must include assays of complement fractions (C3, C4, CH50), and the activity of the ADAMTS-13 protein and the inhibitory protein of the alternative complement pathway (and a test for a mutation of the corresponding gene).

The clinical features of HUS and TTP tend to overlap and it can be difficult, on admission, to distinguish between these two diagnoses. The neurological signs are variable, ranging from simple confusion to coma with generalized convulsions and focal deficits, aphasia, diplopia, facial paralysis, etc. Renal involvement is also heterogeneous, ranging from normal renal function to anuric acute renal failure. Laboratory test results can confirm the diagnosis, but often only retrospectively.

Prognosis

The prognosis of TMA prior to plasma therapy in the ICU was extremely poor. Plasma therapy has considerably improved the prognosis of these usually fatal diseases (3-month mortality greater than 90% for acute forms of TTP without treatment) [27]. In a recent retrospective study of 64 patients admitted to the adult ICU for TMA, the mortality rate was 35% [11] and Coppo et al. reported a mortality rate of 25% in intensive care patients with TMA [1].

Table 4. Main non-infectious circumstances (triggering factors or etiologies?) associated with TMA

- Pregnancy: antepartum and post-partum
- Cancers, hemangioma, hemangio-endothelioma
- Solid organ transplantation (kidneys, liver), hematopoietic stem cell transplantation
- Connective tissue diseases:
  - polyarteritis nodosa
  - systemic lupus erythematosus
  - acute scleroderma
  - ankylosing spondylitis
  - Sjögren syndrome
- Drugs:
  - penicillin, mitomycin C, cyclosporine
  - heparin, lidocaine, penicillamine D
  - tacrolimus, oral contraceptives, quinine, ticlopidine (non-exhaustive list)
- Toxins:
  - cocaine

The mortality related to TMA in intensive care is partly dependent on the treatment used. In recent studies [31–33], the survival rate of patients treated with plasma exchange is 78 to 90%. In the study by Pene et al. [11], the mortality at 90 days was 22% when plasma exchange was performed and 59% in the absence of plasma exchange. The study by Rock et al. [33] confirmed the superiority of plasma exchange compared to fresh frozen plasma (FFP) infusion in terms of survival and response to treatment (78 versus 49%). The use of plasma exchange is positively correlated with survival.

Overall, TMA syndromes with severe organ dysfunction requiring admission to the ICU are associated with a high mortality. The main cause of death in these patients in the ICU is multiple organ failure (MOF). An infection associated with TMA syndrome has a major impact on the outcome of these patients. The presence of a neurological deficit, possibly evaluated by the Glasgow Coma Scale, is the main negative prognostic factor correlated with mortality [11, 32, 34, 35]. Other prognostic factors have also been demonstrated: the SAPS II score, need for vasoactive support, bilirubin level, LDH kinetics on the 3rd day of treatment [11]. Predictive scores for TTP have been established, but their value has not yet been validated [35].

Unlike other diseases usually observed in intensive care, the development of acute renal failure or the need for dialysis in a context of TMA are not correlated with mortality [11, 33, 36]. According to Lara et al. [32], renal failure is correlated with a risk of relapse of TMA, which has a poor prognosis, but the renal prognosis of TTP is generally good.

The survival rates of post-diarrheal HUS are excellent in response to symptomatic treatment alone, and admission to the ICU is only rarely necessary. The mortality of post-diarrheal HUS varies between 3 and 8% and is related to gastrointestinal and neurological lesions. The short-term renal prognosis is good (improvement of renal function over several days to several weeks). However, one quarter to one third of children still present renal sequelae 10 years later (proteinuria and/or renal failure) with end-stage chronic renal failure in 3 to 4% of cases.

Atypical HUS in children has a poor prognosis, frequently resulting in end-stage chronic renal failure and a high post-transplantation recurrence rate. Atypical HUS in adults is a serious disease in which the prognosis depends on the etiology of HUS. The concomitant presence of advanced cancer, HIV infection, mitomycin C therapy and post-partum etiology carries a very poor prognosis. The intensive care mortality rate of atypical HUS is 10 to 20%. Twenty-five to 50% of these patients will require chronic dialysis. Only 50% of survivors are completely cured without sequelae. In patients with underlying nephropathy, chronic renal failure persists after the acute episode in 70% of cases. Long-term recurrences are possible, especially when the cause of HUS persists, but are less frequent than for TTP.

Management

TMA always requires emergency treatment. Admission to intensive care, in addition to the usual criteria of organ failure, must be proposed in all patients with severe thrombocytopenia (less than 50,000/mm^3), due to the high frequency of organ dysfunction at the acute phase of the disease.

Symptomatic Treatment

Symptomatic treatment is required in all forms of TMA. TMA disorders always have an unpredictable course. All available measures of 'aggressive intensive care' must, therefore, be proposed, even in the case of severe neurological signs.

Patients with respiratory failure require mechanical ventilation. This corresponds to 59% of all patients admitted to the ICU for TMA [11]. When possible, non-invasive ventilation should be preferred to invasive ventilation due to the hemorrhagic and infectious risks associated with invasive ventilation. Patients with severe renal failure require dialysis (47% of TMA patients in intensive care) and 32% of patients require vasopressor support. Hypertension must also be treated, preferably by angiotensin converting enzyme (ACE) inhibitors. The target blood pressure is 120/80 mmHg.

Folate supplementation is systematically prescribed (intense folate-consuming bone marrow regeneration). Anemia must be corrected by packed cell transfusion to achieve hemoglobin concentrations greater than 8 g/dl. Platelet transfusions should be avoided, except in the case of uncontrolled bleeding, as they may increase the thrombotic process in the microcirculation. Anticonvulsants should be prescribed to patients with a history of epilepsy. Any triggering or associated factors must be treated. In particular, antibiotics should be initiated whenever an infection is suspected (except for cases of post-diarrheal HUS, in which antibiotics can worsen the HUS [37]). Persistence of an occult bacterial infection can lead to persistence of TTP, making it refractory to conventional treatment.

Specific Treatment: Plasma Therapy

Indication. This treatment is not indicated in post-diarrheal HUS, as plasma therapy does not modify survival (which is excellent in response to symptomatic treatment alone). Plasma therapy must be urgently initiated in patients with TTP, as its efficacy in terms of survival has been demonstrated. Plasma therapy is used in adults

with HUS by analogy with the management with TTP, although no studies are in favor of either plasma exchange or FFP transfusion. It is essential to control underlying predisposing factors.

Fresh frozen plasma transfusion or plasma exchange? Recent studies have demonstrated the superiority of plasma exchange over FFP transfusion in terms of survival and response to treatment of TTP [36]. The use of plasma exchange is independently correlated with survival. The efficacy of plasma exchange depends on the volume of plasma administered to the patient (plasma exchange is able to administer three times more plasma than FFP transfusion alone [11]). Two pathophysiological mechanisms can explain the efficacy of plasma exchange: plasma exchange removes a 'pathogenic' component from the patient's plasma (ULvWF or auto-antibodies) and compensates for the deficient protease (by providing large quantities of plasma). In cases of TTP not associated with an inhibitor, FFP transfusions may be sufficient. When plasma exchange cannot be started immediately (for technical reasons, or while waiting for transfer to a specialized center), large-volume FFP transfusion (20 to 30 ml/kg/day) can be started prior to plasma exchange, but it is associated with a risk of fluid overload, protein-overload proteinuria, hyperproteinemia, and hyperviscosity syndrome. The raised oncotic pressure can also accentuate renal insufficiency.

The plasma exchange technique is based on the daily exchange of one to two plasma masses fully compensated by FFP. A plasma exchange session lasts about 3 hours for 50 to 80 ml/kg of FFP. It is performed via a central venous catheter, rarely placed in a subclavian vein due to the risk of bleeding, and requires anticoagulation at effective doses during the session. A systematic calcium supplement is started during plasma exchange (risk of hypocalcemia due to the toxicity of citrate).

Plasma exchange is time-consuming, expensive and invasive and carries a risk of immunological pulmonary edema (almost eliminated by solvent/detergent-treated plasma). The duration of treatment is variable. Plasma exchange is continued daily until restoration of a normal platelet count ($>150{,}000/mm^3$) for at least 48 hours with a reduction in LDH levels and reticulocyte counts. The frequency of plasma exchange must be progressively decreased. Daily plasma exchange must be reintroduced at the slightest sign of relapse. In the future, monitoring of ADAMTS-13 protein inhibitor levels could guide the frequency of plasma exchange.

During relapses (30% of cases of TTP), the initial treatment in the acute phase is the same as for the first episode. Repeated relapses may represent an indication for splenectomy, during a period of remission. Refractory TTP, defined by no improvement of the platelet count on day 5 of treatment, requires twice-daily plasma exchange and adjuvant therapies (vincristine, polyvalent immunoglobulins, cyclophosphamide: see below) [38, 39].

Second-line Adjuvant Therapy

High-dose corticosteroid therapy is effective in 56% of purely hematological forms of TTP [31], although its efficacy has not been clearly demonstrated by randomized trials. In the absence of a contraindication (active infection), and despite a low level of evidence, methylprednisolone (1 mg/kg/day for 3 weeks) can be administered immediately following plasma exchange.

The therapeutic value of platelet aggregation inhibitors, vincristine, high-dose immunoglobulins, splenectomy, immunosuppressives, unfractionated heparin, fibri-

nolytics, prostacyclin, and vitamin E in the intensive care management of TMA has not been demonstrated.

Staphylococcal protein A columns could be effective in TTP, especially in a context of cancer, but they have not been evaluated in patients with an inhibitor [40]. A possible treatment for the future would be purified or recombinant protease infusion, which could replace plasma therapy.

Other TMA syndromes

Pregnancy-associated TMA

Various types of TMA can occur during pregnancy and the post-partum period: TTP, HUS, HELLP syndrome. Many complications have been described including acute necrotic pancreatitis, myocardial infarction, gastrointestinal ischemia, etc. The treatment of TTP during pregnancy is based on plasma exchange. Pregnancy does not modify the response to treatment, but the consequences of plasma exchange on the fetus have not been evaluated. There is a risk of relapse during subsequent pregnancies.

Ante-partum HUS requires termination of pregnancy, but has a fairly good prognosis. Post-partum HUS can occur up to 3 months after delivery and has a poorer prognosis. Death may be due to cerebral ischemic or hemorrhagic lesions or cardiac sudden death. Some cases of post-partum HUS are related to factor H deficiency (heterozygous mutation).

The HELLP syndrome (hepatic form of TMA) is a TMA disorder specific to pregnancy. It differs from HUS and TTP by the presence of DIC and liver impairment. Treatment consists of fetal extraction. Plasma therapy has been proposed (3 to 5 post-partum sessions appear to be effective). There is a risk of recurrence during subsequent pregnancies. These patients should preferably be treated in a specialized center.

Hematopoietic Stem Cell Transplantation-associated TMA

In this context, TMA is triggered by various predisposing factors including total body irradiation, infections (immunodepressed patients), drugs (tacrolimus, cyclosporine A), and acute graft versus host disease. These predisposing factors induce disseminated endothelial damage. The ADAMTS-13 protein level is normal and the response to treatment is disappointing. Plasma exchange improves the prognosis, which nevertheless remains poor. Management of predisposing factors is an essential aspect of treatment.

Cancer-associated TMA

TMA is essentially associated with secretory adenocarcinomas, such as breast or stomach cancers, or more rarely lung, colon or prostate cancers. Stomach cancers represent more than 50% of all reported cases of cancer-associated TMA. In most cases, the cancer has already been diagnosed and treated, but TTP can also be the first sign of cancer. The pathophysiology of cancer-associated TMA has been poorly elucidated and is associated with variable ADAMTS-13 protein levels. The prognosis is also variable and depends on the underlying cancer.

Drug- or Toxin-associated TMA

Many drugs have been incriminated or suspected including ticlopidine, clopidogrel, cyclosporine A, tacrolimus, interferon alpha, oral contraceptives, quinine, cisplatin, mitomycin C, bleomycin, arsenic, penicillamine D.

Ticlopidine and clopidogrel are associated with severe ADAMTS-13 protein deficiency with the presence of plasma inhibitors. Plasma exchange therapy achieves complete remission in the majority of these patients [41, 42].

TMA associated with mitomycin C is dose-dependent and is observed after a total dose of more than 60 mg (2 to 10% of treated patients). The TMA disorders often appear several months after stopping mitomycin C and are characterized by hypertension and pulmonary edema [43]. Neurological disorders and hyperthermia are rarely present. The prognosis is very serious despite discontinuation of mitomycin and treatment by plasma exchange (mortality of 60 to 70%).

HIV-associated TMA [12]

These forms of TMA generally occur at an advanced stage of HIV infection and, therefore, have a very poor prognosis. Various mechanisms can be responsible:

- development of anti-ADAMTS-13 auto-antibodies responsible for TTP that responds favorably to treatment
- a multifactorial origin (opportunistic CMV infection [44]), drugs (valaciclovir, etc.) with a more variable response to treatment and a poorer prognosis.

Treatment of this type of TMA syndrome must be associated with antiretroviral therapy.

Other TMA syndromes

Other diseases have also been associated with TMA disorders, including catastrophic antiphospholipid syndrome, veno-occlusive disease, DIC, type 2 heparin-induced thrombocytopenia, giant hemangioma, hemangio-endothelioma, and malignant hypertension. These various syndromes must be eliminated before initiating plasma therapy.

Conclusion

Major studies designed to improve our understanding of the pathophysiology of TMA have been conducted over recent years. This improved knowledge opens up new perspectives for more targeted treatment. However, until these innovative treatments become available, early diagnosis of these diseases is essential in order to rapidly initiate specific treatment, as the interval between diagnosis and initiation of plasma exchange is a decisive element in the prognosis of TTP. Treatment must not be stopped too early or too rapidly and must take into account the various associated factors, especially the presence of infection.

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Altered Functionality of von Willebrand Factor in Sepsis and Thrombocytopenia – Potential Role of the vWF Cleaving Protease ADAMTS-13

R. A. Claus, K. Reinhart, and F. M. Brunkhorst

Sepsis, Platelets and Giant Proteins: A New Target for Therapy?

The pathophysiologic course of sepsis involves the release of cyto- and chemokines in addition to the activation of endothelial and neutrophil cells, initiating a cascade of cell-surface interactions. Activation of the coagulation system has been characterized by widespread intravascular fibrin deposition and platelet aggregation (disseminated intravascular coagulation, DIC) with subsequent microvascular and tissue injury, ultimately leading to multiple organ failure (MOF) and death. The contributing role of platelets in the pathophysiology of sepsis and related organ dysfunction is not entirely clear, although the degree and duration of thrombocytopenia, as well as the net change in the platelet count, are important determinants for survival [1, 2]. Of note, the involvement of platelets in sepsis-associated coagulopathy was already studied by many groups more than 25 years ago, focusing on the interaction of platelets with endotoxin, and the role of thrombin generation and DIC on platelet function [3, 4]. However, regarding the various pathogenic mechanisms that have recently been implicated in the activation of coagulation in sepsis, a reassessment of the role of platelets is needed.

A reduction in platelet count may occur in the absence of a significant alteration in coagulation factors (as measured by prothrombin time or activated partial thromboplastin time), indicating that mechanisms other than DIC may be involved [5]. In experimental studies, even when thrombin generation is prevented, platelet counts fall despite the inhibition of coagulant activity. For example, blocking the hemostatic response of primates with LD_{100} *Escherichia coli* sepsis by administering active site inhibited factor Xa, hirudin, or heparin does not prevent the development of thrombocytopenia or death [6, 7]. Platelets may be directly activated by endotoxin or other pro-inflammatory mediators such as platelet activating factor (PAF) [8]. The expression of P-selectin on the platelet membrane mediates the adherence of platelets to leukocytes and endothelial cells at sites of inflammation [9]. Yaguchi et al. found that platelets from septic patients released increased amounts of vascular endothelial growth factor (VEGF), a potent angiogenic factor regulating microvascular endothelial repair and survival [10]. These studies indicate that inflammation *per se* may be associated with platelet consumption. Moreover, bone marrow depression by inflammatory mediators and infectious organisms, and some pharmacologic agents may also contribute to the fall in platelet counts observed in sepsis.

In this chapter, we focus on the potential role of von Willebrand Factor (vWF) in patients with sepsis and organ failure, which is largely underestimated in the literature. vWF is synthesized in endothelial cells and megakaryocytes. The protein

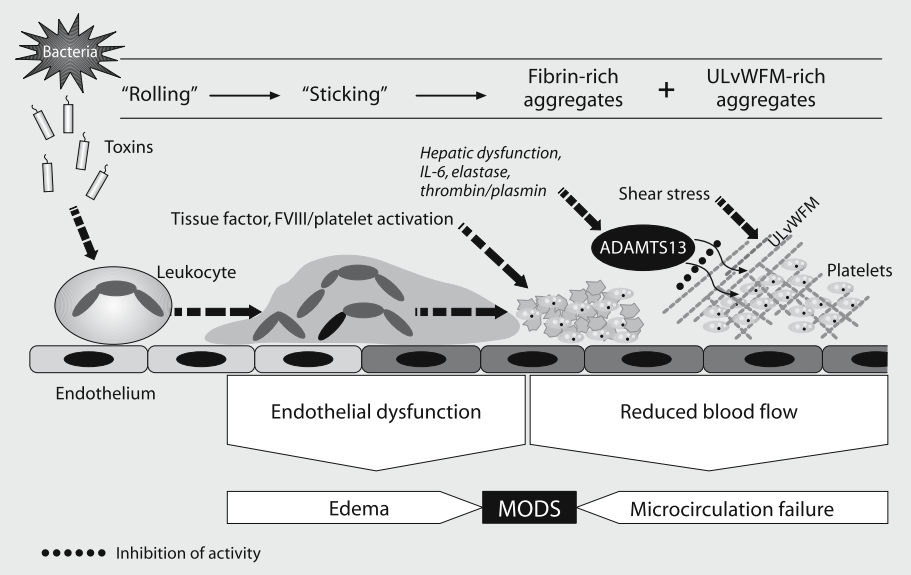

Fig. 1. Simplified illustration of microvascular occlusion in systemic inflammation. Constituents and toxins of circulating bacteria activate leukocytes. Subsequent to rolling and sticking, the release of pro-inflammatory cytokines (i.e. IL-6, TNF-α) results in an exacerbation of endothelial injury and oedema generation. The cross-talk between inflammation and coagulation leads to deposition of fibrin-rich platelet aggregates, *e.g.* due to release of tissue-factor and thrombin generation. Evidence from clinical and experimental studies implies that the release of ultra large multimers of von Willebrand factor (ULvWF) and/or a drop in proteolytic activity of its major cleaving protease ADAMTS-13 triggered by IL-6, elastase, thrombin/plasmin or hepatic dysfunction can generate ULvWF-rich platelet thrombi

circulates in plasma as multimers up to 20,000 kDa in size, and is essential for platelet adhesion and thrombus formation. Deficiency or dysfunction of vWF causes an inherited bleeding disorder, von Willebrand disease [11], whereas high plasma levels are associated with an increased risk of death from severe sepsis [12, 13]. ADAMTS-13 is the main physiological modulator of the size and aggregability of vWF in plasma. In patients with thrombotic thrombocytopenic purpura (TTP), a congenital or immuno-mediated deficiency of ADAMTS-13 reduces or abolishes the degradation of ultralarge multimers of vWF (ULvWF) that cause the formation of intravascular platelet thrombi (thrombotic microangiopathy, TMA) [14, 15], resulting in MOF very similar to severe sepsis [16].

Recent data provide evidence that an altered ADAMTS-13 activity and a subsequent shift in the multimeric pattern of vWF may contribute to thrombocytopenia, intravascular coagulation and microcirculatory failure in patients with severe sepsis (Fig. 1). Nguyen and colleagues [17] reported that children with thrombocytopenia (platelet count $<100{,}000$) associated MOF had reduced or absent ADAMTS-13 activity along with markedly increased plasminogen activator inhibitor (PAI)-1 activity, both reversed by plasma exchange therapy. Bianchi et al. reported decreased ADAMTS-13 activity levels of 0.4 U/ml (range 0.15–0.80 U/ml) in 17 thrombocytopenic patients with severe sepsis or septic shock and six patients with levels between 0.15 and 0.30 U/ml [18]. This is in agreement with findings by Loof et al.

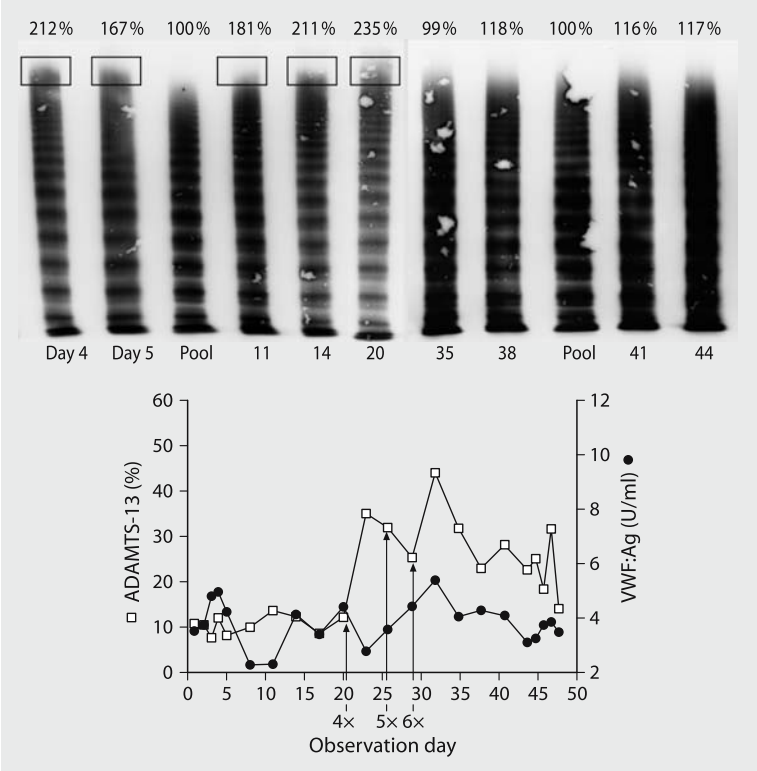

Fig. 2. vWF-multimer pattern in a surviving patient with severe sepsis. Following normalization and separation using 1.2% agarose gel electrophoresis and visualization by immunoblotting the amount of high molecular vWF is analyzed by densitometric methods in comparison to a normal plasma pool from healthy individuals set at 100%. The presence of ULvWF is indicated by boxes (upper panel). ADAMTS-13 activity (open squares) and vWF antigen level (filled boxes) are depicted over time. Activity of ADAMTS-13 is low (10% = 0.1 U/mL) after onset of severe sepsis, and increases over time in parallel to disappearance of ULvWF in plasma. vWF:Ag levels are highly elevated up to six times the normal value. ADAMTS-13 activity increases following administration of fresh frozen plasma (FFP; arrows, numbers of packs). Total numbers of FFP packs is given

[19], who reported a decreased ADAMTS-13 activity of 0.36 ± 0.24 U/ml in 14 patients with DIC and sepsis and Mannucci et al. who found ADAMTS-13 levels decreased to about 0.50 U/ml plasma activity in patients with respiratory tract infections [20]. Unfortunately, neither data on vWF nor detailed clinical information were given in these studies. Since these researchers did not perform vWF multimer analyses, their results are not supported by evidence of diminished vWF proteolysis. Our own results in 11 patients with severe sepsis or septic shock show a decrease in ADAMTS-13 activity from the lower limit of normal of 0.40 U/ml to < 0.30 and < 0.10 U/ml in 97 and 19 patient days, respectively (Fig. 2). Both elevated vWF and decreased ADAMTS-13 generated a significantly higher vWF/ADAMTS-13 ratio and resulted in the appearance of ULvWF in plasma, which is of major clinical relevance, since in the presence of ULvWF spontaneous platelet aggregation and

adhesion leads to a procoagulant state [21]. Moreover, the decrease in ADAMTS-13 was associated with higher sequential organ failure assessment (SOFA) scores and increased 28-day mortality [22]. Very recently, Ono et al. described decreased ADAMTS-13 levels in 109 patients with sepsis-induced DIC [23]. The incidence of acute renal failure and serum creatinine levels in patients with ADAMTS-13 activity levels lower than 0.20 U/ml (incidence: 41.2%, creatinine: 1.81 ± 1.70 mg/dl) was significantly higher than in patients with ADAMTS-13 activity levels >0.20 U/ml (incidence: 15.4%, creatinine: 0.95 ± 0.76 mg/ml) ($p < 0.05$, $p < 0.01$). Additionally, unusually large vWF multimers were detected in 26 out of 51 patients (51.0%) with ADAMTS-13 activity levels <0.20 U/ml. Unfortunately, a closer clinical characterization of patients (i.e., mortality rate, severity scores etc.) was not given in this study.

Clearly, the predictive value of these variables for disease severity and mortality in patients with severe sepsis warrants further evaluation in larger patient cohorts. Assessment of the functional proteolytic activity of ADAMTS-13 and the detection of ULvWF may be of major clinical relevance, since plasma exchange with enzyme containing plasma preparations such as fresh frozen plasma (FFP), cryoprecipitate-poor plasma or the application of recombinant ADAMTS-13 may restore the capacity to cleave ULvWF in the circulation.

This chapter provides recent knowledge about the potential relevance of an altered biofunctionality of vWF for the pathophysiology of severe sepsis and the methodological and laboratory approaches to detect and describe these alterations.

Structure of von Willebrand Factor

vWF is a plasma glycoprotein required for primary hemostasis. As an extracellular adapter molecule, it mediates the adhesion of platelets to subendothelial collagen of a damaged blood vessel and platelet-platelet interactions in high shear-rate conditions. The concentration of mature vWF in plasma is approximately 10 μg/ml, and its half life is about 12 hours [24]. vWF is synthesized in endothelial cells, where it is either secreted constitutively or stored in Weibel-palade bodies for secretion upon stimulation, as well as in megakaryocytes, where it is stored in α-granules that later are partitioned into platelets [25, 26]. Subsequent to the synthesis of a precursor protein, vWF undergoes a number of intracellular processing steps. vWF dimers are initially built by formation of a disulfide bond near the C-terminus, then, by generation of disulfide bonds near the N-termini, the protein multimerizes to a gigantic protein with a molecular mass ranging over 3 orders of magnitude to more than 20,000 kDa [25, 26]. A single molecule may show the extraordinary length of several millimeters.

Each vWF monomer contains a number of specific domains with specific function; elements of note are: the D'/D3 domain, binding to factor VIII; the A1 domain, binding the GPIb-receptor of platelets; the A3 domain, binding to collagen; and the C1 domain, in which the RGD domain binds to platelet integrin αIIbβ3 after its activation. The pro-coagulant activity of vWF exhibits a non-linear function of size, since the larger the multimer, the more effective it is in promoting platelet adhesion exhibiting a critical effect on its function. However, under shear stress conditions in the circulation the protein emerges more vulnerable to proteolytic digestion by limited proteolysis [27].

Regulation of vWF multimer composition in plasma is performed by two major cleaving events: first, ADAMTS-13 proteolytically cleaves in between the A2 domain of each vWF monomer and second, thrombospondin-1 cleaves the disulfide bonds interlinking vWF multimers [28]. In contrast to an irreversible fragmentation of vWF by ADAMTS-13, the activity of thrombospondin-1 may regulate vWF size reversibly employing a reductase activity. Thrombospondin-1 is crucially involved in the vWF cleavage by ADAMTS-13 due to competition with ADAMTS-13 for binding to the vWF A3 domain [29].

Biological Functions of von Willebrand Factor

vWF is involved in maintenance of the plasma coagulation system by binding of coagulation factor VIII as a carrier protein and thereby indirectly contributing to the coagulation process by prolonging this factor's half time in the circulation by stabilizing it from degradation by activated protein C [25, 26]. In case of deficiency, patients have a bleeding disorder called von Willebrand disease. Occurring in up to 1% of the general population, von Willebrand disease is the most common hereditary bleeding disorder, of which several subtypes are recognized. Many cases remain undiagnosed because of the mild nature of bleeding in many patients and the fact that acute phase reactions can mask the diagnosis.

Plasma concentrations of vWF protein are commonly used as an early marker for endothelial injury and dysfunction, which is almost invariably observed in systemic inflammation and infection [12]. In patients with acute lung injury (ALI) and acute respiratory distress syndrome (ARDS), plasma levels of vWF were found to be associated with outcome, illness severity, septic complications and the number of organ-failure free days [30, 31]. Similar to the release of mature vWF upon stimulation by endothelial cell agonists, the plasma concentration of pro-peptide of vWF is elevated in vascular disorders, and a 4–5 fold difference in half-life of processed and unprocessed protein was observed. This raised the question whether the ratio between the proteins may serve as a tool for discrimination between chronic and acute endothelial cell perturbation. In contrast to a parallel increase of both proteins, e.g., in patients with diabetes, patients with acute vascular disorders such as TTP and sepsis exhibited a threefold elevated pro-peptide level consistent with a dramatic endothelial activation at the time of acute exacerbation [32–34]. The growing body of information about the relevance of the vWF protein in inflammation indicates that the biological function is more diverse than was previously thought.

Additional Functional Activity Tests for von Willebrand Factor

In addition to the determination of pure vWF protein analyzing the plasma antigen concentration, the use of vWF collagen-binding assay (vWF:CB) and ristocetin cofactor binding activity (vWF:RCo) provides further information to describe vWF biofunctionality. These conventional assays detect an increased adhesive function of vWF by binding to collagen coated microplates or ristocetin-induced plated agglutination. Both tests are considered to provide an improved analysis of the functionally more important high molecular weight vWF and to correlate more closely

with vWF function and bleeding problems than other assays which measure only total vWF content. In normal plasma, the extent of vWF-activity can be estimated by calculating the ratio between vWF:RCo or vWF:CB, respectively, and the antigen content, which is by definition close to one. A higher ratio indicates a higher thrombogenic activity of vWF. However, in patients with extremely enhanced antigen levels, as observed, e.g., in sepsis, the assay systems for both vWF:CB and vWF:RCo are affected by the absolute vWF concentration to an equal extent. Therefore, conventional analysis of vWF functionality is not sufficient to detect an altered binding activity in patients with supranormal vWF:Ag levels.

Structure of ADAMTS-13

Considerable effort from multiple laboratories has identified a highly specific metalloprotease as the principal enzyme for the processing of vWF multimers [35–38]. The vWF-cleaving protease is the 13th member of the ADAMTS family of proteases, which are named for their characteristic domain structures as A Disintegrin-like and Metalloprotease with ThromboSpondin type 1 motif, ADAMTS-13. Beside a purification procedure from human plasma or commercial factor VIII/vWF concentrate with subsequent peptide sequence analysis [39], a genetic positional cloning strategy starting with a cohort of patients with congenital TMA, linkage analysis and fine mapping resulted in the identification of the gene encoding for ADAMTS-13 [35].

The *ADAMTS-13* gene, comprising 29 exons, predicts a 1427 amino acid protein with a calculated molecular mass of 145 kDa, which is in contrast to the apparent molecular mass for ADAMTS-13 purified from human plasma of approximately 190 kDa, the discrepancy likely being due to glycosylation [36].

Since isolation and cloning of ADAMTS-13, several groups have expressed recombinant protein in cell culture systems. rhADAMTS-13 cleaves vWF by limited proteolysis, verifying that ADAMTS-13 is indeed the 'vWF-cleaving protease' identified in previous studies [36–38]. The protein comprises a signal peptide, a short pro-peptide, a metalloprotease domain, a disintegrin-like domain, a thrombospondin type 1 (TSP-1) repeat, a cysteine-rich domain, a spacer domain, seven additional TSP-1 repeats and two so-called CUB domains [35, 38, 39]. Investigating the structure-activity relationship revealed that: 1) in contrast to other proteolytic enzymes the pro-peptide is not necessary for preservation of the latency of ADAMTS-13 metalloprotease activity [40]; 2) the metalloprotease domain is necessary, but not sufficient for its activity, thus indicating the requirement of additional domains for vWF proteolysis [41]; 3) a single nucleotide polymorphism in the cysteine-rich domain indicates a role as substrate recognition site [39, 41]; 4) the spacer domain is indispensable for activity [42]; 5) TSP-1 motifs are known to mediate protein-protein interactions, in particular among those in the extracellular matrix; and 6) the CUB domains may play an essential role in protein-protein interactions [42]. Due to the multidomain architecture, ADAMTS-13 is able to interact with a wide variety of proteins such as activated integrins.

ADAMTS-13 is predominantly expressed and secreted by hepatic stellate cells [43, 44] in a constitutive manner [45] and in an active form [40]. Mature RNA and active protein have been detected also in platelets [46, 47]. The plasma concentration of the protease ADAMTS-13 is estimated to be 1 μg/ml [48], comparison of

specific activity in plasma with that of recombinant protein has given an independent estimate of approximately 1.6 μg/ml [49, 50]. The half-life in the circulation is approximately 48 to 72 hrs [49, 50], which may be caused by the absence of effective *in vivo* inhibitors.

Biological Functions of ADAMTS-13

The principal and to date only known substrate for ADAMTS-13 is vWF, which is cleaved by limited proteolysis between Tyr(842)-Met(843) of its central A2 domain [51], maintaining the normal size distribution and regulating the biological function of vWF. The cleavage rate of vWF by ADAMTS-13 is markedly increased by mild denaturation or by fluid shear stress [52]. The steady state distribution of multimers reflects an equilibrium between the secretion of large multimers and their extracellular proteolysis into smaller, more inactive fragments, which have been detected in plasma under physiological conditions. A strong decrease in the functional activity of ADAMTS-13 (< 0.10 U/ml), resulting from either genetic mutations [35] or binding of inhibitory auto-antibodies [53–55] leads to accumulation of ultra large multimers with unusually high molecular weight causing excessive platelet aggregation and accompanied by formation of microvascular thrombi of platelets, thrombocytopenia and hemolysis. An impaired proteolysis of vWF by ADAMTS-13 with subsequent formation of ULvWF multimers is the molecular basis of TMA, which leads to the fatal consequences known as TTP [35].

ADAMTS-13 deficient mice exhibit normal embryonic development, perinatal and long-term survival [56]. Despite the complete loss of proteolytic activity in *ADAMTS-13* deficient mice, no difference is evident in the multimer patterns comparing wild-type and knock-out genotype. This demonstrates that in the animal model, ADAMTS deficiency is not sufficient for the development of spontaneous TTP.

Essential links between inflammation and thrombosis were documented by a strong decrease in the ability of ADAMTS-13 to cleave endothelium-derived ULvWF under flow conditions in the presence of the pro-inflammatory cytokine, interleukin (IL)-6 [57]. ADAMTS-13 activity decreased uniformly with increased proteolysis. These results are of major clinical importance since stimulation by IL-6 results in the accumulation of ULvWF in plasma as well as on the surface of endothelial cells with subsequent platelet aggregation and adhesion on the vascular endothelium. In a recent publication, a negative correlation between both the ADAMTS-13 activity level and the antigen level with the plasma level of digests of fibrin proteolyzed by granulocyte elastase has been described [23]. Also, a direct fragmentation of ADAMTS-13 upon incubation with granulocyte elastase was detected and the presence of fragmented ADAMTS-13 verified in plasma of patients with DIC. Moreover, cleavage of ADAMTS-13 by coagulation proteases such as thrombin or plasmin may further account for the decrease in ADAMTS-13 activity at sites of vascular injury under certain pathologic conditions [58].

In healthy individuals, the single intravenous administration of a low dose endotoxin results in a decrease in proteolytic activity, an increase in vWF plasma concentration and the appearance of ULvWF in plasma [59]. This could also be demonstrated for shigatoxin, produced by entero-hemorrhagic *E. coli*, which stimulates the rapid secretion of ULvWF from endothelial cells and decelerates vWF proteolysis by plasma born or rhADAMTS-13 [60]. It is noteworthy that challenge of

ADAMTS-13 k/o mice with shigatoxin resulted in the evolution of a syndrome closely resembling human TTP [56], supporting the concept that toxins of prokaryotic origin or mediators of endothelial injury are required to trigger TMA.

ADAMTS-13: Diagnostic Challenges

Measurement of ADAMTS-13 activity is important for the diagnosis and therapeutic monitoring of microangiopathies. At present, a panel of six assay systems, differing in sensitivity and specificity, is used for determination of proteolytic activity [61, 62]: direct detection of vWF multimer pattern by sodium dodecyl sulfate (SDS)-agarose gel electrophoresis [51] and SDS-polyacrylamide gel electrophoresis [41], two-site immunoradiometric analysis [63], cleavage of minimal specific substrate proteins [64, 65], analysis of residual CB or residual RCo activity [66, 67]. An evaluation of selected assays has been organized in two international collaborative studies [68, 69], with reliable detection of plasma samples with severe ADAMTS-13 activity and strong inhibitory activity; however, samples with moderately reduced activity exhibited less concordant results. Based on dilution series, the most consistent results were obtained by multimer analysis using SDS-agarose gels as well as by analysis of residual CB or RCo activity.

Considering the restricted sensitivity and specificity of the assays determining the residual vWF:CB and the requirement of denaturating conditions, more precise and non-complex assay systems have been developed, which use recombinant synthetic substrates and are applicable in high-throughput techniques. For this purpose, the minimal specific substrate derived from vWF consisted of 73 amino acids around the cleaving site terminally flanked by reporter molecules such as specific protein sequences for subsequent detection of fragments by solid phase techniques, i.e., western blotting or coated microtiter plates [64, 70, 71]. Also, the core peptide was labeled with both a fluorophor and a neighboring quenching molecule, whereby only after cleavage the loss of proximity results in a quantitative increase of the emission rate [65].

The usefulness of the established assays in clinical practice is quite limited because of time consuming procedures, costly assay charges for commercial available test systems, and the requirement of static and denaturating conditions that do not resemble physiological conditions. Furthermore, special equipment is needed and the test systems are not designed for the direct detection of ULvWF multimers in plasma. In addition to activity assays and due to the unknown origin of ADAMTS-13 deficiency observed in a variety of clinical conditions, ELISA kits have recently been available for direct determination of ADAMTS-13 antigen levels.

In healthy subjects, ADAMTS-13 activity features intra-individually over a broad range of values. By consensus from multiple laboratories, the lower limit of normal can be defined around 0.4 U/ml, the upper limit at 2.0 U/ml, against pooled plasma as standard (1.0 U/ml) [15, 20, 62–65]. Comparison of the ADAMTS-13 activities demonstrated differences between gender, suggesting that the ADAMTS-13 activity of women should be significantly higher than that of men. Examination of the effect of age on ADAMTS-13 activities revealed a strong correlation, demonstrating that ADAMTS-13 activity decreases with advancing age, at least after the early 40s [65].

Despite the lack of pathways inactivating ADAMTS-13 activity in healthy individuals, under pathologic conditions a set of mechanisms aborting ADAMTS-13 activity have been described. According to one report, hemoglobin in high concentrations (>0.2 g/100 ml) interferes with ADAMTS-13 activity resulting in proteolytic

inhibition [72]. Another inhibition of ADAMTS-13 by extremely elevated vWF concentration was described [73], which is of special interest due to the variation in vWF levels in a wide range of pathological conditions hampering the interpretation of activity results.

The inhibiting activity of IgG auto-antibodies predominantly directed to the cysteine-rich and spacer domain of ADAMTS-13 are the principal cause of acquired TTP [53–55]. For detection of functional relevant antibodies, heat inactivated plasma from patients is mixed with normal plasma at several dilutions, whereby 1 U of inhibitor is defined to inhibit the proteolytic activity of 1 ml normal plasma [15]. Alternatively a panel of commercial test kits is now available for direct detection of antibodies. It is noteworthy that in a TTP patient, also, non-neutralizing IgG and IgM antibodies have been identified, which function probably by accelerating plasma clearance of the enzyme or by interfering with plasma or cellular surface proteins [54].

Impaired Proteolytic Activity by Vasopressin Administration

Intravenous vasopressin application causes platelet aggregation by enhancing the release of VWF and factor VIIIa from the endothelial cells [74]. In contrast to the widespread use of vasopressin for advanced cardiac life support and treatment of refractory shock the effects of vasopressin on hemostasis and platelets have rarely been investigated. Leone et al. reported a drop in platelet count from 155 ± 67 to 84 ± 72/nl within 24 hours in 17 patients receiving terlipressin for catecholamine-resistant septic shock, together with biochemical signs of altered microcirculation [75]. Dunser et al. described a drop in platelet count from 178 ± 152 to 80 ± 61/nl within 48 hrs in 21 critically ill patients receiving vasopressin at a dose of 4 U/h in contrast to patients who received norepinephrine alone [76] without any change in vWF antigen levels. In our own still unpublished observations, we could demonstrate a decrease in vWF antigen levels in the four hours following vasopressin administration together with a decrease in ADAMTS-13 activity and platelet count.

As outlined above, the plasma levels of vWF regulate ADAMTS-13 activity. This is of special interest when evaluating the behavior of these two proteins in naturally occurring, experimental and clinical situations associated with vWF levels ranging from undetectable to supranormal. In patients with undetectable plasma vWF due to von Willebrand disease (type 3), ADAMTS-13 activity is approximately 35% higher than in a comparable group of healthy individuals with normal vWF. However, proteolytic activity decreased after desmopressin application in order to raise plasma vWF levels and ULvWF were present in plasma [77], supporting the concept of an inverse association between vWF and the activity of its principal cleaving protease [73].

Conclusion

In patients with severe sepsis, ADAMTS-13 may be one of the key players in altered endothelial function and procoagulant activity. Because the methods for detection of an altered vWF-biofunctionality as well as the proteolytic activity of ADAMTS-13 are complex and elaborate there is need for the development of rapid and reliable methods to detect the presence of ULvWF multimers in human plasma.

The potential beneficial effects of protease supplementation appear to result from at least two distinct properties: reconstitution of ULvWF multimer proteolysis may prevent formation of vWF-rich platelet aggregates and lower the tethering and rolling of leukocytes via platelet-decorated vWF-strings. Future studies are needed to elucidate to what extent these properties of ADAMTS-13 are associated with a potential benefit in patients with severe sepsis.

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Coagulopathy in the Critically Injured Patient

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Introduction

Trauma is an increasingly common cause of death of modern society. Death caused by trauma is rapidly surpassing the number of deaths due to stroke or cardiovascular disease. Uncontrolled bleeding is the leading cause of early in-hospital mortality (within 48 h of admission) and the second leading cause of pre-hospital death, accounting for 40% and 45% of the fatalities, respectively [1]. Massive hemorrhage after traumatic injury is frequently a combination of surgical and coagulopathic bleeding. Coagulopathic bleeding results from impairments in platelet function, fibrin formation, or enhanced degradation, or combinations of all these mechanisms. Understanding the exact etiology is crucial for successful management of this pathology. Early coagulopathy post-injury is observed in 25 to 36% of trauma victims upon admission to the emergency department [2, 3] and correlates with the severity of trauma. It is associated with an increased risk of mortality beyond the expected figures from the severity of the injury [3]. Coagulopathy can develop during, and be the result of the 'traditional aggressive' fluid resuscitation of hemorrhagic shock. It can also develop late, due to surgical complications such as sepsis or multiple organ failure (MOF). This chapter describes the pathophysiology of coagulopathy in various phases of trauma and discusses the mechanisms that can contribute to it.

Physiological Hemostatic Response to Trauma

Under normal physiological conditions, hemostasis is achieved through the interaction of blood vessels, formed elements of the blood and enzymatic reactions. The activated coagulation cascade produces a fragile and temporary fibrin clot at the site of injury. The exposure of subendothelial tissues and collagen after vessel injury allows platelet adhesion and aggregation. Moreover, production of thromboxane A_2 at the site of injury causes potent local vasoconstriction and further stimulation of platelet aggregation. The subsequent release of the contents of platelet granules allows further platelet aggregation to occur at the site of injury. Platelet activation also leads to procoagulant activity mediated by the surface coagulation factor (factor Va). Tissue factor exposure at the site of vessel injury forms a complex with FVIIa, which, under normal conditions, circulates in small quantities but is biologically inactive until the complex formation with tissue factor. This leads to generation of small amounts of thrombin (initiation phase). This amount is insuffi-

cient to transform fibrinogen to fibrin but further activates FVIII, FV, FXI and platelets (amplification phase). The cascade then continues on the membrane of the activated platelets, leading to the production of sufficient amounts of thrombin to convert fibrinogen to a fibrin clot (propagation phase). Thrombin also activates FXIII that cross links the fibrin clot to stabilize it and protect the clot from fibrinolysis. It also activates thrombin activatable fibrinolytic inhibitor (TAFI) that further stabilizes the clot against lysis. At the same time, thrombin is inhibited by its potent inhibitor, antithrombin, and in addition binds to thrombomodulin, which activates the protein C system, leading to the neutralization of activated FV and FVIII. Activation of tissue factor pathway inhibitor (TFPI) shuts down further activation of coagulation by the tissue factor/FVIIa complex (termination phase). This complex mechanism enables rapid clot formation at the site of tissue injury with inhibition of clot formation away from the wound.

The last stage of the coagulation system is the elimination phase by the fibrinolytic system which is vital to maintain the fluid characteristics of blood in the intravascular space and to counter blood coagulation. The principal reaction of this system is the activation of plasminogen to plasmin by tissue plasminogen activator (tPA) released from the damaged tissue and urokinase. The fibrinolytic system is regulated by the inhibitory actions of plasminogen activator inhibitor-1 (PAI-1), increased sharply after trauma, and alpha-2-antiplasmin. An imbalance in the production or elimination of plasminogen activators and inhibitors can significantly change the balance of their activities in the plasma and thus have considerable effects on hemostasis, leading to thrombosis – in case of inhibition of fibrinolysis – or bleeding – in case of hyperfibrinolysis.

Conventional laboratory measurements of coagulation and fibrinolysis hardly detect these changes. However, using sensitive assay methods, one can assess thrombin and plasmin activation by measuring fibrinopeptide A (FPA) and fibrinopeptide Bβ15–42 (FPBβ15–42), fibrin formation and its degradation (D-dimer), and the inhibition of fibrinolysis by PAI-1 [4]. The immediate increase in thrombin activity after trauma, as measured by a rise in the level of FPA and FPBβ15–42, is followed by plasmin activity to protect from intravascular thrombi caused by extra fibrin formation. As a result of the secondary fibrinolysis by plasmin, a steep increase in D-dimer levels is noted. To prevent re-bleeding by excessive fibrinolysis, PAI-1 levels increase a few hours after the trauma and this decreases the levels of both FPBβ15-42 and D-dimer. Several days after the trauma, the increase in PAI-1 stops, then the removal of unused fibrin by reactivating plasmin and elevation of D-dimer can be detected.

Pathological Hemostatic Response to Trauma

The coagulopathy in trauma is complex and multi-factorial. It includes factors exposed, released or activated from damaged tissue, hemodilution, hypothermia, acidosis, anemia, complications related to massive blood transfusion and activation of the inflammatory system.

Early Coagulopathy in the Trauma Patient

Nearly one quarter of trauma patients arriving at the emergency department present an established coagulopathy as reflected by a prolonged prothrombin time (PT) and activated partial thromboplastin time (aPTT) [3, 5]. Some of these patients receive only minimal resuscitation in the field and the presence of coagulopathy does not correlate with the amount or type of intravenous therapy administered. This patient population has relatively high injury severity scores (ISS), which might be responsible for the high rate of coagulopathy on admission. As the level of tissue trauma increases, the incidence of coagulopathy increases, and nearly two thirds of patients with an ISS > 45 arrive with a significant derangement in their hemostatic mechanisms. These patients are more likely to die than those without hemostatic derangements, for any given degree of injury. Trauma patients with coagulatory abnormalities develop organ dysfunction and have a longer hospital and intensive care stays. Although a cause and effect relationship has not been established, the findings suggest that trauma results in clinically significant coagulopathy.

A variety of cellular and humoral mechanisms are likely to be involved in this process. Tissue injury leads to the exposure and release of tissue factor, which activates the coagulation pathways. Extensive tissue damage may lead to consumption of coagulation factors and platelets and to excessive fibrinolysis that further decreases coagulation factors and may also damage platelet membranes. The development of acute coagulopathy may, therefore, indicate the loss of regulation of the local inflammatory response and represent the initiation of the systemic inflammatory response syndrome (SIRS) and its sequelae [7]. In a cohort of trauma patients arriving at a Level I trauma center [5], the initial PT and aPTT were measured shortly after arrival at the trauma bay. Twenty-eight percent of the patients had abnormal PTs and 8% had abnormal aPTTs. Such altered levels, independently, predicted mortality. An elevated initial PT above 14.0 s was associated with a 35% increase in mortality, and the aPTT was an even stronger predictor of mortality. Neither prolongation of the pre-hospital period nor the protracted time to laboratory investigations could account for the correlation between death and the initially elevated PT and aPTT. Other studies lend support to the hypothesis that coagulopathy in the injured patient may not always arise from fluid replacement. In 1985, Ordog et al. showed that 97% of a cohort of 180 trauma patients who died had evidence of a coagulation defect before fluid or blood replacement treatment [8]. In a recent report from Israel, soldiers who had sustained a combat injury showed no correlation between the PT or aPTT measured on hospital arrival and the volume of pre-hospital fluid treatment [9]. Although there is no proven cause and effect relationship, it would seem that tissue trauma results in the release of mediators that are responsible for the development of a clinically significant coagulopathy. This response is proportional to the severity of injury. A variety of cellular and humoral mechanisms are involved, and they are detailed below.

Hypothermia. Hypothermia frequently accompanies severe trauma and is associated with a significantly worse prognosis. Hypothermia impairs hemostasis at various levels, including quantitative and qualitative platelet dysfunction, alteration of coagulation enzyme kinetics and disruption of the fibrinolytic equilibrium.

Effects of hypothermia on coagulation factors and fibrinolysis. It is well known that the activity of an enzyme is reduced by approximately 50% for every 10 °C decrease in temperature. PT appears to be the most sensitive to reduced temperature and is significantly increased at temperatures below 35 °C while the aPTT is significantly prolonged at temperatures below 33 °C [10]. The disparity between hypothermic coagulopathy and results from clotting studies was further illustrated when clotting experiments were performed at incrementally lower temperatures (from 37° to 25 °C) on blood obtained from normothermic rats. The PT, aPTT and thrombin time were all significantly prolonged. In contrast, clotting tests conducted at 37 °C in blood obtained from hypothermic rats showed no abnormalities. These findings highlight the disparity between the clinically evident hypothermic coagulopathy and near normal clotting studies, and suggest that altered enzyme kinetics in the coagulation cascade are a major consequence of hypothermia. In a recent study, the effects of hypothermia on coagulation enzymes and platelet function were evaluated. aPTT gradually lengthened with the decrease in temperature but thrombin generation – which reflects the real activity of coagulation enzymes, became significantly inhibited only below 33 °C. Thereafter, thrombin generation markedly decreased with the decrease in temperature while the prolongation of aPTT was much less. This indicates that aPTT does not reflect the real coagulopathic effect of hypothermia [11]. A study correlating hypothermic bleeding time with levels of factor deficiency found small changes in the clotting time of plasma at 33 °C that correlated with a phenotypic expression of 50% to 100% of the normal amount of factor X, a condition that is not associated with significant bleeding [12]. Hypothermia also induces hyperfibrinolysis mainly due to impairments in the intrinsic inhibitors of fibrinolysis, such as PAI-1 or alpha-2-antiplasmin, that occurs at lower temperatures [13].

Effects on platelets. The occurrence of thrombocytopenia during hypothermic bleeding has been known for many years. In early canine studies, platelet sequestration in the liver and spleen was found to contribute to hypothermia-induced thrombocytopenia and quick warming of the animal reversed the sequestration [14, 15]. Although these models highlighted quantitative platelet defects as an important cause of bleeding in hypothermia, studies in other species found that effects on platelet function are also involved in this pathology. The production of thromboxane B_2 was reduced during experimental hypothermia in baboons and human volunteers, and this correlated with increased bleeding time. In both species, rewarming reversed the inhibition of thromboxane B_2 release.

To summarize, the hemostatic defects observed in the mild hypothermic trauma patient result primarily from defective platelet adhesion and aggregation. While clinically significant altered activity of coagulation enzymes and activation of fibrinolysis becomes a compounding factor in severe hypothermia below 33 °C routine coagulation tests are performed at normal temperature and will not fully reflect the degree of the hemostatic defect because of the disparity between aPTT and thrombin generation and because they do not reflect platelet dysfunction caused by the hypothermia.

Metabolic Derangements – Acidosis. Hemorrhagic shock eventually results in intracellular derangements in oxygen consumption, which leads to metabolic acidosis. Correction of acidosis can significantly reduce organ failure rates and mortality compared to patients having persistently elevated lactate levels and reduced oxygen consumption.

There is a strong correlation between the development of coagulation abnormalities and the duration of hypotension. Hypoperfusion is also associated with consumptive coagulopathy and microvascular bleeding, which occur independently of the amount of blood loss [16]. The role of acidosis in the development of clinical coagulopathy is poorly described, although it has been implicated. A prolonged clotting time was observed when lactic acid was added to heparinized human and dog blood *in vitro* [17] and low platelet counts were reported in dogs after the infusion of hydrochloric acid solution [18]. When pH was decreased from 7.4 to 7.0, the activities of the tissue factor/FVIIa complex and the FXa/FVa complex were reduced by 55% and 70%, respectively, as depicted in Figure 1. A decrease of approximately 20% in fibrinogen concentration shortly after induction of acidosis is consistently observed. This decline can be amplified by hemorrhage and resuscitation. The underlying mechanism of the depletion is not clear. Because fibrinogen synthesis in normal animals is approximately 1 to 3% of the total pool size per hour, the rapid 20% decrease in the fibrinogen level is unlikely to be the result of altered synthesis but more likely to be caused by altered sequestration or degradation. Both hypothermia and acidosis induced *in vivo* inhibit thrombin generation. In acidosis, initial thrombin generation is moderately delayed, and after some thrombin is generated, at the subsequent propagation phase, the thrombin generation rate is persistently and drastically inhibited by acidosis. Because of these inhibitions, acidosis might be more detrimental than hypothermia in the development of coagulopathy. The acidotic inhibition of thrombin generation is amplified when hypothermia is present. Thus, correcting blood pH may be a potentially important strategy in reversing clinical coagulopathies. Further studies are required to evaluate the effect of correction of acidosis to coagulation and other physiological/metabolic parameters. Nonetheless, it is reasonable to conclude that the primary detrimental effect of acidosis is the inhibition of thrombin generation. Recently, recombinant activated factor VII (rFVIIa) has been used as adjunct therapy in patients with uncontrolled hemorrhage with variable beneficial effects on survival as well as on blood and blood component consumption [19, 31]. This variability may be explained by the late stage of rFVIIa administration [19], where some patients were already in a state of irreversible shock. Both reports found that the response to rFVIIa is diminished in patients with acidosis [19, 31] which is consistent with the *in vitro* observation of diminished thrombin generation [18]. These suggest that

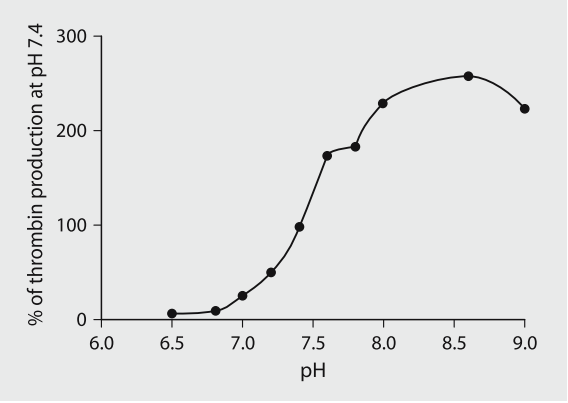

Fig. 1. The effect of pH on thrombin generation. Note the 70% decrease in thrombin generation with the change in pH from 7.4 to 7.0 and the steep increase with the rise in pH from 7.4 to 7.8. From [18] with permission

correction of pH (i.e., bicarbonate infusion) may be vital before administration of rFVIIa in patients with acidosis.

Anemia. An often-ignored effect of red blood cell (RBC) transfusion is the improvement in hemostatic function. Erythrocytes have been shown to modulate the biochemical and functional responsiveness of activated platelets, suggesting that they contribute to hemostasis and thrombosis [20, 21]. RBCs contain adenosine diphosphate (ADP) that can activate platelets; they also activate platelet cyclooxygenase, increase the generation of thromboxane A_2 and may directly increase the thrombin burst through exposure of procoagulant phospholipids [22]. This pro-aggregatory property of erythrocytes can be decreased by an appropriate regimen of aspirin [23]. Another mechanism by which erythrocytes modulate hemostasis is the effect of RBC on the margination of platelets. Under normal circumstances, RBC flow is maximal at the center of the vessel, pushing platelets towards the periphery of the vessel's lumen, thereby optimizing their interaction with the injured endothelium and promoting hemostasis. In rabbit arterioles, platelet numbers are highest near the wall of the vessel [24, 25] and platelets align themselves with their equatorial plane parallel to the vessel wall as they move closer toward the periphery of the vessel [26]. The correlation between the bleeding time and hematocrit levels has been studied experimentally. In non-thrombocytopenic rabbits, the bleeding time varied inversely with the hematocrit – animals with hematocrit above 35% had shorter bleeding times than animals with lower hematocrits [27]. In a rabbit model of cyclic arterial thrombosis and clot lysis, decrease in the hematocrit reduced the cyclic arterial thrombosis rate and increased the bleeding time. Interestingly, normalization of the hematocrit caused thrombosis to reappear. Transfusion of RBCs shortened the bleeding time in anemic thrombocytopenic patients despite persistent thrombocytopenia. Furthermore, perfusion of blood from transfused, previously anemic, thrombocytopenic patients improved the interaction of platelets with the subendothelium in an experimental perfusion model. However, this interaction remained lower than in non-anemic non-thrombocytopenic subjects [28]. Similarly, RBCs shortened the bleeding time and controlled the hemorrhagic diathesis of uremic patients.

In humans with normal renal function and platelet counts exceeding 100 000/mm^3, a modest, but statistically significant, inverse correlation exists between the hematocrit and the bleeding time. Patients with a pre-transfusion hemoglobin concentration of less than 6.0 g/dl had a greater chance of a post-transfusion decrease in bleeding time than patients with hemoglobin concentrations higher than 6.0 g/dl [29]. In healthy volunteers, an acute 15% reduction in hematocrit produced a 60% increase in the bleeding time, while a 32% reduction in platelet count left the bleeding time normal [30]. The clinical significance of these findings remains unclear because the relationship between the bleeding time and perioperative blood loss is highly controversial. Further investigations into the role of hemoglobin concentration or hematocrit on hemostasis are warranted. The data presented above tend to support the concept of a minimal hematocrit value for optimal hemostasis. At present, the optimal hematocrit or hemoglobin concentration for prevention or initiation of treatment of coagulopathy remains unknown. Experimental evidence suggests that relatively high hematocrits, possibly as high as 35%, are required to sustain hemostasis in bleeding patients. Furthermore, reduction of the hematocrit to 20% inhibits platelet adhesion and aggregation to a degree similar to that observed with platelet counts of 20 000/mm^3.

Hyperfibrinolysis and Consumption Coagulopathy. Hyperfibrinolysis may be more common in trauma patients than was previously realized. The failure to detect this condition stems from the absence of routine laboratory tests for fibrinolysis [31]. Recently, rotational thromboelastography (roTEG) has shown that approximately 20% of multi-trauma patients suffering from massive bleeding have marked hyperfibrinolysis. The reproduction of these findings in larger patient series would support the assumption that early administration of antifibrinolytic agents may be beneficial in hemorrhage control in trauma. Treatment with rFVIIa, which reduces clot susceptibility to fibrinolysis partly by the induction of TAFI, may also be of value in hyperfibrinolysis [32]. In trauma, exposure of tissue factor at the site of injury leads to activation of the coagulation cascade at the injury site. Massive injury may cause extensive activation with consumption of coagulation factors and platelets at these sites, leading to consumption coagulopathy. Activation of coagulation results in activation of fibrinolysis, sometimes even with hyperfibrinolysis which further degrades coagulation factors and platelets. This process results in laboratory findings resembling disseminated intravascular coagulation (DIC), such as prolonged PT and aPTT, low levels of platelets and fibrinogen, and high levels of D-dimers and of other markers of coagulation and activation of fibrinolysis. However, in most cases, these findings do not reflect DIC, as there is no evidence of microthrombi formation, and thus, no intravascular clotting [33, 34].

Hemodilution. Dilutional coagulopathy results from the dilution of coagulation factors and platelets caused by the infusion of large volumes of crystalloids, colloids, or blood products that are administered to improve oxygen delivery. The severity of dilutional coagulopathy is determined by both the volume and type of fluid infused. Whereas permissive hypotension and reduced fluid volume in the pre-hospital setting and early in-hospital treatment may decrease the extent of such coagulopathy, newly developed types of fluid, such as hypertonic saline (with or without dextran), new colloids, and artificial oxygen carriers, may exacerbate it.

Crystalloids. In elective surgery, mild hemodilution with crystalloids has been shown to induce a hypercoagulable state [35]. This hemodilution-associated hypercoagulability has been the focus of several investigations, implicating an imbalance in procoagulant/anticoagulant activity in the etiology of the phenomenon. A randomized, controlled, *in vivo* study has confirmed that acute hemodilution of 20% to 30% with normal saline induces a hypercoagulable state, measured by TEG, in surgical patients under general anesthesia. However, it is important to note that changes in hemostatic function will be very different with a more profound hemodilution [36]. The mechanism by which hypercoagulability occurs with mild hemodilution with saline remains intriguing, but the possibility that increased thrombin generation can be one cause for it has been demonstrated. Other mechanisms could also be responsible for this phenomenon, such as the observation that thrombus formation on a collagen-reinforced thrombogenic device inversely correlated with the hematocrit, and that platelet adhesion to an artificial perfusion chamber correlated inversely with blood viscosity [37]. The pattern of TEG changes, predominantly the shortening of k-time and widening of fα, also suggest that the facilitation of platelet interaction with platelet activating surfaces during hemodilution might be a possible mechanism. Further studies are required to define more clearly the mechanism and the significance of hypercoagulability induced by saline hemodilution. In a large prospective cohort study, a U-shaped relationship was found be-

tween hematocrit and the risk of stroke, with the risk of stroke increasing in patients with high or low hematocrit [38]. The relationship between hemodilution, thrombosis and hemostasis is probably more complicated and diverse than previously believed. For practical purposes, the development of hypercoagulability and its potential risks must be considered whenever crystalloid hemodilution between 10% and 30% is clinically employed. The significance of this effect in trauma patients remains unclear. Nonetheless, crystalloid-induced hypercoagulability casts a doubt on studies of the effect of colloids on coagulation that used crystalloids as controls.

Colloids. There is an increasing tendency to use colloid solutions as volume expanders. The different colloids affect coagulation according to their molecular weight. Gelatins have the reputation of barely influencing coagulation other than by their direct hemodiluting effect [39], but dilution of whole blood samples with two gelatin solutions resulted in a reduction of clot quality (less extensive fibrin mesh formation, reduced clot weight and mean shear modulus), compared to dilution with normal saline [40]. The clinical significance of these findings remains unclear. Perhaps the *in vivo* effects on coagulation of gelatin-based plasma substitutes are not clinically important, but, on the other hand, they may have been underestimated, given the difficulties of studying coagulation in the uncontrolled context of trauma and ongoing bleeding. Hydroxyethyl starch (HES) solutions are effective plasma expanders in common use. It has long been known that HES solutions interfere with coagulation, and that their effects vary according to the dose and type of solution administered. Solutions of high molecular weight with longer circulating half-lives cause more profound coagulopathy and compromise platelet function more than lower molecular weight preparations [41]. In addition to their effects on hemostasis, the infusion of large volumes of colloid solutions will result in significant hemodilution. The resulting decrease in hemoglobin and platelet concentrations may compromise primary hemostasis. HES interfere both with platelet function and fibrin polymerization by impairment of fibrinogen polymerization and decrease in fibrinogen concentrations [42]. No adverse events associated with the use of HES solutions for the resuscitation of patients have been reported, inasmuch as the allowed maximal daily dose is not exceeded. Large volumes (up to 5 l) have been infused without major complications. The safety of this practice remains controversial [43]. To summarize, like gelatin solutions, the clinical significance of the effects of HES solutions on hemostasis remain unclear. In massively transfused patients with ongoing bleeding, hemostasis is stressed severely by numerous factors other than the type of non-blood-related fluid used for resuscitation, and the underlying cause of coagulopathy is difficult to ascertain.

Massive blood transfusion. Massive transfusion is a frequent complication of trauma and surgery. It is commonly defined as the replacement of one blood mass within a period of 24 hr. A dynamic definition of massive transfusion, such as the transfusion of four or more red cell concentrates within one hour upon ongoing need, is foreseeable, as is the replacement of 50% of the total blood volume within three hours, which is more relevant in the acute clinical setting. A high percentage of massively transfused patients will show evidence of defective hemostasis, and its incidence will vary according to the clinical context (blunt vs. penetrating trauma, presence of brain injury), the definition of coagulopathy (clinical findings vs. laboratory test results) [44], and the blood products administered to the massively

bleeding patient (fresh whole blood, stored whole blood, packed RBCs, etc.) [45]. For example, prior to the era of blood component therapy, the transfusion of large volumes of stored blood did not result in a hemorrhagic diathesis in young and previously healthy soldiers wounded during the Vietnam war [46]. More recently, it has been shown that abnormalities of PT and the aPTT occur after the transfusion of 12 units of packed RBCs, and that thrombocytopenia develops after the transfusion of 20 units [44]. Despite several attempts at defining reliable laboratory indicators of impending or established coagulopathy, the relationship between laboratory hemostatic abnormalities and abnormal clinical bleeding remains unclear. Most studies of massive transfusion have been conducted in trauma patients, and most are retrospective or uncontrolled observational studies [47], for obvious reasons. Given the variable and complex clinical context, the results of these studies have seldom led to definitive conclusions. Furthermore, factors other than the transfusion strategy and related to trauma itself may have led to the observed hemostatic abnormalities.

Coagulation factors. While numerous studies have measured changes in PT and aPTT in relationship to bleeding and massive transfusion, only few have examined individual hemostatic factors. In 1995, Hiippala et al. showed that the deficiency in fibrinogen concentration developed earlier than any other hemostatic abnormality when packed RBC units and colloid substitutes were used for the replacement of major blood loss [48]. Approximately 90% of the fall in fibrinogen level could be explained by blood loss and the critical level was reached when blood losses exceeded 142% of the calculated blood volume. Blood losses in excess of two blood volumes caused deficiency of prothrombin, factor V, platelets and factor VII, in this order [49]. These observations were made in patients undergoing elective surgery and may not reflect trauma patients. In addition, previous studies on the effects of blood transfusion on coagulation before the era of blood component therapy are not applicable to the current situation where the use of plasma poor RBC units today results in faster and more profound decrease in coagulation factors as compared to the use of whole blood in the past.

Platelets. Since the publication of Miller's classic study on coagulation defects associated with massive blood transfusions [49], thrombocytopenia resulting from hemodilution has been considered to be the most important hemostatic abnormality associated with massive transfusion. Models based on the washout equation (a simple mathematical model of exchange transfusion that calculates the decay of blood components with bleeding and with constant and equal replacement rates) may not apply to bleeding trauma patients whose blood volumes fluctuate, bleeding rates vary with blood pressure, and replacement often lags behind blood loss. Few studies have demonstrated higher platelet counts than predicted by a standard washout equation. This finding implies that platelets are being released into the circulation and counteract the effects of dilution. Sequestered platelets can be released from the spleen and lungs, in addition to the premature release of platelets from the bone marrow. Elevated stress hormones and the administration of catecholamines, a situation more likely to occur in the trauma patient, will influence this release. Thrombocytopenia (defined as a platelet count below 50 000/mm^3) is a late occurrence, and, to great extent, quite variable from patient to patient. In a study of coagulation changes during crystalloid and packed RBC replacement of major blood loss during elective surgery, four out of 12 patients presented with a coagulopathy. All patients with inadequate clinical hemos-

tasis had low platelet counts (83 000/mm^3 or lower), and transfusion of platelet concentrates corrected the problem in the two who had fibrinogen concentrations above 1.0 g/l. Platelet transfusion was ineffective in the two patients with concurrent low fibrinogen concentrations (0.73 and 0.40 g/l). The subsequent transfusion of two and four units of fresh frozen plasma (respectively) normalized hemostasis. Thus, it appears logical to consider that coagulopathy after massive transfusion can result from a combined deficit of platelets and fibrinogen. Focusing only on platelet levels or on concentrations of specific coagulation factors may not lead to the most appropriate therapeutic approach.

Conclusion

The pathophysiology of coagulopathy in the trauma patient is complex and multifactorial. The term 'DIC' is incorrectly used to define trauma-related coagulopathy. Although the routine laboratory results resemble those of DIC because they detect activation of coagulation and fibrinolysis and consumption of platelets and coagulation factors, the pathophysiology of the coagulopathy is quite different. While DIC reflects a hypercoagulable state with loss of localization of the coagulation process, leading to diffuse deposition of fibrin, trauma-related coagulopathy is a hypocoagulable state with fibrin deposition limited to the site of injury. The use of the right terms is not only a semantic issue but has major implications for treatment. In fact this was the main reason for the delay in introduction of rFVIIa to trauma patients a few years after its introduction to hemophilia and bleeding disorders: The use of agents markedly enhancing hemostasis, such as rFVIIa, in patients already suffering from a hypercoagulable state seemed to be risky. Much of the early data in the literature are not applicable due to the change of blood components and transfusion policy in the modern area of trauma patient resuscitation. A multidisciplinary approach involving anesthesiologists, transfusion specialists, hematologists, laboratory specialists and surgeons is required for the diagnosis and treatment of traumatic bleeding.

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Critical Bleeding in Blunt Trauma Patients

O. Chiara, S. Cimbanassi, and S. Vesconi

Introduction

Trauma is a serious global health issue in Western Countries and the leading cause of death during the first four decades of life [1, 2]. Because injury is frequent among the younger population, life-years lost are greater from injury (on average 36 years lost per death) than from cardiovascular or neoplastic disease [3, 4]. In Italy, there are 1,143,305 trauma admissions every year (9.3% of all hospital admissions), with 25,038 (2.19%) trauma patients being admitted to intensive care units (ICUs) (data from Italian Ministry of Health). Trauma deaths before and after hospital admission are 18,000 per year. Acute blood loss has been reported to be the principal cause of immediate or early trauma death [5–8]. In an autopsy study on 255 consecutive trauma deaths [9], hemorrhage alone or combined with severe head trauma, was the cause of death in 70% of cases. Significantly, most of these deaths occurred during the first phases of pre-hospital or hospital care. The length of time between injury and death was less than one hour in 66.5% and from 1 to 6 hours in 24.6% of cases. In European countries, owing to the prevalence of blunt trauma, causes of unstable hemodynamics are mainly pelvic ring and extremity fractures with extensive soft tissue destruction, followed by abdominal injuries (Fig. 1). Advances in trauma care, such as improved transportation systems, hypotensive resuscitation, strategies of damage control in emergency surgery, angiographic embolization procedures, all increase the chances of survival of the hemorrhagic patient. Nevertheless, hemodynamic instability often requires infusion of liters of crystalloid and colloid solutions and transfusion of several units of packed red cells, leading to consumption and dilution of clotting factors. Hypothermia resulting from cold infusions may impair activity of both platelets and coagulation enzymes [10]. Acidosis from decreased perfusion and anaerobic metabolism significantly reduces prothrombin activation. Moreover, hyperfibrinolysis, demonstrated in 20% of multiple trauma patients [11], may be a cause of early rebleeding. Commonly performed laboratory coagulation tests, i.e., platelet count, prothrombin time (PT) and activated partial thromboplastin time (aPTT), often underestimate coagulation impairment, as blood is re-warmed to 37 °C before testing and platelet and fibrinolysis activity are not routinely checked.

When the patient develops the so called 'lethal triad' of hypothermia, acidosis, and coagulopathy, surgical or angiographic restoration of vascular integrity may be insufficient to achieve a definitive control of blood loss, and non-mechanical bleeding from small vessels, usually terminated by spontaneous coagulation, becomes a life-threatening condition [12]. A new, promising approach is the use of recombinant activated factor VII (rFVIIa) in order to prevent or reverse traumatic coagulo-

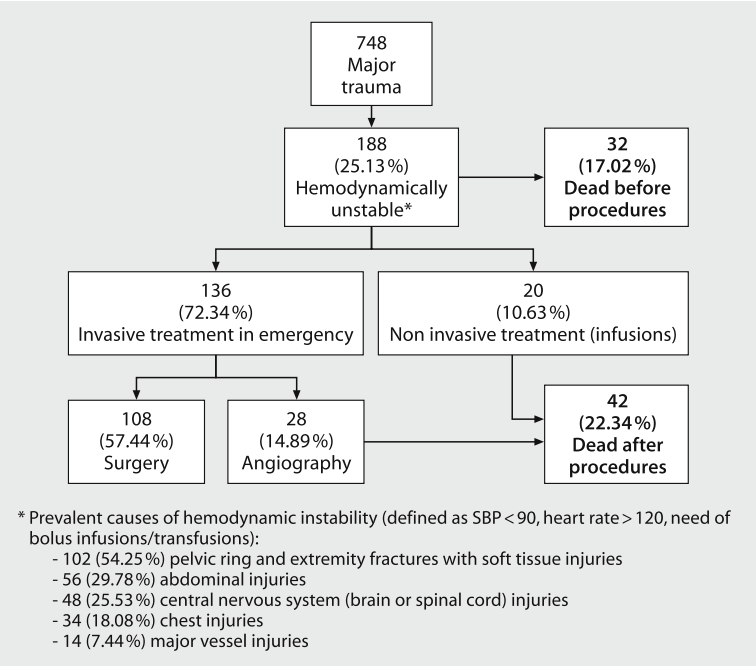

Fig. 1. Epidemiology of major trauma patients admitted to the emergency department: data from trauma registry of the Niguarda Hospital in Milan

pathy. The following paragraphs describe protocols for the treatment of most of the common causes of critical bleeding in severe trauma patients and a strategy for the use of rFVIIa as adjunctive therapy.

Hemorrhage in Pelvic Ring Fractures

A network of arterial and venous vessels lie on the inner wall of the pelvis and can be injured during pelvic trauma. Life threatening hemorrhage is the result of high energy forces with disruption of bone and ligament structures and vessels in proximity. Therefore, severe hemorrhage due to pelvic blood loss is often associated with pelvic biomechanical instability. The considerable force required to disrupt the pelvic ring is often associated with abdominal, thoracic and extremities injuries which may be sources of important bleeding. Clinical evaluation allows the detection of external hemorrhage or significant blood loss from fractures of extremities. Antero-posterior chest x-ray and tube thoracostomy are sufficient to rule out significant hemothorax. Emergency room abdominal ultrasound or diagnostic peritoneal lavage, although accurate in demonstrating free peritoneal blood, may result in false positives from retroperitoneal hematomas that leak blood into the peritoneal cavity [13]. Contrast enhanced spiral computed tomography (CESCT) offers a complete imaging assessment of the abdomen and pelvis with the best sensitivity and specificity, including injuries of intra- and retroperitoneal organs, soft tissues and

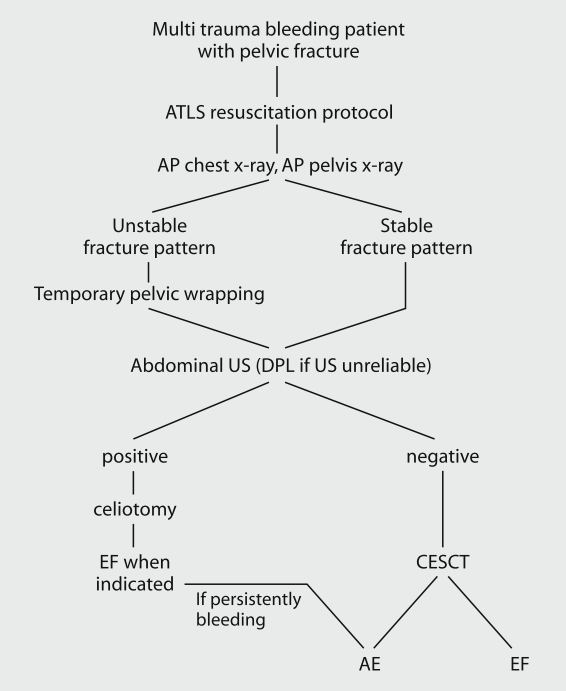

Fig. 2. Management tree for complex pelvic ring disruptions. US: ultrasound; DPL: diagnostic peritoneal lavage; EF: external fixation; CESCT: contrast-enhanced spiral CT-scan; AE: angiography-embolization

bones, but availability and proximity to patient support and monitoring are required if the hemodynamic status is unstable. While abdominal bleeding injuries are best managed immediately with an emergency laparotomy [14], pelvic hemorrhage is optimally treated by angiography/embolization in case of arterial bleeding or pelvic volume closure with external fixation when venous blood loss from bony fragments is the main source of bleeding. CESCT is very useful to differentiate pelvic venous or arterial bleeding and may also help to localize bleeding arterial branches with the 'contrast extravasation sign' [15–17]. All blunt trauma patients with hemorrhage and pelvic bone fractures admitted to our trauma center are managed with a protocol-driven approach based on pelvic fracture pattern, as demonstrated by initial screening radiograph. The orthopedic surgeon classifies the pelvic fracture on plain radiograph, using a simplified Tile/Young & Burgess classification [18]. Fracture patterns are differentiated in two groups: fractures causing biomechanical instability of the pelvic ring (open book and vertical shear); fractures with biomechanical stability (non-displaced fractures of single bones of the pelvis). In unstable fracture patterns, a wide belt is positioned around the pelvis. Abdominal ultrasound scanning establishes the need for celiotomy (Fig. 2):

- If the ultrasound scan shows a significant (more than 1 cm or expanding) amount of free fluid in two or more spaces, celiotomy is performed and abdominal injuries are treated. External fixation is performed at the end of surgery when indicated by fracture morphology. If hemodynamics are persistently unstable, angiography is done after surgery with emobilization as needed.
- If the ultrasound scan is negative or minimally positive, efforts are made to maintain blood pressure and CESCT is performed to detect the presence of

Table 1. Source of bleeding in 87 consecutive pelvic fracture patients

	Unstable pelvic fracture	Stable pelvic fracture	Total	p
n (%)	37 (42.5)	50 (57.5)	87 (100)	
Age	42.14 ± 16.46	40.78 ± 18.06	41.35 ± 17.32	0.710
Injury Severity Score	40.19 ± 13.57	34.65 ± 15.28	36.5 ± 15.7	0.070
Patients with predominant pelvic hemorrhage (%)	32 (87)	3 (6)	35 (40.2)	**< 0.001**
Patients with predominant extrapelvic hemorrhage (%)	5 (13)	47 (94)	52 (59.7)	**< 0.001**
Total deaths (%)	10 (27)	16 (32)	26 (29)	> 0.10
CNS death (%)	2 (20)	4 (25)	6 (23)	> 0.10

venous bleeding or arterial contrast extravasation in the context of pelvic fracture. In case of arterial extravasation from pelvic tissues, angiography/embolization is attempted to control pelvic bleeding. The venous bleeding from bone fractures is treated by pelvic stabilization/compression with external fixation.

Eighty-seven bleeding pelvic fracture patients have been prospectively evaluated using this protocol-driven approach. Predominant hemorrhage from pelvic fracture was observed in 87% of unstable pelvic fracture patients (Table 1) and in only 6% of stable pelvic fractures, while predominant hemorrhage from the abdomen or other extra-pelvic sites was identified in 94% of stable pelvic fractures and in only 13% of unstable pelvic fractures ($p < 0.001$). In 67% of these patients, hemorrhage was severe (8.08 ± 1.02 units of packed red cells, range 5–19). The overall mortality of the study population was 29%. Sixteen of 87 patients (18%) were admitted in extremis and died in the emergency room during initial evaluation without procedures. If we exclude the emergency room deaths, the remaining 71 patients had a mortality of 15% (10 patients), half for central nervous system (CNS) injuries and half for untreatable bleeding from pelvic ring and/or other injuries. These observations demonstrate that the pattern of pelvic fracture is suggestive of the predominant site of bleeding in the majority of multiple trauma patients who survive initial emergency room treatment. Once the need for emergency celiotomy is excluded, CESCT is the best diagnostic tool to decide how to manage the bleeding pelvic injuries. Nevertheless, critical bleeding may be an important cause of death in these patients, as confirmed by other published series [19].

Abdominal Injuries

Death from complex abdominal injuries is most often caused by critical hemorrhage. Because of the high mortality of extensive surgical procedures, the surgical team should switch from the completion of the operation to the much more fundamental goal, which is saving the patient's life. The term "damage control", coined by Rotondo et al. [20, 21], describes a three phase approach to the patient with abdominal exsanguination:

- phase I is an initial surgical procedure aimed at controlling hemorrhage and contamination. Control of bleeding is usually accomplished by packing of each abdominal quadrant and temporary occlusion of the aorta at the diaphragmatic hiatus. Once a degree of hemodynamic stability has been restored, the surgeon explores the abdomen, removing packs in a sequential fashion and sealing bleeding points. Hollow viscus injuries are then closed with simple running sutures or stapled enterectomies, with no attempt, at this time, to re-establish continuity of the bowel. The cavity is then packed to control residual oozing of blood. The abdominal incision is temporarily closed with a running suture or, if edema may impair mechanical ventilation, with an artificial wall such as a non-absorbable mesh or silo bag.
- phase II is the simultaneous treatment of all physiological abnormalities in the ICU. The patient is rewarmed with radiant heaters, warming blankets, a warmed ventilator circuit, and infusions. In selected cases, core rewarming may be attempted using chest tubes and pleural lavage with warm saline. Coagulopathy is treated aggressively with fresh frozen plasma (FFP) cryoprecipitate and platelet transfusions. The coagulation profile must be corrected to achieve an International Normalized Ratio (INR) less than 1.5 and a circulating platelet count of more than 50×10^9/l. Negative fluid balance is achieved with forced diuresis or filtration. Intra-abdominal pressure must be checked frequently using a bladder transducer and reoperation may be necessary to prevent hypertension. Recently, interventional radiological techniques have increasingly been used to improve the chances of bleeding control [22, 23]. In the phase II period, it is often difficult to distinguish active rebleeding from a surgical site from non-mechanical coagulopathic bleeding. Angiography can demonstrate extravasation of contrast from vessels and embolization of bleeding points may prevent an urgent return to the operation room which is a tremendous insult in a patient with reduced physiologic reserve.
- phase III is the definitive reconstruction phase, which is accomplished in the operating room as soon as possible (usually after 48–72 hours) after physiologic deficits and abnormalities have been corrected. Packs are removed, known injuries are reassessed, and a search for injuries that may have been overlooked is performed. Parenchymal injuries are debrided and, if possible, intestinal continuity is re-established. Insertion of tracheostomy cannulas and enteral feeding tubes may be adjunctive procedures at this time. The abdominal incision may be definitively closed or alternatively, in the case of residual contamination and edema or planned surgical procedures, the artificial wall is left in place and delayed closure is programmed, sometime for several months later.

The protocol used in our emergency department is shown in Figure 3. Hemodynamically stable patients or patients stabilized after resuscitation, are studied with CESCT and, in the presence of contrast extravasation, angiography/embolization is attempted to achieve bleeding control. Hemodynamically unstable patients with free peritoneal fluid shown on ultrasound scanning, are immediately transported to the operating room. In the case of class IV shock, resuscitation and operative time >90 minutes, base deficit higher than 10 or pH <7.30, 10 or more units of packed red cells transfused, core temperature less than 35 °C, coagulopathy evidenced by non-mechanical bleeding, a damage control surgical strategy is strongly considered. If the hemoglobin concentration continues to drop after operation, angiography is performed to embolize bleeding vessels. During a 36 month period, 100 trauma pa-

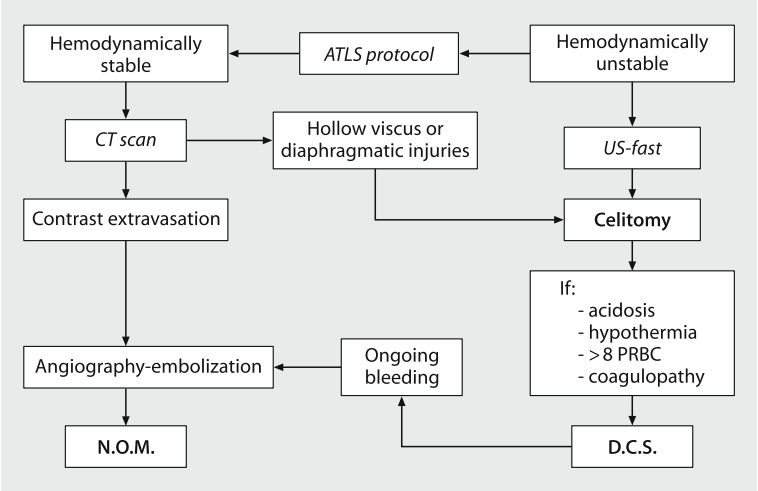

Fig. 3. Management tree for bleeding intraperitoneal injuries. NOM: non-operative management; DCS: damage control surgery; US: ultrasound; PRBC: packed red blood cells

tients with hemorrhage from liver and/or spleen were admitted to our hospital, with an Injury Severity Score of 37.5 ± 13.6, a Revised Trauma Score of 5.11 ± 1.84, and 3.43 associated injuries for each patient. Fifty-three patients underwent surgery, 40 as emergency cases for hemodynamic instability, six for hollow viscus rupture, and seven for failure of initial non-operative management. In five emergency surgery cases, damage control packing of parenchymal bleeding was attempted and two angiography/embolizations were necessary after packing. Overall mortality of this clinical population was 22%; six patients died in the emergency department before any treatment, 6 died after surgery for uncontrolled hemorrhage (three after a damage control approach) and severe physiologic derangement, and 10 died of associated brain injuries.

Injuries of the Extremities

Although hemorrhage from an isolated extremity injury is an unusual cause of death [24], uncontrolled bleeding from limbs or arms in a multiple trauma patient may be devastating, rapidly contributing to profound shock. In our institution, injuries of extremities represent 55% of all injuries and orthopedic procedures account for 59% of all surgical procedures. Major long bone fractures are often associated with extensive soft tissue and vascular injuries and peripheral, potentially ominous sources of ongoing hemorrhage. Recently, a stepwise approach regarding the timing and extent of surgery in a severe trauma patient with extremity injury (Fig. 4) was introduced as “damage control orthopedics” [25]. This strategy is advisable for the management of injuries in all major tissue systems of a limb. In the presence of extensive cutaneous or muscular damage, after an initial debridment of non-viable tissues, temporary coverage may be accomplished by vacuum drainage dressing, delaying definitive closure with a skin graft or a flap. In injured patients

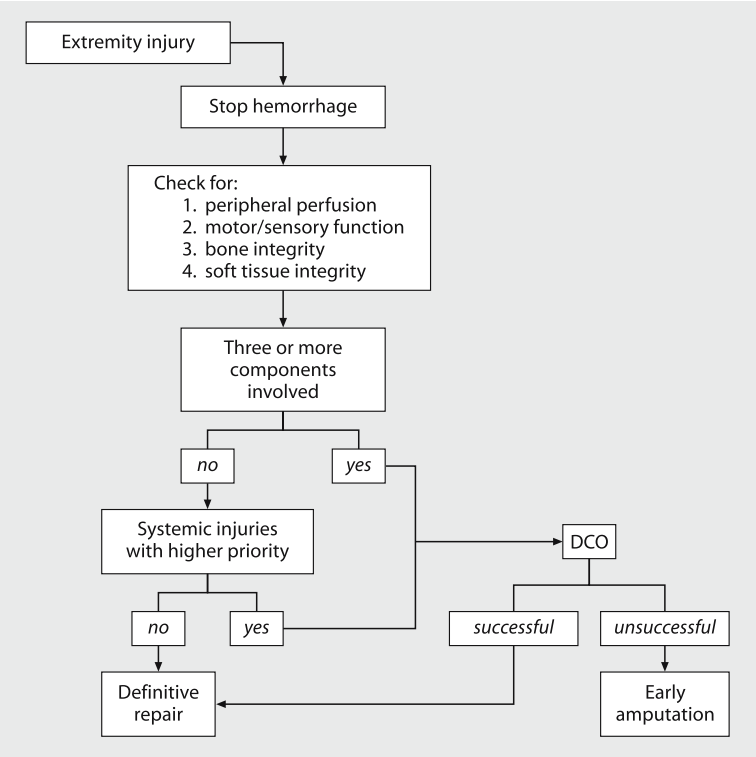

Fig. 4. Management tree of complex injuries of the extremities. DCO: damage control orthopedics

suffering a major long bone fracture, damage control orthopedics is performed using external fixation as a "temporizing device" [26] to achieve benefits of early fracture stabilization during the resuscitative phase, postponing definitive treatment, depending on patient stability, specific bone and soft tissue injuries. External fixation is used routinely as a temporary device for fractures of the humerus, forearms, and tibia in multi-system trauma victims, demonstrating relative or absolute contraindications to open reduction and internal fixation. There is some evidence that external fixation is also feasible for fixing femur fractures in badly injured patients, particularly with closed head injury [27]. In this setting, the advantages of external fixation are represented by a short operative time (average 30 minutes) allowing serial neurological examination, minimal blood loss and short anesthesia time, reducing the incidence of hypoxia and hypotension and thus minimizing secondary brain injury.

With vascular injury in extremity trauma (1.5% of cases in our experience), the related morbidity and limb loss is high. Although distal ischemia is the most frequent manifestation, major hemorrhage – usually from truncal vascular damage – is a potentially life-threatening condition, especially in injuries of limb vessels. Vascular injuries pose two problems: how to control the hemorrhage and how to maintain distal flow. Simple vessel ligation or repair, when possible, are rapid and

lifesaving techniques to stop bleeding. Complex repairs, such as end-to-end anastomosis and graft interposition, are time-consuming procedures [28] and distal perfusion may be supplied by temporary intraluminal shunting (segment of large bore intravenous tube, the Javid, Brenner and Jundt shunts, plastic or Silastic catheters). Shunt patency may be maintained for at least 24 hours without anticoagulation. If bleeding control is achieved by ligation of both arterial and venous vessels, a four compartment fasciotomy is mandatory to prevent compartment syndrome. In the mangled extremity, with involvement of at least three of the major tissue system of the limb (soft tissue, vessels, bone, nerve), the total ischemia time is a major consideration in the decision to amputate. In general extensive soft tissue damage, totally interrupted distal innervation or bone loss > than 6 cm in length are indicative of poor prognosis. Early amputation permits temporary wound closure and reoperation when physiologic stability is achieved. Angiography/embolization may be adjunctive procedures in a damage control approach to extremity injuries, but their use requires further evaluations [28].

Factor VIIa in Traumatic Bleeding

Recombinant FVIIa (NovoSeven®, Novo Nordisk A/S, Bagsvaerd, Denmark) has been used since 1990 in patients with hemophilia A or B with inhibitors to factor VIII or IX for prophylaxis or treatment of acute bleeding episodes [29]. In large doses, rFVIIa requires only factor I (fibrinogen), factor II (thrombin) and platelets to produce coagulation. FVIIa interacts with tissue factor and with activated platelets to produce a 'thrombin burst' and a clot at the site of vascular injury without a systemic effect. This clot has a high thrombin concentration and is more resistant to fibrinolytic enzymes. The results of the first successful experience of rFVIIa in human trauma were published in 1999 [30]. In 2001, Martinowitz et al. [31] demonstrated that administration of rFVIIa in a swine liver trauma model was associated with decreased blood loss and restoration of a normal coagulation profile. Other experimental work from the same investigators confirmed these results [32]. After many anecdotal case reports and small series, the results of the first large clinical series have been published by the Shock Trauma Center of the University of Maryland [33]. Eighty-one coagulopathic trauma patients were treated with rFVIIa: 46 for acute hemorrhagic shock, 20 for bleeding traumatic brain injury (TBI), 9 for hemorrhage in pre-injury warfarin therapy, 6 for acquired or congenital hematologic defects. Dosing was 100 μg/kg in acute dilutional coagulopathy and 50 μg/kg for warfarin reversal, factor deficiency and TBI. The clinical response rate (hemostasis achieved) was 80% with no response in the other 20% (futile administration). In patients who responded to treatment, survival until discharge was 50%. Mortality for the subgroup of TBI patients was 75% (mostly as a consequence of underlying illness) and 55% for warfarin reversal. A Revised Trauma Score less than 4.1, lactate greater than 13 mmol/l, pH <7.1, previous or impending cardiopulmonary resuscitation (CPR), have been identified predictors of futile administration. Recently, results from a phase 2 study of 277 patients have been published [34]. Patients were randomized to receive, after eight red cell units, placebo or 400 μg/Kg of rFVIIa in three doses. In blunt trauma patients treated with rFVIIa transfusion need within 48 hours was significantly decreased and massive transfusion need (>20 units) was reduced from 33% to 14%. In penetrating trauma there

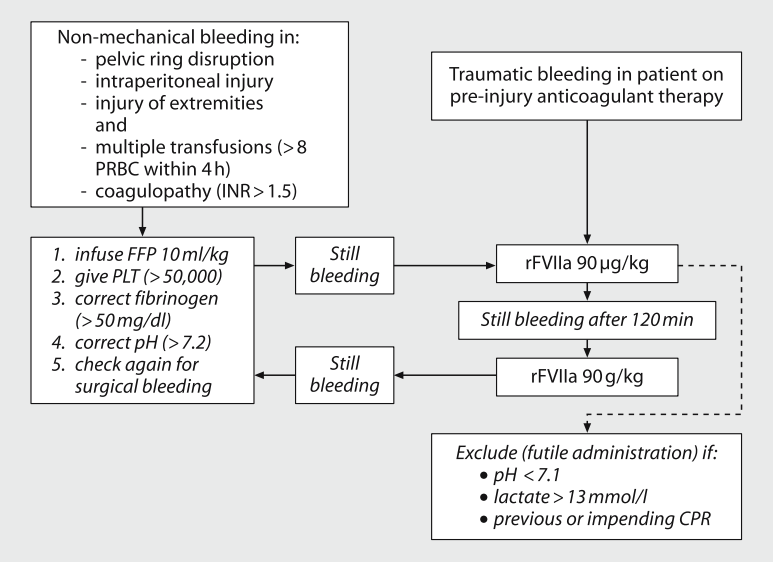

Fig. 5. Protocol for the use of rFVIIa in critical hemorrhage in the blunt trauma patient. INR: international normalized ratio; FFP: fresh frozen plasma; PLT: platelets; CPR: cardiopulmonary resuscitation

was a trend toward reduction of transfusion need, without statistical significance. It is noteworthy that thromboembolic events showed a low incidence (4.33%) with no differences between groups. Using available data from the literature [35], we developed in our department a protocol for the off-label use of rFVIIa in traumatic critical bleeding, as depicted in Figure 5. Multi-transfused (at least 8 red cell units within 4 hours) and coagulopathic (INR >1.5) patients are considered for rFVIIa administration when surgeon and anesthesiologist face a life-threatening non-mechanical bleeding after surgical or interventional procedures under the following clinical conditions: pelvic ring disruptions, complex intraperitoneal injuries, complex injuries of extremities with extensive soft tissue damage. These clinical conditions have been selected because of a well-defined management tree and the incidence of critical bleeding with high mortality. At this moment, we do not consider the use of rFVIIa in TBI and patients with a Glasgow Coma Scale (GCS) of 8 or less or evidence of post-traumatic injury at brain CT-scan (a specific multicenter randomized trial is ongoing). Before dosing, all patients receive FFP at least 10 ml/kg and appropriate replacement if platelets are less than 50×10^9/l and fibrinogen less than 50 mg/dl. Acid-base balance is corrected to a pH higher than 7.2. Thereafter, rFVIIa is given at 90 µg/kg and repeated after 120 minutes if hemorrhage persists. In bleeding trauma patients who were receiving oral anticoagulant therapy pre-injury, early use of rFVIIa is recommended. To avoid futile administration, patients with previous or impending CPR, pH<7.1, lactate >13 mmol/l are excluded.

Conclusion

Hemorrhage in blunt trauma usually derives from many contemporary bleeding sites, which may be stopped with surgery or interventional radiology. Protocol-driven management of specific injuries is mandatory to achieve the best results when a multi-disciplinary team is at work. However, a significant amount of blood loss may derive from small interrupted vessels, not amenable to surgical or angiographic control, especially in the case of traumatic coagulopathy. A pharmacological approach may be indicated to stop this so-called non-mechanical bleeding. Based on available data and on our experience, rFVIIa is a safe and useful adjunctive treatment to standard therapies in the management of critical hemorrhage in a selected trauma population. The drug is extremely expensive, but if it works and if we consider potential reduction of ICU days and, ultimately, life preservation, it may become a cost-effective treatment. However, large, prospective randomized, phase 3 trials are needed to answer some unresolved questions including optimal timing of administration, dosing and number of doses.

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Trauma: Bleeding, Coagulopathy, and Blood Component Transfusion

R. Rossaint and D. R. Spahn

Introduction

One in 10 deaths worldwide is the result of trauma and it is estimated that, by the year 2010, annual trauma-related mortality worldwide will increase to 8.4 million [1]. While resuscitation of trauma patients has improved dramatically in recent years, uncontrollable bleeding still accounts for 39% of trauma deaths and is considered to be the leading cause of potentially preventable death following major trauma [2–4].

Uncontrolled bleeding in trauma results from a combination of vascular injury and trauma-related coagulopathy. Surgery is the definitive treatment for repair of major vessel injury, while arterial embolization may be an effective intervention even in patients with multiple trauma. However, coagulopathy-related diffuse bleeding is more difficult to manage [3, 5–7].

This chapter is based on a detailed recent review [8] and summarizes current understanding of the physiology of coagulation and pathophysiology of coagulopathy; it also considers the interrelationship between massive transfusion and coagulopathy, and attempts to provide some guidance for the therapeutic use of blood components in major trauma.

Coagulation Process after Trauma

Following vascular injury, hemostasis is initiated by a series of interactions between the subendothelial matrix, platelets and coagulation proteins [9, 10]. Disruption to the endothelial lining of vessels exposes the subendothelial matrix to blood and allows for platelet activation and plug formation. These plugs in turn provide a catalytic surface for the recruitment and activation of coagulation proteins.

The entire process of coagulation is initiated by the binding of activated factor VII (FVIIa) to exposed tissue factor (Fig. 1). Factors IX and X are then activated, allowing conversion of small amounts of prothrombin to thrombin. Thrombin generation is amplified by a number of feedback mechanisms – increased generation of FVIIa-tissue factor complexes by FVIIa, FIXa and FXa; thrombin activation of factors V and VIII leading to activation of prothrombin and factor X; and increased generation of FIXa leading to rises in FXIa. The result is that sufficient amounts of thrombin are continuously produced, fibrin is generated and factor XIII is activated, allowing fibrin cross-linking to ensure stable clot formation. The premature lysis of the clot is also protected by activation of thrombin activatable fibrinolysis inhibitor (TAFI) [9, 10].

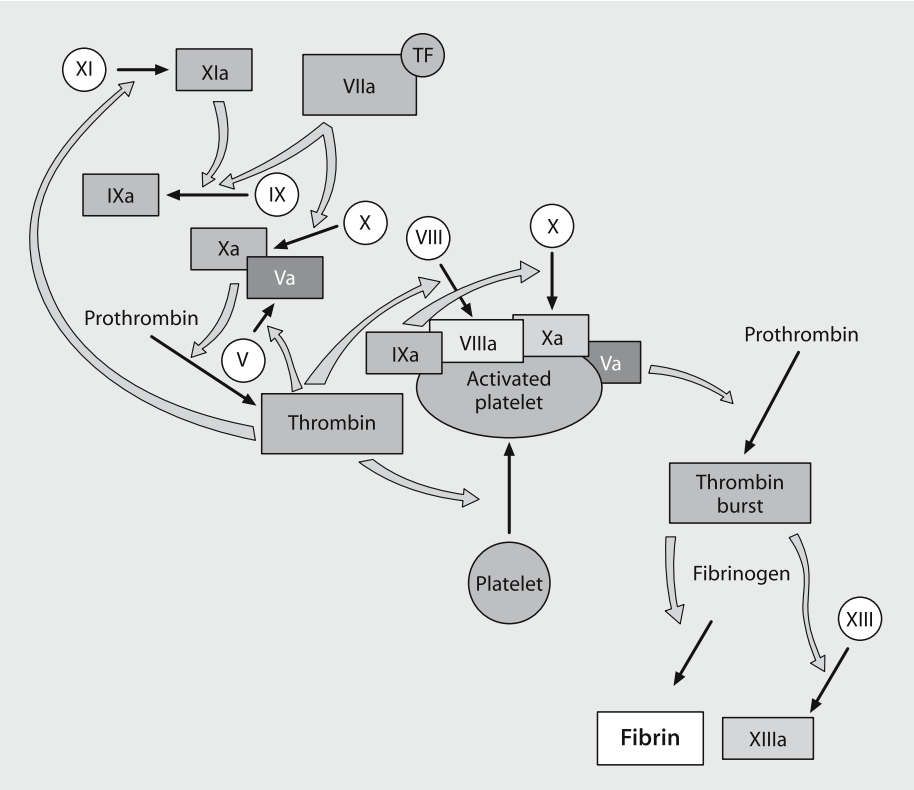

Fig. 1. The coagulation process

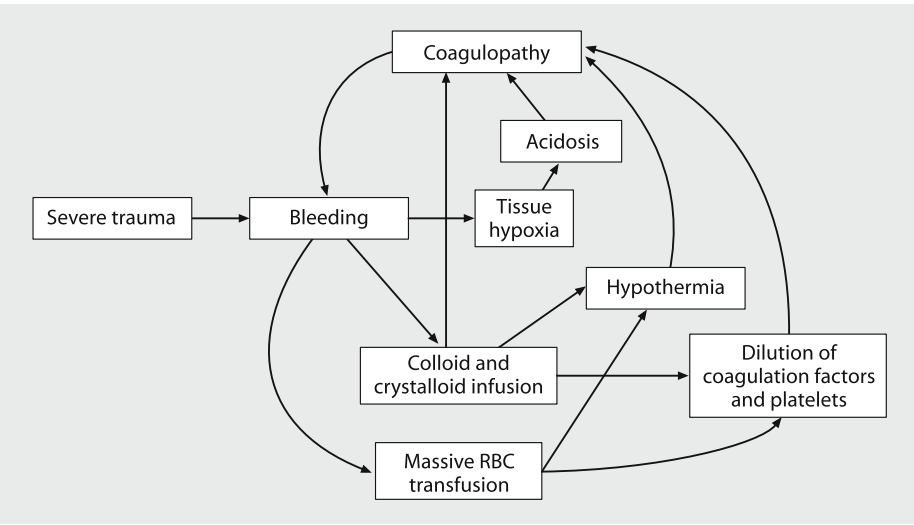

Fig. 2. The interplay between acidosis, hypothermia and coagulopathy in trauma. Adapted from [11] with permission

In massive bleeding following major trauma, the capacity of normal coagulation processes is stretched to the limit and may result in coagulopathy, uncontrolled bleeding and exsanguination even in patients with previously normal hemostasis. Injury severity score (ISS) >25, systolic blood pressure <70 mmHg, acidosis (pH <7.1) and hypothermia (body temperature $<34\,^{\circ}$C) each contribute to coagulopathy [11, 12] (Fig. 2).

The Pathophysiology of Trauma-related Coagulopathy

The factors underlying the development of trauma-related coagulopathy are multifactorial and interrelated and include dilution and reduction of coagulation factors and platelets, dysfunction of platelets and the coagulation systems, compromise of the coagulation system by the infusion of colloid, increased fibrinolysis and hypocalcemia [3, 6, 13, 14].

Furthermore, there is interplay between hypothermia, acidosis and coagulopathy, referred to as the 'lethal triad'. In conjunction, these factors often lead to exsanguination and excessive mortality in up to 90% of cases [6, 15]. Coagulopathy is known to occur early after injury and is a well recognized independent predictor of mortality following severe trauma [7, 16].

Consumption Coagulopathy

A number of factors contribute to trauma coagulopathy, including blood loss, consumption of platelets and coagulation factors, reduction of coagulation factors and platelets, and changes in hemostatic function, which may be linked with acidosis and hypothermia [3, 6].

Tissue damage, anoxia and shock activate the coagulation system and fibrinolysis, resulting in high consumption of platelets and coagulation factors. Continued bleeding further diminishes the pool of coagulation factors and platelets available for adequate coagulation [6]. The infusion of large volumes of crystalloid and colloid is known to reduce circulating concentrations of platelets and coagulation factors, and thrombocytopenia is common after massive blood transfusion [13, 17]. Current practice is to use red blood cells (RBCs) in additive solution rather than whole blood; RBC transfusions, therefore, contain negligible amounts of platelets and coagulation factors.

Coagulopathy Induced by Hypothermia and Acidosis

A fall in body temperature below $34\,^{\circ}$C affects the multiple enzymatic reactions of the coagulation process, which are both temperature and pH dependent [12]. Hypothermia prolongs prothrombin (PT) and activated partial thromboplastin time (aPTT), impairs platelet function and activates fibrinolysis [18]. Hypothermia also impairs thrombin generation and the formation of platelet plugs and fibrin clots, and increases clot lysis [19]. However, routine coagulation tests often underestimate the degree of coagulopathy in hypothermic and acidotic patients because samples are typically rewarmed and buffered during laboratory analyses.

Massive RBC Transfusion and Coagulopathy

Blood component therapy allows for 'tailored' use of RBCs, platelets, plasma and fibrinogen or cryoprecipitate and is designed to avoid the potentially harmful effects caused by transfusion of any surplus constituents [20, 21]. However, the dynamics of blood loss and difficulties in estimating true blood loss after trauma, together with inter-patient physiological differences, makes it difficult to determine the relationship between blood loss, replacement volume and development of coagulopathy. Choosing the correct constituents to restore hemostatic function is, therefore, difficult based on clinical signs, estimates of bleeding or estimates of coagulopathic status [22].

Traditional laboratory tests for signs of coagulopathy are neither practical in trauma settings nor predictive of the true nature of coagulopathy [23]. In addition, as already described, they cannot detect acidosis- and hypothermia-related dysfunctions, both of which are common in trauma. In patients who are already acidotic, massive transfusion of RBCs further increases acid load and worsens coagulopathy [24]. Trauma patients who receive large volumes of fresh frozen plasma (FFP) may develop hypocalcemia (due to chelating citrate present in FFP units), and this again can contribute to a state of severe coagulopathy [8].

Current Issues Concerning Blood Transfusion in Trauma

Trauma patients with critical bleeding typically receive resuscitation comprising infusion of large volumes of crystalloid and colloid followed by RBC transfusion [21]. Although RBC transfusion improves oxygen transport, it does not replace coagulation factors and platelets and can, therefore, contribute further to coagulopathic bleeding following trauma, particularly in cases of massive transfusion [17, 25, 26]. Consequently, current management of coagulopathic bleeding is based on transfusion of FFP, platelets, coagulation factor concentrates (fibrinogen and prothrombin complex) and cryoprecipitate [21].

Blood component replacement therapy carries immunological, infectious and metabolic risks [27, 28]. In patients with severe traumatic injury, there is a known correlation between rising transfusion requirements and an increased risk of multiple organ failure (MOF) and worsened clinical outcome [29–32]. More than 50% of subjects who receive massive transfusion after trauma do not survive their hospital stay [33].

RBC Transfusion

Additional concerns arising from transfusion in the trauma patient relate to the effect of RBC unit transfusion on longer-term clinical outcomes. As already alluded to, RBC transfusion continues to convey some increased risk of transfusion-transmitted infection, and is also associated with a number of acute and delayed non-infectious complications.

Furthermore, for the trauma patient, there is a known relationship between high RBC transfusion requirement and worsened outcome and, in particular, a link with development of post-injury MOF. Organ failure or MOF is a serious post-injury complication which increases intensive care unit (ICU) stay and is associated with increased mortality in trauma patients [29, 31–33]. The relationship appears to be

Table 1. Guidelines for replacement therapy in patients with coagulopathy [8, 21]

Coagulation parameter	Recommended therapy
Prothrombin time > 1.5 times normal	Fresh frozen plasma, prothrombin complex concentrate
Activated partial thromboplastin time > 1.5 times normal	Fresh frozen plasma
Fibrinogen < 1.0 g/l	Fibrinogen concentrate, cryoprecipitate
Platelets $< 50 \times 10^9$/l	Platelets

dose dependent – the higher the number of RBC units transfused, the greater the risk of MOF [29]. For example, a study in over 500 patients found that patients who developed MOF received an average of 13 RBC units in the first 12 hours after injury, as compared with 3.8 units in trauma patients who did not develop MOF [29]. Although the mechanism of increased organ damage due to RBC transfusion has not been firmly established, it is thought that, during storage, bioreactive lipids are generated by RBCs; this may worsen the already heightened systemic inflammatory responses associated with trauma [29]. However, the amount of transfusion required usually also reflects the severity of the injury.

Another common complication in trauma patients receiving transfusion is postoperative infection, with RBC transfusion being an independent risk factor for post-injury infection [27, 28]. Indeed, the infection rate in one cohort of trauma patients was 33% in those who had received at least one unit of RBCs in the first 48 hours of hospital admission, as compared with a 7.6% infection rate in trauma patients who had no requirement for RBC transfusion ($p < 0.0001$) [27]. Another observational study providing data on over 15,000 trauma patients (over 1700 had received a mean of 6.8 RBC units) reported that, after controlling for severity of shock, the need for RBCs in the first 24 hours was clearly associated with increased mortality, admission to the ICU and increased length of ICU and hospital stays, which was thought, in part, to reflect an increased rate of nosocomial infection [31].

A number of theories have been proposed to explain this relationship between RBC transfusion and infection risk, including downregulation of the immune system during exposure to high levels of foreign antigens and exposure to immunosuppressive levels of leukocytes in blood transfusion products [34]. It has also been suggested that rheological changes in RBCs during storage may lead to capillary obstruction by deformed RBCs which, in turn, restricts distribution of prophylactic antibiotics and predisposes to infection and ischemic injury [8, 35]. Development of infection may also relate to the length of time that RBCs are stored prior to use [36].

To date, most of the studies looking at the relationship between RBC transfusion and infection risk post-injury have been observational, due to the difficulties in conducting randomized, prospective trials in the trauma setting [35]. Attempts to study the differences between 'young' (<11 days old) and 'old' (>20 days old) type-specific leukocyte-depleted RBCs in a large study cohort involving 8000 injuries only succeeded in randomizing 24 patients [35].

The shelf-life of RBCs is considered to be around 42 days, with blood banks generally issuing the oldest units first, to avoid wastage. Although it might seem logi-

cal to use the freshest RBCs in trauma patients, inventory management does not allow for such flexibility. Indeed, a more practical solution to the problems posed by RBC transfusion would be to find ways to minimize patient exposure or reduce the amount of RBCs transfused for control of severe bleeding, as suggested by a number of studies in critical care settings [37, 38].

FFP and Platelets

There are no universally accepted guidelines concerning the replacement of platelets, fibrinogen concentrate or the use of FFP and cryoprecipitate in trauma patients. Current recommendations on the use of these potentially hemostatic components tend to be based on personal experience and expert recommendations rather than level I evidence [8].

Current transfusion practices have both advantages and disadvantages in trauma. Transfusion of FFP and platelets prophylactically following the use of a certain number of RBC unit transfusions has been studied [17, 39] but has not clarified the optimal ratio for FFP:RBC or for platelets:RBC, and has not provided conclusive evidence that this practice prevents coagulopathy or improves bleeding.

Transfusion of FFP, platelets or cryoprecipitate has been suggested when there is clinical or laboratory evidence of coagulopathy [13, 25, 39, 40]. However, guidelines for the use of component therapy, such as those recommended by the American Society of Anesthesiology (Table 1), based on evaluating coagulopathic status using clinical signs and laboratory markers [21], have many shortcomings in the trauma setting. Laboratory tests take between 30 minutes to 1 hour to yield results, the hemostatic status of a trauma patient is subject to rapid and precipitous change, and acidosis and hypothermia often confound accurate laboratory assessment of the real coagulation status of the trauma patient.

Blood Component Variability

Donor variations and blood component preparation procedures, including freezing of FFP, affect the levels of coagulation factors found in blood components for transfusion. Consequently, large volumes of FFP are required to correct for a coagulation deficiency [41]. Loss of platelets in preparations is substantial, and, during storage, platelets undergo changes that affect their functional ability. Hence, even transfusion of RBCs, FFP and platelets in a 1:1:1 ratio will not necessarily reconstitute coagulation as seen in normal whole blood [8].

Fresh whole blood has been suggested as an alternative to RBCs but this poses logistic problems to blood banks and requires adequate infection screening; moreover, the transfused blood is likely to contain insufficient platelets and clotting factors to correct massive transfusion-related coagulopathy [42].

Thus, neither whole blood nor blood component replacement therapy in trauma patients is ideal and these measures can only achieve limited hemostasis in patients with trauma-related coagulopathy.

Need for Hemostatic Agents in Trauma

Although there have been steady and significant improvements in the resuscitation of trauma patients with severe blood loss, coagulopathic bleeding remains a major challenge and highlights the need for new approaches to hemostasis as an adjunct to surgical intervention and transfusion of RBCs and blood components [43].

Alternative hemostatic treatments are much needed to help to reduce life-threatening exsanguination. It is hoped that effective hemostatic therapies might reduce the requirement for RBC transfusion and have a positive impact on post-injury complications such as MOF and infection with potential clinical and health-economic benefits.

Recombinant Activated Factor VII (rFVIIa)

Recombinant activated factor VII (rFVIIa) is an agent widely used in the management of bleeding episodes in hemophilia patients with inhibitors, and may also be an effective hemostatic treatment for the management of other coagulation deficiencies characterized by impaired thrombin generation and life-threatening bleeding [44]. This agent has a mode of action founded on physiological coagulation processes [45, 46], and causes localized activation of coagulation at sites of tissue factor exposure such as at injury sites. This may correct the systemic coagulopathy associated with massive blood loss and its management [22, 47–49].

The results of two randomized, controlled studies of rFVIIa for control of bleeding in patients with severe blunt and penetrating trauma have been published and have demonstrated efficacy [50]. Patients in these studies had severe traumatic bleeding (defined as the need for transfusion of six units of RBCs within 4 hours of admission) and received three infusions of rFVIIa (200 μg/kg, 100 μg/kg and 100 μg/kg) at entry – i.e., following transfusion of an eighth RBC unit – and 1 and 3 hours later, or placebo, in addition to local standard surgical treatment to manage hemorrhage. When blunt trauma patients who died within the first 48 hours were excluded from the analyses (an *a priori* decision to exclude possible unpreventable deaths), the reduction in RBC requirements was significant, producing an estimated reduction of 2.6 total RBC units per patient ($p = 0.02$). In this same cohort of blunt trauma patients, the need for massive transfusion – defined as more than 20 units of RBCs – was significantly reduced by rFVIIa treatment from 33 to 14% ($p = 0.03$), a relative risk reduction of 56%. There was also a significant treatment-related reduction in acute respiratory distress syndrome (ARDS). No such statistically significant benefits were observed in penetrating trauma [50]. Adverse event rates between placebo- and rFVIIa-treated groups were similar, with no differences in thromboembolic events observed.

Conclusion

The resuscitation of patients with trauma and hemorrhagic shock has improved over recent years but there is still a need to control bleeding that cannot be corrected surgically, particularly coagulopathic bleeding, in order to reduce the morbidity and mortality associated with major trauma.

Although blood component therapy remains the mainstay of management for coagulopathic bleeding, and RBC transfusion can be life-saving, many patients re-

main at risk of exsanguination, and there are well-documented post-injury problems associated with high rates of RBC transfusion in trauma. Hemostatic agents may offer effective adjunctive control in cases of coagulopathic bleeding, helping to reduce the reliance on transfusion and potentially decreasing the morbidity and mortality burden.

Blood transfusion therapy is unlikely to be replaced completely, but the role of new agents capable of improving bleeding control in emergency settings clearly warrants close consideration and further study.

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Hemostatic Resuscitation

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Introduction

Advances in surgical and critical care medicine frequently parallel the course of armed conflict. Indeed, surgery is a specialty born of warfare and will continue to drive advancements as mankind finds new and more lethal methods of combat. As hemorrhage is far and away the leading cause of potentially survivable death on the battlefield, the methods of resuscitation and blood transfusion continue to evolve. The critical role that blood plays in resuscitation of the critically injured patient was first explored during World War I for the treatment of 'wound shock'. Type O whole blood was collected in sterile glass bottles containing citrate and transfused into patients prior to surgery. During the years following World War I, blood component fractionation became available, blood banking was initiated, and the transfusion of packed red blood cells (RBCs), fresh frozen plasma (FFP) and platelets became a mainstay of the trauma management paradigm. However, in times of war the variable availability of short-lived platelets, FFP, and cryoprecipitate inevitably leads back to the resurrection of fresh whole blood transfusion. Fresh whole blood, though not without some risk, restores the hemostatic mechanism and provides volume and oxygen-carrying capacity.

Several studies have questioned the universal benefit of RBC transfusion for trauma patients, having found immunosuppression and dilutional coagulopathy in patients who receive massive transfusion. Additionally, standard component therapy dilutes platelets and coagulation factors in these patients. Massive transfusion is further hampered by a pressing need for new intravascular methods of preventing ongoing blood. Fluid for optimal resuscitation should be widely available and have a stable shelf life, and combine the safety of blood components with the efficacy, resuscitative and hemostatic effects of fresh whole blood. Such a fluid would truly provide 'hemostatic resuscitation'. Hemostatic resuscitation as an initial resuscitation regimen would restore the hemostatic mechanism, correct, or more importantly, prevent coagulopathy and would also enhance tissue perfusion. In this chapter, we describe the recent widespread use of fresh whole blood on the battlefield, the controversy surrounding massive transfusion of stored packed RBCs and other blood components as well as promising new developments in the arena of blood component substitutes.

Fresh Whole Blood

Combat medicine is an ebb and flow of casualties. Several days may pass when there are no casualties, followed by an onslaught of wounded. Casualties from explosive devices, such as those seen in Operation Iraqi Freedom and civilian terrorist attacks, tend to result in a large number of casualties at one time. This paradigm does not lend itself to component therapy. Packed RBCs, plasma, and crystalloid solutions may be in abundance, but a five-day shelf life prohibits the banking of platelets. The casualties who receive massive transfusions (>10 units packed RBCs) quickly deplete precious stores. FFP bags, not designed to support the temperature (–70 °F) associated with dry ice used during transport, become brittle and break one third of the time during the thawing process. Platelets generated by plasmapheresis became available only two years after the start of the war in Iraq, require significant and dedicated resources and availability remains limited. Limited blood products, manpower shortages, and warming methods increase the vulnerability of wounded soldiers to the onset of a second enemy: hypothermia, acidosis and coagulopathy.

In an attempt to resolve this problem combat surgeons often resort to the 'walking blood bank'. Predetermined donors are mobilized and fresh whole blood is collected, tested, and given to the wounded. Fresh whole blood protocols were followed in post-war Kosovo and in Somalia where over 120 units of fresh whole blood were collected and administered to critically wounded patients [1, 2]. A combat support hospital deployed to Baghdad transfused 598 units of fresh whole blood over a 6 month period [3]. Reports of the rapid positive response of recipients to fresh whole blood are thought-provoking and suggest profound possibilities for fresh whole blood transfusion. In combat, fresh whole blood for massive transfusion becomes a blood bank multiplier, providing within a single unit, RBCs, volume, coagulation components, and functional platelets in a warm fluid.

In 1999, Pearce and Lyons contrasted blood product usage from World War II and Korea to the practice in Vietnam [4]. In World War II and Korea, resuscitation consisted of colloid in the form of concentrated plasma and fresh whole blood. However, the incidence of hepatitis transmission rose to unacceptable levels – as high as 21% in some units in Korea [5]. In Vietnam, tested units of packed RBCs and crystalloid solutions (lactated Ringer's) were used and the incidence of infectious disease decreased. However, complications of the switch from fresh whole blood to stored RBCs became evident in Vietnam as acute respiratory distress syndrome (ARDS) or 'Da Nang Lung' was so prevalent that it became the focus of thousands of studies and book chapters. In comparison, very few cases of ARDS were described during World War II and none during Korea despite the administration of large volumes of whole blood and colloid [4].

These observations from the front lines of the advantages of fresh whole blood transfusion have been borne out in the civilian literature. In particular, two well designed, prospective studies support the clinical testimony of combat surgeons that fresh whole blood improves coagulopathy and decreases blood loss when compared to component therapy. The first study randomized cardiopulmonary bypass (CPB) patients to receive either one unit of fresh whole blood or 10 units of platelet concentrates after surgery [6]. The patients who received one unit of fresh whole blood increased platelet counts by $34 \pm 17 \times 10^9$/l, an increase equivalent to four to six units of platelets. Platelet aggregation response to collagen and epinephrine after fresh whole blood transfusion was superior to that achieved by 10 units of platelets.

Furthermore, bleeding time after administration of one unit of fresh whole blood approximately equaled bleeding time following eight units of platelets. In the second study, the beneficial effects of fresh whole blood on the coagulation cascade were demonstrated in a double-blinded, randomized controlled study comparing the use of fresh whole blood less than 24 hours old ($n = 52$), fresh whole blood between 24 and 48 hours old ($n = 57$), and reconstituted blood using packed RBCs, plasma, and platelets ($n = 52$), in a pediatric population undergoing CPB operations [7]. The 24-hour blood loss was no different between the two whole blood groups. The group that received reconstituted products had an increase in average blood loss that was significantly greater than either of the fresh whole blood groups ($p = 0.03$). After age stratification, the 24-hour blood loss for children less than 2 years old who received reconstituted blood was 85% greater than those who received fresh whole blood ($p = 0.001$). When platelet aggregation times were compared among the three groups, the group that received the reconstituted blood had a greater incidence of abnormal studies in the presence of the agonists ADP, epinephrine and collagen ($p < 0.001$, $p = 0.02$, and $p = 0.007$, respectively). Other recent studies have linked increasingly negative outcomes to each unit of packed RBCs received.

Fresh whole blood transfusion has also been shown to improve hemodynamic and oxygen delivery parameters in animal studies. One such study comparing transfusion of packed RBCs with whole blood in a canine model of hemorrhagic shock demonstrated that animals resuscitated with packed RBCs had significantly less hemodynamic recovery, demonstrated by lower mean arterial pressure and cardiac output ($p < 0.05$) [8]. Resuscitation with packed RBCs elevated total peripheral resistance when compared with resuscitation with fresh whole blood, suggesting vasoconstriction or obstruction of the peripheral vasculature by noncompliant RBCs [8]. This study corroborated findings of an earlier study that showed improved cardiac output and oxygen delivery in dogs resuscitated with whole blood compared to those resuscitated with crystalloid or colloid [9].

In an attempt to explain these observations of improved oxygen delivery, macroaggregated albumin clearance rates from the lungs of hemorrhaged rats were studied. Rats that received fresh whole blood demonstrated improved clearance of the tracer compared to animals resuscitated with either crystalloid or colloid [10]. Another rat model of hemorrhage and resuscitation demonstrated that fresh whole blood for resuscitation provided superior end organ perfusion by preventing ultrastructural kidney damage [11]. In a recent severe porcine hemorrhage study, resuscitation with fresh whole blood resulted in 90% 72-hour survival compared with 27% survival in animals receiving standard of care resuscitation fluids (lactated Ringer's, Hextend, 5% NaCl, 7.5% NaCl-6% Dextran-70 [HSD]) [12]. This was a non-coagulopathic model of controlled hemorrhage (53% bled in 5 minutes), indicating the metabolic benefits of fresh whole blood as a resuscitation solution.

Fresh whole blood transfusion is not without risk. The emergency conditions of the battlefield permit little time to test the blood for infection prior to administration. Aliquots from each unit of transfused fresh whole blood are sent back to the United States for testing. Over 1700 units of fresh whole blood have been transfused in the Iraqi operating room. In the aliquots of this blood, there were two positive confirmatory tests for hepatitis C (1:1000) (RIBA), and one each indeterminate test (Western blot) of human immunodeficiency virus (HIV) and human T-cell lymphocytic virus (HTLV). Compared to the extremely low incidence of transmission of these diseases in component therapy (1:493,000 HIV, 1:641,000 HTLV,

1:103,000 hepatitis C, 1:63,000 hepatitis B) [13], the risk of infectious disease transmission is still very low, but the surgeon must weigh the risk of infection against the risk of poor outcome if fresh whole blood is not received.

Rapid enzyme-linked immunosorbent assay (ELISA) for disease can be accomplished in two hours. ELISA was successfully utilized at one of the large combat support hospitals and has since seen more widespread use [3]. Without ELISA fresh whole blood must be administered sparingly and only under extreme circum-

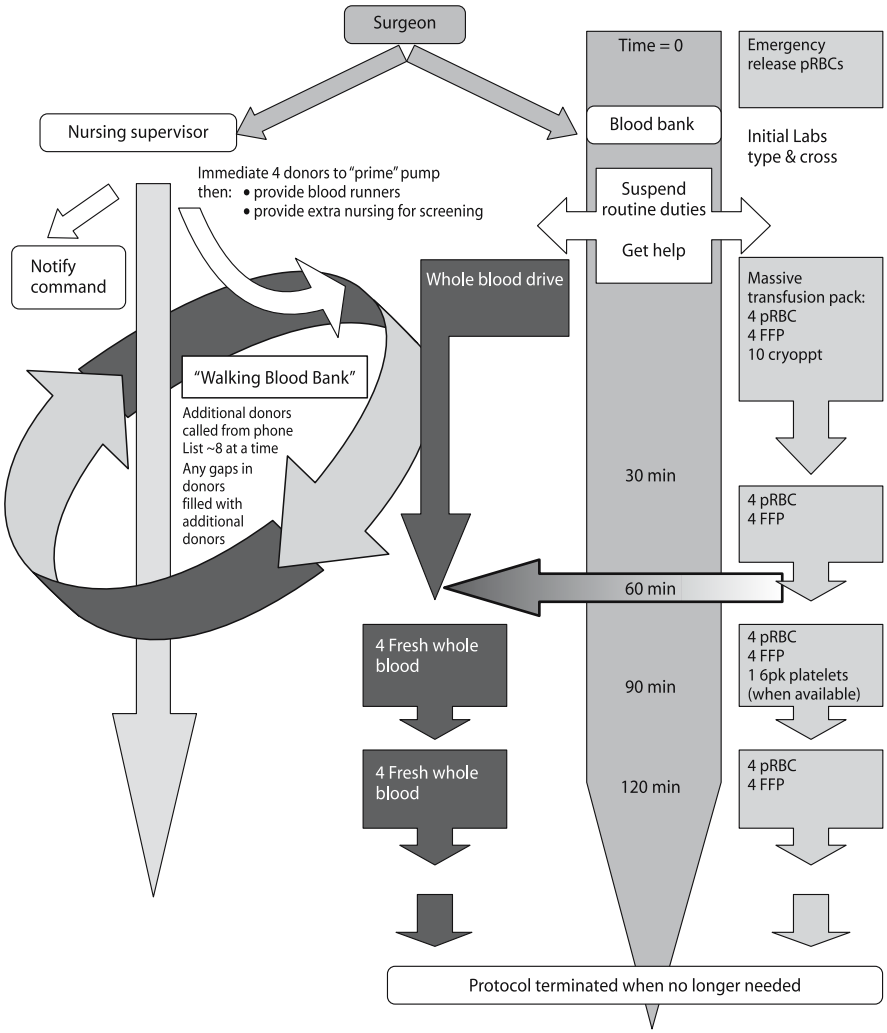

Fig. 1. This flow diagram is the massive transfusion/fresh whole blood transfusion protocol employed by the 31st Combat Support Hospital, while serving in Baghdad, Iraq. This is a two-pronged approach where patients are identified early and the mechanism for obtaining fresh whole blood initiated. Patients receive emergency release packed red blood cells (pRBCs) and when available are transitioned to fresh whole blood. This transition time coincides with the point where platelet transfusion would likely be indicated. FFP: fresh frozen plasma; cryoppt: cryoprecipitate. From [3] with permission

stances and protocols have been established that delineate the clinical guidelines for determining who should receive fresh whole blood. Figure 1 illustrates one such protocol employed at a combat support hospital [3]. Fresh whole blood was requested when large quantities of blood were required in order to save life and when the onset of coagulopathy and the need for platelets was imminent. This protocol illustrates a two-pronged approach in the event a massive transfusion is required. First, uncrossmatched, Type O packed cells are made available as emergency-release blood and the 'massive transfusion' protocol is initiated. At the same time, since there are usually no platelets and nominal FFP and cryoprecipitate available on the battlefield, the 'walking blood bank' of predetermined donors is activated so that required coagulation factors and components can be provided in the form of fresh whole blood. The first unit of fresh whole blood usually becomes available in about 120 minutes and the surgical team transitions to fresh whole blood as an adjunct to the massive transfusion protocol [3]. Many civilian hospitals utilize this template of a 'massive transfusion protocol' where, upon activation, predetermined quantities of packed RBCs, FFP, platelets and cryoprecipitate are sent at scheduled time intervals. The main difference is the effort to obtain fresh whole blood and the transition to whole blood transfusion.

Stored Packed Red Blood Cells

Following a landmark study published in the *New England Journal of Medicine,* the recommendations for red blood cell transfusion when serum hemoglobin levels are less than 10 mg/dl was changed to hemoglobin levels of 7 mg/dl in patients with signs of systemic compromise [14]. This randomized controlled trial compared a practice of liberal transfusion (keeping the hematocrit >30%) with a practice of restrictive transfusion (keeping the hematocrit between 21–27% and only transfusing if the hematocrit fell below 21%). There was no difference in overall mortality. However, less ill patients (APACHE <20) and younger patients (age <55) showed a statistically significantly improved survival with the use of a restrictive transfusion. Of clinical significance, the liberal practice group showed more cardiac complications and the restrictive practice population had lower hospital mortality and a lower adjusted multi-organ dysfunction score.

A growing body of literature underscores the potential risks of stored RBC transfusion. A retrospective study assessed over 15,000 patients for the independent predictors of mortality, intensive care unit (ICU) admission, ICU length of stay, and hospital length of stay [15]. After adjustment for injury severity, blood transfusion was shown to be an independent predictor of mortality, ICU admission, and hospital length of stay. Patients who underwent blood transfusion were nearly three times more likely to die and greater than three times more likely to be admitted to the ICU.

Coagulopathy can be caused by the dilution of coagulation factors by packed RBCs which do not contain any factors. Patients who undergo a massive transfusion (more than 10 units of packed RBCs or receipt of twice the normal blood volume) have prolonged prothrombin time, thrombocytopenia and decreased levels of fibrinogen. Seventy percent of those who receive more than 20 units of red blood cells become coagulopathic, with thrombocytopenia being the most common abnormality [16]. A single unit of packed RBCs is approximately 335 ml with a hema-

tocrit of 55%. A single unit of platelets is 50 ml and contains 5.5×10^{10}/l platelets. A single unit of plasma is about 275 ml with only 80% coagulation activity of fresh whole blood. When all of these components are combined, the result is a fluid with a hematocrit of 29%, 8.8×10^{7}/l platelets and coagulation activity that is 65% of normal [17]. Even when RBCs are administered at a high one-to-one ratio with FFP, dilution of coagulation factors and platelets and iatrogenic anemia is unavoidable.

It is well known that the trauma related to surgery results in the activation of the immune system. Levels of inflammatory cytokines are elevated to a greater extent in patients with severe blood loss when compared to patients with isolated trauma. Additionally, the degree of elevation of pro-inflammatory cytokines correlates with severity of injury [18, 19]. When blood transfusion, which is inherently pro-inflammatory, is combined with the inflammatory response associated with trauma, the result is an exacerbated acute phase response [20]. This exacerbated inflammatory response is associated with increased infectious complications and increased mortality [19]. A biphasic inflammatory response begins, with initial pro-inflammation followed by immunosupression which begins at about 24 hours [18]. It is during the subsequent phase of immunosuppression that the patient is most susceptible to infection. Historically, this immunosuppressive effect was actually used to the advantage of the patient. Prior to the use of cyclosporine, blood transfusion was used in transplant patients as an immunosuppressant, improving outcomes [21].

Several studies further illustrate the disruption of the immune system by RBC transfusion. A prospective trial demonstrated a significantly increased rate of nosocomial infection of 15.4% in 412 patients who received transfusions compared to a 2.9% rate in 1,301 control patients who did not receive transfusions [22]. There was a dose-related response between the number of units of packed RBCs received and the risk of developing a nosocomial infection. Additionally, patients who received transfusions showed increased mortality, length of ICU stay, and hospital stay ($p<0.05$). This single-center prospective trial corroborated the observations of several basic science studies. Detrimental immunomodulation is thought to occur following increased production of both serum and tissue tumor necrosis factor (TNF)-α, interleukin (IL)-1β, IL-6, IL-8, interacting with soluble cytokine receptors [23]. Fransen et al. showed that surgical patients who received packed RBCs compared to those who did not had increased levels of IL-6 ($p<0.01$) and bactericidal permeability increasing protein (BPI) ($p<0.05$), which is a marker of neutrophil activation [20]. In addition to these serum markers of inflammation, patients who received transfusions had increased ventilator time (42 ± 12 vs. 22 ± 2 h, $p<0.025$) and increased ICU days (45 ± 6 vs. 89 ± 21, $p<0.025$) [20]. Transfusion of blood components has also been associated with impaired natural killer cell function and decreased helper-suppressor cell ratios as well as effects on B lymphocytes [24]. Again, there appeared to be a linear relationship between the amount of blood transfused and the degree of impairment [24].

Approximately twenty-four hours is required for transfused packed RBCs to recover their full ability to deliver oxygen, but the precise mechanism of this immunomodulation is a mystery. One theory is that immune system derangement is the result of the storage age of blood. Gastric mucosal pH can be used as an indicator of oxygenation of the gastric mucosa or the organs of splanchnic distribution and studies have shown that direct measurements of gastric mucosal pH are related to the age of the blood transfused to critically ill patients [25, 26]. Two mechanisms

that have been proposed to account for the inability of old transfused RBCs to improve systemic oxygen consumption include a left shift in the oxyhemoglobin dissociation curve due to 2,3-diphosphoglycerate (DPG) depletion with storage, and loss of the RBC compliance and the ability to deform, impeding delivery of oxygen to the microcirculation [27]. Another emerging theory speculates that due to the high affinity of nitric oxide (NO) with free hemoglobin, cell-free ferrous hemoglobin in the plasma is oxidized to methemoglobin and nitrates that rapidly destroy NO [28, 29]. The decreased availability of NO results in regional and systemic vasoconstriction.

The exact mechanisms are uncertain, but it is becoming clearer that the administration of stored RBCs is a clinical decision that should not be taken lightly. The negative repercussions of packed RBC transfusion may become evident in trauma patients, who present in a state of acute inflammation derived from the trauma sustained. The literature supports the theory that the transfusion of packed RBCs can actually result in a deranged inflammatory response and that this may be related to the storage age of the blood. The transfusion of packed RBCs remains the standard of care for restoring RBC concentration and circulating oxygen content during ongoing hemorrhage, but it is not a solution that is devoid of risk.

Hemoglobin-Based Oxygen Carriers

In severe hemorrhage, oxygen delivery to tissues must be restored. Hence, the rationale for hemoglobin-based oxygen carriers is the ability to deliver oxygen to vital organs via plasma components. But the development of hemoglobin-based oxygen carriers to replace packed RBCs has led a rocky course over the past twenty-five years. Several variants of hemoglobin-based oxygen carriers attempted in the past were either abandoned by the developing company or discontinued in phase III trials out of concern for patient safety [30]. Concerns about hemoglobin-based oxygen carriers are their effects on vasoactivity resulting from binding of NO by hemoglobin and subsequent hypertension. Currently, however, in the United States a phase III trial of pyridoxalated hemoglobin (Polyheme, Northfield Laboratories, Evanston, IL.) is 50% completed. This multicenter pre-hospital trial is examining the administration of hemoglobin based oxygen carriers to trauma patients in severe hemorrhagic shock. Hopefully, upon completion of this study, hemoglobin based oxygen carriers will be added to the armamentarium of the caregiver.

An effective and safe hemoglobin-based oxygen carrier appeals to both the civilian and military communities. Polyheme can be stored for 72 hours at room temperature, virtually eliminates any risk of disease transmission, does not require type and cross-matching and provides a fluid which can enhance oxygen delivery [31]. It does not, however, have any restorative effect on coagulation. For military use, Polyheme will require availability at room temperature extended beyond 72 hours. Whether it will be carried in the rucksack of a medic for a limited time or in a civilian ambulance, this potentially lifesaving product deserves further exploration.

Fresh Frozen Plasma

Plasma contains various components including stable coagulation factors, fibrinogen and albumin. Lyophilized plasma was the principle resuscitation fluid during both World War II and the Korean War due to its long shelf life without refrigeration. At the end of World War II and the Korean War, the discovery of infectious diseases as the result of plasma obtained from multiple donors, i.e., pooled plasma, halted this practice. Consequently, the indications for plasma transfusion today no longer include that of a volume expander. FFP is indicated for the correction of coagulopathy associated with factor deficiency (II, VII, IX, X), hemorrhage, reversal of warfarin effect, and antithrombin deficiency.

During massive transfusion of packed RBCs in a patient with exsanguinating hemorrhage, the onset of coagulopathy is usually described as the result of dilution and consumption of coagulation factors, acidosis and hypothermia. The incidence of coagulopathy increases with injury severity. Twenty-one percent of patients with an injury severity score (ISS) of 15–29 are coagulopathic, 41% with an ISS of 30–44, 59% with ISS of 45–59, and 79% of those with an ISS of 60–75 [32]. However, importantly, the most seriously injured patients are coagulopathic upon admission, unrelated to dilution. In a large study of 1,088 trauma patients, 24.4% were coagulopathic on admission [32]. As a general rule, hemostasis is possible as long as coagulation factor activity is kept at 20 to 30% of normal and fibrinogen levels are at least 100 mg/dl. FFP contains plasma clotting factors and fibrinogen; in addition, administration will increase intravascular volume.

Because the incidence of coagulopathy increases with severity of injury, many institutions include the transfusion of single donor FFP in their massive transfusion protocols. Often, the first application of blood products contains six units of type O RBCs and four units of AB negative FFP. Subsequent applications include six units of type specific RBCs and FFP, and a six-pack of platelets with a unit of cryoprecipitate in every other application. Cryoprecipitate provides fibrinogen and some clotting factors which become depleted in a massive transfusion. Volume expansion inherently occurs with the administration of these products and is also augmented with either crystalloid or colloid solutions. The purpose of this protocol is to restore circulating RBCs, volume, clotting factors, and clotting substrate and to sustain the patient while the surgeon stops the source of bleeding.

The onset of coagulopathy in the hemorrhaging patient can be rapid, and the time required to obtain plasma can further propel the patient along the 'bloody vicious cycle' of coagulopathy, acidosis, and hypothermia. Therefore, to prevent a dangerous situation the caregiver must be ready to transfuse blood products based on immediate clinical evaluation rather than delayed laboratory assessment. The current laboratory guidelines for the transfusion of FFP are prothrombin time (PT) more than 1.5 times greater than normal, activated partial thromboplastin time (aPTT) ratio more than 1.5 times greater than normal, fibrinogen less than 0.8 g/l, and coagulation factor levels 30% of normal [17]. Some authors advocate early FFP transfusion at a 1:1 ratio with packed RBCs after hemorrhage of one blood volume, or PT and aPTT greater than 1.5 times normal with ongoing hemorrhage [33]. Even with this aggressive practice of plasma transfusion, hemodilution is unavoidable. As stated earlier, when standard blood products are administered in a 1:1 ratio the resulting fluid has a hematocrit of 29%, 8.8×10^7/l platelets, and coagulation activity that is 65% of normal [17]. The primary risks associated with fresh frozen plasma transfusion are infection, transfusion-related acute lung injury (TRALI), acute aller-

gic and anaphylactic reactions, hemolysis due to anti-A and anti-B, and fluid overload [34]. Of these, the most common serious complication of FFP transfusion is TRALI, thought to occur in a graft versus host mechanism as donor antibodies react with host leukocytes [35]. The rate of infectious disease transmission resulting from FFP administration is similar to that of packed RBCs.

Platelets

It is important that clinicians take into consideration the etiology of thrombocytopenia, platelet dysfunction, risk of bleeding, planned invasive procedures, and the presence of concomitant disorders when deciding whether or not to transfuse platelets. Patients with severe sepsis, regardless of the presence of hemorrhage, usually receive a transfusion of platelets if their platelet count falls below 5000/mm^3. Platelet transfusion should be considered if the platelet count is between 5000–30,000/mm^3, and there are signs of bleeding. If the patient requires surgery or other invasive procedures it is recommended that the platelet count be maintained above 50,000/mm^3. The conundrum presented by these classic recommendations is the highly variable relationship between platelet counts and platelet efficacy.

Platelets pose significant logistical problems. Since the development of platelet aphaeresis technology, it has been well known that platelets stored in the cold have poor recovery and survival *in vivo*. They cannot be refrigerated even for short periods of time, thus platelets are stored at 22 °C. Studies of chilled platelets have shown that this irreversibly alters their morphology as well as the expression of the GPIbα receptor on the platelet, causing rapid clearance of the transfused platelet from the circulation [36]. The changes which occur in the structure and function of stored platelets are known as the 'platelet storage lesion' and are poorly understood. There are few data regarding the function of platelets in the bleeding patient. After room temperature storage of up to 5 days, the risk of bacterial contamination becomes significant. The rate of septic reaction to platelet transfusion in the United States is between 1:10,000 and 1:20,000 [37]. The short shelf-life of platelets combined with an uncertain demand for platelets results in wastage rates as high as 50%.

Significant efforts are being made in the development of novel platelet products and platelet substitutes. Lyophilized platelets are one such product, initially investigated nearly fifty years ago and recently resurfaced [38]. Lyophilized platelets have shown encouraging preclinical results in animal models [39]. A second product, Synthocyte™ (Profibrix, Inc., The Netherlands), is a fibrinogen-coated albumin microsphere that has shown a reduction in surgical bleeding in a thrombocytopenic animal model [40]. The mechanism of action of Synthocytes™ is thought to be due to the cross-linking of fibrinogen which has been directly imbedded into the surface of the microcapsules [41]. Other areas of platelet product development include liposome-based hemostatic agents, thromboerythrocytes, and platelet-derived microparticles.

Fibrinogen

One of the end products of the coagulation system is the production of thrombin which stimulates the sequential proteolytic cleavage of fibrinogen to release fibrinopeptides A and B. The fibrin monomers that result from this process spontaneously polymerize to form an insoluble matrix. This matrix is stabilized by factor XIIIa, which is another product of thrombin generation. Fibrinogen, or factor I, is essential for hemostasis and it is recommended that fibrinogen be administered in the form of cryoprecipitate once fibrinogen levels fall below 100 mg/dl. This number applies to patients with non-surgical bleeding and not necessarily to those with active blood loss.

Cryoprecipitate derived from a unit of whole blood contains 10–20 ml of fluid per unit, providing up to 150–250 mg of fibrinogen, 80–100 units of factor VIII, and 50–60 mg of fibronectin and in a non-bleeding 70 kg patient one pool of cryoprecipitate will increase the fibrinogen levels 45 mg/dl. Cryoprecipitate does not contain all of the necessary coagulation factors and should not be used in place of FFP.

Fibrinogen substitutes are being actively investigated for fibrinogen supplementation. Recombinant fibrinogen, rhFIB, (Pharming Group, Denmark) is currently under investigation by the United States Army as an adjunct for hemostasis. Another group has shown that fibrinogen derived from salmon activates human platelets and may provide a hemostatic function [42]. A third product, Haemocomplettan (Aventis Behring GmbH, Marburg, Germany), is a lyophilized human fibrinogen concentrate that has been shown to correct thromboelastograph abnormalities and improve mortality as seen in rat models of sepsis-induced disseminated intravascular coagulation (DIC) [43]. Fibrinogen substitutes would allow more rapid correction of fibrinogen dilution than cryoprecipitate.

Recombinant Factor VIIa

Over the past several years recombinant factor VIIa (rFVIIa) (Novoseven, Novonordisk, Malov, Denmark) has emerged as a promising therapy for treatment of coagulopathy associated with trauma and massive transfusion. This emergence began as observational experiences [44], translated into pre-clinical studies in animal models [45, 46], and progressed to large, multicenter, randomized and controlled studies which clearly show the safety and efficacy of rFVIIa in severely injured trauma patients. Phase II trials will soon advance to larger phase III studies and rFVIIa holds promise as an adjunct to hemostasis in patients who receive massive transfusions for both blunt and penetrating trauma.

Used as a surgical adjunct rFVIIa reduces blood loss. In a randomized, double-blinded prospective study, surgical patients who underwent a retropubic prostatectomy were given 20 μg/kg rFVIIa, 40 μg/kg rFVIIa or placebo prior to surgery. The higher dose group showed significant decreases in blood loss ($p < 0.01$) and surgical time ($p < 0.05$) and elimination of packed RBC transfusion requirements [47]. On the other hand, studies regarding the proper administration of rFVIIa have elucidated that rFVIIa is ineffective as a 'last resort' [48]. We recommend that the administration of rFVIIa follow a clinical protocol according to set guidelines for dose and other parameters in order to optimize success. One early guideline published

by Dutton et al. outlines the use of clinical "gatekeepers" who are available to assess the patient and clinical circumstances surrounding the administration of rFVIIa [49]. Criteria include evidence of ongoing active hemorrhage, with clinical evidence of coagulopathy, and ongoing utilization of conventional transfusion and hemostatic therapy which must be judged unlikely to succeed. Patients must have received at minimum 10 units of RBCs, eight units of FFP, one unit of platelets, with continued derangement of coagulation studies and ongoing hemorrhage. This approach reserves rFVIIa for the most critically injured.

With its safety demonstrated in a large randomized clinical trial, most clinicians are utilizing rFVIIa earlier in an attempt to prevent massive bleeding and coagulopathy rather than treating them after the fact. In a protocol in May 2004 from the Trauma Consultant to the Surgeon General of the United States, this change from reacting to coagulopathy to actively preventing coagulopathy with rFVIIa was made manifest. It is recommended that combat surgeons "consider rFVIIa administration for use in patients that require damage control procedures, have coagulopathic bleeding, or difficult to control bleeding associated with hypothermia or significant operative bleeding." It is further recommended that the surgeon consider administering two units of fresh whole blood prior to rFVIIa to ensure the presence of functional platelets and coagulation factors.

A significant drawback to the administration of rFVIIa is cost. On average, an 80 kg man receiving a 120 μg/kg dose, costs about $ 8,000. Though this cost appears exorbitant, most studies demonstrate a reduction in blood product requirements, which in turn should translate to lower costs and prevention of subsequent infection and deleterious inflammatory response. Efforts are also being taken to decrease cost by making rFVIIa more efficient. In a rat model of hemophilia, three 'super analogs' of rFVIIa were compared with rFVIIa [50]. The rFVIIa groups received 1, 3, 6 or 10 mg/kg, and the super-analog groups 1 or 3 mg/kg. Two of the 3 mg/kg groups of the super-analogs and the 10 mg/kg group of rFVIIa showed significant improvement in bleeding times ($p < 0.001$) when compared to the hemophilia control group. Average blood loss was reduced in the 3, 6 and 10 mg/kg groups of rFVIIa ($p < 0.05$) and in all groups of the super-analog, including the 1 mg/kg groups ($p < 0.05$). A dose response was seen between the 1 and 3 mg/kg groups of all three super-analogs, with decreased blood volumes in the higher dose super analog groups ($p < 0.001$) [50]. With further research and the development of rFVIIa analogs with greater potency, we hope to see the cost associated with this drug decrease while effectiveness increases.

Where are we Heading?

The potential hematologic and physiologic benefits of fresh whole blood as a hemostatic and resuscitative fluid are clear. This straightforward approach is akin to the damage control procedures so widely accepted in trauma surgery, although it is temporized by inherent logistical problems and the small but real threat of infectious diseases. What is needed is a fluid which contains the oxygen-carrying capability as well as the volume and hemostatic qualities provided by fresh whole blood. For hemostatic resuscitation on the battlefield we also envision a fluid that is stable at room temperature and has a long shelf life. Additionally, a fluid that can transport oxygen, possibly by a hemoglobin-based oxygen carrier could serve

as a volume expander and contain active clotting factors and platelets, like those found in freeze-dried plasma, platelets, fibrinogen and recombinant factor VIIa. Being fielded forward to the point of injury, this fluid could prevent the onset of coagulopathy and decrease blood product transfusion requirements, all in hope of improving survival. Fascinating and promising products on the horizon may make hemostatic resuscitation a possibility.

Conclusion

Component therapy is useful for the majority of patients when blood requirements are minimal and there is no associated coagulopathy. Of concern are requirements for massive transfusion and resuscitation that absorb resources and create a shortfall for patients whose injuries are less severe. Additionally, the conventional massive transfusion model of packed RBCs, plasma and platelets actually further dilutes the patient compared to the blood he or she has lost and thus is not the ideal fluid for patients who require this massive transfusion of products. Fresh whole blood has three vital properties: oxygen carrying capacity, volume, and hemostatic effect. In the austere environment of combat the practice of fresh whole blood transfusion has proven beneficial to patients who are coagulopathic and require massive transfusion. Appropriate use following established guidelines can be beneficial and may even be superior to packed RBCs. A fluid containing the vital properties of fresh whole blood would serve as a bridge to allow a patient to be resuscitated without initiating the 'bloody cycle of death' that is seen all too often in our current paradigm of massive resuscitation.

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Effects of Plasma Substitutes on Coagulation

M. Levi and E. de Jonge

Introduction

Plasma substitutes, such as crystalloid or colloid solutions are frequently used in bleeding patients or in situations with a high risk of bleeding such as trauma or during surgery. There is ample evidence that these agents may affect blood coagulation and platelet function [1], although some authors, referring to thromboelastography studies, have suggested that hemodilution *per se* results in a hypercoagulable state [2]. These findings, however, have been disputed. In particular, all three distinct classes of artificial colloids (i.e., dextrans, hydroxyethyl starches [HES], and gelatins) have been associated with derangements of the hemostatic system, although the clinical significance of these derangements is a matter of debate [3]. In this chapter, we will focus on the anti-hemostatic effects of various volume replacement fluids on platelet function and blood coagulation.

Dextran Solutions

Dextrans are polydisperse glucose polymers produced by bacteria growing in sucrose-containing media. Besides their plasma expanding properties, they also exert an anticoagulant effect. Indeed, dextrans have been shown to be effective in preventing postoperative venous thrombosis and pulmonary embolism [4]. Dextran infusion causes a decrease in the levels of von Willebrand factor (vWF) and associated factor VIII, which is much larger than can be explained by its dilutional effects [5]. Figure 1 shows the results of an *in vitro* experiment on levels of vWF upon incubation with dextran of normal plasma and plasma derived from a patient with mild von Willebrand disease. vWF is the ligand between the platelet surface receptor protein GPIb and subendothelial collagen, playing a pivotal role in platelet adhesion to the vessel wall. Decreased levels of vWF, therefore, may lead to impaired primary hemostasis. Indeed, prolonged bleeding times were observed after infusion of dextran in animals [6] as well as in humans [7]. The prolongation of bleeding time was totally reversed after increasing vWF levels by the intravenous administration of desmopressin (1-desamino-8-d-arginine vasopressin) [8]. Besides their effects on the factor VIII/vWF complex, dextrans also enhance fibrinolysis [9]. It has been shown that fibrinolysis may be enhanced after dextran infusion by increased plasma concentrations of tissue type plasminogen activator (tPA) and decreased concentrations of the physiologic inhibitor of fibrinolysis, plasminogen activator inhibitor-1 (PAI-1). Earlier reports suggested that fibrinolysis is increased by formation of complexes that include dextran, fibrin, and plasminogen activators.

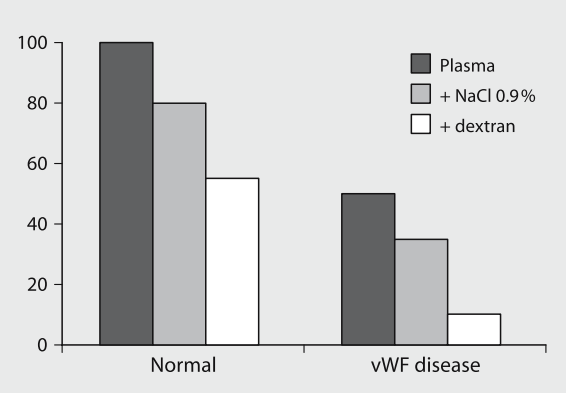

Fig. 1. von Willebrand factor (vWF) activity in plasma of normal healthy subjects and plasma from patients with mild (type III) von Willebrand's disease after dilution with NaCl 0.9% (final concentration 20%) and after incubation with dextran solution (final concentration 20%)

Indeed, clots formed in the presence of dextran are relatively bulky in size, exhibit less tensile strength, and seem more easily disrupted [10]. After administration, the anti-hemostatic effects of dextran on factor VIII/vWF and fibrinolysis may result in an increased bleeding tendency. In clinical studies comparing dextran with unfractionated heparin, low molecular weight heparin, or the heparinoid, Orgaran, as prophylaxis against venous thromboembolism, dextran infusion resulted in increased postoperative blood loss after transurethral prostatectomy or orthopedic surgery, and increased the need for blood transfusion after surgery for hip fracture [11, 12]. In a study comparing intraoperative use of dextran and 4% albumin solution in orthopedic surgical patients, patients treated with dextran needed more blood transfusions [13].

Gelatin-based Solutions

Gelatins are polydisperse polypeptides produced by degradation of bovine collagen. There are two distinct forms of gelatin solutions: succinylated (modified) gelatins, which have NH_3 groups replaced by COO^- groups due to reaction of the basic peptide with succinic acid anhydrase, and polygelines, consisting of polypeptides cross-linked by urea bonds. Although for a long time gelatins were considered not to influence blood coagulation other than by dilution [14], there is now increasing evidence that gelatins do influence platelet function and blood coagulation. *In vitro* blood coagulation in the presence of gelatin was studied using thromboelastography and scanning electron microscopy. Clots produced in the presence of gelatin had decreased weight and strength and loss of the normal reticular network of fibrin strands [15]. Although these results could not be confirmed in another study using thromboelastography, this study also showed a decrease in clot strength after dilution by gelatin as compared with saline dilution [16]. Others have studied the effects of *in vitro* dilution of blood on platelet aggregation. It was found that gelatin impaired aggregation induced by ristocetin and polybrene [17]. Agglutination tests induced by ristocetin and polybrene are specific for binding of vWF to the platelet

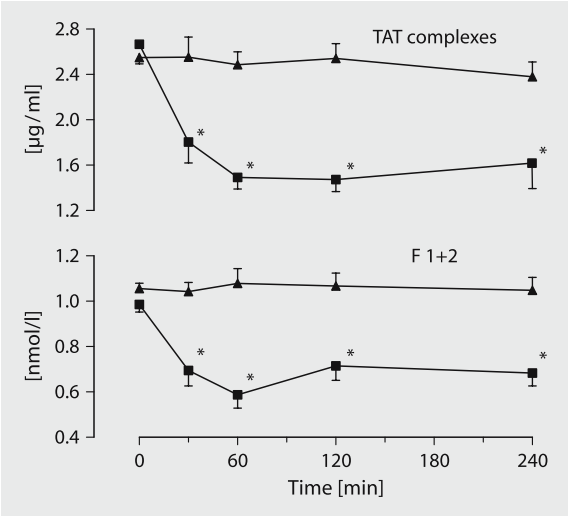

Fig. 2. Thrombin generation in blood (reflected by plasma levels of thrombin-antithrombin (TAT)-complexes (upper panel) and prothrombin activation fragment F1 + 2 (lower panel) after the infusion of 1 liter of gelofusin (■) or 1 liter of NaCl 0.9% (▲) over 60 min to healthy human subjects. Mean values and SEM's are shown, * denotes statistical significance ($p < 0.01$)

receptor GPIb. These findings are supported by the observation that *in vivo* administration of 1 liter of a gelatin-based plasma expander to healthy humans induced a von Willebrand-like syndrome with lengthening of bleeding time, impaired ristocetin-induced platelet aggregation, and decreased levels of plasma vWF. It was suggested that vWF binds to gelatin by means of its collagen binding site. In addition, a decrease in thrombin generation, as measured by thrombin-antithrombin complexes and prothrombin fragment F1 + 2, was observed after gelatin administration, probably caused by hemodilution (Fig. 2) [18]. The clinical relevance of the impairment of hemostasis after gelatin infusion is uncertain. Only one report suggests increased blood loss after cardiac surgery when gelatin instead of albumin was given preoperatively [17]. However, other studies comparing gelatin with HES or HES and albumin found no difference or, in some cases, even an improvement in postoperative blood loss when gelatin was given [19].

Hydroxyethyl Starch-based Solutions

Natural starches cannot be used as plasma substitutes because they are rapidly degraded by circulating amylase and they are insoluble at neutral pH. HES are polymers of glucose units derived from amylopectin and modified by substituting hydroxyethyl for hydroxyl groups on glucose molecules. The substitution results in slower degradation and highly increased solubility. Although HES is generally considered as an effective and safe plasma substitute, the use of this volume expander has been associated with bleeding complications in various clinical settings. Many case reports have been published describing bleeding complications after high molecular weight HES [20, 21]. Also, high molecular weight HES (Hetastarch), which

is the only HES solution that is approved in the United States as a plasma expander, has been associated with increased postoperative bleeding after neurosurgery and in patients undergoing cardiac surgery [22]. It has been suggested that not all HES solutions have negative effects on blood coagulation, but that these effects depend on the average molecular weight of the HES molecules and its kinetics of elimination. HES are very polydisperse solutions of molecules with a broad range of molecular weights from very small to several hundred thousand Dalton. After administration of HES, the low molecular weight fraction is rapidly lost by renal elimination and the large molecules are progressively hydrolyzed, resulting in an average *in vivo* molecular weight that is significantly lower than the average molecular weight of the infused fluid. The rate at which degradation of HES molecules occurs depends on the degree of substitution, that is the proportion of glucose units having a hydroxyethyl group substituted for a hydroxyl group. The rate of degradation and elimination is highest (and thus effects on coagulation are smallest) with low degrees of substitution [3].

Studies in healthy human volunteers have shown that circulating levels of factor VIII and vWF decrease significantly after infusion of 0.5–1 l of high molecular weight HES or medium molecular weight HES [23, 24]. Similar reductions in factor VIII and vWF have not only been found in healthy volunteers, but also in a number of clinical studies [20, 25]. Considerable insight into the influence of HES on blood coagulation comes from observations by Treib et al. [26]. In several 10-day hemodilution experiments in patients, these authors found that a decrease in vWF and factor VIII was only observed when medium molecular weight HES was given that was slowly degradable (i.e., with a high degree of substitution). The infusions resulted in accumulation of HES molecules with higher molecular weights. It was concluded that the negative effects on hemostasis depended on the *in vivo* molecular weight, and that therapy with low-molecular weight HES or easily degradable medium molecular weight HES did not influence blood coagulation. However, we observed a 33% decrease in vWF and a 28% decrease in factor VIII after administration of 1 l of rapidly degradable HES to healthy volunteers [27]. These decreases were more than could be explained by HES-induced plasma dilution. In accordance with the diminished vWF levels, platelet adhesive function, measured by the platelet function analyzer PFA-100 (Dade Behring, Marburg, Germany), was significantly prolonged after HES as compared with albumin 4%, which is compatible with an acquired von Willebrand's syndrome. A possible explanation for these different findings in healthy volunteers as compared with the studies in patients could be that vWF is an acute phase protein, increasing during acute illness and thereby potentially masking a concomitant HES-induced decrease. Furthermore, relatively low quantities of HES were given during the hemodilution experiments (1000 or 500 ml/day) [26]. In circumstances when large volumes of HES are given over a short time period (e.g., in bleeding patients with circulatory shock), HES could potentially induce a clinically relevant coagulation defect. Also, uncertainty exists about the clinical effect of the administration of HES on blood coagulation in patients with already low circulating levels of vWF.

Reductions in the concentrations of other plasma coagulation factors, which could be fully ascribed to plasma dilution after administration of HES, have been reported repeatedly [28, 29]. The prothrombin time is only slightly prolonged after the administration of HES, probably due to dilution of plasma factors [2, 23]. The effects of HES on the activated partial thromboplastin time (aPTT) depend on its molecular weight and the kinetics of elimination. Significant prolongation of the

aPTT up to 40% has been described after repeated infusion of slowly degradable medium molecular weight HES. In contrast, only a minimal prolongation was found after the infusion of low molecular weight HES or easily degradable medium molecular weight HES. The effects of HES on the aPTT can be readily explained by the specific decreases in factor VIII, potentially in combination with some dilution of other plasma factors. High molecular weight HES has been associated with increased fibrinolysis. Infusion of 500 ml medium molecular weight HES in healthy volunteers did not result in changes in plasma levels of tPA, urokinase-plasminogen activator (uPA), PAI, plasmin-antiplasmin complexes (PAPc), or D-dimer when compared with infusion of 5% albumin. The effects of HES on coagulation and fibrinolysis have also been studied using thromboelastography, yielding results that point in the same direction. *In vitro* studies suggested prolonged clot formation time and increases in clot lysis after profound hemodilution with HES [30]. Finally, HES might affect platelet function. Platelet volume decreases after infusion of HES, presumably because of a 'shrinkage' of platelets by the increased plasma colloid osmotic pressure [23]. It has, however, not yet been established whether the decrease in platelet volume after HES administration affects platelet function and bleeding time. Prolonged bleeding times have been reported after high molecular weight HES, and the infusion of medium molecular weight HES has been associated with normal as well as prolonged bleeding times [1]. In contrast, in another study comparing the influence of medium molecular weight HES and albumin on platelet aggregation, no difference was observed between the two colloids [31].

Comparison of Anticoagulant Effects of Various Volume Expanders

It is difficult to make direct comparisons between different colloids in terms of laboratory markers of blood coagulation, as all studies differ in the plasma substitutes used, the amount of colloid infused, and the population studied. Furthermore, some studies looked at effects of short-term fluid therapy (e.g., only during surgery) whereas others considered 10-day hemodilution. Nevertheless, some general conclusions can be drawn. First, the effects of HES on coagulation seem to depend on its molecular weight as well as on the rate of *in vivo* degradation. High molecular weight HES undoubtedly affects blood coagulation, even if given over a limited time period. Slowly degradable medium molecular weight HES and HES with a high degree of substitution also impair coagulation after repeated administration, probably due to accumulation of macromolecules. In contrast, most studies consider easily degradable medium molecular weight HES (with a low degree of substitution) or low molecular weight HES to have minimal, if any, influence on blood coagulation when compared with albumin. The effects of modified gelatin solutions seem to be similar to the effects of easily degradable medium molecular weight HES and albumin.

Parallel to the observations on laboratory markers of coagulation, administration of high molecular weight HES may lead to increased blood loss. No study has documented an increased bleeding tendency after medium molecular weight HES with a low degree of substitution when compared with albumin, suggesting that rapidly degradable medium molecular weight HES can be given safely during surgery. Only one study has been published that compared slowly degradable medium molecular weight HES with albumin in orthopedic patients. In this small study, no

difference in postoperative blood loss was found. Studies comparing gelatin with albumin or medium molecular weight HES have generally found no difference, except for one study that observed a decreased blood loss after gelatin when compared with medium molecular weight HES and one that found an increased blood loss when compared with albumin, but only in a subgroup also treated with aprotinin [17, 32]. Thus, it seems that dextran and high molecular weight HES can induce an increased bleeding tendency, whereas rapidly degradable medium molecular weight HES and gelatin are probably safe in this respect. There are, however, two major limitations to this conclusion. First, no studies have addressed the risk of bleeding after repeated administration of colloids. Theoretically, this could easily increase the risk of bleeding, especially with infusion of slowly degradable HES. Second, these conclusions are probably only valid in subjects with normal levels of vWF or even increased levels due to the acute phase response in acutely ill patients. Because all artificial colloids can lead to decreased levels of vWF, they should be given cautiously to patients who are known to have even mild forms of von Willebrand disease. In those patients, especially when they are bleeding, crystalloid solutions or alternatives like plasma or albumin, although associated with other serious complications, could be considered.

Conclusion

All plasma expanding solutions have effects on the coagulation system. These effects may go beyond simple dilution of coagulation factor concentration. Most plasma substitutes cause a decrease in von Willebrand factor, with or without an associated reduction in factor VIII plasma levels, and may, thereby, also cause effects on thrombin generation. If large amounts of plasma substitutes are given to patients with an already compromised coagulation status, the anti-hemostatic effects of these agents may become clinically significant, although marked differences between various plasma substitutes may exist.

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Blood Transfusions

Transfusion as a Risk Factor for ALI and ARDS

J. Belsher, H. Khan, and O. Gajic

Introduction

Transfusions are regularly employed in the care of the critically ill. While the transmission of infectious agents and clerical errors have long been major concerns, a more common non-infectious complication of transfusion, namely acute lung injury/acute respiratory distress syndrome (ALI/ARDS), has been neglected. Indeed, during the past several years, post-transfusion ALI/ARDS (transfusion-related acute lung injury – TRALI) has become the leading cause of transfusion-related death in the United States [1]. It likely occurs much more frequently than previously estimated. With skyrocketing health care costs and the continuous emphasis on improvement in patient care, it is of paramount importance to better define this transfusion-related phenomenon and to design effective strategies for its prevention. Several investigator groups have been on the leading edge in the quest to clearly elucidate this disease entity and define its prevalence.

Popovsky and Moore published the first case series describing TRALI in 1985 [2]. The 36 patients studied were defined to have TRALI if the following criteria were met within six hours of transfusion: respiratory distress, hypoxemia, and bilateral parenchymal infiltrates on chest roentgenogram in the absence of congestive heart failure. More recent data suggest that the process may occur more rapidly than previously thought, within one hour of transfusion in up to 90% of cases [3]. Although the majority of patients require mechanical ventilation, the resolution is typically rapid, with most cases resolving within 96 hours [1, 2].

Incidence rates of TRALI have varied according to patient populations and case definitions. Most literature generally refers to a range of anywhere between one per 1200 transfusions in observational studies to one per 500,000 transfusions in passive surveillance systems [3]. This is felt to be a gross underestimate as the majority of cases are typically not recognized nor reported. For example, Kopko et al. [4] did a retrospective review of 36 patient charts on the Food and Drug Administration's (FDA) recommendation in a look-back investigation of transfusion-related fatalities. Of the 36 patients who were transfused fresh frozen plasma (FFP) from one particular donor (a 54-year old multiparous female), 13 (36.1%) had transfusion reactions. Of the eight severe reactions involving ALI/ARDS, TRALI was considered in the differential diagnosis of only two.

Several reasons contribute to the widely different estimates of the incidence of TRALI. Most importantly, until recently [1, 3], there has not been a consensus on a uniform case definition of TRALI, with some studies utilizing stricter definitions than others. Additionally, various studies have explored different patient populations, likely making the figures inapplicable in all settings. Finally, the figures typically have

excluded transfused patients with any other known cause of ALI/ARDS including sepsis, shock, trauma and aspiration, though the transfusion itself may have had a contributory or even primary role in the development of the ALI/ARDS ('multiple hit hypothesis'). As a result, patients who have developed ALI/ARDS in the setting of massive transfusion typically have not been considered as having TRALI [1, 3]. In this chapter, we will review the evidence for transfusion as a risk factor for ALI/ARDS rather than concentrating on case series and the case reports of TRALI.

Massive Transfusion as a Risk Factor for ALI/ARDS

A literature search revealed three observational studies [5–7] in which both the risk factor (multiple transfusions) and outcome (ALI/ARDS) were studied in a defined patient population free of ALI/ARDS at study inception (Table 1).

Table 1. Massive transfusion as a risk factor for ALI

Study	Design	Study patients	Multiple transfusion definition	ALI/ARDS Definition	Odds ratio	95% CI
Pepe [5]	Prospective observational	136	> 10 units whole blood or packed red cells within 12 hours	▪ $PaO_2 < 75$ torr with $FiO_2 \geq 0.5$ ▪ new diffuse bilateral infiltrates-pulmonary artery occlusion pressure < 18 mmHg	2.05	0.96–4.36
Hudson [6]	Prospective observational	695	≥ 15 units within 24 hours	▪ $PaO_2/FiO_2 \leq 150$ or $PaO_2/FiO_2 \leq 200$ on positive end-expiratory pressure ▪ diffuse infiltrates involving all lung quadrants ▪ pulmonary artery occlusion pressure < 18 mmHg or no clinical evidence of congestive heart failure	2.24	1.47–3.41
Gong [7]	Prospective observational	189	≥ 8 units packed red cells within 24 hours	▪ Intubated on positive ventilation ▪ $PaO_2/FiO_2 \leq 200$ mmHg ▪ otherwise unexplained bilateral infiltrates ▪ pulmonary arterial occlusion pressure ≤ 18 mmHg or no clinical evidence of left atrial hypertension	0.89*	0.39–2.05

* Many of "control" patients received submassive transfusion, see Table 2 below [8]

CI: confidence interval; ALI: acute lung injury; ARDS: acute respiratory distress syndrome

Pepe et al. [5], studied 136 patients during an 18-month period in the late 1970s/early 1980s at Harborview Medical Center in Seattle, Washington. This group used the term "multiple emergency transfusions" to define those patients who received >10 units of whole blood or packed red blood cells within 12 hours. The primary outcome studied was ARDS defined as: $PaO_2 < 75$ torr with $FiO_2 \geq 0.5$; new diffuse bilateral infiltrates (all lung fields involved) on chest roentgenogram; pulmonary artery occlusion (PAOP) pressure <18 mmHg; and no other explanation for the above findings. Of the 136 patients with one of eight identified predispositions (sepsis syndrome, aspiration of gastric contents, pulmonary contusion, multiple emergency transfusions, multiple major fractures, near-drowning, pancreatitis, and prolonged hypotension), 42 met the "multiple emergency transfusions" criteria either alone or in combination with one of the other predispositions. Nineteen of the 42 developed ARDS yielding an odds ratio of 2.05 (95% confidence interval [CI] 0.96–4.36).

Hudson et al. [6], also from Harborview Medical Center, studied 695 patients who presented to their 38-bed intensive care unit (ICU) from 1983 through 1985. This group also used the term "multiple emergency transfusions" but this time defined it to include those patients who received ≥15 units of blood within 24 hours. The primary outcome studied was ARDS defined as: $PaO_2/FiO_2 \leq 150$ or $PaO_2/FiO_2 \leq 200$ on positive end-expiratory pressure (PEEP); diffuse infiltrates that involved all lung quadrants on chest roentgenogram; PAOP <18 mmHg (if available) or no clinical evidence of congestive heart failure; and no other explanation for the above findings. Of the 695 patients with one of eight identified predispositions (sepsis syndrome, aspiration, drug overdose, near-drowning, pulmonary contusion, multiple transfusions, multiple fractures, and head trauma), 115 met the "multiple emergency transfusions" criteria either alone or in combination with one of the other predispositions; 46 of the 115 developed ARDS yielding an odds ratio of 2.24 (95% CI 1.47–3.41).

Gong et al. [7], studied 189 patients admitted to the neurological, cardiac, medical and surgical ICUs of Massachusetts General Hospital between September 1999 and March 2001. This group used the term "multiple transfusions" to define those patients who received ≥8 units of packed red blood cells within 24 hours. The primary outcome studied was again ARDS defined as: intubated on positive ventilation; PaO_2/fraction of inspired oxygen ratio of ≤200 mmHg; bilateral infiltrates seen on chest radiographs not fully explained by masses, effusions, or collapse; and PAOP ≤18 mmHg or no clinical evidence of left atrial hypertension. Of the 189 patients with one of five identified predispositions (sepsis, septic shock, trauma, multiple transfusions and aspiration), 28 met the "multiple transfusions" criteria either alone or in combination with one of the other predispositions. Ten of the 28 developed ARDS yielding an odds ratio of 0.89 (95% CI 0.39-2.05). However, in this cohort, many of the 'control' patients were exposed to submassive transfusion with significant findings [8] (see below).

In summary, two of these three studies describe a strong association between exposure to multiple transfusions and development of ALI/ARDS. However, causality is difficult to infer in the absence of proper accounting for confounding factors and lack of description of dose-response relationship. Different study populations, definitions, time periods and transfusion exposure further limit the comparison between these particular studies.

Submassive Transfusion as a Risk Factor for ALI

While massive transfusion has typically been identified as a risk factor for ALI/ARDS in various studies, the relationship between submassive transfusion and development of ALI/ARDS has not been as fully studied to date. In a retrospective cohort of 332 critically ill patients mechanically ventilated for 48 hours or longer, 180 of whom were transfused, 80 subsequently developed ALI/ARDS [9]. Any number of transfusions and large initial tidal volumes were identified as the most important risk factors for the development of ALI/ARDS. In a multivariate analysis, the odds ratio for development of ALI/ARDS in the transfused patients was 2.97 (95% CI 1.56–5.9) (Table 2). The authors went a step further and evaluated specific transfusion factors such as type and storage age of blood products [10]. They observed that transfusion of FFP, but not packed red cells, was independently associated with development of ALI/ARDS. Not surprisingly, FFP transfusion has accordingly been implicated in a majority of the case series and case reports of TRALI to date [1, 11]. However, the aforementioned study was limited by its retrospective design and a causal role could not be determined [9, 10].

In an observational prospective study [8] by Gong et al. 688 patients admitted to Massachusetts General Hospital with one of four risk factors for ALI/ARDS (sepsis, trauma, aspiration or multiple transfusions) were studied for a genetic predisposition to ALI/ARDS. The authors observed that any amount of transfusion was independently associated with increased odds of developing ALI/ARDS (odds ratio 2.19, 95% CI 1.42–3.36). In particular, those transfused ≤3 units had increasing rates of ALI/ARDS. The results of this study may not be applicable to all ICU patients as the authors studied only those with known risk factors for ALI/ARDS.

Table 2. Submassive transfusion as a risk factor for ALI

Study	Design	Study patients	Transfusion definition	ALI/ARDS Definition	Odds Ratio	95% CI
Hebert et al. [13]	Prospective interventional (randomized controlled trial)	418 restrictive 420 liberal	Any number of units*	Not specified	0.64 1.5	0.40–1.03 0.97–2.49
Gajic et al. [9]	Retrospective observational	332	Any number of units	Standard AECC definition [23]	2.97	1.56–5.9
Gong [8]	Prospective observational	688	Any number of units	Standard AECC definition	2.19	1.42–3.36
Croce et al. [12]	Retrospective observational	5260	Any number of units	PaO_2/FiO_2 < 200 mmHg, PPV, bilateral infiltrate on CXR, no evidence of CHF & Ppk > 50 cm H_2O	3.42	4.02–34.12

* All patients in the liberal group and 70% of patients in the restrictive group were transfused

CI: confidence interval; ALI: acute lung injury; ARDS: acute respiratory distress syndrome; AECC: American European consensus conference; PPV: positive pressure ventilation; PpK: peak airway pressure

Recently, Croce et al. in a retrospective review [12] of 5260 patients who were admitted to a trauma ICU with an injury severity score (ISS) <25 demonstrated a much higher risk of ALI/ARDS in those who were transfused (2.8%) compared to those not transfused (0.2%). The calculated relative risk increase for ALI/ARDS in those who received any transfusion was approximately 92.9%. In their multivariate logistic regression analysis, transfusion was independently associated with the development of ALI/ARDS (odds ratio 3.42, 95% CI 4.02–34.12). Whether there was a causal relationship between transfusion and ALI/ARDS cannot again be determined by this retrospective study.

Perhaps the most significant evidence for a causal relationship between transfusion and the development of ALI/ARDS comes from the landmark study of the Canadian Critical Care Trials Group [13]. In this study, 838 critically ill patients who were admitted to ICUs for more than 24 hours were randomized to either a liberal strategy (hemoglobin threshold for transfusion 10 g/dl) or restrictive strategy (hemoglobin threshold for transfusion 7 g/dl). On average, the restrictive group received 2.6 units while the liberal group received 5.6 units of blood. ALI/ARDS developed in 11.4% of patients in the liberal group and 7.7% in the restrictive group. The odds ratio for the development of ALI/ARDS was 1.56 (95% CI 0.97–2.49) in patients assigned to the liberal as opposed to restrictive transfusion group.

Pathogenesis of ALI/ARDS Related to Transfusion

The relationship between transfusion and ALI/ARDS has in the past been thought to be directly attributable to the underlying illness, where transfusion was simply a marker of the severity of the underlying illness. However, several distinct mechanisms have been described to explain the potential direct causal role of transfusion in the development of ALI/ARDS. Two of the leading theories include passive transfer of either anti-leukocyte antibodies or biologic response modifiers in a primed susceptible host. Both of these mechanisms have been reproduced in animal models of TRALI [14–16] (Fig. 1). These two pathogenetic mechanisms may not be mutually exclusive and both seem to require additional predisposing conditions leading to pulmonary endothelial activation [17]. Risk factors such as trauma, sepsis or major surgery may serve as the initial priming events ("multiple hit hypothesis") [2, 17].

Passive Transfer of Anti-Leukocyte Antibodies

This traditional theory of TRALI pathogenesis is described in connection with passive transfer of anti-leukocyte antibodies from multiparous or sensitized donors [2, 4, 18]. The antibody-mediated increase in pulmonary capillary permeability leads to pulmonary edema and ALI/ARDS. The antibodies accompanying donor blood are thought to be directed against a variety of leukocyte antigens in the host's system including HLA class I, HLA class II and specific polymorphonuclear (PMN) antigens. During pregnancy, mothers are typically exposed to alloantigens that prime their immune system. The rate of alloimmunization correlates directly with parity as described by Insunza et al. [19], where the percentage of female donors with anti-leukocyte antibodies after one, two or three prior pregnancies was approximately 9%, 18% and 23%, respectively. In the original case series of TRALI, Popovsky and Moore [2] found anti-leukocyte antibodies in 85% of the donors whose blood was implicated in the development of TRALI.

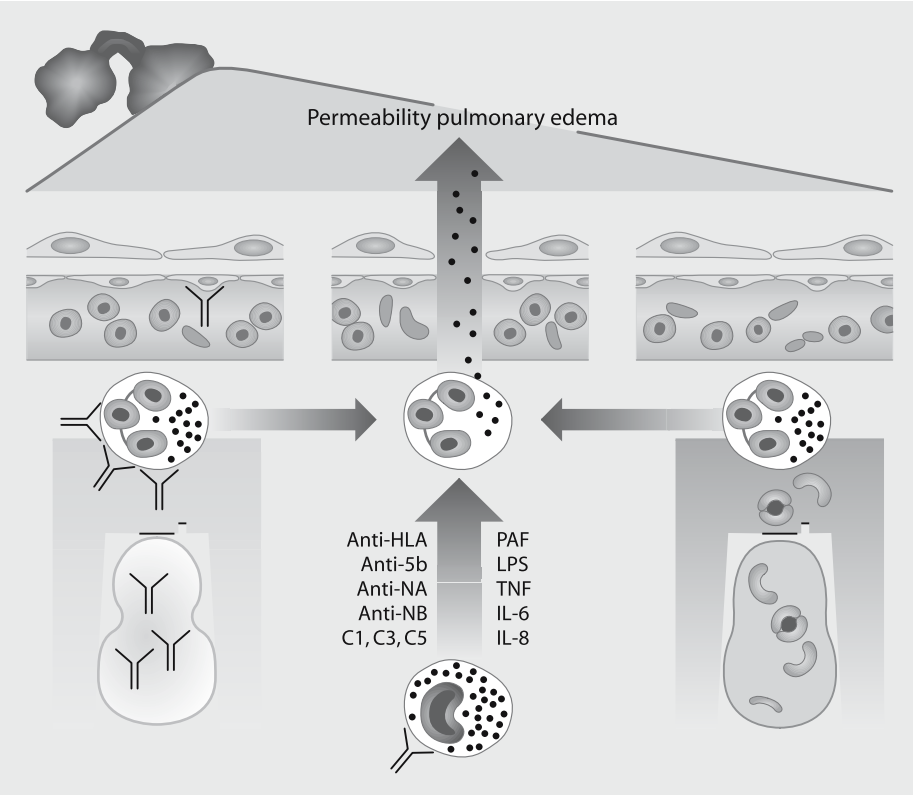

Fig. 1. Pathogenesis of ALI/ARDS related to transfusion. Anti-leukocyte antibodies and/or biologically active lipids from cell membrane fragments in donor blood trigger an inflammatory response and injury to the alveolar-capillary membrane. Activated macrophages secrete inflammatory cytokines that further perpetuate the inflammatory reaction. C: complement; HLA: human leukocyte antigen; IL: interleukin; LPS: lysophosphatidyl choline; PAF: platelet activating factor; TNF: tumor necrosis factor; 5b, NA, NB: neutrophil antigens. From [24] with permission

The strongest evidence yet for the role of multiparous donors in the pathogenesis of TRALI comes from a recent clinical trial [18]. In a randomized cross-over design, 100 patients requiring at least two units of FFP were infused with blood from both a nulliparous and multiparous donor. After receiving the unit of multiparous blood, patients were found to have a greater decrease in their PaO_2/FiO_2 ratio and increased levels of circulating tumor necrosis factor (TNF)-α.

Bray et al. [20], explored the incidence of HLA antibodies in various blood components. 308 units of packed red blood cells, platelets, FFP and cryoprecipitate were randomly selected. Anti-leukocyte antibodies were found to be present in a surprising 22% of these units. FFP and cryoprecipitate were found to have the highest incidence (29% and 24% respectively) while packed red blood cells had the lowest (12%). Why then does the medical community not see a higher rate of TRALI given this relatively high incidence of anti-leukocyte antibodies in donor blood? This mechanism may require specific antigen-antibody pairing between the donor and

recipient as well as achieving a certain concentration threshold of anti-leukocyte antibodies. Accordingly, the amount or concentration of passively transferred antibodies is likely a key determinant of the severity of the reaction [16]. Additionally, preexisting endothelial activation likely serves to potentiate the antigen-antibody reaction [17].

Biologic Response Modifiers in Stored Cellular Blood Products

Biological response modifiers such as pro-inflammatory cytokines (interleukin [IL]-6, IL-8 and TNF-α) and lysophosphatidylcholines (lyso-PCs) are known to accumulate during storage of cellular blood products. These mediators trigger an inflammatory cascade leading to injury of the alveolar-capillary membrane and consequently pulmonary edema [14]. In the study by Silliman et al. [21], it was demonstrated that the levels of neutrophil priming activity, IL-6 and IL-8 increased with the duration of storage time in the transfused patients who ultimately developed TRALI. It is believed that the primary culprit in the development of these circulating inflammatory mediators present in stored blood are residual leukocytes. These leukocytes act on erythrocyte membranes causing the release of bioactive lipids which accumulate in greater concentrations the longer the blood product is stored. It remains to be seen if universal pre-storage leukoreduction, currently employed in many countries, will lead to a decreased incidence of TRALI [3].

An additional mechanism that may cause or contribute to the development of TRALI is depletion of nitric oxide in stored blood products which may result in pulmonary vasoconstriction and a consequent increase in hydrostatic pressure [22]. This is especially true with multiple transfusions. While each of the mechanisms described above seems plausible, it is doubtful that one explains the entire process. To the contrary, it is likely that in many instances there is an interplay between several mechanisms. Better understanding of underlying TRALI mechanisms is necessary before it is possible to design effective prevention strategies.

Conclusion

The last several years have seen an accumulation of evidence that TRALI is an important complication of blood transfusion and is likely occurring much more frequently than previously estimated. Most data support a causal relationship though there are admittedly confounders given the prevalence of this disorder in patients with other predispositions to ALI/ARDS, including sepsis, shock, trauma and aspiration. Both massive and submassive transfusion have been implicated as risk factors. It is apparent from the studies to date that each additional unit likely increases a patient's risk in an additive fashion thus mitigating any false reassurances if no reaction is witnessed after the first few units are transfused.

The implications of this disorder are far and wide given the regular and widespread use of blood transfusions around the world. While most attention in the past has focused on the risk of transmitting infectious diseases, it is imperative that some of the light now be shifted to better defining and understanding the true prevalence of TRALI. The moral, ethical and financial issues of potentially preventive strategies (screening or deferral of particular donors, decrease in storage time and pre-storage leukoreduction) are substantial.

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Red Blood Cell Desialylation in Critically Ill Patients: An Underestimated Cause of Anemia

M. Piagnerelli, K. Zouaoui Boudjeltia, and M. Vanhaeverbeek

Introduction

Anemia is a common pathology in critically ill patients and about one third of intensive care unit (ICU) patients receive a red blood cell (RBC) transfusion at some point during their ICU stay [1]. At ICU admission, the mean hemoglobin concentration of critically ill patients is 11 g/dL, while in 60% and 30% of such patients, the mean hemoglobin concentration is less than 12 and 10 g/dL, respectively [1, 2].

Causes of anemia are multifactorial and include blood losses (from trauma, phlebotomy, surgical procedures and occult gastrointestinal bleeding . . .), decreased RBC production due to blunted erythropoietin response to anemia, apoptosis of erythroid colony forming cells induced by inflammatory mediators, and alterations of the iron metabolism [3]. Effects of hemodilution on the development of anemia are probably less important. Indeed, in human healthy volunteers, Lobo et al. [4] observed a decrease in hematocrit of 6.5 and 7.55% after infusion of 2 litres of 5% dextrose or 0.95% saline, respectively, over 1 hour. In critically ill patients, Margarson and Soni [5] observed a similar decrease (maximum 45%) in the hematocrit of 26 non-septic and 66 septic patients, 120 minutes after infusion of 200 ml of albumin 205%.

Another probably underestimated cause of anemia in ICU patients is the increased destruction of RBCs by the reticuloendothelial system, where decreased sialic acid RBC membrane content is the trigger for RBC uptake. In this chapter we will discuss new insights into desialylation process of the RBC membrane and its possible effects on RBC rheology and metabolism, especially in septic patients.

RBC Membrane

The RBC membrane is composed of proteins (52% in weight), lipids (405%), and carbohydrates (8%). Lipids, including phospholipids, glycolipids and cholesterol, are arranged as a bilayer and distributed unevenly between the two leaflets of this bilayer, constituting a transverse membrane asymmetry. Indeed, the glycolipids and the choline phospholipids (phosphatidylcholine, sphingomyelin) are oriented towards the outer surface of the membrane, while the aminophospholipids (phosphatidylserine, phosphatidylethanolamine and phosphoinositolphospholipids) are oriented towards the cytoplasmic surface (Fig. 1) [6].

Membrane proteins are divided into two classes, depending on their relation to the lipid bilayer. 'Integral', membrane spanning proteins, constitute the first family, especially band 3 protein, membrane channels or transporters, glycophorins (in

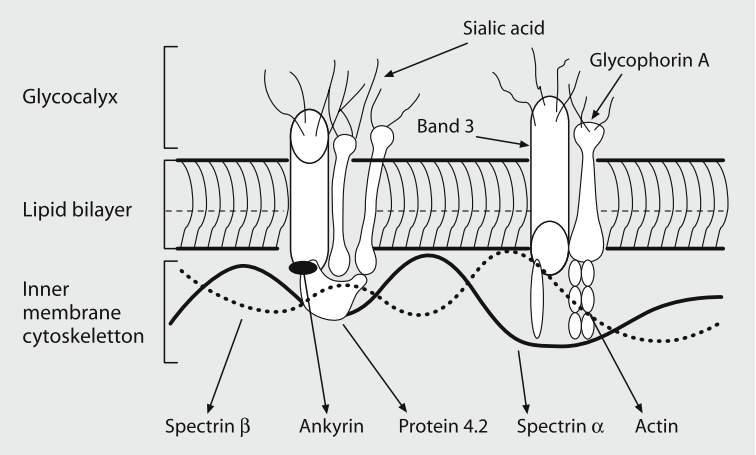

Fig. 1. Schematic representation of the RBC membrane. The membrane is divided into three parts including extracellular, lipid bilayer and intracellular compartments. The extracellular compartments represent the glycocalyx with sialic acid and form a fuzzy coat of thickness of 6 nm. The lipid bilayer is constituted of more than 400 molecular species differing in structure, volume, geometry and charge distribution as well as lateral, transversal and rotational mobility. The intracellular compartment (the cytoskeleton) plays an important role as a stabilizer of the bilayer's integrity

particular A and C) and glycoproteins; moreover, carbohydrate moieties coupled to 'integral' proteins form an additional leaflet, also called 'glycocalyx'. Sialic acids, in particular N-acetylneuraminic acid, an acidic carbohydrate, are bound to glycophorin A and are responsible for 60 to 90% of the surface negative charge of the RBC membrane. Second, the 'peripheral' proteins constitute the inner membrane skeleton, and include spectrin (α and β subunits), actin, protein 4.1, protein 4.2, tropomyosin, adducin, myosin and ankyrin (Fig. 1) [6–8].

Membrane elasticity, and thereby deformability, depends on the structural interactions between the outer plasma membrane and the underlying protein cytoskeleton.

Role of Sialic Acids

Sialic acids (sialon in Greek word: saliva), less commonly called neuraminic acids, is the designation given to a family of over 40 naturally occurring 9-carbon ketosugar acids derived from N-acetylneuraminic acid [9]. The most abundant derivative of sialic acids present in humans and in RBCs is N-acetylneuraminic acid [9] and for these reasons in this chapter, sialic acid represents N-acetylneuraminic acid.

Sialic acids have evolved to mediate a diverse range of cell-cell and cell-molecule interactions including: stabilizing the conformation of glycoproteins and cellular membranes (due to the negative charge), contributing to cell-to-cell interactions and serving as a chemical messenger in tissue and body fluids, regulating transmembrane receptor function and controlling the half-lives of circulating glycoproteins and cells [9, 10].

The RBC glycocalyx is dominated by the carbohydrate domains of glycolipids and integral glycoproteins. These oligosaccharides contain, besides neutral hexoses, pentoses and N-acetylhexosamines, fully ionized sialic acids.

Glycophorin A, the most important transmembrane protein, is highly glycosylated, with approximately 60% of its weight attributable to carbohydrates. Most of the carbohydrate is in the form of 15 O-glycosidically linked tetrasaccharides. The two sialic acid residues of each of these many O-glycosidically linked oligosaccharides account for 60 to 90%, depending on the species, of the negative charge of the RBC membrane surface [11] and account for the fact that RBCs normally repel each other and do not aggregate [6, 8].

While the effects of sialic acids on RBC aggregation are well described, the effects of sialic acids on RBC shape have not been frequently studied. Grebe et al. [12] observed with the micropipette technique that decreased RBC sialic acid content reduces the mean RBC curvature.

RBC sialic acid membrane content also has a primordial role on RBC survival. Indeed, several authors have observed that treating RBCs with neuraminidase – the degrading sialic acid enzyme – facilitates their uptake by the reticuloendothelial system. More than 25 years ago, Durocher et al. [13] showed a rapid clearance of ^{51}Cr desialylated erythrocytes in rats and rabbits, with sequestration by the liver. Simchon et al. [14] observed in rats that more than 70% of neuraminidase-treated RBCs disappeared from the circulating blood in 30 min compared with less than 2% of normal RBCs and the relative distributions of neuraminidase-treated RBCs to normal RBCs, as determined from radioactivity counting, were significantly greater than 1 in the spleen (5.65 ± 0.97, mean $\pm$ SD), the liver (2.84 ± 0.21), the lung (1.48 ± 0.31), and the kidney (1.49 ± 0.27), indicating a preferential trapping of neuraminidase-treated RBCs in these regions. In the same model, a study of regional blood flow determined by microspheres indicated that the reduction in blood flow occurred in the organ where the neuraminidase-treated RBCs were trapped [14].

Reviewing these different studies, we hypothesized that an acute decrease in the sialic acid content of RBC membranes may occur in critically ill patients, especially in septic patients.

RBC and Sialic Acids in Critically Ill Patients: *in vivo* Studies

First, we investigated the relationship between RBC membrane sialic acid content and RBC shape estimated by a flow cytometry technique [15]. We compared blood samples from 19 ICU patients in the first 24 hours of severe sepsis or septic shock; with RBCs from 20 patients without sepsis (on the first day of surgery or of admission in ICU for cerebral hemorrhage) and with RBCs from 20 healthy volunteers. We excluded patients with hematologic pathology, with recent RBC transfusion or with different pathologies known to induce alterations in RBCs (diabetes mellitus, cirrhosis, terminal renal failure). We measured the sialic acid content of RBC membrane proteins using high-performance liquid chromatography after lysis of RBCs; the RBC glycophorin A content was estimated on flow cytometry with an antiglycophorin antibody (monoclonal mouse anti-human glycophorin A antibody) labeled with fluorescein-isothiocyanate. To determine the RBC shape by flow cytometry, we adapted the technique from Rolfes-Curl et al. [16]. Briefly, in iso-osmolality, biconcave RBCs from volunteers appear essentially as two populations of cells, and the forward light scatter channel histograms show a typically bimodal distribution of

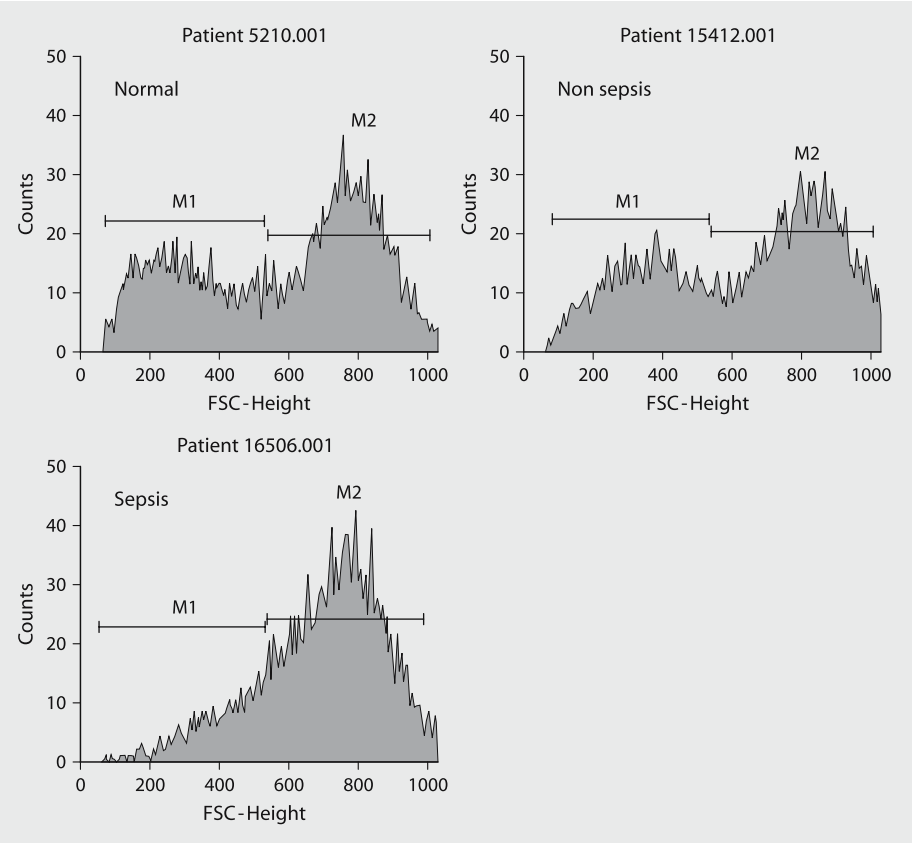

Fig. 2. RBC forward light scatter distribution in isotonicity. Example of a RBC analysis in a healthy volunteer (above left), in a non-septic patient (above right), in a septic patient (bottom) (from [15] with permission)

RBCs (Fig. 2). On this histogram, it is possible to calculate the second moment of Dissymmetry of Pearson (PCD; $3\times[\text{mean-median}]/\sigma$) which expresses the sphericity of the RBC. The PCD value obtained in healthy volunteers is around – 0.7 and a PCD value of zero represents a perfectly spherical RBC shape.

We observed that the sialic acid content was significantly lower in septic patients compared to non-septic patients (1.98 ± 0.79 versus 2.20 ± 0.39 μg/100 μg membrane protein; $p = 0.01$) and healthy volunteers (2.71 ± 1.00 μg/100 μg membrane protein; $p < 0.001$). RBC glycophorin A contents were similar in the septic and non-septic ICU patients. The PCD values were significantly reduced in all ICU patients suggesting a right shift in the histogram (septic -0.48 ± 0.2; non-septic: -0.52 ± 0.23 and volunteers -0.70 ± 0.15). Typical histograms for all populations studied are shown in Fig. 2.

Moreover, RBCs from septic patients failed to modify their shape in hypo-osmolar solution. Interestingly, we also observed a significant correlation between the PCD and the RBC sialic acid membrane content in the ICU patients ($r^2 = 0.15$; $p = 0.015$).

With this study, we concluded that in the first 24 hours of severe sepsis or septic shock, RBCs are characterized by a more spherical shape, a decreased capacity for sphericity in hypo-osmolar solution, and a reduction in the RBC sialic acid membrane content.

In view of these results, we hypothesized that if the desialylation could occur on RBCs, it could also occur in circulating proteins from critically ill patients. To test this hypothesis, we measured the transferrin sialic acid content and the free sialic acid concentrations of serum in ICU patients with or without sepsis. Transferrin, an acute-phase N-glycosylated glycoprotein, plays an important role in iron transport. Human serum transferrin contains two biantennary glycans, each consisting of 0 to 4 molecules of sialic acid; its sialic acid content is heterogeneous with a high concentration of tetrasialotransferrin (4 sialic acid) and low amounts of disialo-, trisialo- penta- and hexasialotransferrin. We hypothesized that serum levels of carbohydrate deficient transferrin (CDT, disialotransferrin) may increase rapidly in septic patients. Blood samples were obtained from critically ill patients with ($n = 15$) and without ($n = 14$) documented sepsis and compared to healthy volunteers. The different forms of transferrin were studied by capillary zone electrophoresis; serum sialic acid concentrations were measured by an enzymatic colorimetric assay. There was a significant increase in the proportion of CDT in septic compared to non-septic patients and volunteers (18.3% [1.3–30.5] vs 0.7% [0.5–0.9]; $p < 0.01$ and 0.9% [0.5–1.1]; $p < 0.05$). Conversely, tri- and tetrasialotransferrin levels were lower in septic patients. Total and free sialic acid concentrations were significantly higher in septic patients than in healthy volunteers (for total sialic acid: 82 [69–99] versus 23 [20–29] mg/dL and for free sialic acid: 9 [8–11] versus 4 [1–6] mg/dL, respectively all $p < 0.05$).

To confirm the rapidity of the desialylation process, we studied the time course of free sialic acid concentrations in a sheep model of septic shock induced by peritonitis [17], and noted that serum free sialic acid was already increased after 15 hours (Fig. 3) [17].

We concluded that sepsis is very rapidly associated with decreased sialic acid content on circulating transferrin and with an increase in blood free sialic acid concentrations. In view of these rapid modifications and the long half-life of transferrin (16 days), the most likely explanation is degradation of transferrin by a circulating neuraminidase.

RBC and Sialic Acid in Critically Ill Patients: *in vitro* Studies

Effect of Neuraminidase on RBC Shape

Several experiments were performed to test the hypothesis that changes in carbohydrate chains of RBC membrane proteins impair their shape and their cytoplasm biochemistry. For this, RBCs from healthy volunteers were treated with several concentrations of neuraminidase and RBC shape was estimated by a flow cytometry technique [15]. Neuraminidase treatment induced a rapid modification of the RBC shape. A dose-response effect on the RBC shape was observed during 10 hours, with a saturation phenomenon between 10 and 24 hours [18]. We also observed higher concentrations of free sialic acid in the milieu after 10 hours of neuraminidase incubation, proving the desialylation of the RBC membrane. Moreover, the

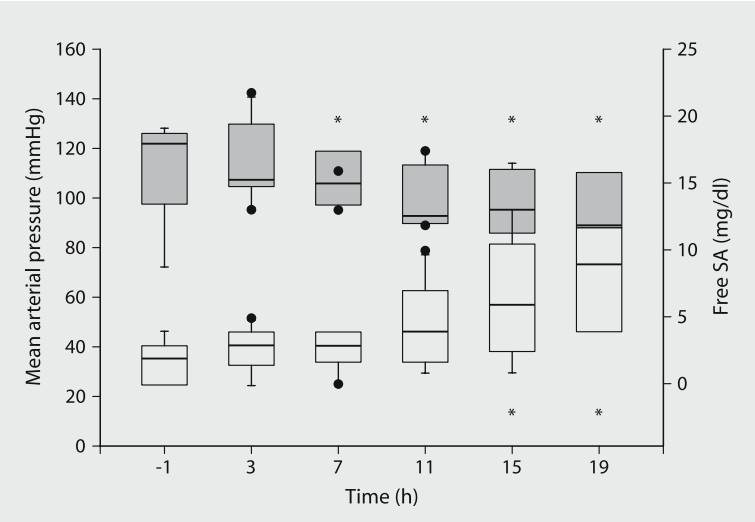

Fig. 3. Time course of mean arterial pressure (gray) and free sialic acid (SA) concentrations (white) in a model of septic shock induced by cecal ligature and perforation. Surgical procedure corresponds to time 0 (T0). Box and whisker plots represent median (central line), 25th and 75th percentiles (boxes) and ranges (whiskers). * $p < 0.05$ versus baseline (from [17] with permission)

free sialic acid concentration was correlated to the sphericity of RBC assessed by the PCD value [18].

Effect of Neuraminidase on Intraerythrocytic Lactate and 2,3-Diphosphoglycerate

In RBC samples from healthy volunteers, intraerythrocytic 2,3-diphosphoglycerate (DPG) was measured by colorimetric determination after 10 hours of incubation with or without neuraminidase in RPMI [19]. The endpoint of the assay was the formation of nicotinamide adenine dinucleotide (NAD). Intraerythrocytic lactate was also measured in the supernatant with the lactate PAP (Biomerieux) on a LX20 Beckman.

The neuraminidase treated RBCs increased their intraerythrocytic 2,3-DPG and lactate concomitant with a shape change assessed by the flow cytometry technique. Interestingly, the intraerythrocytic 2,3-DPG was significantly correlated to the sphericity of RBCs [19].

An increase in intraerythrocytic 2,3-DPG could be important to explain the RBC rheologic alterations reported in sepsis. However, two studies report contradictory results. Suzuki et al. [20] showed that a moderate increase in 2,3-DPG (5 to 15 mmol/l) altered RBC deformability through an increase in RBC internal viscosity; in contrast, Waugh [21] reported that this effect was observed only in supraphysiologic conditions (> 20 mmol/l).

Lactate is the final product of RBC glycolysis. Desialylation may alter RBC lactate metabolism in different ways: first, lactate production could be increased substantially and therefore could exceed the lactate transport capability of the RBC mem-

brane. Second, the increase could have a direct effect on the transporters (the specific proton-linked monocarboxylate transporter 1, the Band 3-mediated transporter) [22]. Another possibility is that the free sialic acid removed from the RBC membrane could be taken up by the RBC and transformed into pyruvate, the precursor of lactate. Indeed, Bulai et al. [23, 24] showed that free sialic acid could be incorporated in a RBC transport system in the cytosol and cleaved to form pyruvate and N-acetylmannosamine by a cytosolic sialate pyruvate-lyase.

RBC glucose uptake is dependent on a glycosylated membrane transporter (GLUT1). Englund and Lundahl [25] reported that there were about 2.1 sialic acid residues per transporter. The effect of neuraminidase treatment on GLUT1 function is unknown.

Although the effects of desialylation on the RBC remain unclear, effects on other cell types have been reported. Indeed, it has been demonstrated that neuraminidase treatment of myocytes increases the cell calcium content affecting the T-Ca^{2+} channel [26]. Other interesting studies have reported that the digestion of adipocytes with neuraminidase has no effect on the binding of insulin to its receptor [27–29], but it produces a total loss of the cell's ability to respond to insulin as measured by a variety of assays. This suggests that the sialic acid membrane could modulate different membrane transporters and could affect the intracellular physiology [30].

Sources of Neuraminidases

Neuraminidases are a family of glycohydrolytic enzymes that remove sialic acid residues from glycolipids, glycoproteins, gangliosides and polysaccharides [31]. The most widely studied neuraminidase is from influenza virus, where the enzyme is involved in viral replication and released from infected cells.

In mammals, these enzymes have been proved to be involved in several cellular phenomena, including cell proliferation and differentiation, membrane function, and antigen masking. Neuraminidases are present in several tissues, organs and cells with a typical subcellular distribution: they are the lysosomal, the cytosolic and the plasma membrane-associated neuraminidases [32]. In contrast to other cells, RBCs from different species possess only the plasma membrane bound neuraminidase form [33].

The human RBC neuraminidase is principally linked to the plasma membrane by a glycosylphosphatidylinositol anchor: indeed, Chiarini et al. [34] showed that using a phosphatidylinositol-phospholipase C (PIPLC), the neuraminidase from human RBC ghosts could be released. It is not known whether a PIPLC that can release neuraminidase is present in erythrocytes and, if so, which conditions could trigger the enzyme to start functioning.

Bacterias are another possible source of neuraminidase. It could be an advantage for microorganisms, which catabolize host sialic acid for nutritional purposes (carbon, nitrogen and energy), to express neuraminidases. Adhesion to epithelial cells is necessary for bacteria to colonize host mucosal surfaces [35]; this adhesion is the result of the interaction of a number of surface-exposed or secreted bacterial proteins with host cells and molecules. Neuraminidase can be an important factor promoting adhesion to host epithelial cells in *Streptococcus pneumoniae* and oral actinomyces. *Pseudomonas aeruginosa,* a common bacteria in sepsis, expresses and releases significant quantity of neuraminidase [36]. Although the *in vivo* function

of bacterial neuraminidase has been a topic of considerable speculation, no definitive studies have been conducted.

Conclusion

Desialylation of the RBC membrane by neuraminidase may alter RBC shape, RBC capacity for deformability, and RBC biochemistry. Some of these alterations are observed early in clinical situations. These RBC modifications are reproduced *in vitro* within a few hours. Moreover, desialylation could facilitate uptake by the reticuloendothelial system, as observed in the senescent process. Further studies including the measurement of neuraminidase activity are needed to understand the process of RBC sialic acid decrease in critically ill patients. As a potential consequence, blockade of neuraminidase activity could represent an interesting therapeutic option to limit anemia and improve RBC rheology in critically ill patients.

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Cardiopulmonary Resuscitation

Clinical Predictors of Physiological Deterioration and Subsequent Cardiorespiratory Arrest among Hospitalized Patients

N. D. Hartman, B. B. Mehring, and W. J. Brady

Introduction

Hospitalized patients who experience sudden, or unanticipated, physiological deterioration and subsequent cardiorespiratroy arrest have very poor outcomes. Studies have reported varying results in mortality following cardiac arrest, but most have historically placed the level of survival to discharge at around 15%, with some recent studies placing the figure closer to 30% [1, 2]. In addition, the institutional and patient costs of an arrest event are quite high [3]. Patients who undergo an unexpected arrest consume extensive personnel resources, receive more medications and other therapies, and spend more time in intensive care units (ICUs). One study has estimated that in-hospital cardiopulmonary resuscitation (CPR) programs cost $ 400,000 per life saved [4]. Other investigations have concluded that much of the morbidity and mortality associated with such events is preventable [5]. Studies have estimated that up to 84% of patients who go on to a cardiorespiratory arrest have measurable evidence of deterioration in the eight hours prior to their event [6]. If these symptoms and signs could be correctly identified and acted upon, a portion of these less than optimal outcomes might be avoided.

The institution of various medical emergency teams (MET) into hospitals has demonstrably improved outcomes for patients at risk for severe deterioration [7–9]. For instance, investigators have demonstrated a marked reduction in ICU admissions among patients who were seen by the MET compared with patients who were not [8]. Some reviews have questioned whether the efforts to employ METs can find justification in the literature, but no one has demonstrated any possible harm brought by the implementation of such programs [10]. More recent studies confirm the utility of these METs. One such study found that the MET reduced the number of unexpected cardiac arrests by 50% [11]. Another investigation found that MET deployment reduced the negative impact from various clinical scenarios, ranging from respiratory failure to stroke, sepsis, and renal failure [7]. Such findings provide justification for the recommendations, made by departments of health in various countries, that hospitals implement these programs as rapidly as possible.

The premise behind METs is quite simple. These teams are dispatched based upon both subjective and objective criteria: 1) subjective concern of the healthcare team; and/or 2) certain physiological criteria. These objective criteria usually include abnormal vital sign findings, including hypotension, tachycardia, bradycardia, or tachypnea; mental status alterations are also a consideration of import. In addition, most deployment activations include a provision for complaints such as chest pain or shortness of breath. In many cases, an 'early warning score', which attempts to capture the patient's risk of deterioration, is assigned. The members of the team,

which generally consist of critical care providers including physicians, nurses, and other therapists, then re-assess the patient and provide a plan for care. This extra attention appears to result in improved outcomes. Often the only intervention these patients require is the administration or adjustment of oxygen therapy [12]. The question remains, however, as to how to predict which patients should receive this additional scrutiny.

While the concept of some form of early warning score seems supportable, no proven, standardized system exists with which to evaluate the risk of cardiorespiratory arrest [13, 14]. Examples of successful systems exist, including the modified Early Warning Score used in the United Kingdom. This scoring system has a demonstrable link to markers of mortality and ICU admissions, as demonstrated in a study by Subbe et al. [15]. This chapter will attempt to review the relevant literature and discern patterns which may help build a comprehensive predictive model for in-hospital cardiac arrest.

Illness on Presentation

Partly because of the outstanding work of the American Heart Association, many envision sudden death only occurring because of underlying cardiac diseases. Campaigns to place cardiac defibrillators in public buildings and instruct CPR to laypeople surely have and will save many lives, but can occasionally lull the public into believing that sudden medical problems can usually be traced to the heart. Many hospitalized patients who undergo de-compensation do present with complaints related to the heart, yet clinicians must realize that a multitude of other underlying pathologies carry a nearly equal cause for concern. For example, one study noted that respiratory illnesses alone made up about 38% of their studied cases of arrest, while isolated cardiac disease only accounted for about 10% [16]. Patients with multiple problems made up another 27% of arrest cases.

The critical importance of cardiac disease should not, however, be forgotten. Many studies have noted the prominence of chest pain in patients who experience arrest. Fifty six percent of arresting patients in another study presented to the hospital with chest pain, and 44% were eventually diagnosed with a myocardial infarction (n=292) [2]. An additional 12% in that study were admitted for planned surgery. Renal disease and the presence of cancer have also been found to be significant risk factors for mortality in cardiorespiratory arrest patients [17]. Older age has also been associated with greater mortality and likelihood of cardiac arrest, as well as other markers of physiological deterioration [14]. Further, although one might expect that patients would improve once admitted to the hospital, it seems that most patients who experience arrest do so after the first 24 hours of admission [5]. In short, many pathologies and clinical courses can lead to deterioration and unexpected arrest, thus clinicians must remain vigilant for warning signs in all patients with severe illness.

Presenting Symptoms

Throughout the course of a hospital stay, patients are likely to report a number of different physical symptoms. In the setting of the serious illnesses mentioned above, some of these symptoms should give clinicians cause for concern of possible deterioration. In multiple studies, certain complaints have been found to signifi-

cantly correlate with cardiac arrest events. It seems that chest pain is the most common presenting chief complaint for eventual arrest patients, followed by dyspnea [2]. One study found that the onset of chest pain was among the most significant antecedents to arrest [18]. In other studies, the symptom that most often signals an in-hospital deterioration is "shortness of breath," or a similar complaint [16]. Respiratory symptoms, along with associated clinical observations of respiratory distress, form perhaps the most important sign of impending systemic problems. In addition to breathing problems and chest pain, altered mental status remains a key indicator of deterioration. Significant changes in mental status include not only onset of coma, but also notable lethargy and agitation. Gastrointestinal complaints are also common among patients who will arrest [16]. Clinicians must recognize these themes when judging the likelihood of deterioration in their patients.

Alterations in Vital Signs and Other Clinical Signs

Detecting alterations in vital signs is a critical method which can be employed by clinicians to identify patients who are at risk for decompensation. In large part, the literature is in agreement that the most critical sign in predicting arrest is respiratory status. Rates above 30 breaths per minute(/min) or below 6/min are significantly correlated with physiological deterioration [19]. In addition, oxygen saturation below 90% also occurs much more frequently among patients who will arrest than among patients who will not. These facts, combined with the above finding that respiratory illnesses and symptoms often lead to arrest, should help the clinician justify enhanced concern over respiratory status. An alteration in mental status, occasionally defined as a change in Glasgow Coma Score (GCS) of 2 or more, is also an important signal for deterioration. One study of an MET found that the most common reasons for alerting the team were a fall in GCS, a fall in oxygen saturation, and an increased respiratory rate – these factors were important indicators of deterioration in this population as well [12].

Cardiac indicators are also key to predicting trouble for hospitalized patients. A drop in systolic blood pressure below 90 mmHg indicates a decline in cardiac function and is an important indictor of a future arrest. In fact, respiratory failure and hypotension were found in one study to be the most common causes of the actual arrest event [1]. In further review of the cardiac issues, abnormal heart rates have also been associated with arrest events. While tracking admissions to the ICU, one study found that abnormal heart rates occurred with about the same frequency as did respiratory issues [20]. These findings were confirmed in a similar study on inpatient mortality [21]. Exactly what constitutes abnormal heart rate may vary somewhat by patient, but one study considered any rate above 130 beats per minute (bpm) or less than 50 bpm as abnormal [19]. Temperature alterations, thus far, have not been found to be a significant indicator.

One major problem in studying these values retrospectively is that the record of vital signs is often incomplete. This data problem in turn complicates the ranking of the clinical variables. In addition, some fluctuation in vital signs should be expected in most patients. Patients are often given therapies that aim to promote such changes in their vital signs. Changes outside of the clinician's expectations are noteworthy, and any of the aforementioned vital sign variations should give the clini-

cian cause for alarm. A model that attempts to predict which patients are at risk for arrest should reflect just that fact as well – objective and subjective variables are of importance in identification of impending deterioration and subsequent cardiorespiratory arrest.

Diagnostic Investigations

The results of diagnostic studies demonstrate a less reliable set of criteria. In general, these tests are less specific and less reliable methods for differentiating patients at risk for arrest. At least one study has found that these physiological indicators cannot, on their own, identify seriously ill patients [8]. A few trends in these tests, however, could help bolster a model that relies on vital signs, patient illness, symptoms, and other clinical signs. A British study examined biochemical markers as one means to determine the condition of patients admitted through the emergency department; the investigators were unable to develop a method using laboratory markers alone yet they did note a trend using several variables (clinical and laboratory). The result was a "parsimonious" model that included only age, heart rate, and serum studies (phosphate and albumin) [22]. Another study of ICU patients found notable variations in serum pH, serum creatinine, and white blood cell count [20]. Further, a survey of patients undergoing cardiac arrest found mean laboratory values outside the normal range for several routine tests, including low serum pH, elevated serum glucose, high blood urea nitrogen, elevated serum creatinine, and low hematocrit. Of these, hematocrit was the only test that showed a statistically significant difference [16]. By taking a few of these factors into consideration, investigators may be able to include laboratory values into a predictive model.

Comprehensive Model

The goal in reviewing patients who undergo cardiorespiratory arrest should be to develop a model that will help predict which patients should be given further attention and care. In this way, many of the problems associated with these events and their aftermath are potentially preventable. Though not all studies agree on which factors should form a significant part of this model, overall trends are apparent. An on-going study of arrest patients at our institution, the University of Virginia, appears to support these comments. This study is examining approximately 200 patients who experienced unanticipated cardiorespiratory arrest, comparing them with 200 patients of similar acuity who did not deteriorate. The goal in such a study is to discover a simple and sensitive model that can be used by clinicians to identify at-risk patients. The forthcoming findings of this study should uphold many of the trends discussed above. Such a building body of evidence should allow investigators to identify a model that can include each of these elements.

In our review, the markers that are associated with sudden mortality merit reiteration. Patients presenting with respiratory, cardiac, renal or neoplastic diseases should be watched closely. Further, those complaining of chest pain, shortness of breath, changes in mental status, or gastrointestinal issues, in the context of the above diseases, warrant attention. Significant variation in vital signs, including abnormal respirations, decreased oxygen saturation, low blood pressure, or varying

heart rate should also be taken as a signal of impending deterioration. Finally, certain abnormal laboratory values can support a suspicion of decompensation risk.

Several attempts have been made to establish physiological scoring systems in the hospital setting. One of the best known versions is APACHE (acute physiology and chronic health evaluation), a system used to evaluate ICU patients on admission for the purpose of determining their in-hospital prognosis. This score includes points for deviations in vital signs as well as health history, ultimately assigning the patient a score between 0 and 71. It has been found to be very effective in predicting length of ICU stay and other related outcomes, but has not consistently been shown to be useful outside of the ICU setting or in predicting sudden death [23, 24]. Further, its complexity and the amount of time consumed in taking the score make it cumbersome for use in rapidly decompensating patients [25]. Another system, used in Great Britain and called the Modified Early Warning Score, is far simpler (Table 1). This score uses only deviations in heart rate, systolic blood pressure, respiratory rate, temperature and response to stimulus to predict the likelihood of deterioration [26]. Its simplicity makes it more useful in treating patients whose physiological status changes, and it is far easier for less senior staff to use. Unfortunately, its helpful effects are difficult to demonstrate and it cannot account for many important clinical indicators [13]. Perhaps a model of intermediate com-

Table 1. Modified early warning score [13]

Variable	3	2	1	0	1	2	3
Systolic blood pressure (mmHg)	<70	71–80	81–100	100–199		≥200	
Heart rate (beats/min)		<40	41–50	51–100	101–110	111–129	≥130
Respiratory rate (breaths/min)		<9		9–14	15–20	21–29	≥30
Temperature (°C)		<35		35–38.4		≥38.5	
Neurological score				Alert	Reacting to voice	Reacting to pain	Unresponsive

Table 2. Predictors of cardiac arrest

- Alteration in mental status
- Dyspnea/respiratory distress
- Tachypnea (>30 /min)
- Decreased oxygen saturation (<90%)
- Hypotension (<90 mmHg systolic)
- Chest pain
- Laboratory abnormalities
  - low serum pH
  - low hematocrit
  - elevated BUN
  - elevated serum glucose
  - elevated serum creatinine

plexity could best serve the dynamic needs of at-risk patients throughout the hospital. Table 2 shows a list of possible elements to include in an early warning score model.

Taken alone, these clinical predictors are quite non-specific. As a whole, they may form the basis of a simple, easy applied screening model to predict, and therefore prevent, severe de-compensation and unanticipated cardiorespiratory arrest among hospitalized patients.

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Cardiocerebral Resuscitation: A Better Approach to Out-of-Hospital Cardiac Arrest

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Introduction

A new approach to resuscitation of individuals with out-of-hospital cardiac arrest due to ventricular fibrillation or pulseless ventricular tachycardia was implemented in Tucson Arizona in 2003 and in 2004 this approach was further modified and implemented in the Rock and Walworth counties of Wisconsin [1–3]. This approach is now called Cardiocerebral Resuscitation. At the time of its development, it was a dramatic departure from the then traditional technique of cardiopulmonary resuscitation (CPR) endorsed by the American Heart Association and the international community in "Guidelines 2000" [4]. This new approach to out-of-hospital cardiac arrest is extremely important, for when the principles of cardiocerebral resuscitation were utilized in the pre-hospital care of adults with a witnessed arrest and an initially shockable rhythm, a marked and statistically significant improvement in survival was observed (Kellum et al. personal communication).

Cardiocerebral resuscitation was developed because standard CPR has resulted in sub-optimal survival in the setting of out-of-hospital cardiac arrest. With the exception of the application of early defibrillation, survival rates are not only dismal but they have also remained essentially unchanged over decades in spite of periodic updates of standards and guidelines.

The poor survival may relate to the fact that CPR was conceived as an appropriate and sufficient intervention for two pathophysiologically distinct disorders: respiratory arrest and cardiac arrest. Cardiocerebral resuscitation challenges many of the tenants of CPR, but it does so only for the purpose of improving survival in cases of cardiac arrest. Cardiocerebral resuscitation is not appropriate for respiratory arrests. When this distinction is acknowledged, there is a wealth of information from both animal and human studies that indicates that substantial improvements in the treatment of cardiac arrest are possible [1, 2]. These facts were utilized to develop the technique of cardiocerebral resuscitation.

The purpose of this chapter is to present the technique and scientific rationale underlying cardiocerebral resuscitation and to promote its acceptance by emergency care providers worldwide.

Limitations of Cardiopulmonary Resuscitation

The Guidelines for CPR have been utilized for decades. In spite of their international scope and periodic updates, with the exception of improved survival with early use of automated external defibrillators (AEDs), the guidelines for CPR

have done little to improve the dismal survival rates of out-of-hospital cardiac arrest.

The definition of 'survival' from cardiac arrest has varied. Survival rates, however expressed, are very low and with few exceptions have remained stagnant over the last few decades [5]. Overall survival rates from non-traumatic out-of-hospital cardiac arrest in Tucson, Arizona have been unchanged at about 6% over the past decade. Survival in larger cities such as Chicago, Los Angeles, and New York is closer to 1% [5]. Most survivors are found in the subset of patients with a witnessed arrest and an initially shockable rhythm. In this group, survival in Tucson Arizona has been $10 \pm 2\%$ for the past decade while in Los Angeles it was 6% [5] and in Rock and Walworth counties in Wisconsin it was 20% (personal communication). Recently more emphasis has been placed upon clinically relevant neurologically normal survival at hospital discharge instead of return of spontaneous circulation or hospital admission as survival endpoints.

Newer Concepts of Ventricular Fibrillation

The three-phase time-sensitive model of cardiac arrest due to ventricular fibrillation (VF) is helpful in understanding some of the features of cardiocerebral resuscitation [6]. This model divides untreated VF cardiac arrest into three successive phases: electrical, circulatory and metabolic. A compelling insight that this model provides is that interventional priorities are time-sensitive and should be determined by an understanding of the unique pathophysiology of an arrest due to VF. An intervention that may be crucial at one phase of an arrest may become less crucial and even harmful if performed at another phase in VF arrest.

The first or electrical phase lasts for about four minutes from the onset of collapse. During this period, the most important intervention is prompt defibrillation. This is why AEDs have been employed so successfully in a wide variety of settings, including airplanes, airports, casinos, and in the community where prompt defibrillation could be accomplished [7–10]. While the heart is fibrillating during this time the myocardium has not yet used up its energy stores nor undergone serious cellular damage and is therefore not only responsive to the defibrillation shock but is also able to generate a perfusing rhythm.

The second or circulatory phase lasts for a variable period of time, but possibly from minute 4 to minute 10 of a VF arrest. During this time, the lack of myocardial perfusion during prolonged myocardial cell contractions results in waning myocardial energy stores. Therefore, an important intervention is to restore myocardial blood flow by the generation of adequate coronary perfusion pressure by chest compressions prior to defibrillation. Immediate defibrillation is inappropriate in this phase because it seldom generates a perfusing rhythm and often results in ventricular asystole of pulseless electrical activity. Chest compressions generate myocardial perfusion and, because they improve VF amplitude, no doubt help to restore energy stores to the point that the heart can respond adequately to the defibrillation shock.

The third or 'metabolic' phase follows the 'circulatory' phase. Survival during this phase is uncommon and innovative therapies are needed – the most promising of which is the employment of hypothermia techniques. Hypothermia in the resuscitated but comatose patient has shown encouraging results for limiting neurological dysfunction [11, 12].

An appreciation of these three phases helps one put into context some of the recent findings in resuscitation research.

What is Cardiocerebral Resuscitation?

Cardiocerebral resuscitation, as its name implies, was developed to improve neurologically normal survival following resuscitation of patients with cardiac arrest and a shockable rhythm. It acknowledges the important role of early defibrillation, but in the absence of early defibrillation, the usual situation in the vast majority of patients with out-of-hospital cardiac arrest, the emphasis is on restoring and maintaining cerebral and myocardial blood flow. The perfusion pressures typically generated by chest compressions are quite marginal relative to those developed by the beating heart and, therefore, any interruption of chest compressions markedly decreases the chance for neurologically normal survival. Accordingly, in cardiocerebral resuscitation, any intervention that interrupts chest compressions, even for assisted ventilation, is strongly discouraged.

This approach is based on the findings that the major determinant of neurologically normal survival from prolonged cardiac arrest is not the blood gas composition, the acid-base balance, nor the frequency or strength of defibrillation shocks, but rather the cardiac and cerebral perfusion pressures generated during chest compressions [1, 13–17]. Adequate perfusion must be generated relatively early in the arrest because delays in its establishment results in few, if any, favorable outcomes [18].

The importance of chest compressions to cerebral perfusion should have been intuitively obvious, but was forcefully brought home to us when listening to a tape of dispatch directed out-of-hospital CPR. After some time, a woman returned to the phone and asked, "*Why is it that every time I press on his chest he opens his eyes, and every time I stop to breathe for him he goes back to sleep?*" [19]. This profound observation confirmed years of CPR research – chest compression is essential for cerebral perfusion and any interruption, even for ventilation, is deleterious.

What does Cardiocerebral Resuscitation Advocate?

The individual components of cardiocerebral resuscitation will be discussed in detail below along with the scientific rationale for their inclusion. Figure 1 is an algorithmic overview of the protocol used in Rock and Walworth counties in Wisconsin.

Bystanders are to call 911 as soon as possible. If an AED is available its use is encouraged. Laypersons are taught simplified continuous chest compression CPR and instructed to continue uninterrupted chest compressions until help arrives. Dispatchers answering 911 calls give continuous chest compression instructions to callers and it is recommended that dispatchers provide metronome guidance. The technique of continuous chest compression is ideally taught with emphasis on a metronome guided rate of 100 per minute. Additionally, full chest recoil after each compression is specifically emphasized.

Law officers and emergency medical service (EMS) rescuers equipped with AEDs are to defibrillate immediately if they witness the collapse. Otherwise, they are to

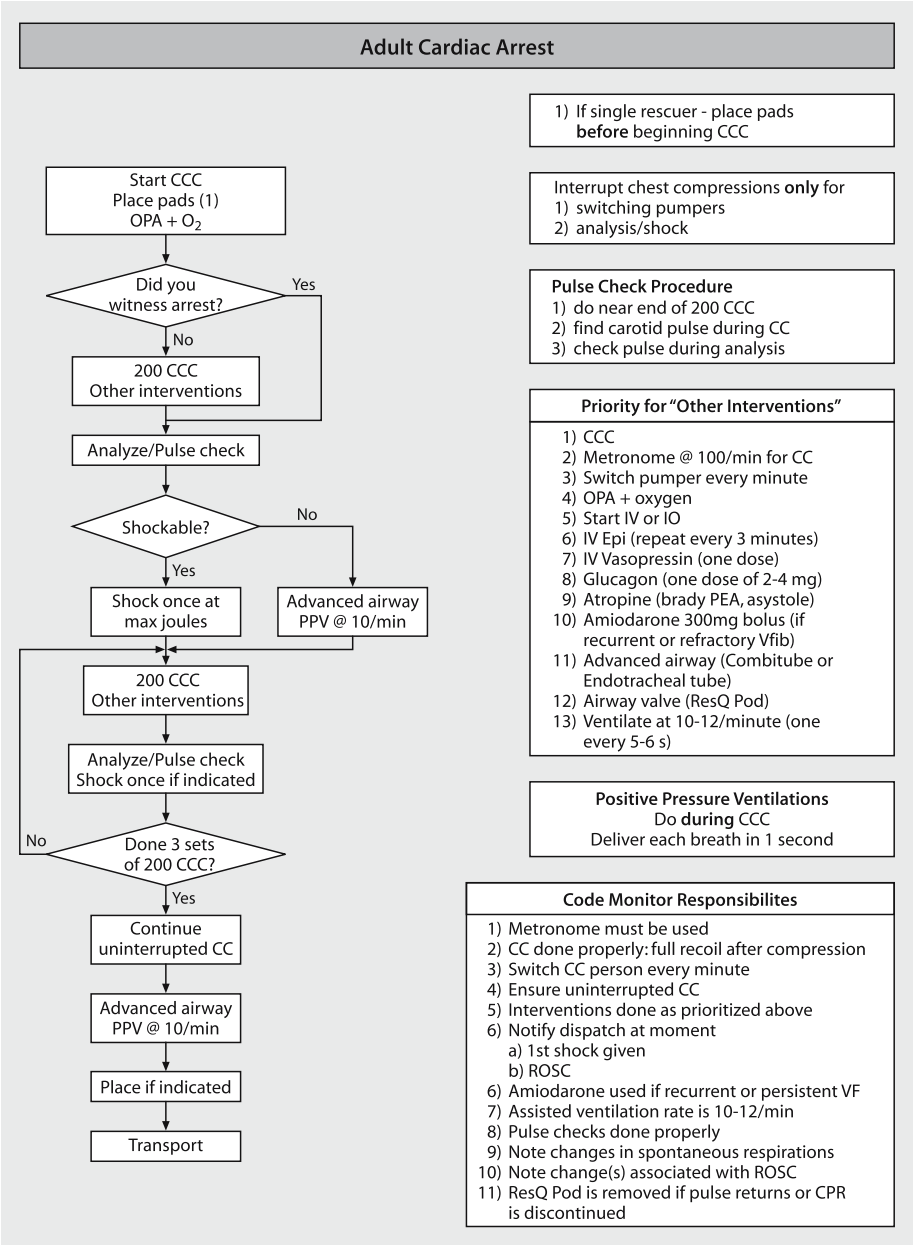

Fig. 1. Algorithmic overview of the protocol for adult cardiac arrest used in Rock and Walworth counties in Wisconsin. CCC: continuous chest compressions; Epi: epinephrine; PEA: pulseless electrical activity; ROSC: return of spontaneous circulation; OPA: oro-pharyngeal airway; VF: ventricular fibrillation

perform continuous chest compression for 200 compressions before defibrillation. If a shockable rhythm is present, a single shock, rather than stacked shocks, is delivered and continuous-chest-compressions are immediately resumed. Pulse checks are not done following the shock but rather during the rhythm analysis period. If only one rescuer is on scene, AED pads are attached before continuous chest compression are initiated. Intravenous administration of vasopressors and other medications is encouraged as soon as possible but without interruption of continuous chest compressions.

Initial airway management consists of insertion of an oropharyngeal airway and provision of oxygen by non-rebreather mask. Rescue breaths and assisted ventilations are not performed until return of spontaneous circulation or until after three cycles of 200 continuous chest compression-rhythm analysis-shock are completed.

If the first analysis reveals a non-shockable rhythm or the arrest was not witnessed by a bystander, then ventilation and invasive airway interventions are performed after that analysis. In this case, continuous chest compression is recommended while other interventions are applied. Ventilations are to be performed at a rate of 10 per minute and are done without interruption of continuous chest compression with each breath delivered over at least a two second interval. Again, this protocol is only applicable to individuals with a presumed cardiac arrest, i.e., an adult with unexpected sudden collapse without normal breathing.

Why Chest Compressions before Defibrillation?

Immediate defibrillation is the treatment of choice during the first or 'electrical' phase of a VF arrest; that is during the first 4 minutes. Unfortunately, rescuers seldom arrive during this phase of cardiac arrest secondary to VF. As mentioned above, immediate defibrillation during the 'circulatory' phase is counterproductive, usually producing either asystole or pulseless electrical activity. Two human studies and multiple animal studies have demonstrated that this detrimental effect of defibrillation can be avoided if the heart is perfused by chest compressions before applying the shock [20, 21]. Adequate coronary perfusion is a prerequisite to successful defibrillation and currently this can only be achieved by chest compressions.

Why Single Shocks?

Stacked shocks and the analysis periods interrupt chest compressions during the circulatory phase of VF for unacceptable periods of time [22]. In a recent study from Seattle, the average interruption was 29 seconds [22]. Interruptions as brief as 10–15 seconds significantly reduce the efficacy of a shock and increase the incidence of post-resuscitation myocardial dysfunction [23, 24]. This might be acceptable if subsequent immediate analyses detected persistent VF, but this is usually not the case. Again, from the recently published study from Cobb and associates in Seattle, "For the first sequence of shocks, automatic external defibrillators defibrillated or terminated ventricular fibrillation with the initial shock in 83.6% … of cases" and "The initial shock defibrillated only 63% of patients using the monophasic truncated exponential waveform, 84% of patients using monophasic damped sine waveform, and 92% of patients using biphasic waveforms [22]." What are the implications of these findings? These authors concluded, "Because the activities of rhythm reanalyses, stacked shock, and initial post shock pulse checks had low yield

with regard to the balance between achieving or detecting a pulse and initiating CPR, one consideration would be to eliminate these activities from the resuscitation algorithm [22]." Cardiocerebral resuscitation does this [1, 2].

Why Eliminate Post-shock Pulse Checks?

In the circulatory period of VF, a pulse is rarely present after the defibrillation shock. A recent study reported that a pulse was detected with the initial post shock pulse check in only 2.5% of cardiac arrest victims [22].

Why 200 Chest Compressions Immediately Following the Shock?

The Sarver Heart Center CPR Research Group of the University of Arizona noted in our experimental laboratory that following prolonged VF arrest, that is, in the circulatory phase of VF arrest, the shock almost always defibrillated the subject into a non-perfusing rhythm. Because researchers had immediate access to the hemodynamics of the instrumented swine, they would immediately restart chest compressions to restore coronary perfusion pressures. With this approach, pulseless electrical activity was likely to revert to a perfusing rhythm [1]. In fact, the way to produce pulseless electrical activity in the research laboratory for study is to produce ventricular fibrillation and leave the subjects in VF for a prolonged period of time and then defibrillate. These animals then develop asystole or pulseless electrical activity.

Why Uninterrupted Chest Compressions?

Chest compressions provide perfusion to the heart and brain. Not only is perfusion immediately curtailed when chest compressions are stopped, but the effectiveness of subsequent chest compressions is transiently diminished. It takes several compressions to generate the perfusion pressures that existed before chest compressions were interrupted [25]. This is particularly relevant to repeated interruptions for rescue breathing or assisted ventilations. But it is also relevant to interruptions for any reason including for pulse checks, rhythm analysis, stacked shocks, intubation, patient reassessment and intravenous line placements and with pacing without perfusion. This is a major reason why cardiocerebral resuscitation advocates continuous uninterrupted chest compressions and emphasizes a minimal discontinuance interval before defibrillation.

Does the Technique of Chest Compression Matter?

Not only are the number and rate of chest compressions important, but the technique of chest compressions is also important [26]. Incomplete chest recoil after each compression compromises perfusion because it interferes with the generation of a negative pressure in the chest cavity and, therefore, venous blood returning to the heart is reduced. As a consequence, cardiac output is reduced. Aufderheide and associates noted incomplete chest recoil occurred in 46% of simulated resuscitations performed by paramedics. They also found significant decreases in both coronary perfusion pressure and cerebral blood flow when incomplete chest recoil ex-

isted [26]. Accordingly, lifting the hands from the chest after each chest compression is essential to assure complete chest recoil.

Furthermore, when incomplete chest recoil was combined with excessive ventilations, perfusion was severely compromised [26]. Unfortunately, excessive ventilation rates (as will be documented below) are extremely common by both in-hospital physicians and out-of-hospital paramedics.

Why No Ventilations in the Initial Treatment of Cardiac Arrest?

Why does cardiocerebral resuscitation encourage only bystander continuous-chest-compression CPR without mouth-to-mouth rescue breathing for witnessed cardiac arrest in adults?

A significant problem contributing to the dismal survival rates of out-of-hospital cardiac arrest is the lack of bystander-initiated basic CPR. Although the majority of out-of-hospital cardiac arrests are witnessed, the prevalence of bystander-initiated CPR varies considerably from city to city, but is such that, on average, less than one in three witnessed cardiac arrests are afforded bystander- or citizen-initiated CPR [27–29]. One survey indicated that only 15% of lay Americans would definitely do mouth-to-mouth resuscitation on a stranger [29]. Anonymous surveys have shown that lay individuals are not the only ones reluctant to provide mouth-to-mouth resuscitation on strangers – so are certified CPR instructors, nurses and physicians [30–33]. In certain cultures, this percentage is even less. In Japan, only 2% of students and 3% of nurses said they were willing to perform mouth-to-mouth rescue breathing on a stranger [34]. Numerous studies have documented increased survival with bystander CPR and yet it is not being done [27, 28, 35, 36]. Even where chest-compression-only CPR is not taught nor advocated, reported survival with bystander chest-compressions-only CPR and standard CPR are similar [37–39].

During the initial phase of a VF cardiac arrest, the pulmonary veins, heart and the entire arterial system are filled with oxygenated blood [40]. The goal of cardiocerebral resuscitation is to maximize the delivery of this oxygenated blood to the brain and the heart by encouraging bystander initiated chest-compression-only CPR. However, cardiocerebral resuscitation also recognizes that oxygen is not the only important component of blood during organ perfusion. To illustrate this point, recall that patients with severe cyanotic congenital heart disease have very poor oxygen saturations and yet their heart and brain are well functioning.

Though not willing to extend the recommendation for chest-compression-only CPR for everyone doing bystander-initiated CPR, the AHA "Guidelines 2000" stated on page I-43 that, "If a person is unwilling or unable to perform mouth-to-mouth ventilation for an adult victim, chest-compression-only CPR should be provided rather than no attempt at CPR being made" [41, 42]. Unfortunately, here-to-fore, during American Heart Association and Red Cross sponsored CPR courses, at least in our areas, chest-compression-only CPR is rarely, if ever, mentioned.

Bystander Initiated CPR with Rescue Breathing is not Optimal

It was indeed a surprise to find that so called 'rescue breathing' not only prevents bystander initiated CPR, but also when performed the way it has here-to-fore been taught, it is actually deleterious. Although "Guidelines 2000" recommended two

breaths with at least 2 seconds per breath before each 15 chest compressions, it takes lay individuals an average of 16 seconds and younger medical students 14 seconds to deliver the recommend two 'rescue' breaths [43, 44]. Using a simulated out-of-hospital cardiac arrest protocol, continuous chest compression CPR was compared with CPR where each of the two breaths required 16 seconds before each 15 chest compressions. Survival in this study was 80% with continuous chest compression CPR vs. 13% for 'standard' CPR. The survival with simulated out-of-hospital cardiac arrest protocols applying continuous chest compressions averaged 73% [1, 2]. The survival rate of 13% for the 'standard CPR' group was of intense interest, for in Tucson, Arizona the average survival rates of individuals with out-of-hospital cardiac arrest due to VF over the past decade were similar [45].

Data support the need for more than 80 compressions per minute to achieve optimal blood flow during CPR [23, 46, 47]. Obviously if every set of 15 compressions is interrupted for 14 to 16 seconds to deliver the recommended two ventilations, the individual will not receive enough chest compressions to survive. This has been recognized by those responsible for updating CPR guidelines. Unfortunately any changes in guidelines that require rescue breathing will not change the reluctance of bystanders to perform bystander initiated CPR [48].

Based on the above data, one aspect of the Cardiocerebral Resuscitation program begun in November 2003 in Tucson is the "Be A Lifesaver" component for the public [1, 2]. In the Walworth and Rock Counties of Wisconsin it is called "Call and Pump." These programs encourage citizens to call 911 and then initiate continuous chest compressions without mouth-to-mouth ventilation for out-of-hospital witnessed unexpected sudden collapse in adults until the paramedics/firefighters arrive. The purpose of this initiative is to increase the incidence of bystander- or citizen-initiated CPR. Dispatchers answering Emergency Medical Services calls (911 in the United States) often give chest compression only instructions, and in the Wisconsin program, metronome guidance of 100 per minutes is also provided.

A major advantage of this approach is that individuals can be taught continuous chest compression CPR in a relatively short period of time. Tucson's "Be A Lifesaver" and Wisconsin's "Call and Pump" recommendations are for witnessed unexpected sudden collapse in an adult – a condition that is almost always due to cardiac arrest. The related web sites are www.heart.arizona.edu or www.CallandPump.org.

What is the Role of Gasping or Agonal Respirations?

Another observation is that if a subject collapses with VF, gasping lasts for a variable period of time. Gasping is both fortunate and unfortunate. It is fortunate because when chest compressions are promptly initiated, the subject is likely to continue to gasp and provide self-ventilation. In fact, Kouwenhoven, Jude and Knickerbocker in one of their early programs indicated that ventilation was not necessary during chest compression because the subject gasped (Kouwenhoven WB, Jude JR, Knickerbocker GB. Demonstration of the technique of CPR for New York Society of Anesthesiologist 1960s. Copy of demonstration provided on CD by J.R. Jude). However, gasping may be unfortunate as most lay individuals interpret this as an indication that the individual is still breathing, and do not initiate bystander CPR nor activate the EMS as soon as they should. This needs to be an educational point for lay or public education.

Ventilations by Emergency Medical Systems Personnel can be Deadly!

The two major problems with assisted ventilations by the EMS are the deleterious effects of early intubation and hyperventilation.

As noted above, any interruption of chest compressions during the circulatory phase of VF arrest markedly decreases the chances of survival. In addition to the excessive delays caused by AEDs noted above, when one adds the delay time for the average intubation, the victim has little chance of survival. Even in children, where respiratory arrest is more common, it has been shown that the use of bag-mask ventilation is better than intubation [49]. Accordingly, early in EMS resuscitation efforts, intubation should be avoided.

In addition, as noted above, all positive pressure ventilations increase intrathoracic pressures and thereby reduce venous return. In the setting of cardiac arrest, this can result in a significant reduction in cardiac output that in turn compromises both cardiac and cerebral perfusion [50, 51]. This is more than a theoretical issue; studies in trained and retrained paramedics showed that once assisted ventilations are initiated, they are almost always performed at rates that severely compromise perfusion. Indeed, a recent publication by Aufderheide and Lurie entitled "Death by hyperventilation" highlighted this real problem [51]. In-hospital, as well as field observations, documented that these personnel delivered an average of 37 breaths per minute rather than the 12–15 minutes recommended by the guidelines [52].

Cardiocerebral resuscitation protocols minimize the chances for excessive ventilations. Cardiocerebral resuscitation advocates placement of an oropharyngeal device and oxygenation by non-rebreather mask in the initial phases of treatment and deliberately delaying positive pressure ventilations in patients with an initially shockable rhythm (Fig. 1). Assisted ventilations are not begun until return of spontaneous circulation or three series of 200 chest compressions and shock (if necessary) have been completed. While the first person with a duty to respond applies the defibrillator pads and begins chest compressions, the second emergency trained and equipped EMS person places an oropharyngeal airway and a non-rebreather mask with high flow oxygen.

Conclusion

This chapter describes cardiocerebral resuscitation, a new approach to out-of-hospital witnessed arrest in adults due to VF or pulseless ventricular tachycardia. We have reviewed the studies that led us to institute this new methodology for witnessed unexpected sudden cardiac arrest in adults. It is dramatically different from Guidelines 2000 and in all probability significantly different from Guidelines 2005. Guidelines 2005 had not been published when this chapter was due for submission.

Compared to historical controls where Guidelines 2000 CPR was used, cardiocerebral resuscitation resulted in dramatic improvements in neurologically normal survival in patients with witnessed out-of-hospital cardiac arrest and shockable arrhythmias (personal communication Kellum). While these finding were in an observational study, they were so dramatic and in accordance with our animal research studies, that we recommend this approach for all out-of-hospital cardiac arrests with a shockable rhythm until a different protocol is proven to be better.

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Induced Hypothermia for Neuroprotection: Understanding the Underlying Mechanisms

K. H. Polderman

Introduction

In the past few years, the use of therapeutic hypothermia as a tool to mitigate neurological injury has gained a firm foothold in many intensive care units (ICU) throughout Europe and, to a lesser degree, in the United States. Currently, in the adult setting its most widespread use is in patients who remain comatose after cardiac arrest. Several studies using historical controls, followed by two randomized controlled trials, have demonstrated that use of induced hypothermia following cardiac arrest improves neurological outcome in patients with witnessed arrests and an initial rhythm of ventricular fibrillation (VF) or ventricular tachycardia (VT) [1, 2]. These benefits were observed in spite of the fact that the speed of induction of hypothermia (cooling rates) was relatively slow, especially in the larger of the two studies; target temperatures were achieved only after an average period of 8 hours in the multicentered Hypothermia after Cardiac Arrest (HACA) trial [1]. In the second study, where cooling was initiated very early (in the ambulance during the patients' transport to the hospital, by administering refrigerated fluids), cooling rates were much faster, although it still took about 2½ hours to reach target temperature [2]. Regarding the observed benefits, the HACA trial reported an absolute increase in rates of favorable neurological outcome of 16% (relative increase 41%); an absolute increase of 23% (relative increase 88%) was reported in the second study. A meta-analysis by Holzer et al. [3] concluded that the number needed to treat to achieve one additional patient with a good neurological outcome was 6, a number that compares very favorably to many other interventions both inside and outside of the ICU setting. Preliminary evidence suggests that there may be benefits in patients with witnessed cardiac arrest regardless of the initial rhythm [4]. Based on the results of these studies the International Liaison Committee on Resuscitation issued a recommendation that hypothermia be used in patients following witnessed out-of-hospital cardiac arrest if the initial rhythm was VT or VF, and to consider its use for other rhythms and for in-hospital cardiac arrest [5].

Favorable effects of hypothermia in mitigating post-anoxic cerebral injury have also been observed in a number of non-randomized and in two multicentered randomized studies in newborn infants with perinatal asphyxia. In the two RCTs, hypothermia was initiated 5 to 6 hours after birth and applied for a period of 72 hours; significant improvements in the rates of favorable neurological outcome were observed, with the greatest benefits being observed in patients with less severe injuries [6, 7].

In a separate but closely related issue, it is becoming clearer that the development of fever in patients with neurological injury is an independent predictor of

adverse outcome, and that fever may cause significant additional neurological injuries in these patients. Thus, maintaining normothermia may help mitigate or prevent some of these injuries [8, 9].

In contrast to these positive findings, studies in patients with traumatic brain injury have produced conflicting results [9, 10], and studies looking at short-term induction of hypothermia in the peri-operative setting have been negative [11]. These negative findings in some studies have led to heated debates on the role of hypothermia in general, and have hindered the introduction of hypothermia even in those cases where the evidence is strong.

Historical Perspective

To understand better the reasons for the negative results of some studies and the positive results of others, we should look firstly at the physiological aspects and at the processes that we are trying to influence when we use therapeutic hypothermia. A better understanding of the mechanisms underlying brain injury and hypothermia's potential protective effects will help us to properly use hypothermic treatment and avoid the (potentially severe) side effects of hypothermia. A lack of such understanding has almost certainly contributed to the failure of some of the hypothermia trials to date. This is illustrated by some of the early experiences with induced hypothermia in the 1950s and 1960s, when it was used in the treatment of cardiac arrest and traumatic brain injury and in the perioperative setting during cardiac surgical and neurosurgical procedures. At that time it was thought that hypothermia exerted its effects exclusively by reducing brain metabolism, with concomitant decreases in oxygen and glucose demand. Based on this assumption of the underlying mechanism, patients were mostly treated with deep hypothermia ($\ll 30\,^{\circ}C$). The core temperatures that were actually achieved varied considerably both between different patients and within the same patient, because the available cooling and rewarming methods were not very reliable and the patients' temperatures could, therefore, not be easily controlled. The most frequently used cooling methods were placement of slabs of ice, ice pads and refrigerated water on the patient's skin. Because no intensive care facilities were available, the treatment was applied in general wards. Duration of cooling also varied considerably.

In spite of this lack of precise and well-controllable cooling methods and the use of temperatures below $30\,^{\circ}C$ (which is associated with a much greater risk of severe side effects compared to temperatures $\geq 30\,^{\circ}C$ [8, 9]), some of these studies appeared to show benefits compared to 'expected outcome' or historical controls. However, the reported benefits were variable and uncertain; in addition, there were significant problems in patient management and severe side effects. These problems led to the discontinuation of prolonged hypothermia as a medical treatment following cardiac arrest, traumatic brain injury and most other indications at that time, although hypothermia continued to be used in the peri-operative setting. These experiences graphically illustrate the difficulties involved in trying to improve patient outcome with induced hypothermia.

However, interest in hypothermia treatment was rekindled in the early 1980s by the positive results of a large number of animal experiments in various types of brain injury. Hypothermia was found to be effective in models for global ischemia, focal ischemia, traumatic brain injury and ischemic or hemorrhagic stroke. Many important insights regarding the most effective use of hypothermia, as well as more

detailed knowledge regarding the mechanisms underlying hypothermia's protective effects were gained from these experiments. The most important breakthrough was the realization that neurological outcome could be improved by using mild-to-moderate (31–35 °C) rather than deep hypothermia (≤30 °C), with far fewer and less severe side effects. The reason for this was the observation that protective effects of hypothermia were not, or at least not mainly, due to a decrease in oxygen and glucose consumption in the brain. To understand this we need to look in some more detail at the complicated processes that occur in the brain as a whole and in individual brain cells following a period of ischemia or trauma, and at the way in which temperature (either hypo- or hyperthermia) affects these developments.

Post-resuscitation Disease and Secondary Injury

Numerous destructive processes begin in the minutes to hours following an initial (ischemic or traumatic) injury. These processes, collectively known as 'post-resuscitation disease' when they occur after cardiac arrest, or as 'secondary injury' in patients with traumatic brain injury, can continue for hours to several days after the initial injury. Even when this has finally subsided, the whole cascade can be re-triggered by new episodes of ischemia. A key realization from the animal data described above was that all of these processes are temperature dependent; they are all stimulated by fever, and can all be mitigated or blocked by mild to moderate hypothermia. The wide-ranging effect of hypothermia on all of these mechanisms may explain why it has proved to be clinically effective, whereas studies with agents that specifically target only one of the destructive processes have been far less successful.

The second key development that set the stage for a successful 'comeback' of hypothermia as a clinical treatment was the advent of ICUs. Invasive monitoring of various body functions, appropriate sedation and analgesia, controlled mechanical ventilation and circulatory support, tight metabolic control – all of these play a role in allowing hypothermia to affect neurological outcome in a positive way. The side effects of hypothermia can be far better managed, and temperature can be much better controlled in the cooling, maintenance, and re-warming phases, in an ICU setting. In addition, the methods for inducing and maintaining hypothermia at the desired level have improved significantly since the 1950s.

So, how did all this impact on the potential effects of hypothermia on the destructive mechanisms following brain injury? From the abovementioned studies and numerous other investigations, it has become clear that the key factors determining success or failure of hypothermia treatment are the speed of induction of hypothermia, the duration of cooling, the speed of re-warming, and the prevention of side effects [8–10]. The overall equation is complicated by the fact that the contributions of different destructive mechanisms to the ongoing post-resuscitation/secondary injury may differ; these differences may exist between different types of injury, between different patients, or even within the same patient over time. Thus the available 'window of opportunity' for therapeutic hypothermia and other interventions may vary; the same may apply to the required duration of hypothermia treatment in order for it to be effective.

For all these reasons, knowledge and a better understanding of these underlying mechanisms may help us to better target our treatments and help improve out-

come. These mechanisms, and the way in which they are affected by hypothermia and fever, will be the focus of the remainder of this article.

Destructive Processes Following Ischemia or Trauma and Effects of Temperature (Fig. 1)

Decrease in Cerebral Metabolism

As discussed above, when hypothermia was first applied in a clinical setting it was presumed that its protective effects were due to a slowing of cerebral metabolism, leading to a decrease in glucose consumption and oxygen demand. Indeed, during induction of hypothermia cerebral metabolism decreases by between 5% and 8% for each °C reduction in body temperature [12–14]. However, in the 1980s numerous animal experiments showed that marked neuroprotective effects could be realized using very mild hypothermia (31–34 °C). These effects could not be explained by effects on brain metabolism alone [13]. Thus it became clear that although a decrease in metabolism and oxygen demand is probably one of the mechanisms underlying hypothermia's neuroprotective effects, other mechanisms must be equally or more important. Attention then focused on finding and elucidating these other mechanisms.

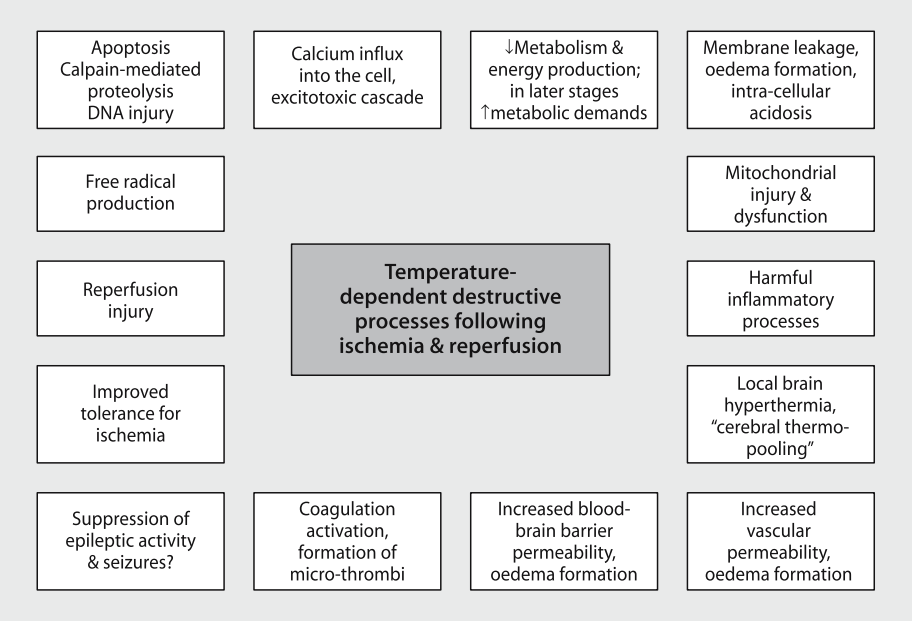

Fig. 1. Depiction of temperature-dependent destructive mechanisms occurring at the cellular level following ischemic or traumatic injury

Apoptosis, Calpain-mediated Proteolysis and Mitochondrial Dysfunction

When an area of the brain has experienced a traumatic injury or an episode of ischemia and reperfusion, several things can happen to the brain cells in that area. If the injury is extremely severe or long lasting, acute cell death will ensue, leading to widespread necrosis in the affected area. Alternatively, the cells may survive the injuries and in time recover. This recovery can be complete, with all the normal cell functions and inter-cell communications being restored; in that case the affected brain area will function normally, as it had before the traumatic or ischemic event. Recovery may also be partial, with some cell functions and interactions with other brain cells being impaired; this will lead to a diminished functional status of the affected brain area. Finally, the cells may also enter a path leading to apoptosis or programmed cell death. This implies that in theory a cell could have recovered from the initial injury with all its normal functions intact, but that in the event the period of trauma or ischemia has triggered a 'suicide program' that methodically shuts down and kills the cell in the days following initial injury.

Whether a cell will become apoptotic is determined by a number of cellular processes, including mitochondrial dysfunction and disorders in cellular energy metabolism as well as the release of a number of so-called caspase enzymes. Importantly, during the early stages the process of apoptosis can still be reversed; cells on the road to apoptosis can still turn back from the brink and make a full recovery.

Numerous studies have shown that hypothermia can interrupt the apoptotic pathway and thus prevent cell injury from leading to apoptosis [16–18]. Hypothermia probably exerts its effects in the early stages of the apoptotic process, by influencing a large number of different mechanisms which are all involved in the initiation of apoptosis. Hypothermia inhibits the activation of caspase enzymes, prevents or mitigates mitochondrial dysfunction, decreases the overload of excitatory neurotransmitters, and modifies the severe disorders of intracellular ion concentrations occurring after ischemia/reperfusion (see below). All of these processes are key elements in initiating and maintaining the apoptotic process; therefore, interruption or inhibition of these processes can prevent or reverse the apoptotic process provided the treatment is initiated in time. In this regard, it is important to realize that apoptosis is one of the destructive processes that begins relatively late in the post-perfusion phase or after trauma, and continues for a period of 48–72 hours or even longer. Thus the time window for therapeutic interventions such as hypothermia to specifically influence the process of apoptosis may be relatively large; for this reason influencing the apoptotic pathways could play an important role in human neuroprotection and in mitigating post-traumatic and post-resuscitation injury.

Ion Pumps and the Neuroexcitatory Cascade

As already touched upon above, there is a large body of evidence suggesting that hypothermia inhibits harmful excitatory processes occurring in brain cells during ischemia and reperfusion. This so-called neuroexcitatory cascade has been extensively investigated in hundreds of experimental brain injury studies [12, 19–23]. Thus many details are now known about these processes.

When oxygen supply to the brain is interrupted, levels of high-energy metabolites such as adenosine triphosphate (ATP) and phosphocreatine in brain cells decrease within seconds. The breakdown of ATP and the switch of intra-cellular meta-

bolism to anaerobic glycolysis leads to an increase in intracellular levels of inorganic phosphate, lactate, and H^+, resulting in both intra- and extracellular acidosis and an influx of calcium (Ca^{2+}) into the cell. Loss of ATP and acidosis also inhibit the mechanisms that normally deal with excessive intracellular Ca^{2+} by sequestration of Ca^{2+} from the cell, further aggravating intracellular Ca^{2+} overload. These problems are compounded by the failure of ATP-dependent Na^+-K^+ pumps and K^+, Na^+, and Ca^{2+} channels, leading to an additional influx of Ca^{2+}. The excess Ca^{2+} can induce mitochondrial dysfunction (increasing intracellular calcium influx yet further in a vicious cycle) and activate numerous intracellular enzyme systems (kinases and proteases). In addition, immediate early genes are activated and a depolarization of neuronal cell membranes occurs, with a release of large amounts of the excitatory neurotransmitter, glutamate, into the extracellular space. This leads to prolonged and excessive activation of membrane glutamate receptors, further stimulating Ca^{2+} influx through activation of Ca^{2+} channels in a vicious cycle. Under normal circumstances neurons are exposed to only very brief pulses of glutamate; when exposed to glutamate for prolonged periods of time the neurons remain in a permanent state of hyperexcitability (the 'excitotoxic cascade'), which can lead to additional injury and cell death. Moreover, high levels of glutamate can be neurotoxic, especially in energy-deprived cells. Glutamate receptor activation can persist for some time after reperfusion, even when glutamate levels have returned to normal, which may be another important mediator of brain cell death. In this way, ischemia and reperfusion lead to an interruption of a delicate balance between calcium influx and sequestration at the cellular level.

Numerous animal experiments have clearly demonstrated that key destructive processes of the neuroexcitatory cascade (such as calcium influx, accumulation of glutamate, and the release of its co-agonist glycine) can be prevented, interrupted or mitigated by application of hypothermia. Even a relatively small decrease in temperature can significantly improve ion homeostasis, thereby preventing the destructive effects of intracellular calcium overload. Conversely, the occurrence of fever can trigger and/or stimulate these destructive processes.

It is not clear how long the 'window of opportunity' to interrupt this particular cascade is. The disruptions in Ca^{2+} homeostasis begin in the first minutes after injury, but continue in the hours or sometimes even days following the initial injury or period of ischemia. Moreover, the processes can be re-triggered by each new episode of ischemia. Thus, in theory, there could be a significant time window for therapeutic interventions to block or interrupt the neuroexcitotoxic cascade, although it may be more difficult to completely prevent it because the processes start within minutes (unlike the apoptotic process, which begins only after several hours). However, some animal experiments suggest that the excitatory processes can be blocked or reversed only when hypothermia treatment is initiated in the early phases of the neuroexcitatory cascade. Other studies have reported somewhat wider time frames, ranging from 30 minutes to up to 6 hours. Thus, the exact time frame available to modify these post-ischemic destructive processes remains unclear, and may vary between different types of injury and between different species. Some authors have suggested that the therapeutic window for hypothermia could be extended by combining it with other treatments, such as caspase inhibitors or other experimental compounds.

Immune Response and Inflammation

An episode of ischemia/reperfusion or trauma will induce a significant and protracted inflammatory response, beginning ±1 hour after injury and continuing for several days. Pro-inflammatory mediators such as tumor necrosis factor-alpha (TNF-α) and interleukin-1 (IL-1) are released in large quantities by astrocytes, microglia, and endothelial cells following ischemia and reperfusion; levels of these mediators increase ± 1 hour after reperfusion and remain elevated for up to 5 days [12, 24]. This stimulates the migration of activated leukocytes across the blood-brain barrier (via chemotaxis), leading to an accumulation of inflammatory cells in the injured brain and to the appearance of adhesion molecules on leukocytes and endothelial cells. Simultaneously, there is an activation of the complement system beginning in the very early stages after brain injury, which further stimulates the passage of neutrophils and (in later stages following injury) of monocytes/macrophages [24]. These inflammatory responses occur especially during the reperfusion phase, and are accompanied by free radical production (see below). The inflammatory response can cause significant additional brain injury, both through the phagocytic actions of macrophages, through synthesis of various toxic products, and by further stimulating immune reactions in what may become a vicious cycle.

It should be realized that this inflammatory response is to a certain extent physiological. The cascade of inflammatory processes outlined above may have a 'dual role', in the sense that while some inflammatory mediators are neurotoxic, others have been shown to have neuroprotective properties [24, 25]. Nevertheless there is strong evidence suggesting that certainly an excessive inflammatory response, with a disproportionate and persistent production of pro-inflammatory cytokines and massive leukocyte infiltration, can significantly increase the risk and extent of brain injury [24–26]; the IL-1 family, especially, appears to play a major role in this regard [26]. It is noteworthy that this effect is to a certain extent time dependent; the destructive aspects of inflammation outweigh the potential beneficial effects especially during the later stages of injury. The time interval before the inflammatory process begins (at least one hour, usually longer), and the (variable) time delay before the process actually becomes destructive, implies that again there may be a substantial time window for therapeutic interventions to interrupt or mitigate this process before it becomes destructive.

Numerous animal experiments and some clinical data have shown that hypothermia can suppress ischemia-induced and post-traumatic inflammatory reactions. For example, it has been shown both in animal experiments and in clinical studies that hypothermia decreases the levels of pro-inflammatory cytokines following brain injury [27–29]. Apart from its effect on cytokine levels, hypothermia also prevents or mitigates reperfusion-related DNA injury, lipid peroxidation, and leukotriene production, as well as decreasing the production of nitric oxide (NO), a key agent in the development of post-ischemic brain injury. In animal experiments the extent of brain injury and infarct size can be significantly attenuated if *any* of these processes is mitigated or interrupted [19]; as hypothermia can affect *all* of these steps there is (at least in theory) a huge potential for improving neurological outcome. Moreover, as the inflammatory response begins relatively late (>1 hour) following ischemia and reperfusion, and because the destructive processes take some time to fully develop and continues for prolonged periods of time following injury, there seems to be a definite therapeutic window for application of hypothermia to affect this specific mechanism.

Free Radical Production

A destructive process that is closely linked to, but distinct from, the mechanisms discussed above is the release of free oxygen radicals following ischemia and reperfusion. These free radicals, which are also a part of the inflammatory response (see above), can oxidize and severely damage all cellular components (lipids, proteins including enzymes, and nucleic acids), thereby impairing the normal function of the cells and decreasing the capacity of the cell to protect itself from the other destructive mechanisms listed above. The presence and levels of mediators such as superoxide (O_2^-), peroxynitrite (NO_2^-), hydrogen peroxide (H_2O_2), and hydroxyl radicals (OH^-) plays an important role in determining whether injured cells will recover or die [30, 31].

To a certain degree, the production and presence of free radicals is a normal phenomenon even under normal circumstances, and brain cells have a number of enzymatic and non-enzymatic anti-oxidant defense mechanisms to prevent this type of injury. However, the amounts of free radicals produced after an episode of ischemia/reperfusion is so great that these defensive mechanisms are highly likely to be overwhelmed.

Although induction of hypothermia does not completely inhibit free radical production, the amounts of free radicals that are produced are significantly decreased during hypothermia [30, 31]. This allows the endogenous protective (anti-oxidant) mechanisms to better cope with the free radicals that are released, decreasing the likelihood that the defensive mechanisms will be overwhelmed and thus preventing or significantly mitigating the peroxidation of lipids, proteins, and nucleic acids. If the suppression of free radical production is sufficient this will allow the cell to recover, rather than suffering permanent injury and/or dying.

The degree of suppression of free radical production is directly linked to the degree of hypothermia; the further temperature is lowered, the greater will be the decrease in free radical production. This may have direct therapeutic implications; in theory, the greater the severity of injury (for example, a prolonged ischemic episode), the greater will be the decrease in temperature required to sufficiently suppress free radical production.

Vascular Permeability, Blood Brain Barrier Disruption and Edema Formation

Both traumatic injuries and episodes of ischemia/reperfusion will lead to significant disruptions in the blood-brain barrier [32–34]. The combined effects of blood-brain barrier disruption, cytotoxic edema caused by the destructive processes listed above, and disruptions in the normal drainage of spinal fluid can together induce massive brain edema, manifesting itself as an increase in intracranial pressure (ICP). This increase in ICP can itself further increase brain injury, by causing regional or general decreases in brain perfusion, and by blocking the drainage of cerebrospinal fluid (leading to further increases in ICP in a vicious cycle) [10, 35]. Therapeutic interventions such as mannitol administration in traumatic brain injury or stroke can decrease ICP, but can also cause additional blood-brain barrier disruption especially with prolonged usage [33, 36].

Mild hypothermia significantly reduces the disruptions in the blood-brain barrier [32–34]. In addition, vascular permeability following ischemia-reperfusion injury is also decreased during mild hypothermia, which further decreases edema

formation [37]. Additional support for the concept of a membrane- and blood-brain barrier-stabilizing effect of hypothermia is provided by the observation that induction of hypothermia decreases the extravasation of hemoglobin following traumatic brain injury [38].

Ischemia and reperfusion can also affect the integrity of the blood-brain barrier in other ways, including a decrease in the fluidity and integrity of cell membranes and an increase in vascular permeability of microvascular endothelial cells in the brain, which is mediated by vascular endothelial growth factor (VEGF) via the release of NO [39]. These processes of membrane disintegration and hypoxia-induced permeability changes can be mitigated or reversed by hypothermia [39]. Thus, there are multiple mechanisms through which hypothermia can prevent or significantly mitigate the development of brain edema; apart from stabilizing the blood-brain barrier and decreasing vascular permeability, hypothermia also decreases cytotoxic edema via the mechanisms described above, i.e., by decreasing inflammatory responses in the brain, and by improving ion homeostasis and decreasing free radical production in the brain.

In the clinical setting, the development of brain edema is usually assessed qualitatively and quantitatively by measuring the ICP, which can be regarded as a 'final common pathway' for all the destructive processes that can lead to brain edema [10]. The key role of intracranial hypertension and brain edema formation in the development of (additional) neurological injuries in patients with traumatic brain injury and stroke is well recognized [35, 40]. Brain edema formation can also cause additional injuries and critically determine outcome in encephalitis and meningitis, and there is evidence suggesting that it can also play a role in the development of post-hypoxic injury following cardiac arrest [41].

Because intracranial hypertension appears to be both a marker for ongoing neurological injury and a cause of additional injury [10], it seems plausible that treatments that lead to a decrease in ICP will also help improve neurological outcome. In patients with traumatic brain injury, ICP is frequently used as a key parameter to guide treatments and to determine the efficacy of a treatment [35, 42].

Numerous *in vitro* experiments and clinical studies including thousands of patients have clearly established that mild hypothermia can significantly decrease ICP in a wide range of neurological injuries, including traumatic brain injury, stroke, hepatic encephalopathy and many others [8]. Decreasing brain edema and ICP may be yet another mechanism through which hypothermia can mitigate brain cell injury and improve neurological outcome; this would also give hypothermia a relatively wide therapeutic window. It should be noted that although hypothermia has been shown to decrease ICP in many clinical studies, the results in improving survival and neurological outcome have been mixed [8, 10], with one large multicentered trial failing to show any benefit of induced hypothermia [43]. These varying results may be (partly) related to the management of the side effects of hypothermia [9, 10]. These side effects can be severe, especially in patients with traumatic brain injury. The reason for the higher risk of side effects in patients with traumatic brain injury may be a greater susceptibility of the traumatically injured brain to episodes of hypotension, hypovolemia and electrolyte disorders, and perhaps a cumulative increase in these risks due to interactions with other treatments such as mannitol administration [9]. The speed of induction and depth and duration of hypothermia may also play an important role, as there is evidence showing that whether or not a lasting protective effect is achieved is critically dependent on these factors [8, 10, 44].

Intra- and Extracellular Acidosis

The diminished integrity of cell membranes, the failure of various ion pumps, development of mitochondrial dysfunction, inappropriate activation of numerous enzyme systems with cellular hyperactivity, and the disruption of various other intracellular processes, all contribute to the development of intracellular acidosis, a factor which powerfully stimulates many of the abovementioned destructive processes [45]. Ischemia/reperfusion also leads to a significant increase in cerebral lactate levels [46]. Numerous *in vitro* studies have reported that mild hypothermia can reduce both cerebral lactate accumulation and intracellular acidosis [45–48]. Because of the link between acidosis and increased cell injury (including stimulation of necrosis and apoptosis [45]), the hypothermia-related improvement in intracellular acid-base balance is likely to provide yet another mechanism through which hypothermia can exert neuroprotective effects.

It should be noted that the above-mentioned effects apply to intracellular acidosis, and that the effects of hypothermia on extracellular pH are very different. Indeed hypothermia usually leads to a mild extracellular acidosis, caused by increased synthesis of glycerol, free fatty acids, ketonic acids and lactate [9]. This mild acidosis is often observed in patients treated with mild to moderate hypothermia, and is a physiological consequence of cooling. As explained in this paragraph this does not signify intracellular acidosis; rather the opposite.

Cerebral Metabolism

Both traumatic brain injury and an episode of ischemia/reperfusion can induce short-term and long-term changes in cerebral glucose utilization and brain metabolism [49, 50]. Following traumatic or ischemic injury, an initial increase in cerebral metabolism (lasting for several hours) is followed by a persistent decrease in metabolic rate, with a marked depression of mitochondrial oxidative phosphorylation and glucose utilization lasting for several weeks [49, 50]. Various studies have shown that inducing hypothermia in this situation increases the speed of metabolic recovery, with better preservation of high-energy phosphates and reduced accumulation of toxic metabolites. This would provide another potential therapeutic avenue for hypothermia, with a potential wide therapeutic window (at least several hours to affect the initial rise in cerebral metabolism, many days to weeks to mitigate the depression in cerebral metabolism that follows the initial increase).

Brain Temperature and Cerebral Thermo-pooling

Even in healthy individuals the temperature of the brain may be somewhat higher than the measured core temperature. This difference can increase significantly in patients with neurological injuries, ranging from 0.1 °C to more than 2 °C [51–53], although this does not occur in all brain-injured patients. When fever develops (which occurs very frequently in patients with neurological injuries [54–57], see below) the differences between brain temperature and measured core temperature can increase even further. In addition, there may be small differences in temperature between different areas of the brain even in healthy individuals, with a slight warming of the more active areas of the brain; these regional differences increase significantly when brain injury occurs, with injured areas becoming much warmer than non-injured areas [51] due to the destructive 'hyperactivity' of injured cells.

Numerous clinical studies have shown that the development of fever in patients with neurological injuries is linked to adverse outcome and an increase in the severity of neurological injuries [54–57]. Although these observations do not necessarily prove that fever is a cause of additional injury (it could simply be a marker of more severe pre-existing injury, in spite of attempts to correct for severity of injury with multivariate analysis), there are numerous animal experiments showing that (external) induction of hyperthermia in animals with (experimental) neurological injuries significantly increases the severity and extent of these injuries [58–61]. Various destructive mechanisms may underlie this phenomenon; indeed, all of the destructive mechanisms outlined above, which can be mitigated or interrupted by hypothermia, can be increased and stimulated by fever. For example, induction of hyperthermia increases the risk that ischemic areas will become necrotic or apoptotic, even when fever develops (or is externally induced) many hours or days after initial injury. Baena et al. reported that transient whole-body warming to 39–40 °C for a period of 3 hours, performed 24 hours after a brief episode of forebrain ischemia in rats, led to a 2.6-fold increase in the extent of neuronal injury in the hippocampus [59]. Similar observations were made in numerous other animal studies [8, 60, 61]. This strongly suggests that fever can be detrimental even when it is of relatively short duration and occurs a long time after the initial injury.

In this regard, it should be noted that these effects become even more pronounced when a period of fever coincides with an episode of cerebral ischemia. Taken together, these observations suggest that ischemic brain cells are extremely susceptible to the deleterious effects of fever, and that although this susceptibility decreases once re-perfusion has occurred and the acute ischemic event has passed, the injured cells remain vulnerable to harmful effects of high temperatures for many days thereafter.

Numerous clinical studies have confirmed that fever is indeed an independent predictor of adverse neurological outcome and increased mortality in stroke, traumatic brain injury and post-anoxic injury [54–57, 62–64]. In a prospective observational study, Azzimondi and associates observed that developing fever was associated with a 3.4-fold increase in the risk of adverse outcome in stroke patients, with a 95% CI of 1.2 to 9.5 [65]. Castillo and associates reported that developing fever within 24 hours after the onset of stroke was independently related to larger infarct volumes (OR 3.23, 95% CI 1.63 to 6.43) and higher neurological deficits (OR 3.06, 95% CI 1.70 to 5.53) at 3 months [66]. Kammersgaard et al. reported that each 1 °C increase of admission body temperature independently predicted a 30% relative increase in long term mortality risk, with a 95% CI of 4 to 57% [67]. Although it remains to be conclusively demonstrated that the relationship between fever and adverse outcome is causal (i.e., that fever causes additional neurological injury rather than just being a marker), the temporal relationship and the animal data discussed above strongly suggest a causal relationship. This view is strengthened by observations from other animal studies showing that inducing hypothermia can prevent fever-related neurological injuries and improves tissue tolerance for ischemia [8, 68]. Thus, there may be an important role for fever prevention in the mitigation of the severity of neurological injuries. The destructive processes outlined above are all temperature dependent, and thus can be increased by fever and decreased/mitigated by mild to moderate hypothermia. Which degree of 'temperature management' (preventing very severe hyperthermia, maintaining normothermia, inducing mild or moderate hypothermia, required duration of these interventions) is

required, will depend on factors such as the type and severity of injury, degree of activation of the destructive mechanism listed above, etc.

Coagulation Activation and Formation of Microthrombi

Various studies have shown that cardiopulmonary arrest and resuscitation are accompanied by a marked activation of coagulation, which can lead to intravascular fibrin formation and clotting with blockage of the microcirculation in the brain and heart [69]. This has led to attempts to improve the circulation and reperfusion by administering anticoagulants following the restoration of spontaneous circulation. The initial results of animal experiments and preliminary clinical observations suggest that such a strategy might be highly successful; administration of heparin or recombinant tissue plasminogen activator [70] improved microcirculatory reperfusion and survival in cats, and early thrombolysis improved cerebral tolerance to ischemia in a rat model [71]. Böttiger and co-workers performed a prospective clinical study in cardiac arrest patients with initially unsuccessful cardiopulmonary resuscitation, and observed that administering thrombolytic agents in the very early stages of cardiopulmonary resuscitation was associated with improved neurological outcome and survival [72].

These observations suggest that inducing an anticoagulation effect following ischemia/reperfusion can improve outcome, probably by improving the microcirculation and removing micro-thrombi. This is relevant to the subject of cooling because hypothermia also affects the coagulation system. The mechanism is a combination of effects on platelet count, platelet function, and the coagulation cascade [9]. This anticoagulation effect could constitute yet another mechanism for hypothermia's neuroprotective and cardioprotective effects. This concept remains speculative, as no studies directly addressing this issue have so far been performed, but seems plausible in light of the evidence outlined above.

Vasoactive Mediators

Hypothermia can influence the local secretion of vasoactive mediators secreted by the endothelium, such as endothelin, thromboxane A2 and prostaglandin I_2. This effect has been observed both in the brain and at other sites. Endothelin and thromboxane A_2 are powerful vasoconstrictive agents, while prostaglandin I_2 is a potent vasodilator. Thromboxane can also induce platelet aggregation. Thromboxane and prostaglandin I_2 play an important role in regulating local cerebral blood flow, and a balance between the two is required to maintain homeostasis [73]. This local homeostasis may be disrupted following an ischemic or traumatic event, with a relative increase in the production of thromboxane [74]. Such a disruption in equilibrium with an increased predominance of thromboxane can lead to vasoconstriction and hence to hypoperfusion and (local) thrombus formation in injured areas of the brain.

Various animal studies and some clinical observations have found that the local imbalance in the production of vasoactive mediators can be modified or corrected by hypothermia [75]. Aibiki and co-workers measured prostanoid levels in patients with traumatic brain injury treated with moderate hypothermia (32–33 °C), and reported that induction of hypothermia led to reduced prostanoid levels and an improved balance between thromboxane A2 and prostaglandin I_2, with a relative increase in the vasodilatory mediator prostaglandin I_2 [75]. In another clinical study,

Chen et al. reported a decrease in production of the vasoconstrictor endothelin-1 associated with hypothermia [76].

However, the regulation of cerebral perfusion (especially in the injured brain) is a highly complex issue. Apart from the (local) temperature, local cerebral circulation can be influenced by many other factors including presence or absence of normal cerebral autoregulation, ventilator settings and blood gas management, concomitantly administered treatments such as mannitol and hypertonic saline, appropriate sedation, etc. Thus, although preliminary evidence suggests that there may be a favorable effect of hypothermia on the secretion of vasoactive mediators these findings are still very preliminary. Further studies will be required to determine the exact nature and impact of hypothermia on the vessel wall, and to assess whether this plays a role in explaining hypothermia's neuroprotective effects.

Improved Tolerance to Ischemia

Induction of hypothermia improves tolerance to ischemia in various animal models [77, 78]. For this reason (and in spite of the lack of solid clinical evidence [8]) hypothermia is widely used in the peri-operative setting, especially during major vascular surgery, cardiothoracic surgery and neurosurgical interventions [8]. An ability to better withstand periods of ischemia would be an important potential protective mechanism, because patients with different types of neurological injury may have periods of ischemia for many days following initial injury. For example, in patients with traumatic brain injury a combination of biological factors (cytotoxic and vasogenic edema) and mechanical factors (blockage of spinal fluid drainage) can lead to the development of cerebral edema and intracranial hypertension in the hours and days following initial injury [8, 10, 35]; this can subsequently lead to (additional) ischemic injury. In patients with subarachnoid hemorrhage, the development of vasospasm in the days and weeks following an initial bleeding episode is a major complication that can lead to significant additional ischemic injury. The risk of ischemia long after admission to the hospital or ICU also applies to patients following cardiopulmonary resuscitation, where there is evidence suggesting that cerebral ischemia can persist for many hours following successful resuscitation even when saturation and arterial oxygen levels are normal [79]. Thus an increased tolerance for ischemia could be yet another mechanism for the beneficial effects of hypothermia on neurological and cardiac outcome.

Suppression of Epileptic Activity

Numerous studies have reported that non-convulsive status epilepticus, i.e., epileptic activity without obvious clinical signs and symptoms, is a frequent complication in patients with various types of brain injury. These include all the major neurological emergencies such as subarachnoid hemorrhage, stroke, traumatic brain injury and post-anoxic encephalopathy. It is as yet unknown whether non-convulsive status epilepticus by itself causes permanent (additional) brain injury; however, there is mounting evidence that non-convulsive status occurring in the acute phases of brain injury, i.e., while the destructive processes outlined above are ongoing, can lead to a significant increase in brain injury.

Evidence from various sources suggests that hypothermia can suppress epileptic activity. Various case reports and case series have reported successful treatment of

grand mal seizures with hypothermia [80, 81]. Animal studies have shown that external induction of hyperthermia increases the extent of epilepsy-induced brain injury; conversely, prevention or prompt treatment of hyperthermia and/or induction of hypothermia decreases epilepsy-induced brain injury in a dose-dependent fashion [82, 83]. Thus anti-epileptic effects of hypothermia provide yet another potential explanation for its neuroprotective effects.

Other Potential Mechanisms

There are a number of other destructive and/or protective mechanism in brain injury that can be affected by temperature. The available data are preliminary, and the exact nature and importance of these mechanisms in brain injury (and the potential role of hypothermia) remain to be determined. Nevertheless, preliminary data provide some interesting clues.

Following an ischemic or traumatic injury of the brain, so-called immediate early genes are activated and a number of cold shock proteins induced [44]. This is part of the cellular stress response to injury, which can provide some protection from both ischemic and traumatic injuries [44]. Hypothermia leads to an enhanced expression of these immediate early genes and cold shock proteins, providing yet another potential explanation for its neuroprotective effects. In addition, hypothermia can suppress so-called spreading depression-like depolarizations, which can increase neuronal damage in various types of brain injury [84]. The role of these events, and the degree to which they can be influenced by therapeutic interventions, remains to be determined. So far, they have been studied mainly in traumatic brain injury and stroke [44].

Conclusion

Hypothermia is by far the most promising treatment of the past few decades for various types of neurological injury. Hundreds of animal experiments in numerous animal models ranging from rodents to higher primates have shown that hypothermia can improve neurological outcome in various types of brain injury, including post-anoxic encephalopathy, traumatic brain injury, subarachnoid hemorrhage and stroke. The reason for its effectiveness appears to be its broad range of effects; virtually all the destructive mechanisms initiated by ischemia/reperfusion or traumatic injury can be favorably influenced by hypothermia (and adversely affected by fever). Although many questions still remain unanswered, much has been learned in the past 20 years.

For clinicians, a better understanding of these mechanisms can help to apply hypothermia treatment more effectively, and to better understand its potential risks and side effects as well as its potential benefits. In the animal studies cited in this article (and the hundreds that were not cited but reported similar findings), one recurring theme seems to be that the clearest benefits of induced hypothermia are observed when it can be induced very quickly (during or soon after injury), *or* when it is applied later after injury but for longer periods of time. The overwhelming majority of animal experiments addressing this issue have reported benefits on neurological outcome associated with cooling; of those studies reporting no or only modest benefits associated with hypothermia, almost all began cooling relatively

late after injury and applied hypothermia for only brief periods of time. Other reasons for treatment failure in animal experiments have included not providing sedation and analgesia during cooling, allowing episodes of hypotension/hypovolemia to occur, and not correcting severe metabolic disorders such as hyperglycemia and electrolyte disorders.

The 'lessons' for clinicians should probably be that the quicker hypothermia is applied, the more effective it is likely to be; that when it cannot be applied quickly, it should be applied for prolonged periods; and that we should take great care to avoid the side effects of hypothermia therapy. This is borne out by the results of the clinical trials to date; most of the studies reporting positive effects on neurological outcome applied hypothermia for prolonged periods of time (24–72 hours) [1, 6, 7], or initiated cooling relatively quickly [2]. The findings in clinical studies in patients with traumatic brain injury have been mixed, but a similar link between duration of hypothermia treatment and effects on outcome has also been observed in this area [10, 85]. Another lesson from the animal studies is that quick re-warming can re-trigger the destructive processes outlined above, and should therefore be avoided.

In summary, there are numerous mechanisms underlying hypothermia's protective effects in brain injury, and perhaps myocardial injury. Different destructive mechanisms may play a more or less prominent role in different types of brain injury; in addition, the relative importance of these mechanisms may vary between patients, and even over time within the same patient. A more detailed understanding of these processes will help us to apply hypothermia treatment more effectively. Perhaps in the near future we will be able to measure some or all of the destructive processes in the brain, to determine the required depth and duration of hypothermia treatment and to monitor the effects of our therapy. Currently, measuring ICP provides us with such a monitoring tool, albeit a very rough one. Potential future targets for monitoring may include systemic and local measurement of the levels of various cytokines and other inflammatory parameters, continuous EEG monitoring, measurement of vasoactive mediators such as endothelin, prostaglandin and thromboxane, measuring intracerebral pH and lactate levels, monitoring local brain temperatures, etc.

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Acute Lung Injury

Hyaluronan in Acute Lung Injury

D. A. Quinn and H. G. Garg

Introduction

The extracellular matrix has not been well studied in acute lung injury (ALI). We have found that hyaluronan, an organizer of the extracellular matrix, may play an important role in the pathogenesis of ALI. Using an animal model of ventilator-induced lung injury (VILI), a form of ALI, we have found high production of the low molecular weight (LMW) forms of HA that act as non-protein, non-cytokine inflammatory mediators in the lung. This chapter will review the mechanisms involved in the production of LMW hyaluronan and the mechanisms of LMW hyaluronan-induced inflammation in VILI.

Hyaluronan and Lung Extracellular Matrix

The lung extracellular matrix is composed of several different components, including collagens, elastin, proteoglycans and stromal cells. The extracellular matrix modulates a variety of biological processes through selective binding and subsequent release of growth factors and cytokines, and through its interaction with cell surface receptors [1].

Proteoglycans are a group of macromolecules that make up the ground substance of the extracellular matrix. Proteoglycans consist of a core protein to which one or more glycosaminoglycan chains (GAGs) are attached. The GAGs in the lung include chondroitin sulfate, dermatan sulfate, heparan sulfate and hyaluronan [2]. Hyaluronan is a negatively-charged, linear GAG composed of alternating *N*-acetylglucosamine and glucuronic acid in repeating disaccharide units, without covalently linked protein core. High molecular weight (HMW) hyaluronan (> 500 kDa) is an important component of the lung interstitium and functions to maintain the structural integrity and compliance of the normal lung. LMW hyaluronan (< 500 kDA) can function as an intracellular signaling molecule in inflammation [3, 4]. LMW hyaluronan has been shown to elicit the expression of pro-inflammatory chemokines such as monocyte chemoattractant protein (MCP)-1α, MCP-1β, keratinocyte derived-chemokine (KC), regulated on activation normal T cell expressed and secreted (RANTES) and interleukin (IL)-8 [5, 6]. LMW hyaluronan binding to CD44 has been found to regulate inflammation through the activation of nuclear factor-kappa B (NF-κB) [7].

LMW Hyaluronan Production

LMW hyaluronan may be produced either by *de novo* synthesis or by breakdown of HMW hyaluronan either by oxidants [8] or by hyaluronidase [3]. Hyaluronan is synthesized by hyaluronan synthase (HAS), a protein on the cell surface that links together *N*-acetylglucosamine and glucuronic acid in repeating disaccharide units and excretes long chains into the extracellur space. HAS exists as 3 isoforms (HAS1, HAS2 and HAS3). The isoforms are distinct from each other in their stabilities, the rates at which they cause elongation of hyaluronan, and the range of size distribution of their hyaluronan products. HAS3 forms LMW hyaluronan, while the products of HAS1 and HAS2 are HMW hyaluronans [9]. All three HAS isoforms have been cloned and sequenced [9, 10]. HAS1, 2 and 3 knockout mice have been generated. The HAS2 knockouts were embryonic lethals, due to abnormal cardiac morphogenesis [11]. The mice deficient in HAS1 or 3 are viable, but their phenotypes have not yet been fully established. The role of HAS in ALI has not been previously explored.

Cell Surface Receptors for Hyaluronan

LMW hyaluronan transduces signals by binding to cell membrane receptors. Several hyaluronan receptors have been identified, but the two most studied that have been shown to be involved in cell signaling are RHAMM (receptor for hyaluronan-mediated motility), also identified as CD168, and CD44 [12]. RHAMM has been found on the cell surface, attached to the cytoskeleton, and within the mitochondria and cell nucleus [12]. CD44 is a transmembrane receptor that plays an important role in cell signaling [3]. RHAMM and CD44 selectively activate specific signaling pathways, and thus perform separate functions [12].

The role of CD44 has been previously examined in ALI secondary to bleomycin injury. Following bleomycin treatment, Teder et al. [13] found that CD44 knockout mice had unremitting lung inflammation, whereas their wild type CD44 counterparts developed lung inflammation that subsequently resolved. Besides contributing to LMW hyaluronan-induced cytokine production, CD44 also appeared to have mediated clearance of hyaluronan. In CD44 deficient mice there was both an increased accumulation of hyaluronan fragments and an impaired clearance of apoptotic neutrophils [13]. Therefore, CD44 may play an important role both in the pro-inflammatory actions of LMW hyaluronan and in its resolution over the course of ALI.

Recently, hyaluronan has also been found to bind to Toll-like receptors (TLRs), a group of innate immune receptors belonging to the IL-1 receptor family [14]. In endothelial cells, small hyaluronan fragments (4–16 oligosaccharides) were found to induce IL-8 production by binding to TLR4 [15]. The role of TLRs in lung disease has not been studied so far.

LMW Hyaluronan in Acute Lung Injury

To investigate the role of LMW hyaluronan in ALI, we have used animals models of VILI, a form of ALI. We used mechanical ventilation of the whole lung with large tidal volumes (20 ml/kg) to produce VILI in normal animals [16, 17]. VILI alone

has been shown to cause a form of ALI characterized by pulmonary edema, release of inflammatory cytokines, and subsequent influx of neutrophils. We have developed a rat model of VILI using tidal volumes of 20 ml/kg [17]. Rats were ventilated for 2 hours with either 7 ml/kg or 20 ml/kg and euthanized either immediately following ventilation or 6 hours after ventilation had been stopped, and the rat allowed to recover. Pulmonary edema, as measured by the ratio of wet to dry lung weight, occurred by the end of 2 hours of ventilation with high tidal volumes. The increase in the chemokine MIP-2, (the rodent functional counterpart of IL-8) and neutrophils in the bronchoalveolar (BAL) fluid did not occur immediately but was present 6 hours after the end of ventilation.

Using this model we found that high tidal volume ventilation of normal lungs also caused increased production of LMW hyaluronan [18]. Rats were euthanized immediately after 2 hours of ventilation with tidal volumes of 20 ml/kg, and the lungs were removed for analysis of hyaluronan. The pattern of total hyaluronan lung deposition was determined by hyaluronan binding protein immunohistochemical staining of lung tissue (Fig. 1 a). Hyaluronan staining was greatest in the air-

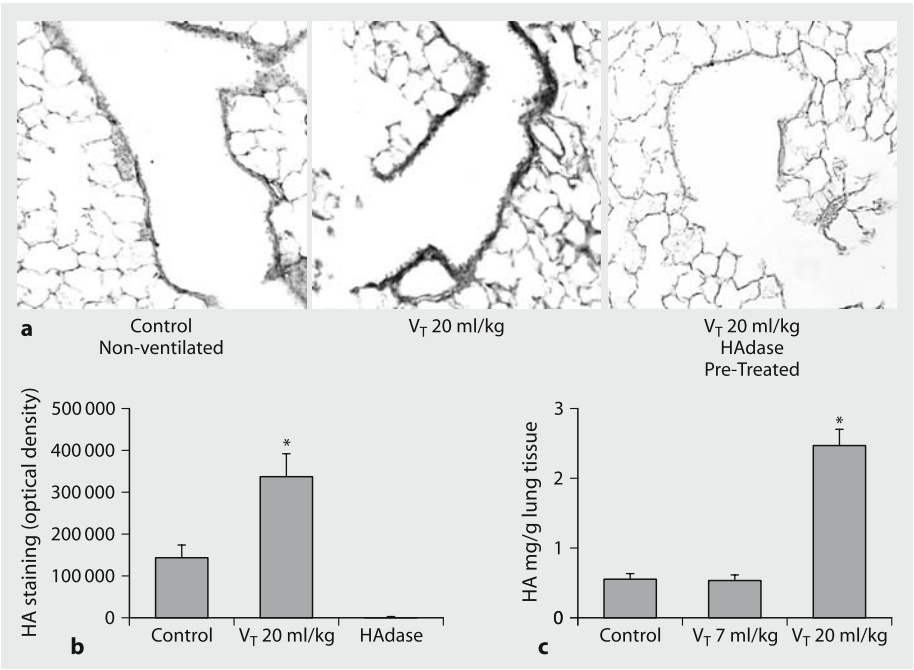

Fig. 1. a Biotinylated hyaluronan binding protein showed increased staining of hyaluronan in paraffin-embedded lung sections from rats ventilated with tidal volume (V_T) 20 ml/kg as compared to control non-ventilated rats. Pretreatment with hyaluronidase (HAdase) eliminated hyaluronan staining, confirming specificity of the hyaluronan stain. **b** The amount of staining was quantified by digital image analysis of 16 random fields per slide. Staining was 2–3 times higher in rats ventilated with tidal volume 20 ml/kg. Control = Non-ventilated. V_T 20 ml/kg = Ventilation for two hours at 85 breaths per minute. HAdase = Slide treated with hyaluronidase prior to staining to remove any hyaluronan on the slide and show specificity of staining. *$p < 0.05$ vs. Control. **c** Ventilation with tidal volume 20 ml/kg significantly increased total hyaluronan production in the lung. *$p < 0.05$ vs. Control and tidal volume 7 ml/kg. (Modified from [18] with permission)

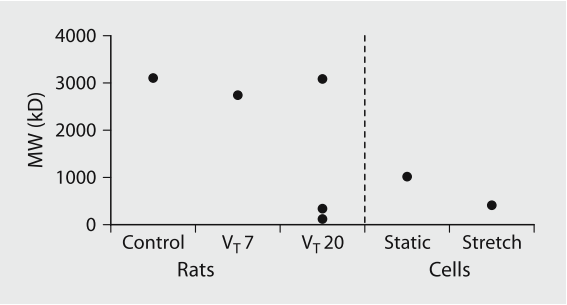

Fig. 2. Molecular weights of hyaluronan. The left side of the graph shows the molecular weights in the ventilated (V_T 7 and V_T 20) and non-ventilated rats (Controls); the right side shows hyaluronan in static and stretched fibroblasts (Cells)

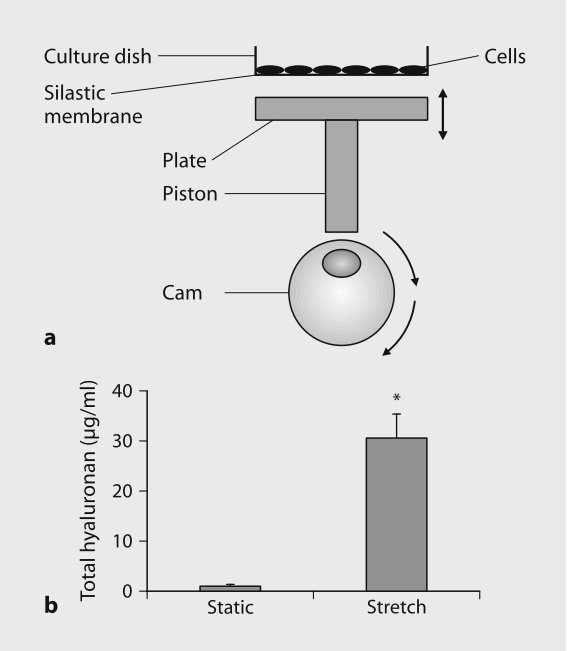

Fig. 3. a *In vitro* model used to apply rhythmic stretch to lung cells. **b** Cyclic stretch of lung fibroblasts caused a significant increase in the total amount of hyaluronan (* $p < 0.001$ vs. Static cells)

ways, but was also increased in the parenchyma. The amount of hyaluronan staining was quantified by microscopic imaging analysis (Fig. 1b). Total amount of hyaluronan was 2–3 times higher in ventilated rats. We confirmed these findings by directly measuring the amount of total hyaluronan in pooled frozen lung tissue from animals ventilated at 7 ml/kg and at 20 ml/kg, and from non-ventilated animals (Fig. 1c). We found there was a significant increase in the amount of total hyaluronan with large tidal volumes [18].

Hyaluronan standards and agarose gel electrophoresis were used to determine the molecular weights of hyaluronan that accumulated in the lungs of animals ventilated at tidal volumes of 20 ml/kg, of animals ventilated at 7 ml/kg, and lungs from control, non-ventilated animals (Fig. 2-Rats). In high tidal volume rat lungs, two LMW forms (180 kDa and 370 kDa) and one HMW form (3100 kDa) of hyaluronan accumulated. This result contrasted with hyaluronan detected in rats venti-

lated at 7 ml/kg (2730 kDa) and in control non-ventilated animals (3100 kDa) in which only the HMW form was found.

To examine the effects of ventilator-induced stretch at the cellular level, we have developed an *in vitro* model of VILI. We used a cell-stretching device that uniformly applies biaxial strain to flexible cell culture membranes (Fig. 3). Primary human fetal lung fibroblasts (IMR 90, Coriell Repository, Camden, NJ) were grown on fibronectin-coated silicone elastomeric membranes and exposed to 15% strain at 60 cycles/min using our cell stretch model. Fibroblasts were used because they are a known source of hyaluronan biosynthesis. *In vitro* cell stretch induced the production of hyaluronan that was readily detected in cell culture supernatant compared to supernatant from control non-stretched cells (Fig. 2). The hyaluronan was analyzed using cellulose acetate electrophoresis of the extract. The presence of hyaluronan in the fraction was confirmed by subsequent treatment of the fraction with hyaluronidase [18]. Stretched human fetal fibroblasts produced LMW hyaluronan (400 kDa), whereas non-stretched fibroblasts produced HMW hyaluronan (1000 kDa) (Fig. 2-cells).

Mechanisms of Cell Stretch-induced LMW Hyaluronan Production

Using our *in vitro* model of mechanical stretch we have confirmed that stretch-induced LMW hyaluronan production is dependent on HAS3 upregulation. We exposed lung fibroblasts to cyclic stretch and measured their subsequent expression of HAS mRNA by reverse transcriptase-polymerase chain reaction (RT-PCR). Stretch upregulated mRNA expression of HAS3, but not HAS1 or HAS2 [18].

LMW Hyaluronan but not HMW Hyaluronan Induced IL-8 Production in Cultured Cells

IL-8 production in static, non-stretched A549 lung epithelial cells was measured in response to LMW and HMW hyaluronans that were purchased from commercial sources (Sigma Chemical Co. 2300 and 370 kDa, and ICN Biochemicals 600 kDA). LMW hyaluronan was isolated from bovine vitreous and HMW hyaluronan was isolated from rooster comb. Cells were exposed for 6 hours. Four hyaluronan standards of molecular weight 2300, 740, 600 and 370 kDa were used. A459 cells were used as a type of lung cell known to produce IL-8. Of the hyaluronan sizes tested, only LMW hyaluronan (< 500 kDa) and HMW hyaluronan significantly increased IL-8 production in a dose-dependent manner that was maximal at 100 ug/ml [18].

To confirm that it was the hyaluronan that was responsible for induction of IL-8 and not a contaminating protein or DNA, specific treatments were employed. Samples were treated with hyaluronidase to remove hyaluronan, DNAse to remove DNA, and heated to denature protein. Treated samples were then assayed for their ability to induce IL-8 production in A549 cells. Only hyaluronidase treatment, and not heat inactivation or DNA degradation, inhibited LMW hyaluronan (370 kDa) induction of IL-8 (Fig. 4). This showed that LMW hyaluronan and not the contaminating protein or DNA was responsible for induction of IL-8. These data support our hypothesis that LMW hyaluronan can stimulate IL-8 production and thus contribute to inflammation in the setting of VILI.

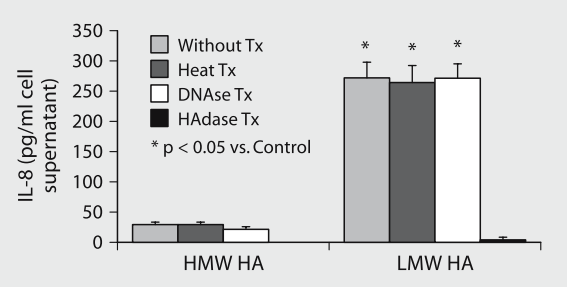

Fig. 4. IL-8 production in A549 cells stimulated with LMW hyaluronan and HMW hyaluronan that had been pre-treated with heat (to denature protein), DNAse (to digest DNA), or hyaluronidase (HAdase, to digest hyaluronan). LMW hyaluronan and not HMW hyaluronan, protein or DNA was thus shown to be responsible for IL-8 production

LMW Hyaluronan and HAS3 in VILI

We further investigated the role of HAS3 and LMW hyaluronan *in vivo*. Although our early *in vivo* data were generated in rats, we were interested in obtaining data from mice, to tap into the understanding of specific gene effects that are afforded by mice, especially via knockout technology. We adapted our rat model of VILI to wild-type mice [19]. We have found that mice are more resistant to the effects of ventilation than rats [17]. Mice did not show a measurable increase in capillary leak until the tidal volume was increased to 30 ml/kg for 5 hours.

With the HAS3 deficient mice, there was no production of LMW hyaluronan with high tidal volume ventilation. The HAS3 KO mice responded to 30 ml/kg ventilation very differently than WT mice [20]. The HAS3 KO mice had significantly less neutrophil infiltration as measured by a myeloperoxidase (MPO) assay (Fig. 5a) or neutrophil count in the BAL (Fig. 5b). They also produced significantly less total hyaluronan (Fig. 5c.) Interestingly, there was no significant difference in the amount of capillary leak as measured by Evans blue dye (Fig. 5d) compared to WT mice. This suggested two important conclusions: that *de novo* synthesis of LMW hyaluronan plays a role in VILI, and that HAS3 is a stretch sensitive enzyme. No other characteristic phenotype of the HAS3 KO mice has been reported previously.

Mechanisms of LMW Hyaluronan-induced Cytokine Production

We hypothesized that LMW hyaluronan produced in VILI induces upregulation of IL-8 via the mitogen-activated protein kinase (MAPK) and NF-κB/IκB pathways. We exposed normal bronchiolar epithelial cells (BEAS-2B, Clonetics, Walkerville, MD), which are important sources of IL-8 production, to LMW hyaluronan from stretched human fetal fibroblasts (IMR-90). LMW hyaluronan increased phosphorylation of JNK (JNK 1 and JNK 2) as early as 15′, an effect that was sustained up to 60′ with no change in the total level of JNK (JNK 1 and JNK 2. LMW hyaluronan increased phosphorylation of ERK 1 but not ERK 2 with no change in total ERK. LMW hyaluronan increased NF-κB expression in the nuclear fraction and decreased

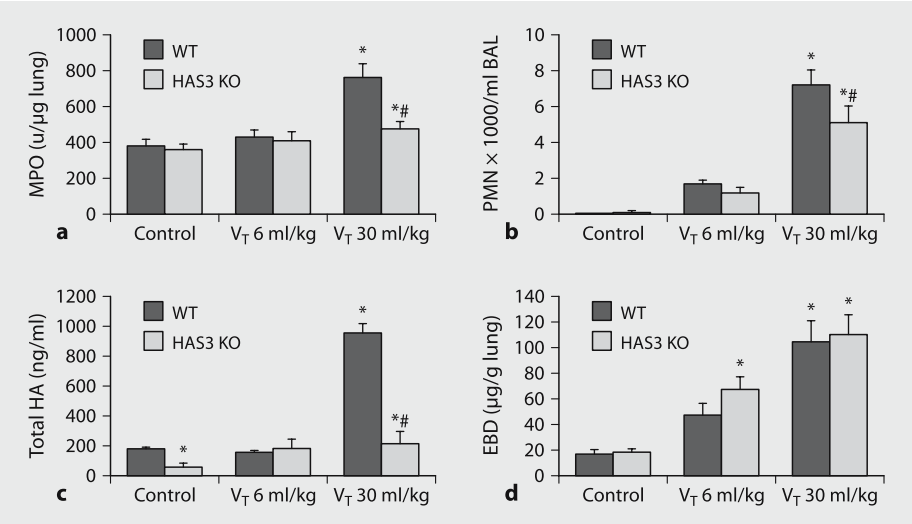

Fig. 5. Effects of high tidal volume ventilation (V_T 30 ml/kg) and low tidal volume ventilation (V_T 6 ml/kg) for 5 hours compared to control non-ventilated mice. Wild type (WT) were compared to HAS3(knock-out) mice. **a** Myeloperoxidase activity (MPO). **b** Neutrophils (PMN)×1000 per ml of BAL fluid. **c** Total hyaluronan (HA) in BAL (ug/ml). **d** Capillary leak as measured by Evans blue dye (EBD ug/g lung). * $p < 0.05$ vs. WT – Control; # $p < 0.05$ vs. WT – V_T 30 ml/kg (Reproduced from [20] with permission)

IκB expression in the cytosol, consistent with NF-κB activation. HMW hyaluronan had no effect on JNK, ERK or NF-κB activation.

To investigate the role of the MAPK and NF-κB in LMW hyaluronan-induced IL-8 production we used specific inhibitors for each. We used the anthrapyrazole JNK inhibitor SP600125, a reversible ATP-competitive inhibitor with greater than 20-fold selectivity versus other kinases in inhibiting the action of p-JNK but not the expression of p-JNK [21]. Using this inhibitor we found that LMW hyaluronan induced IL-8 production was significantly decreased. To inhibit NF-κB activation we used the NF-κB inhibitor. This inhibitor significantly blocked the LMW hyaluronan-induced IL-8 production compared to the NF-κB control peptide that failed to block LMW hyaluronan-induced IL-8 production. There was no significant decrease in IL-8 production using the ERK Inhibitor. Although LMW hyaluronan activated p-ERK, it did not significantly participate in LMW hyaluronan-induced IL-8 production.

Hyaluronan in ALI and Acute Respiratory Distress Syndrome (ARDS)

In BAL fluid from normal human lungs only very low concentrations of hyaluronan are found [22]. However, the total amount of hyaluronan in the BAL fluid increases in several lung diseases and this increase correlates with loss of function and poor prognosis. These diseases include cystic fibrosis [23], asthma [24], alveolar proteinosis [25], sarcoidosis [26], farmer's lung [27] and idiopathic pulmonary fibrosis [28]. Increased amounts of hyaluronan are also found in BAL fluid from smoke-ex-

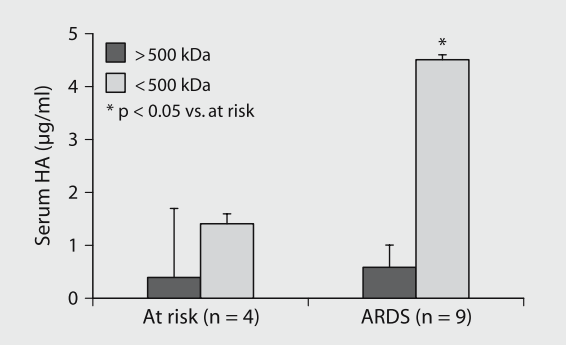

Fig. 6. LMW and HMW hyaluronan in serum of patients at risk for and with ARDS

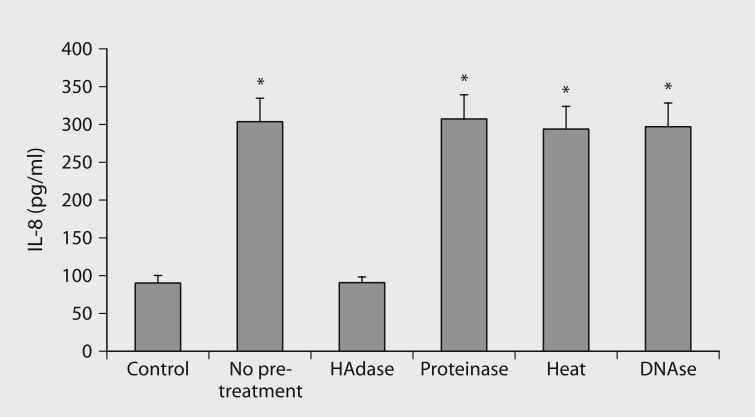

Fig. 7. IL-8 production in human bronchial epithelial cells stimulated with human LMW hyaluronan (100 ug/ml) and in the presence and absence of heat (to denature protein), DNAse (to digest DNA), proteinase (to digest protein) and HAdase (to digest hyaluronan). LMW hyaluronan, and not protein or DNA, was thus shown to be responsible for IL-8 production. Control = No LMW hyaluronan. * $p < 0.05$ vs. Control and hyaluronidase treated

posed firefighters [22] and in lung tissue from rats exposed to smoke from diesel exhaust [29].

Hyaluronan was measured in BAL fluid and serum from 12 patients with ARDS and 28 normal controls [30]. These authors found that the median BAL hyaluronan concentration was 6 times higher in the ARDS patients than normal controls, while the median serum hyaluronan concentration was 20 times higher. In their study, the three patients who died with ARDS had the highest serum hyaluronan concentrations, and two of them also had the highest BAL hyaluronan concentrations. The molecular weight of the hyaluronan present was not determined.

In our laboratory, we investigated the potential role of LMW hyaluronan in humans with ALI and ARDS by measuring the amounts of LMW hyaluronan in BAL fluid and serum. We found LMW hyaluronan in patients with ARDS undergoing bronchoscopy

in our medical intensive care unit (ICU), but not in patients who did not meet criteria for ALI. We then collected blood samples from 4 at risk patients, 9 ALI/ARDS subjects, and 2 healthy controls. Risk factors included: pneumonia, aspiration, sepsis, and intra-abdominal infection. We excluded all subjects with liver failure, steroid use, asthma flares, collagen vascular disease and malignancy, since these factors independently increase hyaluronan production. We found a significant increase in total serum hyaluronan in the patients with ARDS, but not in the patients at risk of ARDS.

We determined the size of the serum hyaluronan in the at risk and ARDS patients using sepharose CL-4B chromatography. We found a significant increase in the amount of LMW hyaluronan (< 500 kDa), but not HMW hyaluronan (> 500 kDa) in ARDS subjects (Fig. 6). In one patient who successfully recovered from ARDS, we observed that the hyaluronan in the serum returned to the HMW form after extubation.

We exposed human bronchial epithelial cells (BEAS-2B, Clonetics, Walkerville, MD), to LMW hyaluronan, which was isolated from the BAL fluid of ARDS patients. LMW hyaluronan isolated from the serum of patients with ARDS caused a significant increase in IL-8 production (Fig. 7), which only hyaluronidase pretreatment prevented, suggesting it was the hyaluronan in the BAL fluid that induced the IL-8 production.

Conclusion

Hyaluronan in HMW forms serves as an organizer of the extracellular matrix of the lung, but LMW forms act as signaling molecules that are involved in the production of inflammatory cytokines. LMW hyaluronan is produced either by breakdown of HMW hyaluronan or by *de novo* synthesis by HAS3. LMW hyaluronan regulates cytokine production through binding to cell surface receptors, CD44, RHAMM and TLRs. We have shown that in VILI, a form of ALI, LMW hyaluronan is produced through upregulation of HAS3 and plays an important role in production of chemokines and neutrophil infiltration found in VILI. LMW hyaluronan-induced IL-8 production in lung cells is dependent on NF-κB and JNK/AP-1 activation. Our clinical studies of LMW hyaluronan in patients at risk of ALI and patients with ALI/ARDS suggest that LMW hyaluronan may play an important role in the pathogenesis of ALI/ARDS and may offer targets for new treatment modalities.

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Aspiration-induced Lung Injury: Experimental and Human Studies

M. A. Matthay, G. Mednick, and Z. A. Matthay

Introduction

Aspiration of gastric and oropharyngeal contents is an important clinical cause of acute lung injury (ALI) that has been recognized for more than 50 years. Epidemiologic studies of ALI, as well as clinical trials, have identified aspiration-induced lung injury as one of the major causes of ALI and the acute respiratory distress syndrome (ARDS) [1, 2]. The most important etiological factor for aspiration-related lung injury is a depressed level of consciousness [3]. One group of investigators found that an altered level of consciousness was the major predisposing factor in 68% of cases [4]. Like other clinical disorders that predispose patients to the development of ALI, aspiration of gastric contents may be associated with other clinical risk factors including primary pneumonia, sepsis, hypotension, and drug overdose [2].

Various experimental studies have been done to understand the pathogenesis and pathophysiology of aspiration-induced lung injury. Most of these studies have used low pH hydrochloric acid solutions to simulate the clinical problem of aspiration of gastric contents. The studies have been done in large and small animal models and considerable information has been derived, some of which has substantial clinical relevance.

The purpose of this chapter will be to review some of the experimental studies that have provided insights into the mechanisms of aspiration-induced lung injury. The second purpose will be to identify the potential utility of the experimental models for relevance to clinical setting. The third purpose is to consider the results of these studies for their potential application to clinical treatment strategies for aspiration-induced lung injury.

Experimental Models

Experimental studies have indicated that the instillation of low pH hydrochloric acid solutions results in a dose related and pH-dependent ALI. The severity of the injury is directly related to three variables: (1) the acidity of the instilled fluid, (2) the volume of instilled fluid, and (3) the tonicity of the fluid. Hypotonic fluids cause more lung injury than isotonic fluids. Gastric contents have approximately one-third the osmolality of plasma.

Most clinical and experimental studies demonstrate that there is an initial lung injury associated with airway constriction, arterial hypoxemia, and the develop-

Table 1. Effect of prior hydrochloric acid (HCl) aspiration on the severity of bacterial induced lung injury in mice. Data from [6]

Experimental End Points	Saline/Pneumonia	HCl/Pneumonia
Lung weight (mg of lung tissue/g of mouse)	8.0 ± 0.3	14 ± 1.5*
Neutrophils (10^6/ml of BAL fluid)	1.8 ± 0.2	7.5 ± 1.8*
Protein (μg/ml of BAL fluid)	220 ± 15	800 ± 150*
TNF-α (pg/ml of BAL fluid)	67 ± 6	734 ± 278*
Il-1β (pg/ml of BAL fluid)	2014 ± 585	21,786 ± 5789*

BAL: bronchoalveolar lavage; TNF: tumor necrosis factor; IL: interleukin; data as mean ± SD; * $p < 0.05$

ment of pulmonary edema. In some experimental models, there is evolution of the acid-induced lung injury with the magnitude of the lung injury becoming maximal by approximately 6–8 hours after the instillation of acid [5]. As will be discussed in a later section, the tidal volume and airway pressures that are used to ventilate the animals exert an important influence on the severity of the ALI.

Most studies have been done in anesthetized, ventilated animals, although some studies have been done in non-anesthetized spontaneously breathing animals. The use of genetically modified mice has made it possible to explore the contribution of several specific molecular pathways to the pathophysiology of ALI from acid installation.

One advantage of experimental studies is that it is possible to study the low pH and hypotonic fluid aspiration that occurs in humans. However, some of the features of human aspiration-induced lung injury are incompletely modeled because humans may aspirate material from their upper gastrointestinal tract that has particulate matter. Also, humans may aspirate aerobic and anaerobic bacteria. Usually, experimental models do not include this latter variable in the study design. Also, in most animal models, the co-morbidities that exist in patients, including chronic liver disease, diabetes mellitus, recent surgery, or altered neurologic status, are not replicated. One recent experimental study showed that lung injury is markedly worse in animals if there is initially a sub-lethal aspiration followed 24 hours later by instillation of Gram-negative bacteria (Table 1) [6]. In spite of these limitations, the animal models have been useful for understanding basic mechanisms of lung endothelial and epithelial injury.

Pathogenesis

Several studies have indicated a primary role for the neutrophil as an important cellular component that mediates much of the lung injury from acid instillation. In most of these studies, neutrophil depletion or blockade of neutrophil influx into the lung markedly attenuates lung injury [5, 7]. In addition, there are convincing

data that neutrophil chemotactic factors that attract neutrophils to the airspaces of the lung, primarily interleukin-8 (IL-8), are important mediators of lung injury. IL-8 has been proposed as a major chemotactic factor for recruitment of neutrophils to extravascular sites of inflammation, including those in the lung. Not only is IL-8 a potent chemoattractant for neutrophils, it also up-regulates neutrophil β_2 adhesion receptors on the endothelium, mediates neutrophil migration across activated endothelium, and primes neutrophils for activation. IL-8 is also known to be produced by several cells in the lung, including alveolar macrophages, alveolar type II epithelial cells, bronchial epithelial cells, and pulmonary fibroblasts. Interestingly, there is also good evidence from clinical studies that IL-8 levels in pulmonary edema and bronchoalveolar lavage (BAL) fluid are higher in patients with ALI [8, 9]. In one single center study, there was evidence that elevated IL-8 concentrations may be associated with worse clinical outcomes [10]. In a more recent large multicenter study, elevated plasma IL-8 levels at baseline were associated with a higher risk of death, fewer ventilator free days, and fewer organ failure free days [11].

The effectiveness of anti-IL-8 monoclonal antibody therapy has been demonstrated in experimental studies in rabbits. Neutralization of IL-8 was effective both as a prevention or treatment given one hour after the acid instillation (Fig. 1) [5]. Subsequent studies demonstrated that alveolar epithelial injury in acid-induced lung injury was also mediated in part by IL-8 dependent mechanisms [12]. There is also evidence that neutralization of tumor necrosis factor (TNF)-α can attenuate lung injury for acid aspiration [13]. TNF-α is a major proximal cytokine that can lead to the production of IL-8 by several cells. TNF-α appears earlier than IL-8 in the acute inflammatory cascade.

Inhibition of CD18 or CD11b can be effective in reducing experimental lung injury. In one study, inhibition of these two adhesion molecules reduced lung injury [14]. Interestingly, the beneficial effect was not associated with a reduction in the number of neutrophils in the airspaces, thus suggesting that the neutrophils responded normally to the chemotactic stimuli, but that the neutrophils that were present in the lung did not injure the lung, presumably because they were less activated. Prior investigators had reported that acid-induced lung injury resulted in CD18 independent neutrophil influx in the airspaces [15].

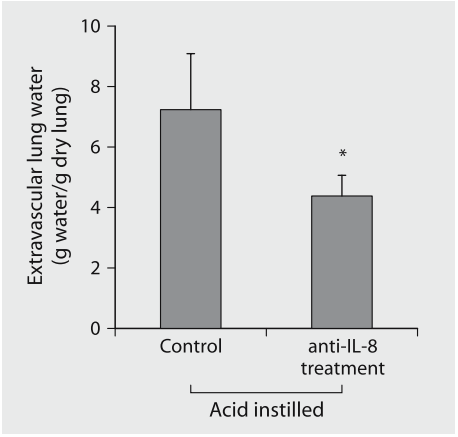

Fig. 1. Extravascular lung water was significantly reduced in rabbits with acid induced lung injury by anti-IL-8 monoclonal antibody treatment. Data shown as mean ± SD; * $p < 0.05$. Data from [5].

One study reported that antibodies to intercellular adhesion molecule-1 (ICAM-1), when administered intratracheally, can both prevent and treat acid-induced lung injury in rats [16]. Anti-ICAM-1 treatment significantly inhibits neutrophil accumulation in the lung and results in improvement in gas exchange and the mechanical properties of the lung. The magnitude of pulmonary edema was also decreased and there was less histologic evidence of lung injury.

Other experimental treatments have been effective in modifying the severity of ALI. Most of the treatments were focused on attenuating the severity of lung endothelial injury. These therapies include hyperosmolar therapy [17] and pre-treatment with keratinocyte growth factor [18].

There is also evidence that injury to the alveolar epithelium can be monitored by the release of a type I cell antigen, RT140. This finding was demonstrated in experimental studies in acid-induced lung injury in rats [19]. More recent work indicates that another type I cell marker may be useful for monitoring lung injury in rats, specifically the receptor for advanced glycation end products (RAGE). In preliminary experiments from our laboratory, administration of nicotine to rats prior to intratracheal instillation of hydrochloric acid (pH 1.2) markedly attenuated the accumulation of protein in the BAL fluid compared to the control rats treated with saline. Nicotine has been shown to reduce mortality from sepsis in mice, apparently from anti-inflammatory effects mediated by the α_7 nicotinic receptor [20]. In these rat studies, there was also a marked reduction in the accumulation of RAGE. The quantity of RAGE was 50% less in the BAL fluid of the nicotine treated versus saline-treated control rats [21]. These studies also demonstrated a reduction in the quantity of BAL protein in the nicotine-treated rats. Thus RAGE concentrations seemed to parallel the severity of lung injury, suggesting it may be useful as a marker of type I cell injury. In separate preliminary data, RAGE levels were higher in the pulmonary edema fluid from patients with ALI than in control patients with hydrostatic pulmonary edema [22].

Treatment Strategies

One of the more interesting potential therapeutic approaches is the use of aerosolized β_2-adrenergic agonist treatment. Recent experimental work has demonstrated that the use of β_2-agonist therapy can reduce the severity of lung endothelial injury and upregulate the active ion transport mechanisms that are responsible for the removal of edema fluid from the distal airspaces in the lung (Table 2) [23]. These

Table 2. Excess lung water in salmeterol versus placebo treated acid instilled rats. Data from [23]

Experimental Condition	Excess Lung Water (ul)
Saline (2 h)	420 ± 60
Salmeterol (2 h)	410 ± 80
Saline (4 h)	300 ± 60
Salmeterol (4 h)	190 ± 30 *

Data as mean ± SD

* $p < 0.05$

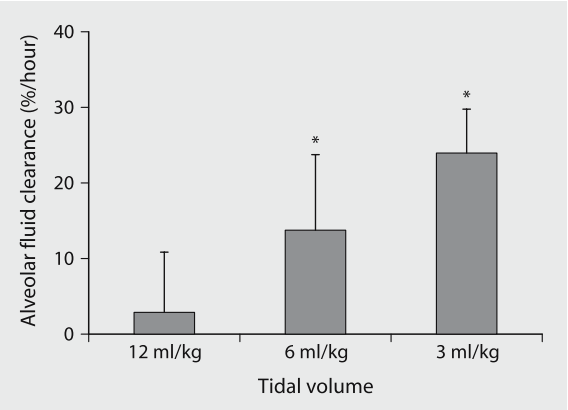

Fig. 2. Alveolar fluid clearance was significantly higher in acid injured rat lungs when they were ventilated with lower tidal volumes. Data as mean ± SD. *$p < 0.05$ by ANOVA. Data from [19].

studies were done in rats over 4 hours. The beneficial effects of the β_2-agonist therapy were not evident at 2 hours but became statistically significant at 4 hours (Table 2).

Furthermore, recent work also indicates that ventilation of acid-induced rat lungs with lower tidal volumes reduces the severity of lung injury in a dose-dependent fashion. Ventilation with 12 ml/kg tidal volume results in severe lung injury in rats but ventilation with a 6 ml/kg tidal volume reduces the injury by approximately 50%. A reduction of the tidal volume to 3 ml/kg resulted in a further reduction in lung injury. The additional improvement with the lowest tidal volume, 3 ml/kg, was explained by improved alveolar epithelial fluid transport function (Fig. 2) [23]. These studies parallel the results of the NHLBI ARDS Network trial in which low tidal volume ventilation decreased mortality in patients with ALI [24].

Conclusion

For clinical management of patients with aspiration-induced lung injury, the first proven treatment is for the patient to receive a lung protective ventilation strategy with a low tidal volume (6 ml/kg/ideal body weight) and a plateau airway pressure < 30 cmH_2O [24, 25]. Clinical application of the results of experimental studies with anti-inflammatory treatments needs to be tested in the clinical setting. In particular, strategies to reduce the biologic activity of IL-8 in the airspaces of the lung are promising, providing that they do not increase the risk of lung infection. The use of aerosolized β_2-agonist therapy is promising because it can both decrease lung endothelial permeability and increase the resolution of alveolar edema. One clinical study demonstrated that therapeutic levels of β_2-agonists in pulmonary edema fluid can be achieved with standard aerosolization in intubated, ventilated patients with ALI [26]. Another strategy to be considered is keratinocyte growth factor (KGF), although experimental studies have demonstrated benefit only when KGF is given as prevention [18]. Nevertheless, KFG might be of value in reducing lung injury and enhancing alveolar epithelial repair, especially if a method were developed to administer KGF directly into the lungs.

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Pulmonary Edema in Organ Donors and Lung Transplant Recipients: Is there a Role for Beta-adrenergic Agonists?

M. B. Covarrubias and L. B. Ware

Introduction

Lung transplantation is a therapeutic option for patients with end-stage lung or pulmonary vascular disease with an average of 1700 bilateral and single lung transplants performed each year in the United States. Since the 1980s, survival rates for lung transplantation have improved due to advances in surgical techniques, availability of newer immunosuppressive regimens, and enhanced post-operative management. Despite better outcomes, low donor lung utilization rates and a high incidence of primary graft failure are challenges that limit the availability of donor lungs and lung transplant recipient survival, respectively. The objectives of this review are as follows:

- to summarize the condition of lungs that are rejected for transplantation using current donor lung selection criteria,
- to outline the mechanisms of pulmonary edema in the brain dead organ donor,
- to outline the mechanisms of primary graft failure in lung transplant recipients,
- to explain the importance of alveolar fluid clearance in resolution of pulmonary edema, and
- to discuss potential therapeutic interventions, specifically β-agonists, for attenuating pulmonary edema in organ donor and lung recipients.

Donor Selection Criteria – are we Missing Good Lungs?

In 2003, the lung transplant waiting list in the United States reached a record high of 3836 registrants, up more than threefold since 1994 [1]. Despite the burgeoning waiting list, the number of lung transplants performed each year has remained relatively stable in the United States since the late 1990s [2]. The lack of growth in the number of lung transplants performed can be attributed to the shortage of donor lungs, leading to prolonged waiting times and high mortality among those on the waiting list [3]. The current donor lung utilization rate in the United States is less than 15%, and most donors are not accepted because they fail to meet predetermined selection criteria [4]. These criteria have not been prospectively studied, and although broadening the selection criteria has been advocated, it has not been widely adopted. Liberalizing criteria to age >55, smoking history >20 pack-years, or allowing for prolonged mechanical ventilation in the donor has been done by several centers without significant adverse clinical outcomes in a series of retrospective studies [5–9]. Duration of mechanical ventilation, length of intensive care

unit (ICU) stay, and 30-day mortality did not differ significantly between recipients receiving lungs from donors meeting 'extended' selection criteria versus lungs procured from donors satisfying 'standard' selection criteria. Other strategies aimed at increasing the supply of donor lungs include improving the rate of consent for organ donation, implementing living lobar transplantation, procuring lungs from non-heart beating organ donors (post-cardiac arrest), and developing aggressive donor management strategies [4].

We recently reported that some lungs that were rejected for transplantation may have been suitable as potential grafts [10]. Twenty-nine pairs of rejected lungs were assessed in our laboratory with physiological, microbiological, and histological methods. The most common reasons cited for rejecting donor lungs in this study were poor oxygenation, a smoking history greater than 20 pack-years, infiltrates on chest radiograph, or suspected infection, sepsis, or aspiration. When all factors for appropriate lung donor selection were taken into account (absent or mild pulmonary edema, intact alveolar fluid clearance, normal or mildly abnormal histology, negative cultures) approximately 41% of rejected lungs in this study would have been potentially suitable for transplantation. Since donor oxygenation was a common cause for rejection of these lungs, we then asked whether there was a reversible cause for hypoxemia evident in these donor lungs. Based on gravimetric lung water measurements, only 25% of donor lungs had no evidence of pulmonary edema (Fig. 1). Approximately 60% had mild pulmonary edema and the remainder had moderate or severe pulmonary edema. Arterial oxygenation in the donor was associated with the degree of pulmonary edema (Fig. 2). Donors with better oxygenation had significantly less pulmonary edema, while donors with worse oxygenation had more pulmonary edema as measured by lung water-to-dry weight ratios (4.4 versus 5.1, $p = 0.008$). These findings suggest that therapies aimed at improving donor oxygenation by ameliorating pulmonary edema might significantly improve donor lung utilization rates.

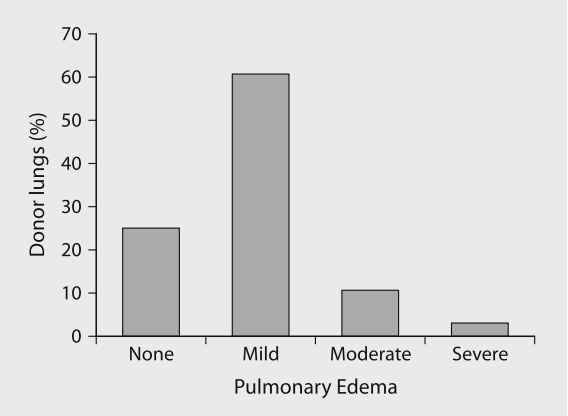

Fig. 1. Severity of pulmonary edema as measured by the lung water-to-dry weight ratio in lungs resected from 29 organ donors whose lungs were rejected for transplantation. Normal = < 4.2, Mild = 4.2–5.0, Moderate = 5.1–6.0, and severe > 6 grams water/grams dry weight (From [10] with permission)

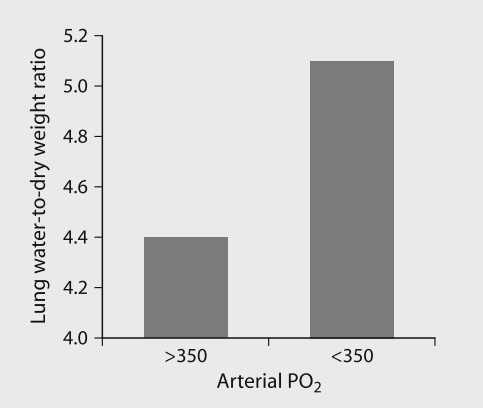

Fig. 2. Relationship between arterial PO_2 on F_iO_2 1.0 and lung water-to-dry weight ratio in lungs from 29 donors whose lungs were rejected for transplantation (From [10] with permission)

Mechanisms of Pulmonary Edema in Brain Dead Organ Donors

Despite new strategies to increase the pool of available donor lungs, brain dead organ donors remain the major source of lung grafts. Pulmonary edema is a common cause of hypoxemia in organ donors, which prohibits the use of lungs as potential grafts. The process of brain death appears to contribute to the development of pulmonary edema through a complex interaction of sympathetic, hemodynamic, and inflammatory changes [11–12]. Activation of α and β adrenoreceptors likely causes neurogenic pulmonary edema by increasing peripheral and pulmonary venous constriction with subsequent elevations in pulmonary capillary pressures. The increase in pulmonary capillary pressure damages the lung microvascular endothelium and disrupts the basement membrane resulting in extravasation of fluid and protein into the airspaces [12]. Concurrently, brain death also induces an inflammatory response, but the exact mechanism is unknown [11]. In humans, blood levels of interleukin (IL)-8 are significantly higher in brain dead compared to ventilated non-brain dead patients [11]. Furthermore, high levels of IL-8 in donor bronchoalveolar lavage (BAL) fluid specimens are associated with primary graft failure and higher rates of early mortality following lung transplantation [13]. Minimizing the donor's inflammatory response prior to lung procurement may improve early outcomes after lung transplantation and increase the lung utilization rate from the existing donor pool.

Investigations to determine if pulmonary edema in patients with neurologic injury is hydrostatic (increased hydrostatic pressures, low protein) or proteinaceous (increased permeability, high protein) have been reported. Smith and Matthay conducted a retrospective review of 12 patients who had pulmonary edema fluid, obtained after the onset of clinical pulmonary edema following a sudden neurologic event, for measurement of edema fluid-to-plasma protein ratio, a measure of alveolar capillary barrier permeability. Patients with a prior history of cardiac disease were excluded [14]. Although a range of edema fluid-to-plasma protein ratios was observed, the most common form of neurogenic pulmonary edema was hydrostatic. Interestingly, the edema fluid-to-plasma protein ratio was associated with the length of time between the initial clinical manifestation of pulmonary edema and collection of pulmonary edema fluid. The shorter the time interval, the more likely that

the edema fluid to plasma protein ratio would be low, consistent with hydrostatic edema, while a longer interval produced fluid that was more proteinaceous. This observation may be explained by active transport of salt and water from the alveoli (alveolar fluid clearance) over time. The data from this study provide evidence that neurally mediated pulmonary venoconstriction is a possible mechanism for the development of neurogenic pulmonary edema in humans [14].

A recent study in a rabbit model found that massive brain injury may additionally predispose potential donor lungs to ventilator-induced lung injury [15]. Although initially similar to control lungs, lungs in brain injured rabbits demonstrated significantly greater changes in ultrafiltration coefficient, weight gain, and alveolar hemorrhage. These changes occurred within half an hour of being exposed to mechanical ventilation using peak airway pressures of 30 cmH_2O and a positive end-expiratory pressure (PEEP) of 5 cmH_2O. The authors suggest that mechanical ventilation may cause or exacerbate acute lung injury (ALI) if alveolar overdistention and repeated alveolar collapse occurs. They emphasize that even if the distending pressures seem harmless in the short term, they may be high enough to promote lung injury if exposure to a sufficient number of cycles occurs in a lung that may be predisposed to injury. If these lungs are then used for transplantation, then the lung recipient may be at higher risk of developing primary graft failure. The major limitation of this study is that the lungs were ventilated after removal from the thoracic cavity so studies in intact animal models are warranted. If intact animal models demonstrate the same result, then the use of protective ventilatory strategies, as implemented in the acute respiratory distress syndrome (ARDS), may be a potential tool to decrease the incidence of primary graft failure in lung recipients.

Primary Graft Failure in Lung Transplant Recipients

Primary graft failure is a common cause of early death after lung transplantation with an incidence of 15–25% and mortality of 28.9% at 30 days [2, 16]. Primary graft failure is histologically and pathophysiologically identical to ALI and ARDS. Onset is usually within the first 72 hours after lung transplantation and is characterized by diffuse alveolar damage and pulmonary edema in the lung allograft, arterial hypoxemia and acute respiratory failure [17]. Ischemia-reperfusion injury is probably the predominant cause of primary graft failure, however, other events occurring in the donor prior to lung procurement may contribute to lung injury including aspiration, pneumonia, mechanical ventilation, cold ischemic storage, brain death, hypotension, and trauma [17]. A number of clinical predictors of primary graft failure have been identified. Risk factors include a recipient diagnosis of primary pulmonary hypertension, donor African-American race, donor female gender, and donor age [18]. Clinical predictors of mortality from primary graft failure in lung recipients include age, the severity of the gas exchange impairment, graft ischemic time, and presence of early hemodynamic compromise [19]. Primary graft failure also has significant impact on hospital length of stay, duration of mechanical ventilation, and length of time to recovery of physical function [16]. Moreover, this form of ALI is a risk factor for bronchiolitis obliterans syndrome, the major complication that limits long-term survival following lung transplantation [20].

Alveolar Fluid Clearance

Active sodium transport is the primary mechanism that drives fluid clearance from the distal air spaces of the lung [21]. Studies of alveolar fluid clearance in the *ex vivo* human lung demonstrate that fluid clearance occurs primarily via the active transport of Na^+ via Na^+ channels on the apical membrane of alveolar epithelial type II cells that is driven by the Na^+-K^+-ATPase on the basolateral membrane. Although aquaporins are present in the lung, there is no evidence that these channels regulate fluid clearance [21]. In animal studies, alveolar fluid clearance has been confirmed to be driven by active transport mechanisms. However, the basal rate of fluid clearance differs among species. This difference in the rate of fluid clearance may be attributable to the number or activity of apical Na^+ channels or the density of the Na^+-K^+-ATPase in the alveolar epithelium among species [21].

In humans, the rate of alveolar fluid clearance can be measured by aspirating serial samples of undiluted pulmonary edema fluid from mechanically ventilated patients with pulmonary edema. The rate of alveolar fluid clearance can be calculated from the change in protein concentration in the edema fluid over time [22]. Faster rates of alveolar fluid clearance are associated with more rapid improvements in oxygenation, a shorter duration of mechanical ventilation, increased survival in patients with ALI and ARDS, and improved oxygenation in patients with hydrostatic pulmonary edema [22–24]. Additionally, in patients with reperfusion pulmonary edema after lung transplantation, patients with intact alveolar fluid clearance had more rapid resolution of radiographic infiltrates, less severe histological abnormalities, more rapid improvement in oxygenation, and a shorter duration of mechanical ventilation [25]. Thus, alveolar fluid clearance is an important variable in determining clinical outcomes across a wide variety of critically ill patients with acute pulmonary edema including those following transplantation.

Role of β-Adrenergic Agonists in Accelerating Alveolar Fluid Clearance

Although several pharmacologic agents have been shown to increase the rate of alveolar fluid clearance and to enhance the resolution of pulmonary edema, inhaled β-adrenergic agonists are the most appealing for clinical application because of their ease of administration and lack of major side effects [21]. In experimental studies, β-agonists accelerated the rate of alveolar fluid clearance in a variety of animal models and in the *ex vivo* human lung [21, 26–27]. We recently studied the rate of alveolar fluid clearance with or without the β-agonist terbutaline (10^{-6} M) in 31 rewarmed, resected human lungs from donors whose lungs were rejected for transplantation [28]. The rate of alveolar fluid clearance was measured by instilling 5% albumin into a subsegment of an isolated human donor lung that had been resected, transported at 4 °C, and rewarmed to 37 °C. Samples of the instillate were sequentially aspirated for measurement of alveolar fluid clearance. In the terbutaline group, mean alveolar fluid clearance was $7.1 \pm 1.3\%$ per hour in the first hour, significantly higher than the mean alveolar fluid clearance of $4.8 \pm 2.4\%$ per hour in the control group (p 0.05, Fig. 3). These findings indicate that alveolar fluid clearance can be stimulated in the donor lung with 10^{-6} M terbutaline, a concentration that is on the plateau of the dose response curve for upregulating alveolar fluid clearance in the *ex vivo* human lung [26]. Albuterol, administered in standard

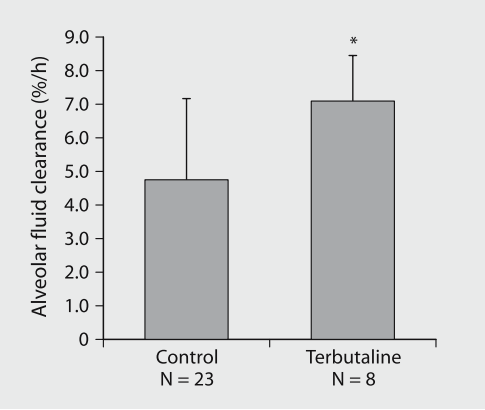

Fig. 3. Terbutaline stimulates alveolar fluid clearance in the *ex vivo* human donor lung. Lungs were harvested from brain-dead organ donors, cooled to 4 °C, and transported. After lungs were rewarmed to 37 °C, alveolar fluid clearance was measured with or without the addition of 10^{-4} M terbutaline. Values are means + SD; *N* = no. of donors. **P* < 0.01. (From [28] with permission)

doses through a ventilator circuit, reaches the distal airspaces in patients with acute pulmonary edema in similar concentrations with levels in the 10^{-6} M range measured in the pulmonary edema fluid [29]. Until recently, there have been no clinical studies of β-agonists in patients with acute pulmonary edema. In a small study of patients with ALI that has only been presented in abstract form, intravenous salbutamol significantly decreased extravascular lung water compared to placebo, with a trend towards improved survival [30].

In addition to upregulation of alveolar fluid clearance, β-agonists may have other beneficial effects in the lung including anti-inflammatory properties that decrease lung endothelial damage and microvascular permeability [31]. Although the majority of evidence for the anti-inflammatory effects of β-agonists has come from animal studies, a recent human study confirmed these findings. Maris et al. reported that inhaled salmeterol attenuated the pro-inflammatory response in the lung to inhaled endotoxin in normal human volunteers including decreased neutrophil influx, decreased neutrophil degranulation, and decreased release of pro-inflammatory cytokines [32]. Thus, inhaled β-agonists have a number of properties that may be therapeutic for both organ donors and lung recipients with acute pulmonary edema.

In order to test the hypothesis that inhaled β-agonists can improve pulmonary edema, donor oxygenation and donor lung utilization in organ donors, we have recently undertaken a randomized double blind placebo controlled study of inhaled albuterol versus placebo in 500 consecutive organ donors managed by the California Transplant Donor Network, the organ procurement organization that serves Northern California. This study has just begun enrolling donors and should be completed in 2 years.

Conclusion

Pulmonary edema is a common problem in both brain dead organ donors and lung transplant recipients. Based on prior studies of the physiology of the donor lung, there is strong scientific rationale for rigorously testing a strategy aimed at accelerating alveolar fluid clearance and reducing pulmonary edema in organ donors. Use of β-agonists to accelerate alveolar fluid clearance in lung recipients might also be therapeutic but further study is needed. Other therapeutic options that warrant investigation include protective ventilatory strategies, diuretics, anti-inflammatory agents, and medications or preservation techniques that preserve or stimulate alveolar fluid clearance in the organ donor. Along with improving donor lung utilization rates, these measures might result in better lung transplant recipient outcomes.

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Respiratory Support

Current Concepts of Airway Management in the ICU and the Emergency Department

C. Byhahn, D. Meininger, and B. Zwissler

Introduction

Effective airway management is a central part of emergency medicine, and many consider it as an undisputable core skill for emergency physicians [1]. The failure to establish and maintain adequate gas exchange can be catastrophic and may have important medicolegal implications. Emergency airway management in the field and in the emergency department is often challenging for the physician in charge. Trauma patients pose specific airway problems: unfavorable conditions (e.g., darkness, inadequate space, limited access to the airway, poor patient positioning, unknown assisting personnel with different levels of training, etc.) contribute to failed endotracheal intubation as much as patient peculiarities, e.g., oropharyngeal or pulmonary hemorrhage, facial trauma, or immobilized cervical spine. All these factors, as well as poor skills of physicians themselves, result in a difficult airway in 7–10% of patients who require emergency endotracheal intubation in the field or in the emergency department [2–4].

Airway management in the intensive care unit (ICU) can also be challenging, but mostly for reasons other than merely establishing an airway. Particular lung diseases or ventilation strategies require lung separation, either to prevent spread from the diseased to the healthy lung (e.g., blood, pus, etc.), or to independently ventilate both lungs with different ventilation strategies (e.g., after unilateral lung transplantation).

Regardless of the fact that, during the last year, a number of clinical studies and a recent meta-analysis [5] have advocated performing early tracheostomy within the first 48 hrs of intubation, delayed tracheostomy on days 7–10 of intubation is still the prevailing practice of most intensivists. Apart from new aspects in tracheostomy timing, another innovative tracheostomy technique is recently being introduced – balloon facilitated percutaneous tracheostomy.

The Unanticipated Difficult Airway during Emergency Endotracheal Intubation

Endotracheal intubation is classified as difficult when successful placement of an endotracheal tube by conventional laryngoscopy a) requires more than three attempts or b) takes longer than 10 minutes. While the prevalence of difficult intubation ranges from 3.0–8.5% in elective patients, emergency endotracheal intubation is more likely to be difficult (7–10%), regardless of whether intubation is attempted

Table 1. Alternative devices for managing the difficult airway

Common supraglottic airways
▮ Laryngeal mask airway
▮ Intubating laryngeal mask airway
▮ Laryngeal tube
▮ Esophageal tracheal combitube
Different laryngoscope blades
▮ McCoy laryngoscope
▮ Dörges universal laryngoscope blade
▮ Straight blades (Miller, Foregger, Philipps, Henderson)
▮ Videolaryngoscopy
Fiberoptic devices
▮ Bonfils intubation fiberscope
▮ Bullard laryngoscope
▮ Flexible fiberoptic bronchoscopy

in the prehospital or hospital environment. Several factors have been identified contributing to this higher prevalence. Amongst them are lack of practice, limited availability of alternative airways, difficult environmental conditions such as poor lighting, unfavorable patient positioning and poor accessibility of the airway (e.g., in entrapped patients). In addition, patient-related challenges, such as facial trauma, soft-tissue injuries of the neck, suspected cervical spine injuries, and oropharyngeal or pulmonary bleeding can lead to a difficult airway situation. Indications for emergency endotracheal intubation in Central Europe are mostly cardiorespiratory disorders (80% of cases) [6]. The most frequent indication for airway intervention is cardiac arrest.

Most endotracheal intubations in the emergency setting are performed successfully. In a small percentage of cases, an airway cannot be secured. When this happens, emergency physicians may find themselves in a dangerous situation. The ability to quickly apply a rescue technique to establish an airway is essential to prevent hypoxemia and subsequent death. A variety of techniques has become available either to sufficiently insufflate oxygen, or to intubate the patient's trachea with devices other than a standard laryngoscope (Table 1).

Bag-Valve-Mask Ventilation

Bag-valve-mask (BVM) ventilation provides immediate ventilation and oxygenation. In elective patients, the incidence of difficult BVM ventilation – defined as an arterial oxygenation $<90\%$ during ventilation with 100% oxygen – is 5%, whereas impossible mask ventilation is very rare ($<0.1\%$) [7]. Under emergency conditions, however, BVM ventilation performed by both emergency physicians and health-care professionals may be insufficient due to lack of experience with this technique, resulting in an increased risk of gastric inflation with subsequent regurgitation and pulmonary aspiration. The incidence of significant pulmonary aspiration was 12.4% and 29.0%, respectively, under BVM ventilation in cardiac arrest victims [8, 9]. Beyond poor manual skills and too large tidal volumes, a sudden decrease of the lower esophageal sphincter tone after cardiac arrest (from a normal of 20 cmH_2O to less than 5 cmH_2O within 6 minutes after cardiac arrest) leads to gas-

tric regurgitation [10]. BVM ventilation should therefore only be used as an initial manner to buy time until a more appropriate airway management technique is available and finally established.

Supraglottic Airways

Laryngeal Mask Airway

A variety of supraglottic airway devices has become available in recent years (Table 1). The most popular and best evaluated item is the laryngeal mask airway (LMA), which was invented in 1985 [11] and is used in many anesthetic procedures. Beyond its routine use in the operating room, numerous studies have documented the usefulness and efficacy of the LMA in airway emergencies and under difficult conditions, such as cardiac arrest, in trauma patients, and even its efficacy and safety when used by non-physicians [12]. The LMA does not protect the trachea from aspiration of regurgitated gastric contents, but has been demonstrated to lower the incidence of gastric insufflation, regurgitation, and significant pulmonary aspiration when compared to BVM ventilation. Its insertion is easier to learn than endotracheal intubation, can be taught successfully on mannequins, and is superior to BVM in delivering adequate ventilation. Success rates of insertion are $>95\%$, and there is evidence that the LMA may avoid some of the morbidity seen with emergency endotracheal intubation [13, 14]. The LMA is available in different sizes from infants to adults and, recently, even disposable LMAs are available.

Intubating Laryngeal Mask Airway

The Intubating Laryngeal Mask Airway (ILMA) is an advanced version of the LMA allowing a special endotracheal tube to be passed through the ILMA into the trachea [15]. The device follows a two-step concept. First, it can be used as a rescue device in the unanticipated difficult airway to allow oxygenation and ventilation. Secondly, the ILMA serves as a conduit for endotracheal intubation without the need to visualize the vocal cords. A high cumulative success rate $>95\%$ for endotracheal intubation with a maximum of three attempts is reported when conventional endotracheal intubation using a Mackintosh blade has failed. Disposable ILMAs in different sized have just become available.

Esophageal Tracheal Combitube

The Esophageal Tracheal Combitube (ETC) is a double-lumen airway device designed for emergency ventilation when visualization of the vocal cords and thus endotracheal intubation is not possible [16]. With the ETC, the patient can be successfully ventilated regardless of whether the ETC is inserted into the trachea or into the esophagus. On blind insertion through the mouth the tube is more likely to pass into the esophagus ($>95\%$) than into the trachea ($<5\%$). If the ETC is placed in the esophagus, the distal balloon seals the esophagus and prevents regurgitation, while the proximal cuff seals the oropharyngeal space. Ventilation is achieved through a number of perforations situated between both cuffs. If placed in the trachea, the ETC functions as a standard endotracheal tube. The most common

reason for ventilation failure in this setting is too deep placement of the ETC, so that the perforated pharyngeal portion of the tube has entirely entered the esophagus. Pulling the ETC 3–4 cm back usually resolves this problem. If the tube passes into the trachea, the distal balloon is inflated, and ventilation is initiated via the distal lumen with no need to inflate the pharyngeal, proximal balloon.

The ETC is only available in two sizes and cannot be used in patients < 122 cm in height, is not reusable and is expensive. Beyond that, the device requires a certain amount of training that needs to be continuously refreshed. Despite its popularity amongst US health-care providers, it has not gained a similar level of popularity in Europe.

Laryngeal Tube

The laryngeal tube is a single-lumen reusable or disposable tube inserted blindly into the esophagus. It has two cuffs sealing the pharyngeal airway and the esophageal inlet to an airway pressure of up to 40 mbar. Both cuffs are inflated through a single pilot tube and balloon. The ventilation outlet is situated between the cuffs [17]. There are three black lines on the tube near a standard 15 mm connector, which indicate adequate depth of insertion when aligned with the teeth. The device is available in all sizes from newborns to tall adults. A further development of the basic tube is the laryngeal tube with suction port, which is a second, small bore lumen not intended for ventilation, but for suctioning off gastric contents or inserting a gastric tube. The laryngeal tube is a technically simple, easy-to-use device with which ventilation can be initiated as fast as with BVM ventilation [18]. Improved efficacy of ventilation and fewer requirements for manual skills as compared to BVM ventilation may contribute to the replacement of BVM ventilation by the laryngeal tube in situations when basic ventilatory life support is provided by heath-care professionals untrained in more sophisticated emergency airway management.

Evaluation of Supraglottic Airway Devices

According to the 2000 International Liaison Committee on Resuscitation (ILCOR) guidelines, endotracheal intubation is recommended as the gold standard for emergency airway management [19], although its contribution to outcome after cardiac arrest still remains controversial [20]. Acceptable alternatives to tracheal intubation and BVM ventilation include supraglottic airway devices, especially for those practitioners with limited experience in endotracheal intubation [19].

According to evidence based criteria, the ETC and the LMA have been evaluated to be easier to place than an endotracheal tube with similar complication rates, ventilation with both devices is comparable to that obtained with endotracheal intubation and definitely superior to BVM ventilation, and these devices are effective in case of failed endotracheal intubation [19].

It is beyond doubt that emergency physicians must be trained in the use of at least one of the supraglottic devices described above, and an alternative supraglottic device must be readily available in an emergency when endotracheal intubation fails.

Modified Laryngoscope Blades

Various modified laryngoscope blades are available to improve visualization of the glottic aperture and have proved useful when the Macintosh technique fails. The McCoy laryngoscope has an adjustable blade tip controlled by a lever on the handle, designed to lift the epiglottis without excessive leverage. It is definitely valuable in Cormack and Lehane grade III situations, but there is some doubt regarding its efficacy in the most difficult grade IV patients.

Straight blades are often valuable in infants and small children. Unlike curved blades, they can be used to directly lift the epiglottis, thus improving the view to the glottic aperture. However, the paraglossal straight blade technique requires increased neck mobility, which is undesirable in trauma patients with anticipated cervical spine injuries.

The Dörges universal laryngoscope blade (Fig. 1) has a low blade profile of only 16 mm, which makes introduction easier in patients with restricted mouth opening. Beyond that, the design of the blade allows its use in all patients >10 kg body weight, thus replacing the traditional Macintosh size 2–4 blades. The most obvious benefit of this blade is a significant reduction in the number of blades that need to be held available for emergency intubation, and thus significant reduction of costs [21].

The video laryngoscope is a laryngoscope blade with a camera lens on its tip. The blade is fixed to the handle that incorporates the camera unit. Two armed wires connect the laryngoscope handle to an electric light source and a video screen. This technique has been described to improve the visualization of the larynx to Cormack and Lehane grade I in patients with Cormack and Lehane grades III and IV under conventional laryngoscopy during elective endotracheal intubations. In emergency situations, however, the lens may be in contact with blood, secretions, and gastric contents, thereby worsening the indirect visualization of the glottis on the video screen. Beyond that, no wireless video signal transmission is possible yet, a 110/220 V light source is needed, and the technique requires a heavy video monitor, which significantly limits its potential value in emergency situations.

Fig. 1. The Dörges Universal Laryngoscope Blade has a low profile (16 mm) and can be used from children >10 kg to adults. (Reproduced with permission from Karl Storz GmbH, Tuttlingen, Germany)

Fiberoptic Devices

Two fiberoptic devices have a place in current emergency airway management strategies: Flexible fiberoptic bronchoscopes, and the Bonfils intubation fiberscope. The Bullard laryngoscope, a curved, rigid, laryngoscope device with an eyepiece attached to its handle, requires a high level of training, and has been widely replaced by the former devices.

Flexible Fiberoptic Bronchoscopy

Fiberoptic endotracheal intubation in the awake, spontaneously breathing patient represents the gold standard for establishing a secure airway in patients with anticipated difficulties to conventional endotracheal intubation. Despite its unequalled value in elective patients under controlled in-hospital conditions, flexible fiberoptic bronchoscopy has some important limitations in airway emergencies. First of all, flexible fiberoptic bronchoscopy is restricted by its availability. Flexible fiberoptic bronchoscopes are expensive and, therefore, often not available in emergency departments or in the out-of-hospital environment, regardless of the fact that some flexible fiberoptic bronchoscopes can be used with a small battery-powered light source and thus independent of a large 110/220 V cold light source. Apart from limited availability, the lens often gets fogged or soiled with secretions or blood, which makes visualization of anatomical landmarks difficult, if not impossible. Another important limitation is the fact that most emergency patients in whom flexible fiberoptic bronchoscopy would be potentially indicated because of unanticipated airway difficulties are unconscious, thus have decreased or no muscle tone, which results in difficult anatomy for fiberoptic intubation (i.e., the tongue is adjacent to the pharyngeal wall in patients who are in the supine position). Considering this, flexible fiberoptic bronchoscopy is of minor value to resolve intubation difficulties in emergency situations.

Bonfils Intubation Fiberscope

The adult Bonfils intubation fiberscope is a reusable, rigid, straight fiberoptic device with a 40° curved tip; 40 cm long and 5 mm in diameter. A flexible eyepiece is mounted on the handle of the scope. The fiberscope has a connector that fits onto the 15 mm tracheal tube adapter and thereby allows oxygen insufflation (Fig. 2), which also prevents fogging of the distal lens during the intubation procedure. A 110/220 V cold light source or a small battery handle (powered by two 1.5-V alkaline batteries) can be attached to the stylet handle. The tip of the Bonfils intubation fiberscope is positioned just proximal to the tip of the attached endotracheal tube (Fig. 2, inset), thereby preventing the lens from being soiled with blood or secretions to a significantly larger extent than those of a flexible fiberoptic bronchoscope. In recent studies, the Bonfils intubation fiberscope has proved its superiority and efficacy in patients with both predicted and unanticipated difficult airways requiring endotracheal intubation [22, 23].

Apart from its use in the operating room, the battery handle allows the Bonfils intubation fiberscope to be used in almost any situation, anywhere, i.e., in the field or in the emergency room, especially when definitive endotracheal intubation is required (e.g., in patients prone to regurgitation and aspiration).

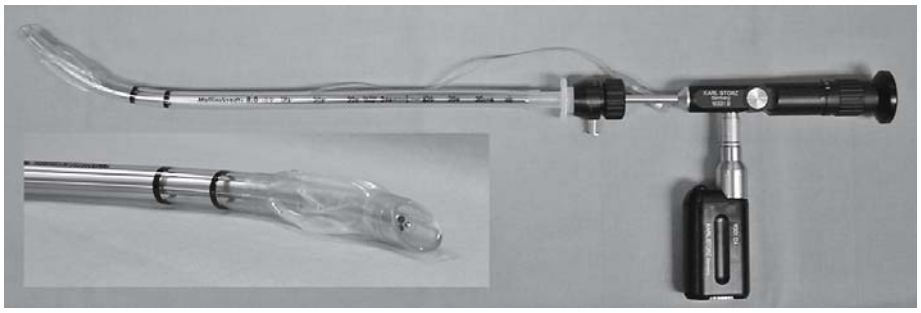

Fig. 2. Battery-powered Bonfils Intubation Fiberscope armed with an 8.0 mm ID endotracheal tube. The tip of the lens must be inside the endotracheal tube (see inset)

Many trauma victims are susceptible to cervical spine injury until this diagnosis has been ruled out by radiological examination. The cervical spine is therefore, initially, immobilized by a rigid collar according to the Advanced Trauma Life Support (ATLS) protocol. Limited mouth opening and absent neck extension contribute to poor views on direct laryngoscopy (Cormack and Lehane grades III and IV) in 64% of patients with a rigid cervical collar [24]. Using an ultrasound-based motion system of the cervical spine, the potential benefit of the Bonfils intubation fiberscope was demonstrated during elective endotracheal intubation under general anesthesia in the hospital: the range of cervical spine movement, especially neck extension, was much greater with a standard Mackintosh blade ($23^\circ \pm 10^\circ$) than with the Bonfils intubation fiberscope ($6^\circ \pm 5^\circ$) [25]. The potential benefit of the Bonfils intubation fiberscope in trauma patients with suspected cervical spine injury is currently being evaluated by in-hospital and in-the-field trials.

Airway Considerations in the Intensive Care Unit

The algorithm for managing both anticipated and unanticipated difficult airway in the ICU is not different to that recommended by the American Society of Anesthesiologists. There are, however, particular situations in the ICU and specific considerations regarding the patient's airway that require particular attention and skills of the intensivist in charge.

Lung Separation

The indications for single-lung ventilation are classified either as absolute or as relative. The absolute indications include life-threatening complications such as massive bleeding and abscess formation, to prevent spread to the non-diseased lung. Broncho-pleural and giant unilateral bullae may rupture under positive pressure ventilation, and modest invasive ventilation is mandatory. Finally, during bronchoalveolar lavage (BAL) for alveolar proteinosis or cystic fibrosis, prevention of contralateral lung drowning is absolutely necessary. When lung separation becomes indicated in the ICU, most patients are already intubated with a standard endotracheal tube.

Techniques for single-lung ventilation can be accomplished in two different ways. The first involves the use of a double-lumen endotracheal tube. The second method involves blockade of a mainstem bronchus to allow lung collapse distal to the occlusion (bronchial blockers). Although double-lumen tubes are still the most common device used during lung separation techniques, bronchial blockade technology is on the increase.

Double-lumen Endotracheal Tubes: A double-lumen endotracheal tube is still the most frequently used device for single-lung ventilation and is considered as the gold standard for lung separation. The cuffed tip of the tube with its distal lumen is advanced either into the left or the right main bronchus, while the cuffed proximal lumen is placed in the trachea. If both cuffs are inflated and the tracheal lumen is occluded, only the left (left endobronchial double-lumen endotracheal tube) or right lung (right endobronchial double-lumen endotracheal tube) is ventilated. If the bronchial lumen is occluded, it is vice versa.

The main advantage of a double-lumen endotracheal tube over any other devices for lung separation is its large bore lumina that allow for adequate suctioning, fiberoptic bronchoscopy of both lungs, and application of independent ventilation strategies to each lung. However, placement is difficult, if not impossible, in pa-

Table 2. Characteristics of various techniques for endobronchial blockade

Device/Technique	Advantages	Disadvantages
▮ Endobronchial intubation	– Easy, fast, and cheap technique	– No suction of the isolated lung possible – No PEEP can be applied to the isolated lung
▮ Fogarty Catheter	– Can be used through the endotracheal tube in situ, no risk of airway loss	– Difficult to position – Tends to dislocate
▮ Univent Tube	– Intubation with a single lumen tube – Ventilation does not need to be discontinued during blocker placement	– Placement of the integrated blocker usually requires fiberoptic control – Small lumen does not allow for adequate suctioning of viscous secretions or blood
▮ Arndt Endobronchial Blocker	– Can be used through the endotracheal tube in situ, no risk of airway loss – Continuous ventilation possible during blocker placement – Can be placed in almost any part of bronchial tree	– Flexible fiberoptic bronchoscopy mandatory – Small lumen does not allow for adequate suctioning of viscous secretions or blood – Re-positioning difficult in case of dislocation
▮ Cohen Endobronchial Blocker	– Can be used through the endotracheal tube in situ, no risk of airway loss – Continuous ventilation possible during blocker placement – Can be placed blindly in an emergency (e.g., pulmonary hemorrhage)	– Placement usually requires fiberoptic control – Small lumen does not allow for adequate suctioning of viscous secretions or blood – Expensive

PEEP: positive end-expiratory pressure

tients with a difficult airway (i.e., Cormack and Lehane grades III and IV). In patients with a standard endotracheal tube already in place, the tube needs to be removed before re-intubation with a double-lumen endotracheal tube can be attempted. Particularly in ICU patients, there is a certain risk of airway loss during tube exchange due to airway edema, hemorrhage, etc. Therefore, a variety of techniques and devices for endobronchial blockade have become available. Table 2 gives a comprehensive overview of currently available techniques and devices for blocker-facilitated lung separation.

Endobronchial Blockade: Endobronchial advancement of the single-lumen endotracheal tube in place is restricted to life-threatening emergencies to buy time until more appropriate lung separation techniques become available.

The Fogarty occlusion embolectomy catheter is a device designed specifically to be used as a vascular tool; however, there are well documented reports of its use for lung isolation [26]. The occlusion balloon of the catheter is considered a high-pressure, low-volume cuff that requires 0.5–10 ml of air to achieve occlusion of a bronchus. The Fogarty catheter has an incorporated stylet that can be preshaped at the distal end to facilitate its guidance into the left mainstem bronchus. Advantages of the Fogarty catheter over a double-lumen endotracheal tube are as follows: it can be advanced through the lumen of an existing standard endotracheal or tracheostomy tube; and it can be used as a rescue device when lung separation is insufficient in patients who are already intubated with a double-lumen endotracheal tube. Disadvantages of the Fogarty technique are that the catheter is a vascular device, and neither designed nor certified as a bronchial blocker. Further it is made of natural rubber latex, which is contraindicated in patients with latex allergy. The lack of a communicating channel in the center makes suction or oxygen insufflation impossible. Although the incorporated stylet facilitates insertion into a bronchus, it cannot be coupled to and navigated with a fiberoptic device. Keeping the catheter in a stable position once its balloon has been successfully advanced into a mainstem bronchus is also difficult, with a high risk of dislocation. Both endobronchial intubation and the use of a Fogarty catheter are, therefore, not reliable techniques for lung separation.

The Univent tube is a single-lumen endotracheal tube with a channel enclosing a moveable bronchial blocker that can be used to block the left, right, or any specific secondary bronchus [27]. The channel that encloses the bronchial blocker has a diameter of only 2 mm which increases the anterior-posterior external diameter of the Univent tube, making the device larger than a single-lumen endotracheal tube of corresponding internal diameter. Because of its relative ease of placement, the Univent tube has been used in patients with hemoptysis or bleeding diathesis, and can be used during rapid sequence induction. By deflating and withdrawing the bronchial blocker the Univent tube can be converted to a conventional single-lumen endotracheal tube. Bronchoscopic guidance is usually required to successfully place the bronchial blocker. A definitive advantage of the Univent tube over a double-lumen endotracheal tube is that its placement is easier in patients with a difficult airway; however, sufficient suctioning of the isolated lung is impossible, and the blocker also tends to dislocate.

Wire-guided endobronchial blocker (Arndt-blocker): Another technique to achieve lung separation is the wire-guided endobronchial blocker, which is considered an independent bronchial blocker. Invented by the US anesthesiologist George A.

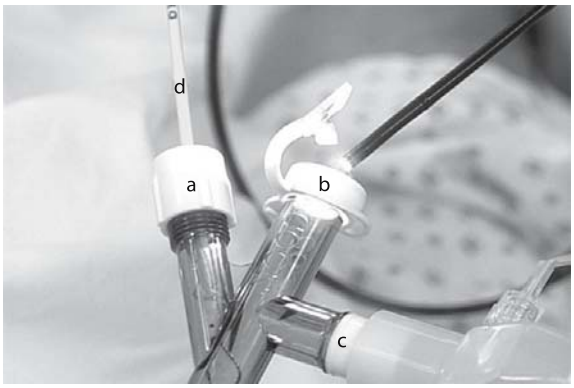

Fig. 3. Wire-guided endobronchial blocker with Arndt Multiport Airway Adapter®. **a** Blocker port with Touhy-Borst type valve; **b** bronchoscopy port; **c** ventilation port; **d** blocker with monofilament guide loop at its tip inserted into the airway adapter (Reproduced with permission from Cook Inc., Bloomington, IN, USA)

Arndt in 1999 [28], the wire-guided endobronchial blocker is a cuffed catheter available in different sizes (5, 7, and 9F outer diameter) and lengths (50, 65, and 78 cm). Its inner lumen contains a flexible nylon wire passing through the proximal end of the catheter, extending to the distal end, and then exiting as a small flexible wire loop. The wire loop of the Arndt blocker is coupled with a pediatric bronchoscope and serves as a guidewire to introduce the blocker into a bronchus under direct visualization. A special adapter with separate ports for the ventilator tube and the pediatric bronchoscope is attached to the endotracheal tube. Ventilation can therefore be maintained during bronchoscopy and placement of the blocker. The blocker itself is introduced through a third port with a Touhy-Borst type valve, locking the blocker in its final position and maintaining an air tight seal (Fig. 3).

There are advantages to using the wire-guided endobronchial blocker over the double-lumen endotracheal tube or Univent tube in patients who are already tracheally intubated, who present with a difficult airway, or who require single-lung ventilation during acute trauma to the chest. In addition, it can be used as selective lobar blocker, e.g., in patients with previous pneumonectomy who require a selective one lobe ventilation. Because the wire-guided endobronchial blocker only requires a single-lumen tube, its use eliminates the need for tube exchange if mechanical ventilation is contemplated in the postoperative period. One limitation of the wire-guided endobronchial blocker is that with the removal of the wire-guided loop it cannot be reinserted and thus intraoperative repositioning of the blocker can be difficult, especially during left mainstem bronchus intubation. Also the small diameter of the suction channel, which allows oxygenation and positive end-expiratory pressure (PEEP) application to the isolated lung, increases the time required for the lung to collapse and makes suctioning difficult.

Figure 4 shows the thoracic computed tomography (CT) scan of a patient with bronchopleural fistula secondary to a tear in the distal portion of the right lower lobe bronchus. The wire-guided endobronchial blocker was used to selectively isolate the right lower lobe, and the fistula closed within 24 hrs without further intervention [29].

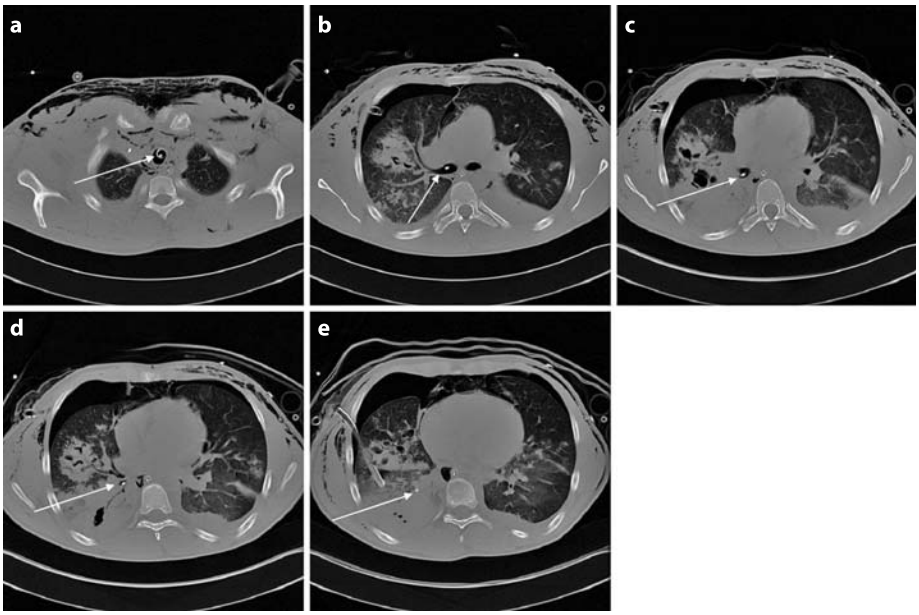

Fig. 4. Thoracic CT scan of a patient with bronchopleural fistuala in whom the wire-guided endobronchial blocker was advanced from the trachea (**a**) into the right lower lobe bronchus (**e**)

Because fiberoptic control and guidance is mandatory for optimal placement of the wire-guided endobronchial blocker, the device is of little value in situations with difficult bronchoscopy conditions, such as in severe pulmonary hemorrhage.

Tip-deflecting endobronchial blocker (Cohen blocker): The 9F tip-deflecting endobronchial blocker, invented by thoracic anesthesiologist Edmund Cohen from New York in 2004, is closely related to the wire-guided endobronchial blocker, but does not have a guide wire loop coupled to a bronchoscope, rather an adjustable tip that can be deflected by 60 degrees with a small wheel, the rotation of which is transmitted to the blocker's tip by wires located inside the catheter. The tip-deflecting endobronchial blocker is intended to be placed under fiberoptic visualization; however, blind placement into the left or right main bronchus seems also possible in emergencies. Compared to the wire-guided endobronchial blocker, the tip-deflecting endobronchial blocker can only be placed into a main bronchus and thus is restricted to isolating an entire lung, and not a specific portion thereof.

Recent Developments in Percutaneous Tracheostomy

Tracheostomy Timing

Percutaneous dilational tracheostomy has become an established treatment modality for long-term airway access in the ICU during the past two decades. However, the optimal time to perform a percutaneous dilational tracheostomy has been based largely on personal experience and judgment rather than on medical evidence. In

2004, Rumbak and colleagues published a milestone study evaluating the benefits of early (< 48 hrs of intubation) versus delayed (> 14 days of intubation) percutaneous dilational tracheostomy in medical ICU patients [30]. They found significant advantages of early percutaneous dilational tracheostomy in terms of facilitated weaning, shorter ICU length of stay, and incidence of nosocomial pneumonia. A recent meta-analysis of five prospective-randomized trials comparing early versus late tracheostomy in the ICU confirmed these results [5]. Based on the results of this meta-analysis that included Rumbak's data, intensivists should no longer delay tracheostomy in critically ill patients with an anticipated duration of intubation of more than 10–14 days.

Balloon Facilitated Percutaneous Tracheostomy

Five different percutaneous dilational tracheostomy techniques are currently available: the classic Ciaglia technique using multiple dilators, the forceps dilational technique, the retrograde, translaryngeal technique, the single-dilator dilational Blue Rhino technique, and the PercuTwist technique using rotating dilation with a self-cutting screw. A potential disadvantage of all of these techniques is the requirement of two independent steps: stoma creation by dilation, and subsequent cannula placement.

Balloon facilitated percutaneous tracheostomy is an innovative technique basically invented by the pioneer in percutaneous dilational tracheostomy, Pasquale Ciaglia, shortly before his death in 2000, and refined by the pulmonologist Michael Zgoda from Kentucky, USA [31]. The novel approach to percutaneous dilational tracheostomy combines both steps – dilation and cannula placement – into a single

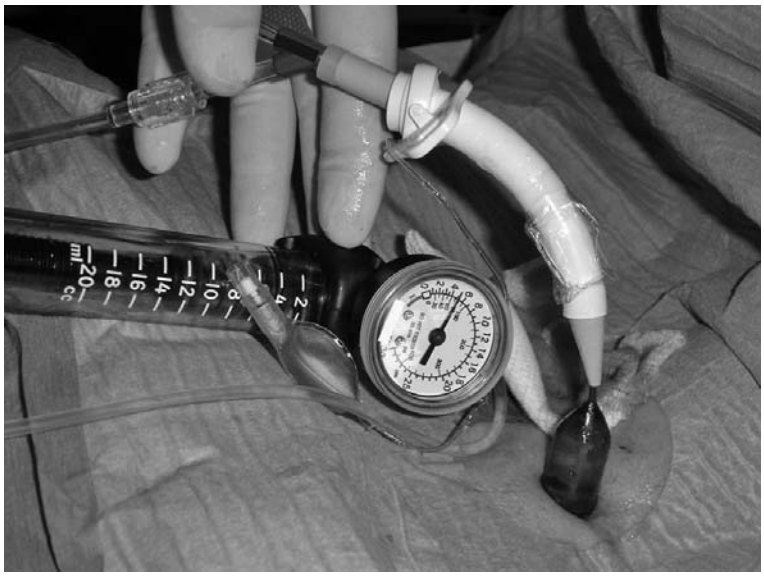

Fig. 5. Balloon facilitated percutaneous tracheostomy. An angioplasty balloon is inflated to 5 atm to dilate the stoma. After balloon deflation, the tracheostomy device is passed further into the trachea to advance the tracheostomy cannula

step. The initial steps of the procedure are identical to any other technique: bronchoscopically guided tracheal puncture and subsequent guide wire placement. Thereafter, a special device is passed over the guide wire. The distal portion of the device is an angioplasty balloon filled with saline solution to create sufficient pressure to dilate the stoma. The proximal portion of the device is basically a loading dilator armed with a tracheostomy cannula. Once the distal portion of the balloon is seen inside the trachea, the balloon is inflated for 60 seconds (Fig. 5). Thereafter, the balloon is deflated, and the tracheostomy device is introduced further into the trachea, thus advancing the proximal portion of the device, which carries the cannula, into the trachea. Finally, the guide wire and tracheostomy device are removed, leaving only the cannula *in situ*. Once its correct intratracheal position has been bronchoscopically confirmed, the ventilator circuit can be connected to the cannula.

Experience with balloon facilitated percutaneous tracheostomy is currently limited to animal [31] and cadaver studies [32, 33] and a few documented clinical cases [34]. There are potential benefits of this first real one-step percutaneous dilational tracheostomy technique, which will have to be confirmed in larger clinical trials before a definitive recommendation regarding the clinical impact of balloon facilitated percutaneous tracheostomy can be made.

Conclusion

Concurrent failure of tracheal intubation and mask ventilation may ultimately result in death or brain damage. Therefore, these two basic techniques are the most important that any emergency physician has to learn. An increasing number of airway devices and techniques has been developed to manage the unanticipated airway in the field or in the emergency department. Successful use of any alternative technique to endotracheal intubation requires a high level of continuous training especially when endotracheal intubation has failed. The skills and experience of a rescuer performing basic or advanced airway management determine whether these maneuvers result in effective oxygenation or serious complications, such as severe neurological impairment or even death [35].

Recently published data and a meta-analysis clearly advocate abandoning the prevailing practice of performing tracheostomy in critically ill patients around day 10 of endotracheal intubation, and supports performing early tracheostomy instead. Balloon facilitated percutaneous tracheostomy is a recently introduced, one-step tracheostomy technique that needs further clinical evaluation.

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Use of Continuous Positive Airway Pressure in Critically Ill Patients

S. M. Maggiore, A. Arcangeli, and M. Antonelli

Introduction

Positive end-expiratory pressure (PEEP) or continuous positive airway pressure (CPAP) are probably the most frequently used ventilatory treatments in critically ill patients. In a recent international survey, PEEP was used in more than 90% of patients with acute respiratory distress syndrome (ARDS) and in more than 50% of patients with an exacerbation of chronic obstructive pulmonary disease (COPD) [1]. PEEP is a technique in which airway pressure is maintained above atmospheric pressure at end expiration by pressurization of the ventilatory circuit, whereas during CPAP, pressure is applied to spontaneous breathing throughout the entire respiratory cycle. Many pathological conditions benefit from the application of PEEP or CPAP, as shown by the pioneering work of Poulton and Oxon [2] and Barach and collegues [3] who demonstrated that application of positive pressure to the airway can effectively treat patients with cardiogenic pulmonary edema. Nowadays, CPAP or PEEP are used in various forms of acute respiratory failure to improve respiratory mechanics, gas exchange, and cardiac performance.

Pathophysiology

In patients with acute exacerbation of COPD, hypercapnia and respiratory acidosis are consequences of hypoventilation, often requiring mechanical ventilation to be reversed. Alveolar hypoventilation develops in spite of an increased respiratory drive and respiratory muscle activity, which are ineffective in increasing tidal ventilation due to the impairment of respiratory mechanics. The result is rapid shallow breathing, with low volume ventilation not compensated by the increase in respiratory rate because of the concomitant increase in dead space ventilation. The mechanical derangement of the respiratory system in patients with acute exacerbations of COPD is mainly due to dynamic hyperinflation, which reduces the force-generating capacity of the diaphragm, and increased airway resistance. When the load becomes excessive, respiratory muscle fatigue can develop. In these patients, it was initially thought that PEEP produced little or no additional benefit. Subsequently, it was recognized that, particularly in the presence of airflow obstruction, the lungs may fail to deflate to functional residual capacity at end-expiration [4]. As a consequence, alveolar pressure remains positive to an extent that depends on the volume of trapped air, a phenomenon referred to as intrinsic PEEP (PEEPi). In this context, application of external PEEP replaces the amount of pressure that must be gener-

ated by the inspiratory muscles to offset PEEPi, needed to initiate inspiratory flow or trigger the ventilator [5]. This benefit is evident during spontaneous breathing or with any patient-triggered mode of ventilation: CPAP or PEEP has been shown to reduce dyspnea, work of breathing, respiratory drive, inspiratory muscle effort, and to improve triggering function and patient-ventilator interaction [5–9]. However, if PEEP exceeds PEEPi, it may further hyperinflate the lungs, increasing the risk of barotrauma, with additional mechanical disadvantage to the diaphragm, and hemodynamic impairment [9]. PEEP should be titrated according to a precise evaluation of the level of PEEPi, which is difficult to evaluate when respiratory muscle activity is present [10].

Hypoxemic acute respiratory failure is caused by intrapulmonary shunt and venous admixture, ensuing from an acute reduction of aerated lung volume due to lung edema and/or atelectasis. As a consequence, functional residual capacity is decreased and respiratory mechanics impaired (i.e., reduced lung compliance). The reversal of hypoxemia requires interventions that recruit more aerated lung units for ventilation. In this context, PEEP can improve arterial oxygenation by increasing functional residual capacity, by reducing venous admixture, improving respiratory mechanics, and reducing the work of breathing [11, 12].

In cardiogenic pulmonary edema hypoxemia is often associated with hypercapnia secondary to respiratory muscle fatigue. The reduction of lung compliance and the increase in airway resistance increase the work of breathing [13]. Thus, the inspiratory muscles have to generate large negative swings in pleural pressure leading to an increase in left ventricular transmural pressure and afterload [14]. The reduction in cardiac output compromises oxygen delivery to the respiratory muscles, and may create a vicious cycle. In this setting, the application of CPAP has several beneficial effects on the cardiovascular system. A CPAP-induced increase in intrathoracic pressure decreases left ventricular transmural pressure (i.e., afterload), and improves left ventricular performance [14]. This effect of CPAP on afterload is primarily related to the improvement in respiratory mechanics that leads to a reduction in the inspiratory negative swings of intrathoracic pressure [13].

Clinical Applications of CPAP

Hypercapnic Acute Respiratory Failure

The application of CPAP in acute exacerbations of COPD reduces the threshold load imposed on the inspiratory muscles by PEEPi, although it does not reduce hyperinflation and its related negative effects on the diaphragm's force-generating capacity. The addition of CPAP to spontaneous breathing is effective in reducing dyspnea, decreasing the work of breathing, and improving cardiac function. During triggered modes of ventilation, PEEP reduces the patient's inspiratory effort and ventilatory drive, and facilitates triggering of the ventilator by reducing both inspiratory effort and the delay between onset of inspiratory effort and initiation of ventilator assistance delivery, improving patient ventilator interactions, and reducing ineffective inspiratory efforts [5–8, 15]. Despite a large body of physiologic studies, no randomized, clinical trial has evaluated the effectiveness of CPAP in improving outcomes in ventilated patients with COPD. Nevertheless, many trials have demonstrated the effectiveness of non-invasive ventilation with 4–5 cmH_2O PEEP and pressure support in patients with COPD exacerbation and acute hypercapnia [16, 17].

Patients with acute severe asthma have high airway resistance, dynamic hyperinflation and PEEPi, which increase respiratory muscle load and decrease force-generating capacity. Although some flow limitation may be present, the main cause of dynamic hyperinflation and PEEPi is the prolonged time constant, due to the high expiratory resistance. These patients are generally less responsive to external PEEP [18]. In the absence of flow limitation, PEEP increases hyperinflation and alveolar pressure [19]. In addition, some alveolar units may be entirely occluded by mucus plugs and thus completely excluded. Low levels of CPAP may decrease breathlessness [20]. In patients with asthma exposed to aerosolized histamine, Martin et al. found that CPAP 12 cmH_2O reduced both esophageal and transdiaphragmatic pressure swings, but inspiratory work of breathing did not decrease because minute ventilation simultaneously increased [21]. In a series that included 17 patients with acute asthma, Meduri et al. reported that CPAP 3–5 cmH_2O combined with pressure support via a face mask improved gas exchange and avoided endotracheal intubation in all but two patients [22]. In a randomized trial of 30 patients treated in an emergency department for acute asthma, Sorosky et al. found that non-invasive ventilation with low CPAP levels produced greater and faster improvements in lung function with shorter ICU lengths of stay than did standard therapy [23]. Although these few data suggest that low levels of non-invasive CPAP with pressure support might be beneficial in less severe and selected asthmatic patients, further evaluation is required.

Hypoxemic Acute Respiratory Failure

In patients with acute lung injury (ALI) or ARDS, application of PEEP/CPAP increases the amount of aerated lung volume, improves respiratory mechanics, reduces intrapulmonary shunt, ameliorates gas exchange, stabilizes unsteady lung units, and reduces the risk of ventilator-induced lung injury (VILI) [12, 24–27]. From its introduction almost forty years ago [24], PEEP remains a cornerstone in management of patients with ALI/ARDS. In fact, no randomized trial has evaluated ventilator strategies without PEEP in patients with ALI/ARDS, except in the very early phase. In a study performed on spontaneously breathing patients with hypoxemic acute respiratory failure, including a subgroup of patients in the early phase of ALI, Delclaux et al. found that application of CPAP via a face mask improved oxygenation, but did not improve the need for endotracheal intubation, length of hospital stay, or mortality [28]. Early use of non-invasive CPAP, with or without pressure support, may benefit specific high-risk patients, such as immunocompromised patients [29, 30]. CPAP is also useful during fiberoptic bronchoscopy, a procedure that can worsen oxygenation and respiratory mechanics. In a double-blind trial, Maitre et al. compared mask CPAP to oxygen therapy alone during bronchoscopy in severe hypoxemic spontaneously breathing patients [31]. During, and immediately after the procedure, oxygenation was well preserved in the CPAP group, whereas it fell in the oxygen only group. CPAP prevented subsequent respiratory failure necessitating ventilator support. Similar good results were obtained by Antonelli and co-workers by applying PEEP with pressure support through a helmet [32] (Fig. 1).

Postoperative hypoxemia is mainly caused by atelectasis, which develops in 30–50% of cases, and leads to acute respiratory failure requiring intubation and mechanical ventilation in 8–10% of patients [33]. The application of CPAP (5 to 10 cm H_2O) via a face mask, alone or combined with pressure support, reduces atelectasis and im-

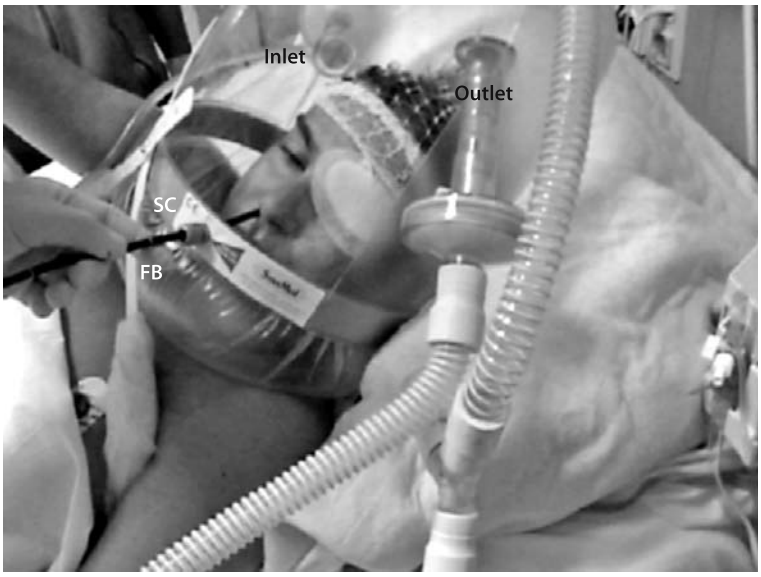

Fig. 1. Fiberoptic bronchoscopy during helmet CPAP combined with pressure support. Inlet and outlet are the two ports of the helmet connected to the gas source and PEEP valve through the respiratory circuit. Heat and moisture exchangers are placed in the respiratory circuit not as humidifiers, but in order to reduce the noise that may be generated by the system. FB = Fiberoptic bronchoscope; SC = seal connection of the helmet with adjustable diaphragm, to allow ventilation during the procedure, without air leaks. The picture was taken with the patient's permission

proves oxygenation, without increasing surgical complications, such as anastomotic leaks [34–35]. By reducing atelectasis, CPAP may decrease bacterial growth in the lung, mitigating bacterial translocation into the bloodstream, and normalizing alveolo-capillary permeability [36]. Despite well recognized physiologic benefits, only very recently have randomized trials assessed the effects of CPAP on outcome variables [37, 38]. In 70 patients, Fagevik Olsen et al. reported that, compared to breathing exercises, use of CPAP after thoraco-abdominal resection of esophageal cancer resulted in a lower intubation rate [37]. Squadrone et al. randomized patients who developed hypoxemia following major abdominal surgery to either oxygen alone (FiO_2 50%) or oxygen plus CPAP 7.5 cm H_2O delivered through a helmet [38]. The study was interrupted after 209 patients were enrolled, because the intubation rate was lower in the CPAP group than in the oxygen group (1 vs 10%). The patients who received CPAP at the end of treatment had higher oxygenation and lower rates of pneumonia, infection, and sepsis than the oxygen group [38].

Cardiogenic Pulmonary Edema

It has been known for a long time that acute respiratory failure secondary to cardiogenic pulmonary edema can significantly benefit from the application of positive pressure ventilation [2, 3]. Applying mask CPAP in severe cardiogenic pulmonary edema is associated with fast and marked improvement in hemodynamics and respiratory mechanics. Over the last two decades there has been growing interest

in the delivery of CPAP via a face mask, as a means of avoiding endotracheal intubation. Seven randomized controlled trials have compared CPAP versus standard medical treatment [39–45], and four trials have compared CPAP versus bi-level positive airway pressure (i.e., PEEP plus inspiratory pressure support, BiPAP) [44–47]. The seven studies comparing CPAP (7–10 cm H_2O on average) versus standard treatment included a total of 191 patients receiving CPAP and 197 control patients. CPAP produced a greater and more rapid physiologic improvement in all studies but one [44]. Five studies reported a prompter and greater improvement in oxygenation with CPAP [39–43]. Bersten et al., who included patients with the most severe respiratory acidosis, observed an improvement in pH and $PaCO_2$ with CPAP [40]. CPAP produced a lower rate of therapeutic failure and intubation in four studies [40, 41, 43, 45] and decreased hospital mortality in one [44]. The four studies comparing CPAP (10 cm H_2O) with BiPAP included a total of 82 and 87 patients in the CPAP and BiPAP groups, respectively. Mehta et al. found a more rapid reduction in $PaCO_2$ and other physiologic variables with BiPAP than with CPAP [46], whereas the other three studies did not find differences in physiologic variables [44, 45, 47]. Three studies did not reveal differences in any clinical outcome variable [45–47], while Crane et al. found a higher hospital survival in the CPAP group than in the BiPAP group [44]. The incidence of myocardial infarction was similar between groups in three studies [44, 45, 47].

Interfaces for Non-invasive CPAP

Non-invasive CPAP can be administered through a nasal mask, face mask, or helmet. The nasal mask, usually employed in home ventilation, is comfortable and well-tolerated. However, critically ill patients are often mouth-breathers. Leaks through the mouth make the technique less effective and cause an increase in nasal resistance. Full face or facial masks are preferable in the acute setting because they minimize leaks and improve the effectiveness of the technique. However, they are less comfortable and less tolerated than nasal masks, and are associated with a greater incidence of skin lesions. The helmet (Fig. 1) has been proposed recently in an attempt to improve tolerance [48–49]. In healthy subjects, Patroniti et al. found that non-invasive CPAP delivered by helmet was as effective as mask CPAP in increasing end-expiratory lung volume without the need of a reservoir bag [50]. However, high gas flow rates were necessary to maintain a relatively low inspiratory CO_2 concentration. In 11 patients with hypoxemic acute respiratory failure related to cardiogenic pulmonary edema, Tonnelier et al. found that CPAP delivered through a helmet was as effective as facial mask CPAP in improving gas exchange and clinical parameters, even in cases of severe respiratory acidosis and hypercapnia [51]. Moreover, it allowed CPAP administration for a longer period of time, without any adverse events or clinical intolerance.

Conclusion

CPAP is a simple and effective ventilatory treatment for acute respiratory failure related to specific conditions. CPAP (10 cm H_2O) should be considered as the first-line ventilator treatment for achieving prompt physiologic improvement and lower rates of endotracheal intubation in severe cardiogenic pulmonary edema. CPAP is

effective for managing postoperative hypoxemia, enabling improved outcomes in selected patients, provided it is applied for a sufficient period of time. CPAP is also useful during fiberoptic bronchoscopy in hypoxemic patients to prevent subsequent acute respiratory failure. Although application of non-invasive CPAP cannot be generally recommended in the early stage of hypoxemic acute respiratory failure related to ALI/ARDS or pneumonia, CPAP may be beneficial in high-risk, immunocompromised patients. Surprisingly, no study has yet evaluated the effectiveness of early use of CPAP alone in improving outcome in patients with acute exacerbation of COPD. In contrast, use of PEEP/CPAP is often detrimental and is not advisable in other conditions, such as in patients undergoing mechanical ventilation for acute severe asthma.

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Non-invasive Respiratory Support in Pre-term Neonates and Pediatric Patients with Respiratory Failure

P. Pelosi, G. Chidini, and E. Calderini

Introduction

Ventilation is one of the most expensive therapies in neonatal and pediatric intensive care units (ICUs) [1] and a considerable morbidity is associated with its use. The cost of the treatment of preterm neonates, infants and children with respiratory failure, who require mechanical ventilation, is extremely expensive and increases with the length of stay [2]. Moreover, the financial burden caused from respiratory morbidity for preterm infants who subsequently develop bronchopulmonary dysplasia (BPD) should also be noted. This expense largely reflects nursing and respiratory therapist time, which may be much less in different health care systems worldwide.

During the last decade, non-invasive respiratory support has been used increasingly in different pathologies causing acute or chronic respiratory failure. However, most of the experience originates from the adult population. Increasing interest is developing on the use of different forms of non-invasive respiratory support both in preterm neonates, infants and children with acute and chronic respiratory failure.

In this chapter we will discuss:

- the main pathophysiological characteristics of respiratory failure in preterm neonates, infants and children;
- technical aspects of non-invasive respiratory support, such as continuous positive airway pressure (CPAP) and positive pressure ventilation (PPV);
- clinical data available concerning the use of non-invasive respiratory support;
- new technical developments in the use of non-invasive respiratory support.

Pathophysiological Characteristics of the Respiratory System

Major differences exist in the characteristics of the respiratory system of preterm neonates, infants and very young children compared to adults [3]. With increasing age, these differences tend to be less marked and the basic characteristics of the respiratory system become more similar. The neonate is characterized by a relatively stiff lung and a very compliant chest wall. Thus, the lung tends to collapse and without unopposed forces it would lead to a functional residual capacity of only 15–20% of the total lung capacity. However, infants are able to maintain a functional residual capacity of at least 40% of total lung capacity by different physiological maneuvers, such as laryngeal breaking [4], maintenance of the post-inspiratory tone in the muscles of chest wall [5], and the use of respiratory rates fast enough

to make the expiratory time less than the time constant of the respiratory system. The neonate's ability to generate adequate tidal volume is partially impeded by the compliant chest wall, thus the work of breathing is increased, by wasting forces on the chest wall distortion instead of producing effective alveolar ventilation. Furthermore, this contributes to muscle fatigue and likely accentuates normal growth.

Other factors may contribute to hamper the neonate's respiratory system, such as high flow resistance of the nasal airway and small airways, increased propensity to hypertrophy of the adenoids and tonsils, a reduced zone of apposition of the diaphragm, horizontal ribs, and, in the very young, the presence of immature muscles.

In addition, metabolism is increased, doubled compared to adults, resulting in a dramatic rise in the alveolar ventilation/functional residual capacity ratio compared to adults (5/1 vs 1.5/1) [6]. Moreover, in preterm neonates, surfactant deficiency, which manifests as a diminution of surface tension, leads to further collapse of alveolar segments [7].

The diaphragms of preterm neonates have a lower number of high-oxidative fibers; making fatigue of this muscle probable [8]. Muller et al. [9] using electromyography (EMG) via surface electrodes demonstrated that the diaphragms of normal preterm neonates and infants operate very close to the threshold of diaphragmatic fatigue.

The hypoxemic response is also attenuated in infants and apneas are more frequent than in adults [10]. Thus, combined increased thoracic compliance and surfactant deficiency, especially in preterm neonates, leads to a loss of functional residual capacity, effectively meaning that the infant is attempting to achieve adequate gaseous exchange in a smaller compartment of ventilated lung. In addition, ventilation of lungs below a normal functional residual capacity can result in cyclical opening and closing of lung units, and resulting injury [11]. This has been termed low-volume injury or atelectotrauma, leading to inflammatory changes associated with severe alterations in the lung structure [12].

Thus, it appears that the respiratory system of preterm neonates and infants is extremely weak, and even the presence of a minor abnormality can cause a rapid deterioration in respiratory function. The rationale for respiratory support is to increase and maintain the functional residual capacity, prevent atelectasis (augmenting surfactant production in the case of preterm neonates), support the easily fatigable ventilatory muscles, and provide respiratory stimulation (against apnea); and in doing so, provide gaseous exchange of both oxygen and carbon dioxide.

Bronchopulmonary Dysplasia in Preterm Neonates and Infants

BPD, is characterized by early interstitial and alveolar edema which progresses to persistent inflammation and fibrosis. As the survival rate of preterm babies improves, the numbers of infants with BPD also rises [13]. Infants with BPD have a higher mortality and morbidity; they receive more ventilation, drugs, oxygen and intensive care and have higher hospital readmission rates in the first year of life than infants of similar gestational age who do not develop BPD [14]. The advent of antenatal steroids and surfactant has altered the definition of BPD from the previous stress patterns of early ventilatory support in hyaline membrane disease, coupled with long exposure to ventilation and oxygen.

Several authors now report a different picture ('new BPD') in extremely low body weight neonates [15] in the first days to weeks of life, infants having only modest or no respiratory support, which then becomes necessary later. Infants with chronic pulmonary insufficiency of prematurity (CPIP) appear to form the current 'epidemic' of the new BPD.

Conventional mechanical ventilation via an endotracheal tube has undoubtedly led to the improvement in neonatal survival in the last thirty years. However, prolonged use of an endotracheal tube and mechanical ventilation may cause upper airway damage, alter normal mucociliary flow, lead to infection and predispose the infant to BPD. Intubation can also cause fluctuations in oxygenation and blood pressure that may have potentially harmful consequences to the cerebrum.

Although multiple factors contribute to BPD, intubation and mechanical ventilation of preterm neonates is the single most important predictor of subsequent BPD [16]. Recognition of this has, in part, contributed to the general term of ventilator-induced lung injury (VILI), which can be applied to both adults and children.

VILI appears to have very similar risk factors to BPD, namely volutrauma, barotrauma and atelectasis or end-expiratory alveolar collapse. These mechanical stresses are most likely transduced into a final common biological signal via the presence of toxic reactive oxygen species and associated inflammation. These are certainly not mutually exclusive, as the inter-relation between volume and pressure indicates. Since the structural abnormalities of injured lungs cannot be easily reversed, preventative measures, like non-invasive respiratory support aimed at minimizing the incidence and severity of BPD are very attractive.

Hypoxemic Respiratory Failure in Infants and Children

Hypoxemic respiratory failure is characterized primarily by hypoxemia with low oxygenation and normal to low capnia. The predominant mechanism in hypoxemic respiratory failure is uneven or mismatched ventilation and perfusion in regional lung units. In infants and children this kind of respiratory failure mainly occurs in disorders characterized by airway obstruction such as status asthmaticus and bronchiolitis. Furthermore, the presence of pneumonia due to different etiologic agents can lead to hypoxemic respiratory failure. Bronchiolitis occurs mainly in children less than 2 years old. Respiratory syncytial virus (RSV) is estimated to be the most frequent etiologic cause, responsible for over half of bronchiolitis cases [17]. RSV bronchiolitis involves mainly small airways but also lung interstitium. On the other hand *Streptococcus pneumoniae* can be considered the most frequent agent of pneumonia, although other microorganisms can also play a relevant role. Other possible causes include non-infectious ones, such as the presence of lobar atelectasis, mainly occurring in the postoperative period. The onset of hypoxemic respiratory failure is more frequently acute.

Hypercapnic Respiratory Failure in Infants and Children

Hypercapnic chronic respiratory failure is characterized by the presence of alveolar hypoventilation associated with normal or reduced oxygenation. The predominant mechanism in hypercapnic chronic respiratory failure is the reduced ventilation caused by depressed neuronal ventilatory drive (central hypoventilation disorders), acute or chronic upper airway obstruction (obstructive sleep apnea), neuromuscular weakness (Duchenne muscular dystrophy and spinal muscular atrophy), rib

cage abnormalities, marked obesity and parenchymatous conditions (advancing cystic fibrosis). The onset of hypercapnic respiratory failure may be insidious and may develop when respiratory muscle fatigue occurs. Thus, it is more frequently associated with chronic stages of respiratory failure.

Continuous Positive Airway Pressure and Positive Pressure Ventilation: Technical Aspects

Since its introduction more than thirty years ago [18], CPAP has continued to develop with a large number of different delivery systems and flow drivers. The physiological benefits of CPAP and main clinical indications are shown in Tables 1 and 2. Fundamentally, the delivery of CPAP requires three components [19]:

- A flow generator;
- An airway interface;
- A positive pressure system.

Flow Generation

Two major varieties exist; constant flow and variable flow (demand). The flow generator should also warm and humidify the inhaled gases. Constant flow is usually provided by an infant ventilator; this limits costs because of the dual use of a single piece of equipment. Most often, the amount of flow is set by the clinical team. Alternatively, variable flow devices use a dedicated flow generator. Here the 'expira-

Table 1. Physiologic benefits of CPAP

Produces more regular breathing pattern
Establishes and maintains functional residual capacity
Decreases upper airway resistance
Results in progressive alveolar recruitment, inflates collapsed alveoli and reduces
Intrapulmonary shunting
Decreases upper airway collapsibility
Reduces obstructive apneas
Promotes the release and conservation of surfactant on the alveolar surface
Distending pressure increases lung volume and lung weight in immature animals

Table 2. Clinical indications for CPAP

Respiratory support of the recently extubated infant
Management of apnea of prematurity
Treatment of hyaline membrane disease
Hypoxemic respiratory failure
Prevention and treatment of post-operative respiratory complications
Alternative to mechanical ventilation in resource-scarce areas?

tory' limb of the circuit is open to the atmosphere and the infant can draw extra gas from this limb to support inspiratory efforts. This device has gained widespread acceptance in Europe and North America. Despite the theoretical advantages of the variable flow device, there are no consistent data showing clinically meaningful benefit over constant flow devices [20].

Airway Interface

A bewildering array of interfaces between the circuit and the infant's airway have been used: single prongs, binasal prongs (short and long), nasopharyngeal prongs, endotracheal tubes, head boxes, pressurized plastic bags, nasal cannulae, and face masks.

The route most commonly used today is nasal CPAP, which was introduced in the early 1970s [21]. In preterm neonates, a Cochrane Systematic Review suggests that short binasal prongs are more effective at preventing re-intubation compared to single nasal prongs. Nasal prongs are very easy to apply and comparatively non-invasive to the airways. The infant can still be nursed and handled with uninterrupted CPAP. Binasal prongs can, however, cause nasal excoriation and scarring [22].

The use of nasal cannulae has been found to be effective in the treatment of apnea of prematurity, however their use may still be associated with nasal mucosal trauma and bleeding [23].

In general, nasal masks are effective interfaces for non-invasive intermittent positive pressure ventilation (IPPV) in most pediatric patients even with a mouth leak and have the advantage of producing less anxiety in small children. Nasal-oral masks have the advantage of eliminating significant oral gas leaks and should be considered in acute setting when oral leaks appear to limit the effectiveness of non-invasive IPPV administered by nasal mask. The main concern regarding the use of nasal oral masks in the acute setting is the potential for significant gastric distension and the tendency for children to vomit and potentially aspirate gastric contents.

Nasal pillows or cushions are a third type of interface which may be effective in children who do not tolerate mask interfaces. They may be also used in the event of skin breakdown at the nasal bridge.

Positive Pressure System

At its simplest, expiratory pressure is provided by a fluid column (bubble CPAP), more frequently by resistance at the expiratory valve of the ventilator, by a Benveniste device – pressure generation at nasal level [24] – or by generating CPAP in the immediate vicinity of the nasal airway by converting kinetic energy from a jet of fresh gas. The aim is to achieve a constant distending pressure throughout the respiratory cycle to maintain functional residual capacity.

Bubble CPAP is a form of oscillatory pressure delivery in which mechanical vibrations are transmitted into the chest secondary to the non-uniform flow of gas bubbles across a downstream underwater seal. Its proponents pointed to the generation of waveforms similar to those produced by high-frequency ventilation, as recorded by a transducer at the airway. In preterm lambs, bubble CPAP results in lower indicators of acute lung injury (ALI) (neutrophils and hydrogen peroxide) than does mechanical ventilation in the first two hours of life [25]. Bubble CPAP

has the advantage of being simple and inexpensive. Studies are required to identify the most effective pressure source for supplying a continuous distending pressure.

There are no compelling data about the optimal pressures for CPAP in infants [26]. Traditionally, pressures of 4–6 cmH_2O have been used. Some investigators, however, claim that higher pressures should be used and some studies have used pressures as high as 10 cmH_2O. We suggest a tailoring of the pressure to the infants needs: increasing oxygen requirements, low volume lung fields on chest radiograph, increase in apneic episodes should prompt a judicious increase in the distending pressure by 1 cmH_2O increments to a maximum of 10 cmH_2O. Few clinical studies have addressed this question, although older physiologic studies would support this approach.

Positive pressure systems can be used to deliver PPV. Most bilevel ventilators available for commercial use are remarkably adept at delivering CPAP but more problems arise when PPV is used. A significant barrier to effective CPAP in young or very small patients is the inability to achieve sufficient inspiratory flow to trigger the inspiratory pressure support feature. This problem can be solved by replacing the connector circuit between the mask interface and ventilator from standard tubing supplied by the manufacturer to a connector tube that is shorter and less compliant. However, this is a departure from standard procedure that is not recommended by the manufacturer. Another common problem with PPV is the effect of significant air leaks around the interface. In the presence of a significant leak the inspiratory pressure target is never achieved, resulting in a very long insufflation time as the unit delivers massive amounts of inspiratory flow in an attempt to attain the preset inspiratory pressure. In some modern ventilators, an adjustable inflation time can be set to limit this problem. However, the majority of positive pressure ventilators used for pediatric use have a fixed inspiratory and expiratory flow trigger and both bench studies and clinical studies have shown significant differences in the trigger sensitivity and performance of the various bilevel pressure devices [27]. The clinical impact of these ventilator differences vary between patients with a significant impact on respiratory effort shown in some patients but not in others. These discrepancies may be explained by the different devices tested but also by the patients' diseases. A bench study showed that the performance of a home bilevel ventilator decreased when the respiratory effort increased [28]. Furthermore leaks can also affect the quality of the trigger increasing the inspiratory trigger delays by slowing the decline in mask pressure [29].

Furthermore a crucial role is represented by the pressurization rate which can markedly affect work of breathing and dyspnea, and has been shown to be markedly different among different ventilators [30, 31]. It must be underlined that these studies were either bench studies or clinical studies performed in adult patients. However, the respiratory effort of these adult patients is far less important than that measured in the infants. Moreover, the breathing pattern of these infants is also different with a higher respiratory rate and a smaller tidal volume which could promote patient ventilator asynchrony.

We, therefore, believe that it is essential to perform non-invasive ventilatory support in infants and children using high performance ventilators with inspiratory and expiratory lines able to correct gas inflation for possible leaks. Furthermore, it is essential that different modalities of ventilation, including pressure time or flow cycled can be delivered, in association with the possibility of regulating inspiratory trigger in flow or pressure, inspiratory-expiratory triggering, and pressure rise time.

Clinical Data

Indications for CPAP in Preterm Neonates

CPAP is now used for a variety of neonatal conditions. It is effective in supporting the recently extubated infant [32] and for treating apnea of prematurity [33]. Increasingly, it is being seen as an alternative to intubation and ventilation in the treatment of hyaline membrane disease [34]. In an historical case series, the team at Columbia University has consistently demonstrated a decreased prevalence of BPD [35] compared to other Neonatal ICU centers. This has been credited to a management strategy emphasizing early and routine use of CPAP, for the treatment of hyaline membrane disease, and more limited use of intubation, surfactant and mechanical ventilation, but has never been subject to a randomized controlled trial [36].

Conditions where CPAP may not be useful include upper airway abnormalities (e.g., Pierre-Robin syndrome), severe cardiovascular instability and intractable apneic episodes.

Upper airway obstruction due to congenital abnormalities of the larynx and trachea can cause severe respiratory distress in infancy. Laryngomalacia is the most frequent congenital abnormality of the larynx and the most common cause of stridor in newborns and infants. Non-invasive CPAP and PPV have been used in this setting [37, 38].

The use of CPAP requires meticulous attention to the infant's airway. One needs the correct prong size and the infant's neck must be properly positioned to avoid excessive flexion or extension. The airway requires frequent suction to clear accumulated secretions (there are no data available to support how frequent airway suction should be), constant observation of breathing patterns and standardized and rigorous training of physicians, respiratory practitioners and/or nursing staff.

One needs to allow the early and judicious use of CPAP, allow both the $PaCO_2$ (permissive hypercapnia) and the FiO_2 to rise and to tolerate and treat apneic episodes. Many infants, with higher mortality and morbidity, are denied access to neonatal intensive care in the developing world because 'scarce' financial resources are directed towards more viable infants. In a prospective study from South Africa, Pieper et al. conducted a quasi-randomized control trial of CPAP for very low body weight infants denied access to the neonatal ICU compared to the standard therapy of headbox oxygen [39]. Although the CPAP was initially placed by respiratory therapists, care was continued by nursing staff with no intensive care or CPAP experience. The infants who received CPAP in these circumstances had a significantly improved short term survival (<24 hours), with trends towards improved long-term survival. None of the infants in the study received surfactant therapy. Further studies are warranted to define if the routine early use of CPAP in areas of diminished neonatal resources provide an alternative to conventional mechanical ventilation and reduction in costs and waste of resources.

Indications for Intermittent Positive Pressure Ventilation in Neonates

In addition to CPAP, neonatal ICUs have adopted nasal IPPV, via nasal prongs, with and without synchronization, as an alternative non-invasive strategy for respiratory support [40]. Nasal IPPV may improve patency of the upper airway by creating intermittently elevated pharyngeal pressures and by intermittent inflation of the phar-

ynx, activating respiratory drive. Lung inflation, by artificial ventilation, provokes an augmented inspiratory reflex, i.e., Head's paradoxical reflex, in certain preterm infants. Moretti et al. [41] demonstrated improved efficiency of breaths obtained by synchronized nasal IPPV as compared to nasal CPAP leading to larger tidal volumes (V_T) and minute volume. In response to the increase in V_T, respiratory rate decreased by a statistically significant amount and the $PaCO_2$ reduced.

Physiologically synchronized nasal IPPV may offer advantages over non-invasive CPAP, by improving tidal and minute volumes and by activating respiratory drive which is poorly controlled in extremely low birth weight infants. From the three randomized control trials published, synchronized nasal IPPV appears to provide superior respiratory support for recently extubated preterm infants [42–45]; the number needed to treat being 3 infants to prevent one extubation failure. A trend towards lower rates of BPD in infants randomized to synchronized nasal IPPV was noted in the two trials reporting this outcome [42, 45], but did not reach statistical significance, the trials not being powered for this outcome.

These trials report only short term benefits of synchronized nasal IPPV over nasal CPAP, and are not powered to detect benefit of synchronized nasal IPPV for clinically relevant long-term outcomes such as BPD and death. Do the advantages of synchronized nasal IPPV over CPAP in the short term following extubation lead to real and meaningful clinical outcomes in the longer term?

There are no studies describing the use of synchronized nasal IPPV in the first line management of hyaline membrane disease.

Indications for Nasal/mask Continuous Positive Airway Pressure in Infants and Children

The experience of non-invasive CPAP in infants and children is scarce and so far case series without any control group constitute the vast majority of the available knowledge, especially in the acute setting. Furthermore, many of the published case series reported results from the treatment of groups with acute respiratory failure of different etiologies and severity, making it even more difficult to draw conclusions in respect of any specific disease. Finally, there are no generally accepted guidelines for non-invasive ventilation in infants and children.

Soong et al. [46] investigated 10 infants with an average age of 6 months and severe bronchiolitis treated with CPAP by nasal prongs and found an improvement in respiratory rate and gas-exchange. A recent study in infants and young children with a mean age of 10 months and chronic upper airway obstruction showed that CPAP and bilevel positive airway pressure (BiPAP) delivered by nasal mask were associated with a significant and comparable decrease in respiratory effort but patient-ventilator asynchrony was more frequent during BiPAP ventilation [47].

Indications for Nasal/mask Positive Pressure Ventilation in Infants and Children

Data regarding the effect of PPV in infants and children in the acute setting are astonishingly scanty and mainly characterized by case reports or studies with small numbers of patients. In 1993, Akingbola et al. [48] published a case report describing the effectiveness of nasal IPPV in two pediatric patients with acute respiratory distress due to leukemia, to prevent intubation after extubation. Since that time nasal IPPV has been applied in pediatric patients with a variety of respiratory dis-

orders associated with acute hypoxemic and chronic hypercapnic respiratory failure. Marino et al. [49] reported the effectiveness of prolonged nasal IPPV to stabilize oxygenation and avoid intubation in one case of acute respiratory failure with lung infiltrates due to leukemia. Fortenberry et al. [50] reported an intubation rate lower than expected (11% vs 42%) in a group of 28 patients with pneumonia and neurologic disorders. Akingbola et al. [51], in 9 patients with pulmonary edema, atelectasis and pneumonia found an improvement in oxygenation but not in ventilation. Teague et al. [52] in 26 patients with status asthmaticus reported improvement in oxygenation in 70% of patients but a high rate of intubation (26%).

Nasal IPPV has also been found to be effective in improving ventilation and oxygenation in children with upper airway obstruction [53] and following corrective spinal repair [54].

Nasal IPPV has been reported effective also in chronic disorders. Padman et al. [55] reported improved dyspnea scores and oxygen saturation in 43 patients with neuromuscular disease, obesity and encephalopathy with an intubation rate of 9%. In another study, Niranjan and Bach [56] combined the use of nasal IPPV with expiratory support (manual and assisted coughing) in 10 children with neuromuscular disease pointing out that patient cooperation was critical for success. Rosen et al. [57] reported the effectiveness of nasal IPPV in five patients with obstructive apnea post-tonsillectomy with no intubations. Bimkrant et al. [58], in 25 patients with spinal muscular atrophy, found that nasal IPPV allowed 80% of cases to be weaned from an invasive airway and, similarly, Bach et al. [59] reported that nasal IPPV was successful in 11 very young children with severe skeletal and bulbar weakness due to spinal muscular atrophy. Padman et al. [60] reported the possible use of nasal IPPV in seven patients with advanced cystic fibrosis as a bridge to lung transplantation. However, another study [61] showed that, in stable patients with advanced cystic fibrosis, nasal IPPV did improve respiratory gas exchange but long term acceptance of nasal IPPV by this population was poor. Nasal IPPV can have also a role in the early management of acute chest syndrome in children with sickle cell anemia by restoring lung volume and thereby preventing atelectasis [62].

However, in the majority of these studies, both in premature neonates, infants and children the overall rate of endotracheal intubation was relatively low, due to the light to mild respiratory insufficiency of the majority of the patients included and no control group. It is, therefore, not possible to conclude that the application of non-invasive ventilation in infants and children with severe hypoxemic respiratory failure can prevent endotracheal intubation [63].

Continuous Negative Extrathoracic Pressure (CNEP)

During the polio epidemics of 1930 to 1960, negative pressure ventilators in the form of the 'iron lung' saved many lives. However, by the 1950s the greater efficiency of PPV through a tracheostomy or endotracheal tube had superseded the negative pressure devices.

Despite one randomized controlled trial on CNEP [64] in neonatal respiratory failure, showing a small benefit, the devices have failed to gain widespread acceptance and have been superseded by the more effective nasal CPAP. Nevertheless, in infants and children, CNEP has been found to be superior to the use of oxygen alone with regard to avoiding intubation and has been compared favorably to the use of nasal CPAP [65, 66].

New Technical Developments

Recently, the use of helium oxygen mixtures (heliox) and the developments of new interfaces has been introduced into the field of non-invasive respiratory assistance. The beneficial effects of Heliox have been attributed essentially to lower density compared with an air oxygen mixture reducing the driving pressures required under turbulent flow conditions and preserving laminar flow at higher flow rates. Therefore, heliox can reduce the work of breathing by means of a marked reduction in airway resistance. A recent study [67] reported that in infants with moderate to severe acute bronchiolitis, heliox therapy rapidly enhanced their respiratory clinical status and the improvement was maintained as long as heliox therapy was maintained. More interestingly, the authors reported a reduction in pediatric ICU length of stay. Long term prospective studies are required to corroborate these findings and establish the proper role of heliox in the therapeutic schedule of bronchiolitis. Furthermore, the association between heliox therapy and non-invasive respiratory support should be evaluated in physiological and clinical trials.

As discussed above, non-invasive respiratory support is usually applied by nasal or facial mask but these interfaces may result in air leaking, discomfort, need of sedation and pain which can lead to discontinuation in ventilatory treatment [68]. Thus, improving the interface between the patient and the ventilator would be expected to be crucial to achieve a prolonged and effective application of non-invasive ventilation.

In an attempt to improve tolerance, a new interface that has been previously successfully employed in adult patients in several trials [69, 70] has been developed. It consists of a new helmet, specifically designed for infants, with an original fixation system that should minimize risks of cutaneous lesions and improve tolerance (Castar© Starmed, Italy).

The helmet is made of transparent latex free polyvinylchloride and is secured to a soft collar that adheres to the neck of the child. This collar allows a sealed connection to be obtained during continuous positive pressure, avoiding air leakage. The system is secured by two braces to the pants.

Athough several sizes are commercially available, for infants between 5–10 kg, the neck collar is a maximum 27 cm diameter with a volume of approximately 6 l. The two ports of the helmet are connected to a fresh gas flow source and to a PEEP mechanical or water valve. Elevated fresh gas flows must be used to avoid rebreathing [71] and a reservoir balloon (15 l) can be inserted in the inspiratory limb to minimize pressure swings. No active humidification should be applied during treatment. A specific seal connector is used to allows the passage of a nasogastric tube when needed. Moreover the transparency of the system allows the infants to interact with parents and environment (Fig. 1). The helmet is specifically designed for CPAP. Piastra and Antonelli [72] used the helmet during positive pressure non-invasive ventilation in four severely hypoxemic children with a mean age of 14 years with acute leukemia who showed an immediate improvement in oxygenation without complications. A rigid helmet has been recently used to deliver CPAP also in preterm neonates for apnea and/or mild respiratory distress resulting in similar success at improving gas exchange and better tolerability compared to a nasal mask [73].

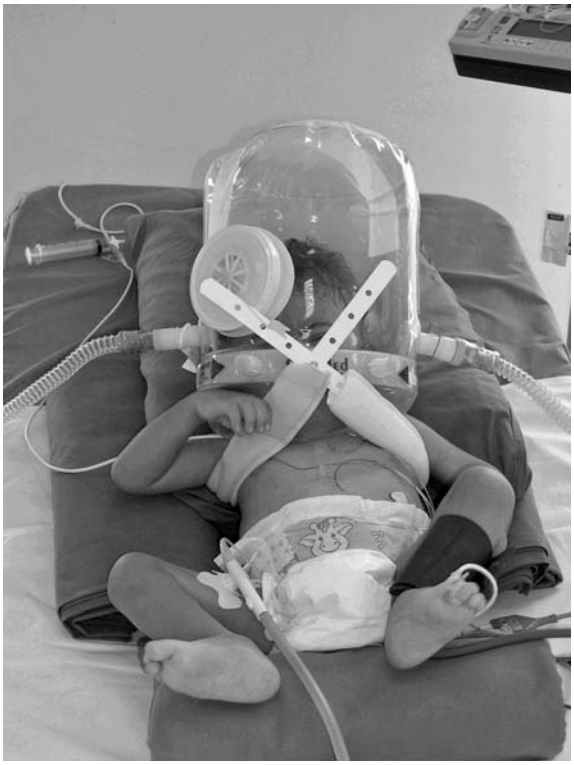

Fig. 1. Infant receiving CPAP with helmet

Conclusion

In conclusion, non-invasive ventilatory support in the neonatal and pediatric ICU has become an option in the last few years and is being applied increasingly. A few uncontrolled trials and case series indicate that the technique can be useful for pediatric patients with a wide spectrum of respiratory disorders including hypoxemic and hypercapnic conditions. The development of more recent techniques and new interfaces can further improve the success of non-invasive respiratory support in this setting. However, the evaluation of the impact of non-invasive respiratory support on morbidity and mortality by appropriate prospective randomized trials and the development of evidence based guidelines for diagnosis, treatment organization and follow up are warranted in the near future.

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Fast and Slow Compliance: Time, in Addition to Pressure and Volume, is a Key Factor for Lung Mechanics

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Introduction

Static lung mechanics are considered state of the art in spite of the fact that they only provide a narrow view and do not represent the mechanical behavior of the lung during on-going tidal ventilation. Static measurements are usually cumbersome to perform and are uncommon in clinical practice. There is now ample proof of the importance of choosing a protective ventilatory strategy, which has been defined as ventilating with pressures between the lower and upper inflection point (LIP, UIP) [1, 2]. Determination of these two inflection points demands static or at least quasi static measurements. The definition of true static conditions is that a sufficiently long end-inspiratory and end-expiratory pause is used to not only stop gas flow in the airways, but also equilibrate visco-elastic forces of the lung tissue. It has been shown that this equilibration time is short and the tracheal pressure decreased as little as ~ 2 cmH_2O during the five seconds after instigation of an end-inspiratory pause [3]. This pressure fall is small compared to the pressure fall that occurs within milliseconds immediately after closing the inspiratory valve of the ventilator. The initial pressure drop is a result of obtaining no-flow conditions in the patient's airways and the time is correlated to the endotracheal tube and patient airway resistance (R in cmH_2O/L/s), the breathing circuit compliance (C in l/cmH_2O) and the flow immediately before closing the valve:

$$t = \text{time constant} = R \times C$$

In a typical case, the breathing circuit has a compliance of 0.5×10^{-3} l/cmH_2O and a tube resistance of 6 cmH_2O/l/s which gives a time constant of 3 ms. In this case the flow will decrease by 95% in three time constants, i.e., ~ 10 ms. After a minimal time, the pressure in the ventilator and in the alveoli will be equalized, disregarding the visco-elastic forces, that will lag behind through the breath during tidal ventilation. During a long end-inspiratory pause the visco-elastic forces will cause the pressure in the alveoli to fall as compared to the pressure immediately after closing the inspiratory valve and *vice versa* during a prolonged end-expiratory pause.

Thus, there are different time perspectives regarding lung mechanics: dynamic measurements, no-flow measurements, measurements including equilibrated visco-elastic forces, and finally measurements including time needed for opening collapsed lung units.

Fast Compliance

Three different methods have been proposed for determining the pressure-volume relationship during tidal ventilation, i.e., fast compliance: The slice method [4, 5], the dynostatic algorithm (DSA) [6, 7], and the stress index [8, 9]. The slice method and the DSA both give an alveolar pressure volume curve during on-going ventilation.

The Slice Method

The slice method is a three step procedure where first the tracheal pressure is calculated from the ventilator pressure and flow and an algorithm for the endotracheal tube resistance and then a tracheal pressure-volume loop is obtained. For reasonable precision of the measurements the pressure volume loop is divided into six slices and the least square method is applied for each slice to give a compliance value for each slice from the bottom to the top of the tidal volume. Thus, this method has the capacity of calculating volume-dependent compliance breath by breath: fast compliance. The advantage of the method is that it is based on easily available data for computation and can be used in both volume and pressure control ventilation. The disadvantage is that tube resistance may differ from the algorithm values in clinical practice and that the end parts of the breath – at the bottom and at the top are difficult to calculate with good precision. Also, any inflection point will be pre-positioned between the slices irrespective of where it is in reality.

The Dynostatic Algorithm

The DSA is based on direct tracheal pressure measurements for obtaining a tracheal pressure-volume loop. Assuming that the expiratory and inspiratory resistances at the same inspiratory and expiratory volume are reasonably similar, the alveolar pressure can be calculated by using the equation of motion at a number of isoplanes of the tracheal pressure-volume loop and an alveolar pressure-volume curve can be obtained displaying the 'fast' volume-dependent compliance breath by breath. The advantage of the DSA is that it is independent of tube resistance and also of changes in the resistance of the airways during inspiration and expiration. Also, the pressure-volume curve can show the correct position of any inflection points as it is based on at least twenty isovolume planes for each breath. A further advantage is that it can be used in both volume and pressure control mode. The disadvantage is that it demands a tracheal pressure line inserted through the tube, but this is at the same time an advantage as the pressure in the trachea can be measured without interrupting ventilation, i.e., peak tracheal pressure and intrinsic positive end-expiratory pressure (PEEP) can be monitored continuously.

The Stress Index

The stress index method is not a method that directly results in a conventional pressure-volume curve as it calculates the shape of the inspiratory pressure-time curve during on-going volume control ventilation according to the formula:

$$\text{airway pressure} = a \times \text{time}^b + c\,,$$

where coefficient b (stress index) describes the shape of the curve. Coefficient $b < 1$ indicates tidal recruitment and $b > 1$ indicates hyperinflation. This stress index curve can easily be transformed into a more conventional inspiratory pressure-volume curve by exchanging the time with volume as the inspiratory flow is constant. By exchanging the Y- and X-axis an inspiratory airway pressure-volume curve is obtained. Displaying the stress index based on this airway pressure-volume curve, a $b < 1$ indicates hyperinflation and a $b > 1$ indicates tidal recruitment. In Figure 1, the original stress index is graphed above the stress index converted to a pressure-volume plot. The lower graph is easier to compare with conventional pressure-volume curves and it is obvious that instead of the stress index, compliance could be calculated for different parts of the curve to obtain volume-dependent compliance values. The advantage of the stress index is that it is based on easily available data for computation of the index. The main disadvantage is that it requires volume control ventilation and only considers the inspiratory part of the breath. There may also be some doubts as to whether airway resistance is constant during inspiration.

These three methods for dynamic volume-dependent compliance monitoring give pressure-volume curves that disregard the effects of the visco-elastic forces. This may lead to an underestimation of the level of an LIP in comparison with an LIP determined with a slow inflation inspiratory pressure-volume curve as the time that the alveolar pressure is below the LIP during tidal breathing is much shorter, in a typical case 1.5 seconds as compared to over 6 seconds during slow inflation

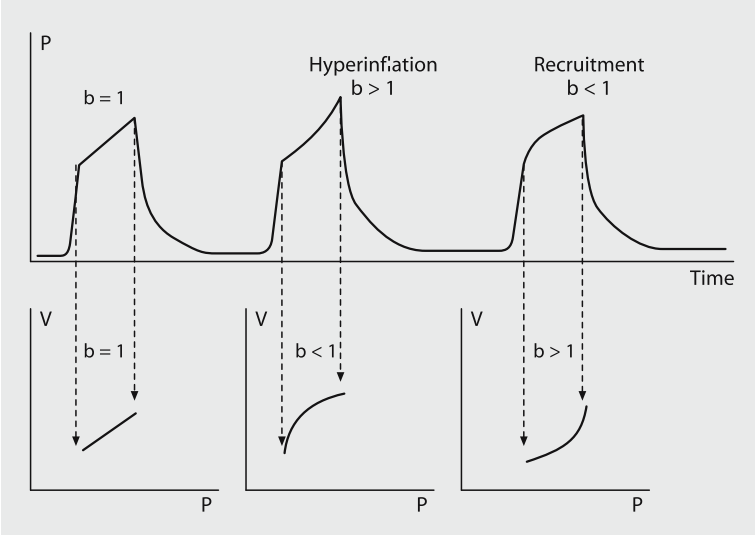


Fig. 1. Upper panel showing airway pressure vs. time during volume control (constant flow) ventilation. Stress index $b > 1$ indicates hyperinflation and $b < 1$ recruitment. In the lower panel the pressure-time curve part obtained during constant flow is converted into a conventional inspiratory pressure-volume (P/V) curve. As pressure now is on the x-axis, the $b > 1$ indicates recruitment and $b < 1$ indicates hyperinflation. From the lower panel it can be seen that compliance could be calculated to obtain 'fast', volume-dependent compliance

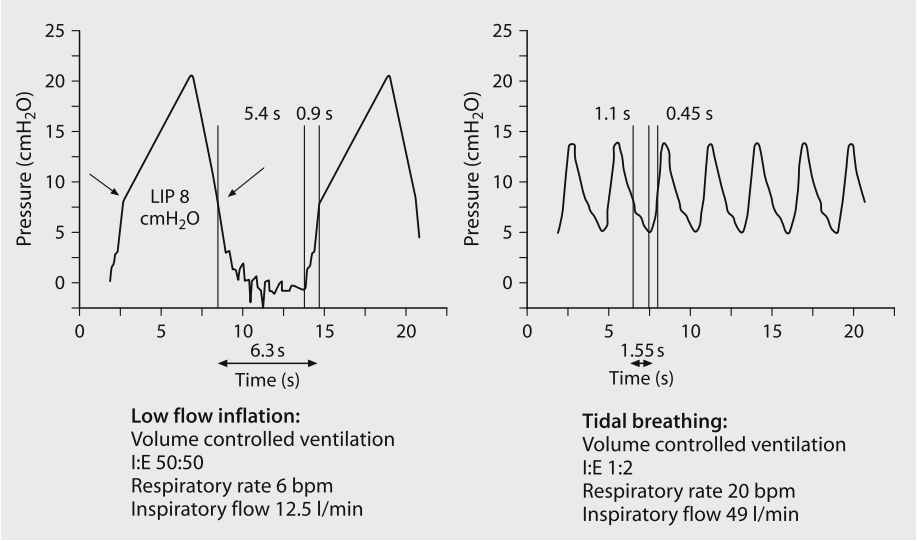


Fig. 2. Alveolar pressure vs. time in a patient with acute lung injury during slow inflation (left panel) and during tidal breathing (right panel). In this patient the lower inflection point (LIP) is ~ 8 cmH_2O which can be seen as the inflection in the pressure trace (indicated by arrows) during inspiration and expiration. During the slow inflation, the pressure is below the LIP for more than 6 seconds. During therapeutic tidal ventilation at a rate of 20 breaths/min the corresponding time is only 1.5 seconds and this is too short a time for collapse to occur. From [10] with permission

(Fig. 2) [10]. Taking into account that the time needed for alveolar or terminal bronchiolar collapse may be up to 4 seconds [11], the LIP during normal tidal breathing may not be seen even when the PEEP level is below a LIP determined with the slow inflation method.

Slow Compliance

In 5 patients with acute respiratory failure we measured conventional quasistatic compliance and functional residual capacity with a modified nitrogen washout/washin technique [12] at two different PEEP levels. The increase in functional residual capacity was 386 ± 265 ml when PEEP was increased 3 ± 1 cmH_2O. If conventional quasistatic compliance had been used for predicting the increase in functional residual capacity as the result of such a PEEP increase, the functional residual capacity would only have increased by 149 ± 35 ml. In fact, the compliance of the lung calculated as the $\Delta FRC/\Delta PEEP$ was much higher, 108 ± 59 ml/cmH_2O, than the conventional quasistatic fast compliance, 44 ± 9 ml/cmH_2O. This indicates that the mechanical properties of the respiratory system differ when subjected to short and long procedures.

If, when increasing PEEP, the delivered tidal volume is larger than the increase in functional residual capacity caused by the step change one would expect the first tidal volume after the PEEP change to be enough to top up the functional residual capacity to the new level unless there is a difference in fast and slow mechanical properties of the lung. But when we increased or decreased PEEP, it took several

breaths, 8–9, to reach the new functional residual capacity level. Figure 3 shows a typical breath by breath increase in functional residual capacity after increasing PEEP and a stepwise decrease when lowering the PEEP level [13]. In a patient with a large functional residual capacity and high compliance the procedure may take even longer.

In a study on one lung-lavaged piglet, body weight 2 kg, using the electric impedance tomography (EIT) technique, Frerichs et al. studied the effect of increasing PEEP stepwise by 5 cmH_2O from 0 up to 30 cmH_2O and then downwards with the same steps during volume control ventilation with 10 ml/kg [14]. The EIT permits regional analysis of ventilation and in this study, four regions, 1, 2, 3, and 4 from ventral to dorsal were studied (Fig. 4). At zero end-expiratory pressure (ZEEP), the peak pressure was 20 cmH_2O and tidal inflation was only seen in the two most ventral regions. At a PEEP of 10 cmH_2O, tidal ventilation of the third region could be seen but not until a PEEP of 15 cmH_2O was applied with a peak pressure of 28 cmH_2O did tidal ventilation start in the most dorsal regions. Thus, although the peak pressure was 20 cmH_2O at ZEEP and exceeded the 15 cmH_2O level that was needed to create tidal ventilation in the most dorsal region, such a tidal ventilation was not seen. When the 15 cmH_2O level was reached it was no longer possible to see the different PEEP steps in the three most dorsal regions as continuous recruitment was achieved. A careful view of the pressure tracing shows a fall in the peak pressure at each PEEP level as a consequence of the recruitment. As predicted by Hickling et al. in a mathematical model (with pressure control ventilation), stepping down the PEEP ladder maintains tidal ventilation at lower PEEP levels also in the dorsal regions more than going up the PEEP ladder [15].

Odenstedt et al. have recently investigated the time factor of changes in lung mechanics during recruitment maneuvers using EIT in lung lavage pigs with a body weight of $\sim$28 kg [16]. During the EIT calibration procedure, when PEEP was in-

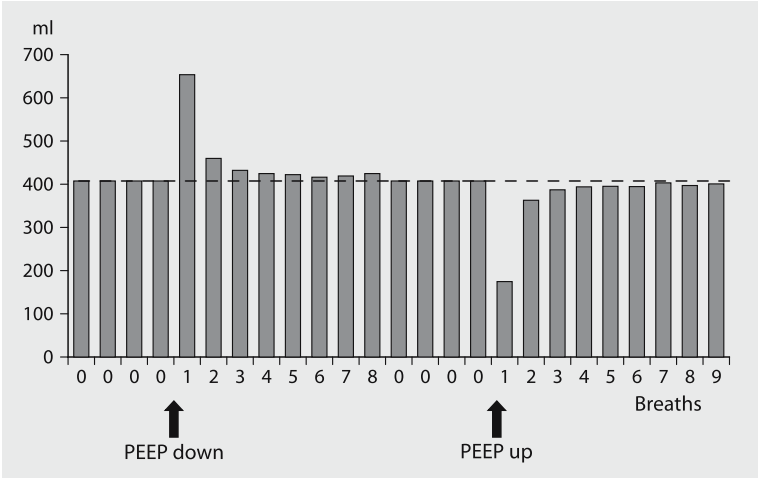
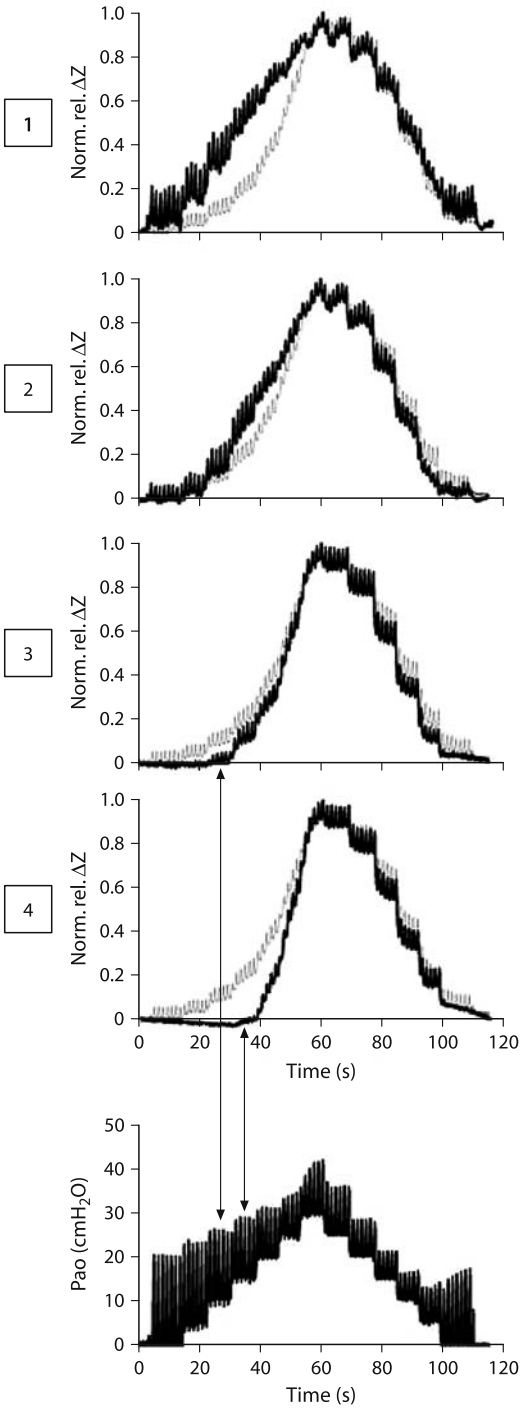


Fig. 3. The expiratory tidal volumes following a decrease in PEEP and an increase in PEEP of 7 cmH_2O. The main emptying and filling of the functional residual capacity occurs during the three first breaths but continues for several more breaths. In a patient with higher compliance the process would be even longer


1
2
3
4
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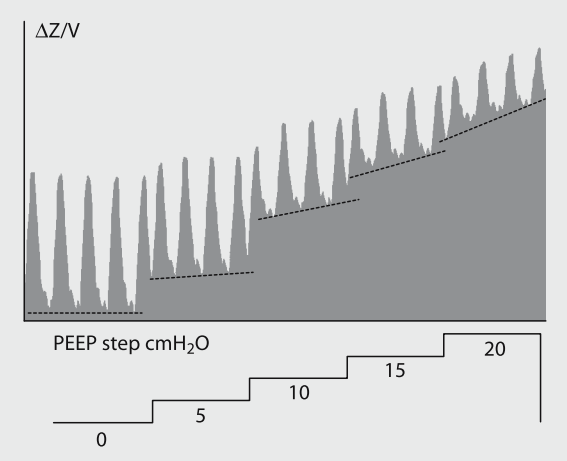

Fig. 5. Global electric impedance tomography signal during stepwise, 5 cmH_2O, increases in PEEP in a pig with surfactant depletion of the lungs through lavage. Note the progressive increase in end-expiratory lung volume (indicated by dashed lines) at increased PEEP levels

creased stepwise by 5 cmH_2O, it was clearly seen that 12–15 seconds at each PEEP level did not create a lung mechanics steady state. In Figure 5, a global EIT tracing shows an increasing end-expiratory lung volume at each step and the increase is more pronounced the higher the PEEP level. This is a clear indication that recruitment is a time dependent process, which was verified when two conventional, rapid, high pressure recruitment maneuvers were compared to a slow recruitment maneuver with lower airway pressure applied intermittently over a longer period (PEEP increased to 15 cmH_2O and a prolonged 7 second end-inspiratory pause twice a minute for 15 minutes). During the slow recruitment maneuver there was first a rapid increase in end-expiratory lung volume which was followed by a slow increase until the end of the recruitment maneuvers. After the recruitment maneuvers, PEEP was set to 10 cmH_2O (Fig. 6). Fifteen minutes after the recruitment

Fig. 4. Electric impedance tomography signal from four regions where 1 is the most ventral and 4 the most dorsal during an up and down PEEP ladder with steps of 5 cmH_2O. The grey trace superimposed on each of the regions is the global EIT curve. The lowest panel shows the airway pressure. Note that during the stepwise increase in PEEP, the regional ventilation of the ventral region occurs at a much lower PEEP than the global EIT signal indicates. The two arrows indicate that tidal ventilation does not occur in the 3rd region until a PEEP level of 10 cmH_2O is applied. A PEEP level of 15 cmH_2O is needed for tidal ventilation to occur in the most dorsal region in spite of the peak pressure during ventilation at lower PEEP levels that should have been enough to cause tidal ventilation in these parts of the lung already at ZEEP. During decreasing levels of PEEP, there is tidal ventilation in the most dorsal region even at a PEEP level of 5 cmH_2O, which indicates that the critical closing pressure of the alveoli are much lower than the opening pressure. In the pressure tracing, the peak pressure level falls during all the 'PEEP up steps', as an indication of recruitment and during the 'PEEP down steps' the peak pressure increases as a sign of derecruitment. At the highest PEEP level, the peak pressure rises rapidly breath by breath without a corresponding increase in EIT volume signal. This is probably a sign of hyperinflation or compression of gas in the lungs. From [14] with permission

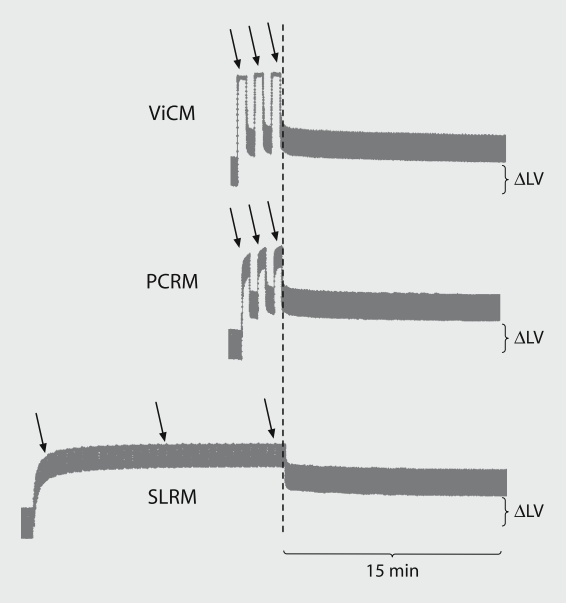

Fig. 6. Global electric impedance tomography recordings in a lung lavaged pig before, during and after three different recruitment maneuvers. The ViCM is a vital capacity maneuver at a pressure of 40 cmH_2O for 30 seconds repeated three times, with a recovery period of 30 seconds at a PEEP of 10 cmH_2O between the recruitment maneuvers. The PCRM is a recruitment maneuver in pressure control ventilation with PEEP at 20 and peak pressure at 20 cmH_2O over PEEP, repeated as for the ViCM. During the slow, moderate pressure recruitment maneuver (SLRM), PEEP was increased to 15 cmH_2O and an end inspiratory pause of 7 seconds applied twice a minute for 15 minutes. After all the recruitment maneuvers, PEEP was set at 10 cmH_2O and the pig was ventilated in volume control mode with the same tidal volume as at baseline. For each repetition of the ViCM and the PCRM the lung volume increases, especially in the PCRM as an indication that not even during these high pressure is recruitment instant (indicated by arrows). In the SLRM, there is a moderately rapid increase in end expiratory lung volume during the initial 2–3 minutes and then a more slow increase throughout the 15 minutes used for the recruitment maneuver. This indicates clearly how time consuming it is to 'remould' the lung mechanics when acute lung injury is present

maneuvers there was no difference in end-expiratory lung volume but compliance was significantly higher with slow recruitment maneuvers compared to the more forceful vital capacity recruitment maneuvers.

Conclusion

In view of this slow lung mechanics phenomenon, it is worth considering whether a slow inflation procedure to obtain a quasistatic inspiratory pressure-volume curve has a duration that is sufficient to give adequate information of the slow 'moulding' process of the lung. It may be better to use a stepwise up and down PEEP ladder during on-going ventilation where lung mechanics are evaluated with a combination of functional residual capacity measurements and breath-by-breath measure-

ments of volume-dependent compliance. The PEEP ladder functional residual capacity measurements would give data on the slow compliance phenomenon, and the volume-dependent compliance measurements would provide information on the fast compliance. In combination, these measurements may improve the rationale for setting PEEP and tidal volume to minimize lung damage.

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Does Ventilator-induced Lung Injury Initiate Non-pulmonary Organ Dysfunction?

L. Brander and A.S. Slutsky

Introduction

The mortality rate and costs associated with acute respiratory distress syndrome (ARDS), the most severe form of acute lung injury (ALI), remain excessively high [1]. Although the most obvious clinical abnormalities in ALI/ARDS are referable to the lung, the most common cause of death is not due to hypoxia but to multiple organ dysfunction syndrome (MODS) [2]. MODS is often irreversible with a mortality rate higher than 60%. We currently lack a specific treatment of the syndrome and modern technology, such as hemodialysis, only allows temporary substitution of organ function, providing a bridge to recovery. Better understanding of the pathophysiology leading to the development of MODS in mechanically ventilated patients should help in the development of approaches to interrupt the cascades leading to MODS.

Most ALI/ARDS patients require mechanical ventilation. Employing low tidal volumes (V_T), limited airway pressures, and positive end expiratory pressure (PEEP) during ventilation of injured lungs has not only been shown to attenuate ventilator-induced lung injury (VILI) [3], but has also been shown to decrease non-pulmonary organ dysfunction [4, 5], and mortality in patients with ALI/ARDS [4]. Despite intense research, the mechanisms by which mechanical ventilation ultimately culminates in MODS and death in these patients and how a protective ventilatory strategy would reduce mortality and MODS are only incompletely understood. The coexistence of ARDS and dysfunction of non-pulmonary organs in critically ill patients makes it difficult to determine whether there is a direct causal relationship between mechanical ventilation and the development of MODS. Nevertheless, in view of experimental and human data, it is reasonable to assume that in many instances, injurious mechanical ventilation potentates the adverse effects of an underlying critical illness and worsens non-pulmonary organ dysfunction.

With this chapter, we wish to review and integrate some of the most pertinent concepts likely to be crucial in the translation of VILI to non-pulmonary organ dysfunction. We structure the chapter into

- mechanical ventilation-induced and propagated lung and systemic inflammatory response,
- mechanical ventilation-induced pro-apoptotic pathways,
- mechanical ventilation-induced impairment of global hemodynamics and oxygen delivery (DO_2),
- mechanical ventilation in ALI/ARDS and kidney dysfunction,
- mechanical ventilation in ALI/ARDS and dysfunction of organs in the hepatosplanchnic region.

Mechanical Ventilation-induced and Propagated Lung and Systemic Inflammatory Response

Two primary mechanistic factors, summarized as mechanotrauma, contribute to the evolution of VILI:

1) exposure of lung tissue to high airway pressures (Paw) resulting in high transpulmonary pressures and overdistention of alveolar regions, and
2) cyclic and repetitive alveolar recruitment and derecruitment (collapse) resulting in alveolar shear-stress forces [6].

Alveolar overdistension and shear-forces can stimulate lung and immune cells to produce and release inflammatory cytokines and chemokines – a process termed biotrauma [3]. Concomitant disruption of tissue and cell integrity as well as disruption of lung epithelial and endothelial barriers enables spill-over of inflammatory mediators into the bloodstream resulting in initiation, exacerbation, or propagation of a systemic inflammatory response [7]. The concept that VILI initiates and propagates a systemic inflammatory response that may eventually contribute to the onset of MODS was already suggested almost a decade ago [8]. Mechanical ventilation of injured lungs as compared to healthy lungs consistently results in release of larger amounts of inflammatory mediators into bronchoalveolar lavage (BAL) fluid and blood, and low V_T as compared to high V_T normally attenuates the release of cytokines and chemokines by the lungs [6]. Given that the vast aerated surface area of the lung is perfused by almost the entire cardiac output, it is conceivable that release of even small quantities of inflammatory mediators per cell could accumulate to a significant amount of these mediators in the circulating blood.

Furthermore, mechanical ventilation-induced disruption of epithelial and endothelial barriers has also been shown to facilitate translocation of intratracheally instilled bacteria and endotoxin from the lung into the blood [9]. Lin et al. instilled *Pseudomonas aeruginosa* intratracheally in rats and ventilated the animals for one hour followed by extubation. The rats ventilated with an injurious strategy tended to have higher 48-h mortality and were more likely to have a positive blood bacterial culture and an impaired host defense reflected by lower blood and lung levels cytokines as compared to animals subjected to lung protective mechanical ventilation before bacteria instillation [10]. Development of bloodstream infections acquired during an intensive care unit (ICU) stay is associated with mechanical ventilation and was identified as an independent risk factor for death from critical illness [11].

Clinical trials support the concept that biotrauma ultimately leads to MODS. Mechanical ventilation of patients with ALI/ARDS with low V_T (about 6 ml/kg predicted body weight) as compared to higher V_T (about 12 ml/kg predicted body weight) decreased serum cytokine levels [4, 12], decreased levels of organ dysfunction [4], and decreased mortality [4]. Furthermore, changes in serum levels of proinflammatory mediators were found to be associated with changes in overall MODS score [5].

Despite an increasing body of evidence supporting the concept that systemic upregulation of an inflammatory response associated with mechanical ventilation ultimately contributes to the pathogenesis of MODS, the specific mechanisms remain undefined.

Activated neutrophils appear to play a central role in generation of the tissue injury characteristic of VILI. The lung contains a large pool of marginated neutro-

phils, and the lungs of patients with ALI/ARDS have a large number of activated neutrophils. In experimental models, induction of neutropenia markedly decreases lung vascular permeability and other indices of lung injury [13]. Postmortem studies in ARDS patients demonstrated pulmonary accumulation of neutrophils, and neutrophil counts in the BAL fluid were higher in non-survivors than in survivors [14].

In addition to lung-born chemo- and cytokines, activated neutrophils circulating in the bloodstream might be an important factor in the pathogenesis of non-pulmonary organ inflammation and dysfunction. Adherence of activated neutrophils to the endothelium creates a microenvironment in which neutrophil derived oxidants, proteases, and cationic proteins are discharged, a process which finally leads to loss of cellular and microvascular integrity, increased vascular permeability, and tissue injury.

Mechanical Ventilation-induced Pro-apoptotic Pathways

Apoptosis is an active, gene-regulated mechanism of cell death that maintains cellular homeostasis under physiological conditions. Increased rate and dysregulation of apoptosis may contribute to the pathogenesis of a number of diseases including ALI and MODS. Increased epithelial cell apoptosis has been detected in an animal model of sepsis as well as in patients dying of sepsis and MODS, trauma and shock [15]. Various pathways and mediators involved in the induction of apoptosis have been described including inflammatory cytokines and chemokines and it has been suggested that apoptosis of parenchymal and microvasculature endothelial cells is important in the pathogenesis of MODS [16].

Binding of soluble Fas ligand (sFasL, a circulating pro-apoptotic factor) to Fas (a cell-surface receptor present on most cells of the body) initiates an intracellular apoptotic signaling pathway. Matute-Bello et al. found that sFasL is released in the airspaces of patients with ALI, and that at the onset of ARDS sFasL concentrations in the BAL fluid of non-survivors were significantly higher than in patients who went on to survive [17]. Intratracheal administration of sFasL induced lung injury and inflammation in rabbits [18] suggesting that activation of the Fas/Fas-ligand system may contribute to the epithelial damage that occurs in ARDS. Albertine et al. showed that Fas expression is increased in the alveolar epithelium of patients who die with ARDS and that sFasL is measurable in pulmonary edema fluid of patients with ALI [19].

Lung born, pro-apoptotic mediators might decompartmentalize (i.e., spill over into the systemic circulation), and contribute to cell apoptosis in non-pulmonary organs. Imai et al. recently showed increased cell apoptosis in the kidneys after mechanical ventilation with a relative injurious (V_T 15–17 ml/kg, PEEP 0–3 cmH_2O) as compared to a protective ventilatory strategy (V_T 5–7 ml/kg, PEEP 9–12 cmH_2O) [20]. In addition, the authors showed increased apoptosis, when LLC-RK1 rabbit renal proximal tubular cells were incubated with plasma from injuriously ventilated animals, and an attenuation of apoptosis induction when soluble Fas ligand (a pro-apoptotic agent) was blocked with Fas:Ig. The clinical implications of these findings were further assessed by using plasma samples from a previous clinical trial comparing a lung protective mechanical ventilation strategy versus conventional mechanical ventilation [12] where Imai et al. found a correlation between changes in plasma soluble Fas ligand and changes in plasma creatinine concentrations.

Mechanical Ventilation-induced Impairment of Global Hemodynamics and DO_2

The heart is the non-pulmonary organ most directly affected by mechanical ventilation [21] but our understanding of the effects of mechanical ventilation on cardiac function as well as on regional hemodynamics is incomplete partially due to the complexity of the interaction as well as to limited accessibility of the involved systems and vascular beds. Interventions to facilitate mechanical ventilation, such as administration of sedative, analgesic, and occasionally paralytic drugs, frequently impact the hemodynamic system and further complicate the study of heart-lung interactions. Impairment of cardiac function during mechanical ventilation is often reversed or counteracted by means of fluid or administration of vasoactive drugs.

Compromised global, regional, and microcirculatory blood flow and DO_2 is likely to contribute to the pathogenesis of MODS. However, the effect of mechanical ventilation on non-pulmonary organ perfusion has only rarely been studied in animals and critically ill patients with ALI/ARDS [22, 23], and most concepts related to heart-lung interactions during mechanical ventilation stem from studies in animals [24] and patients without lung injury [25]. Interestingly, little is known about the effects of mechanical ventilation on the microcirculation in non-pulmonary organs [26–28].

Ventilation of normal lungs has a different impact on the cardiovascular system compared to ventilation of diseased lungs. The heart-lung system can be conceptualized as two interdependent pressure chambers with asynchronous cycling of their pressure changes. Paw is transmitted to lung tissue and vasculatures, and to intra-thoracic organs. Transmission of Paw to the intra-thoracic structures depends on lung compliance, i.e., with low lung compliance (such as in ARDS) a lower proportion of Paw is transmitted to the pericardium and eventually the cardiac ventricles, which limits application of concepts established with normal compliance of the lungs to patients with injured lungs [21].

Positive pressure ventilation and PEEP help improve arterial oxygenation but also affect the intra- to extra-thoracic vascular pressure gradients such that return of blood flow to the right ventricle is impaired and pulmonary vascular impedance is increased (at least with high PEEP levels) resulting in enhanced right ventricular afterload. The combination of both mechanisms is believed to represent the major determinants of the depression of cardiac output during mechanical ventilation [21].

As impaired oxygenation of arterial blood is the most obvious finding in ARDS, it seems attractive to focus adjustments of mechanical ventilation parameters, such as PEEP levels, on improvement of arterial oxygenation. However, DO_2 is the product of both arterial oxygen content (CaO_2) and cardiac output. If any PEEP induced increase in CaO_2 is counterbalanced by a concomitant impairment of cardiac output, DO_2 will remain unchanged or even decrease [29]. Clinicians managing patients with ALI/ARDS must reconcile the often competing objectives of maximizing CaO_2, optimizing DO_2, and ventilating within ideal respiratory system compliance when adjusting PEEP. In fact, PEEP levels deemed 'optimal' may substantially differ depending on the targeted physiological parameters or the algorithm used. For example, in pigs with oleic acid-induced ALI, when PEEP was increased continuously, the highest arterial oxygen tension was found at PEEP levels of 10–14 cmH_2O, the highest respiratory system compliance was found at a PEEP of 7.5 cmH_2O, and the highest DO_2 was found at a PEEP of 1–6 cmH_2O [30]. In 1975, Suter et al. had sug-

gested titrating PEEP in individual patients to its 'optimal' level based on its effect on DO_2 [31], but this approach requires adequate measurement of cardiac output and iterative reassessment as hemodynamic conditions may change over time. Suter et al. showed that low levels of PEEP in ARDS patients may have no or even beneficial effects on cardiac function [31].

In the recent ARDS Network ALVEOLI study, 549 patients with ALI and ARDS were mechanically ventilated with 6 ml/kg predicted body weight and randomly assigned to either lower or higher PEEP levels, which were set according to different tables of predetermined combinations of PEEP and fraction of inspired oxygen [32]. There were no significant differences in mortality rates or the numbers of ventilator-free days, ICU-free days, or organ-failure-free days between the lower- and higher-PEEP study groups suggesting that clinical outcomes are similar whether lower or higher PEEP levels are used. However, it has been argued that the PEEP/FiO_2 adjustment protocols used in the ALVEOLI study did not take into account physiologic responses in individual patients and might, therefore, have failed to differentiate patients in whom high PEEP actually induces alveolar recruitment and might be beneficial, from those in which high PEEP has no or even adverse effects [33].

Right ventricular dysfunction has been identified as an important prognostic factor in patients with ARDS. Clinical features common in patients with ARDS, such as hypoxemia, high arterial blood PCO_2 levels, destruction of lung tissue, airway collapse, and mechanical ventilation can increase right ventricular output impedance [34]. Vieillard-Baron et al. recently found echocardiographic evidence of acute cor pulmonale in 19 of 75 ARDS patients ventilated with low V_T (8 ml/kg) and identified arterial blood PCO_2 levels as the sole factor independently associated with acute cor pulmonale in a multivariate analysis [35]. Schmitt et al. evaluated the effect of different PEEP levels on right ventricular outflow impedance in patients with ARDS [36]. While zero PEEP and the higher PEEP levels (13 ± 4 cm H_2O) were associated with an increased right ventricular outflow impedance during tidal ventilation, this association disappeared at the lower PEEP level (6 ± 3 cm H_2O) and cardiac output was preserved with the lower but not with the higher PEEP level.

Mechanical Ventilation in ALI/ARDS and Kidney Dysfunction

The kidneys are the most common non-pulmonary organ to fail in patients with ARDS and the presence of acute renal failure has been identified as an important prognostic factor in these patients [2]. In a prospective study, Ranieri et al. found a higher incidence of renal failure in patients who had been ventilated using conventional strategies (V_T 11.1 ± 1.3 ml/kg, PEEP 6.5 ± 1.7 cmH_2O) as compared to those patients ventilated with a lung protective strategy (V_T 7.6 ± 1.1 ml/kg, PEEP 14.8 ± 2.7 cmH_2O) [5]. This observation was confirmed in the large, randomized, and controlled ARDSnet trial [4].

A number of mechanisms have been proposed to explain the effects of positive pressure ventilation on renal function, including a reduction in cardiac output and renal blood flow, redistribution of intrarenal blood flow, stimulation of sympathetic and hormonal pathways, and release of inflammatory or pro-apoptotic mediators by the lungs as a consequence of VILI (see above) [20, 37].

Annat et al. observed a parallel decrease in renal plasma flow, glomerular filtration rate, and urine output during mechanical ventilation in patients, and suggested

a causal relationship between increased intrathoracic pressure, decreased cardiac output, and impaired renal function [38]. Hall et al. suggested that intra-renal blood flow redistribution from the cortical to the juxtamedullary nephrons secondary to the release of vasoactive mediators induced by positive-pressure ventilation may contribute to depression of renal function during mechanical ventilation with PEEP even when total renal blood flow remains unchanged [39].

Maintaining spontaneous breathing has been shown to attenuate the negative effects of mechanical ventilation on the hemodynamic system. Steinhoff et al. observed an improvement in renal function when patients with acute respiratory failure were switched from controlled mechanical ventilation with intermittent mandatory ventilation to spontaneous breathing [40]. In patients with ARDS, Hering et al. recently demonstrate higher cardiac output, DO_2, renal blood flow, and glomerular filtration rate during partial ventilatory support using airway pressure release ventilation (APRV) *with* spontaneous breathing as compared to APRV *without* spontaneous breathing when minute ventilation was kept constant [22]. These observations support the concept that maintaining kidney perfusion is important in preventing renal dysfunction secondary to mechanical ventilation in patients with ALI/ARDS.

Mechanical Ventilation in ALI/ARDS and Dysfunction of Organs in the Hepatosplanchnic Region

Although the impact of breaching skin and mucosal defense barriers on induction of systemic inflammation and infection is well appreciated, the role of other barriers exposed to potential pathogens, such as the extensive gut epithelial surface, is incompletely understood. Intestinal epithelial hyperpermeability, a common condition in critical illness, may facilitate translocation of bacteria and their products from the gut to the systemic circulation. Disruption of epithelial structural integrity in the gut has been associated with injurious mechanical ventilation. Imai et al. found an association between injurious mechanical ventilation and increased rate of epithelial cell apoptosis in vili (but not crypts) of small intestines [20].

Fink recently advanced the concept that epithelial dysfunction may be a common final pathway contributing to organ dysfunction, and that functional disruption of specialized structures in epithelial cells (tight junctions) caused by dysregulated inflammatory processes may be a key factor leading to lung, liver, gut, and perhaps kidney dysfunction associated with ARDS [41]. An abnormal and severe derangement of intestinal permeability has been associated with the development of MODS in 47 patients with prolonged ICU stay [42]. An experimental study by Guery et al. further supports this concept [43]. The authors ventilated rats with either high V_T (30 ml/kg/zero PEEP) or moderate V_T (10 ml/kg/PEEP 2 cmH_2O) for two hours. A subset of animals was injected with anti-tumor necrosis factor (TNF)-α antibodies two hours prior to mechanical ventilation. Lung and gut permeability were assessed after intravenous injection of radio labelled albumin. Mechanical ventilation with high V_T/zero PEEP resulted in an increase in plasma TNF-α concentrations, lung edema (higher lung wet-to-dry ratio), and lung and gut permeability for albumin as compared to mechanical ventilation with moderate V_T/PEEP 2 cmH_2O. Pre-treatment with anti-TNF-α antibodies abrogated the increase in lung edema as well as lung and gut permeability.

Hepatosplanchnic hypoperfusion is thought to be important in the development of MODS. Mechanical ventilation with PEEP mediates its adverse effects on hepatosplanchnic blood flow by diminishing arterial inflow, raising downstream (right atrial) pressure, and by increasing hepatic sinusoidal resistance via mechanical compression of the liver by the descending diaphragm [44]. Furthermore, the architecture of gut vasculature permits shunting of blood resulting in hypoxia at the tips of villi leading to mucosal damage, a common finding in mechanically ventilated patients. Mechanical ventilation of ALI/ARDS patients for greater than 48 h has been shown to be one of the two strongest independent risk factors for clinically important gastrointestinal bleeding in ICU patients [45].

A reduction of hepatosplanchnic blood flow as well as hepatic venous oxygen saturation has repeatedly been shown in animals ventilated with PEEP [44]. Halden et al. found a redistribution of cardiac output to extra-splanchnic organs during incremental increases in PEEP [46].

In patients undergoing major abdominal surgery, applying 10 cmH_2O PEEP reduced mesenteric blood flow and DO_2 while total hepatic blood flow and DO_2 remained unaltered due to a compensatory increase in hepatic arterial blood flow (a mechanism known as hepatic arterial buffer response) [25]. In patients with septic shock, incremental increases in PEEP induce a drop in hepatic glucose production (a marker for hepatic metabolic performance) in parallel to reductions in cardiac output and hepatic venous oxygen saturation [47]. Furthermore, hepatic clearance of drugs that are highly extracted at the hepatic level and therefore primarily depend on hepatic blood flow (e.g., lidocaine) can be impaired by positive-pressure mechanical ventilation [48].

In contrast, Kiefer et al. found no consistent effect of increasing PEEP by 5 cmH_2O (from 5–8 to 10–13 cmH_2O) on cardiac output, hepatosplanchnic perfusion and metabolism (lactate to pyruvate ratio), or liver function (indocyanine green extraction) in six fluid resuscitated patients with ALI [23]. The authors concluded that intestinal perfusion and metabolism is not affected when cardiac output remains stable, which is more likely to be the case if patients are adequately hydrated, and when patients are ventilated within the linear part of the pressure-volume curve.

Variables describing global and regional hemodynamics and DO_2 do not necessarily reflect abnormalities of tissue microcirculation and oxygenation. Impaired microcirculation is likely to contribute to the development of MODS but is difficult to assess in clinical practice. In animal models, PEEP induced reduction of mesenteric arteriolar blood flow (assessed by *in vivo* video-microscopy in rats) [28] and gastric mucosal oxygenation (assessed by lightguide spectrophotometry in dogs) [26] remained impaired despite normalization of cardiac output after fluid administration, suggesting that restoration of normal local tissue perfusion and microcirculation, once impaired, is difficult. Furthermore, hepatosplanchnic perfusion and microcirculation decreased when PEEP and high intraabdominal pressure were combined despite unchanged cardiac output in pigs [27].

In a pig model with oleic acid-induced ALI, systemic blood flow and mucosal-submucosal blood flow (measured with colored microspheres) in the stomach and gut was better maintained during APRV *with* spontaneous breathing as compared to both high and low V_T APRV *without* spontaneous breathing [49]. Spontaneous breathing in patients with ALI/ARDS has been associated with maintained or improved cardiac output and DO_2 [50].

Clinical Implications and Conclusions

The pathogenesis of MODS is complex and only incompletely understood. To date, neither a specific treatment, nor an effective means for preventing its onset is available in clinical practice. Pathways likely to be involved in the translation of VILI into MODS include spill over of lung-borne inflammatory mediators, pro-apoptotic factors, and bacteria and their products into the systemic circulation, activated neutrophils, as well as impairment of global, regional, and possibly local hemodynamics and DO_2 (Fig. 1). Measures to prevent the initiation or to attenuate the propagation of these pathways have the highest likelihood of preventing MODS in the context of VILI.

Although mechanical ventilation is only one of multiple factors contributing to the pathogenesis of MODS, clinical trials have clearly shown that lung protective mechanical ventilation (V_T 6 ml/kg predicted body weight) decreases mortality in patients with ARDS and is associated with a lower incidence of MODS [4]. Combining the results of the two large ARDS Clinical Trials Network studies, a total of almost 1000 patients with ARDS have been treated with a protective, low V_T strategy, resulting in a mortality rate in the ICU of 25 to 31% which provides a benchmark for clinical practice and future clinical trials. Whether high or low levels of PEEP should be used during mechanical ventilation of ARDS patients remains un-

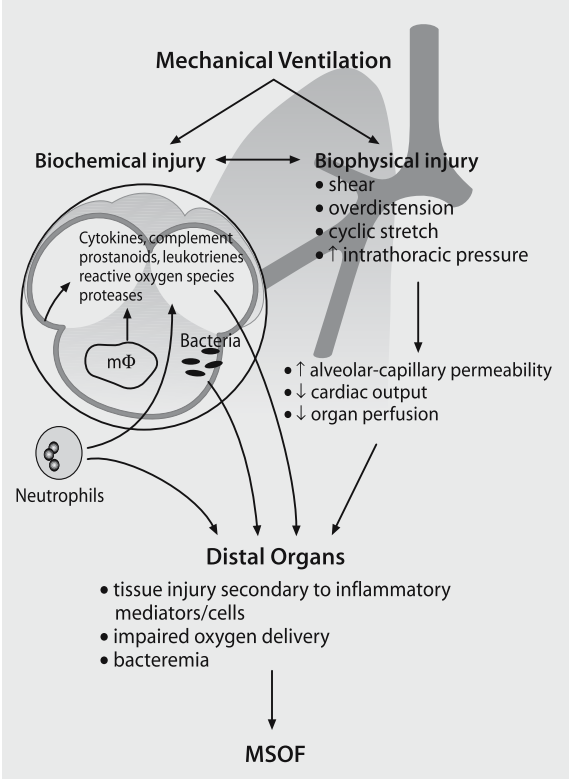

Fig. 1. Postulated mechanisms whereby mechanical ventilation may contribute to multi-system organ failure (MSOF). From [8] with permission.

certain. For now, PEEP titration in daily practice is probably best based on a careful and iterative re-evaluation of its effects on oxygenation, on the compliance of the respiratory system, and on cardiac function and hemodynamics.

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Monitoring in Respiratory Failure

Electrical Impedance Tomography and its Perspectives in Intensive Care Medicine

I. Frerichs, J. Scholz, and N. Weiler

Introduction

Electrical impedance tomography (EIT) is a non-invasive, radiation-free medical imaging technique invented more than 20 years ago. In 1984, the first EIT tomogram (a cross-sectional image of the human forearm) was generated [1]. Merely one year later, in 1985, the first EIT scan of the human chest, clearly showing both lungs, followed [2]. In the following two decades, there was immense development of this method. About twenty research groups, most of them located in Europe, significantly improved EIT hardware and software, identified the major fields of possible future application, and conducted multiple methodological validation studies, as well as experimental animal and clinical studies. Since the early days, the use of EIT in pulmonary applications has been defined as one of the most promising techniques although other applications (e.g., detection or monitoring of breast cancer, pharyngeal and gastric motility, cortical brain activity, pulmonary and peripheral blood perfusion, cardiac performance, urinary bladder emptying, uterus activity) have also been considered [2–4].

In 2000, a review of all EIT research activities related to lung and ventilation summarizing the major results achieved and outlining the perspectives and limitations of EIT in this field was published [5]. Considering the available results of various lung-oriented EIT studies, the conclusion was made that mechanically ventilated intensive care patients would benefit most from the use of EIT in the future. At approximately the same time, it became evident that intensivists were becoming increasingly interested in this technique. Recent editorials, published in leading journals [6, 7], provide good evidence of this rising clinical interest. At the moment, the use of EIT for monitoring regional lung aeration and ventilation is regarded as being of most relevance in intensive care patients.

This chapter briefly presents the basic characteristics of the EIT technique, summarizes the main results of the lung-oriented EIT studies performed so far, and gives an overview of possible clinically relevant applications in intensive care patients.

Measuring principles of EIT

Biological tissues conduct electrical current because they contain ions which act as charge carriers. The number of ions in a defined volume differs among human tissues, which is the reason why large differences exist in the conductivity (or resistivity) of different tissues [8]. For instance, blood and muscle are good conductors,

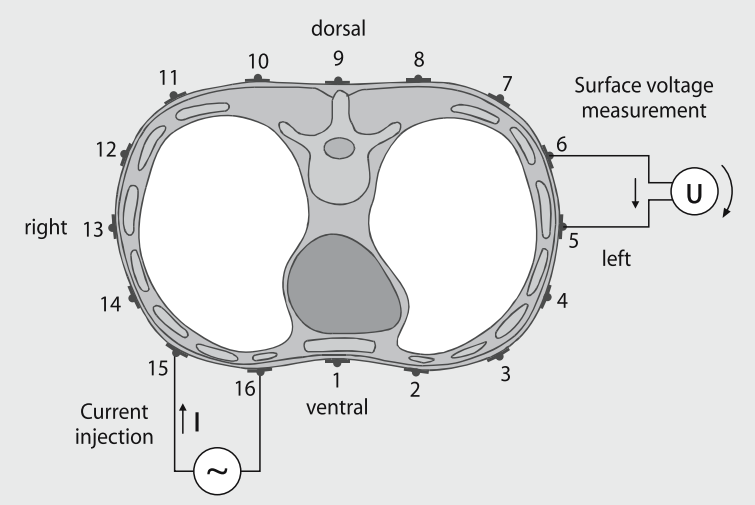

Fig. 1. Basic principle of electrical impedance tomography (EIT). Rotating electrical current (I) injection is performed between pairs of adjacent surface electrodes (denoted by nos. 1–16), and resulting voltages (U) are measured between pairs of remaining electrode pairs. From [36] with permission

whereas bone and fat are poor ones. The idea of imaging the human body with electricity is based on the existence of these dissimilar electrical properties of different tissues and organs.

EIT makes use of this fact and generates cross-sectional images (i.e., scans) of the internal distribution of electrical impedance (i.e., the resistance to alternating electrical current) within a chosen part of the human body. To determine the electrical properties of the tissues contained in the body segment studied, very small alternating currents are repeatedly applied through a set of electrodes placed on the surface of the body and the resulting potential differences measured. The measuring principle of EIT is depicted in Figure 1, showing, schematically, an EIT measurement performed on the chest. The boundary voltage data acquired during the cyclic and rotating application of electrical currents are then processed to generate the EIT images. This process is called image reconstruction.

Generation of EIT images

In general, there are three ways of EIT imaging [9]:
1) imaging the distribution of impedance within the body
2) imaging the frequency variation of impedance within the body
3) imaging the variation in impedance during a physiological process, e.g., breathing or cardiac activity.

The first two approaches are essentially anatomical because they show how different tissues are distributed within a body section under study. The resulting EIT scans reflect the momentary distribution of regional electrical tissue properties. Both of these first two approaches are inferior to other already established imaging

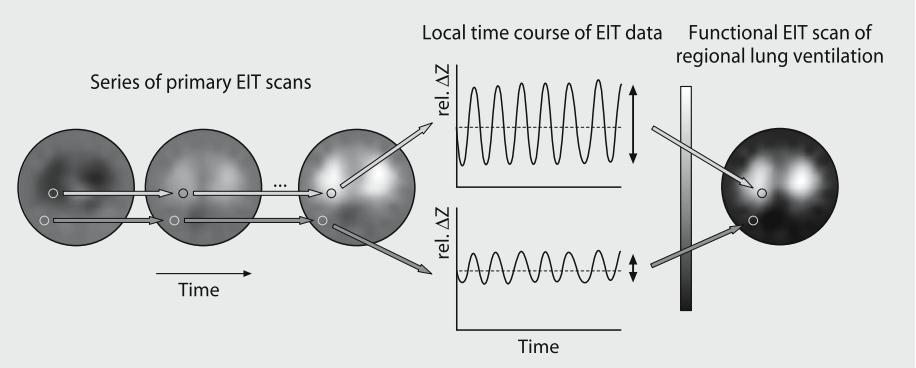

Fig. 2. Generation of a functional EIT scan of regional lung ventilation (right) from a series of EIT scans (left) acquired during tidal breathing. The individual scans in the series show the local changes in electrical impedance within the chest cross-section with respect to the average local electrical impedance determined from all tidal respiratory cycles measured. Expiration decreases the local air content and leads to a fall in regional electrical impedance; inspiration is accompanied by an increase in regional electrical impedance. The fall in local electrical impedance is shown in dark tones and the lungs appear black in the first EIT scan of the series which was acquired during expiration. The rise in local electrical impedance is depicted in light tones and the lungs appear white in the last scan of the series which was collected during inspiration. The local time courses of relative impedance change (rel. ΔZ) in two out of a total of 912 pixels show tidal fluctuations in local electrical impedance synchronous with the breathing rate (center). The differences between the end-inspiratory and end-expiratory relative impedance changes (vertical arrows to the right of both diagrams) are proportional to the local tidal changes in air content. The calculated local end-inspiratory-to-end-expiratory relative impedance changes are plotted in the corresponding pixel locations using a black-and-white scale. In this way, one functional EIT scan of regional lung ventilation is generated from a series of hundreds of primary EIT scans. From [37] with permission

techniques like computed tomography (CT) or magnetic resonance imaging (MRI) because of the lower spatial resolution of EIT scans. The philosophy of the third approach is different as it is oriented on imaging the function and not merely the anatomy. In this respect EIT is superior to the mentioned established methods as it enables the detection of changes in organ function. Moreover, it is suitable for long-term monitoring of such functional changes because of its radiation-free measuring principle.

The first functional EIT images were published in the mid-1990s [10, 11]. The generation of functional EIT scans is based on the acquisition of time series of scans with subsequent off-line or on-line evaluation. In the case of lung imaging, the most soliciting approach is to quantify the ventilation-related changes in regional impedance. This approach, based on the determination of impedance changes occurring between inspiration and expiration, is shown in Figure 2.

However, this is not the only functional approach available. For instance, if a change in regional lung aeration takes place during the EIT scanning period, the corresponding shift in local air content accompanied by a shift in local impedance can be quantified and a functional EIT scan showing this change in local lung air volume generated. Three functional scans of this type are presented in Figure 3 (middle). These scans reveal the acute changes in local aeration occurring in a healthy, spontaneously breathing subject due to the changing gravity effect.

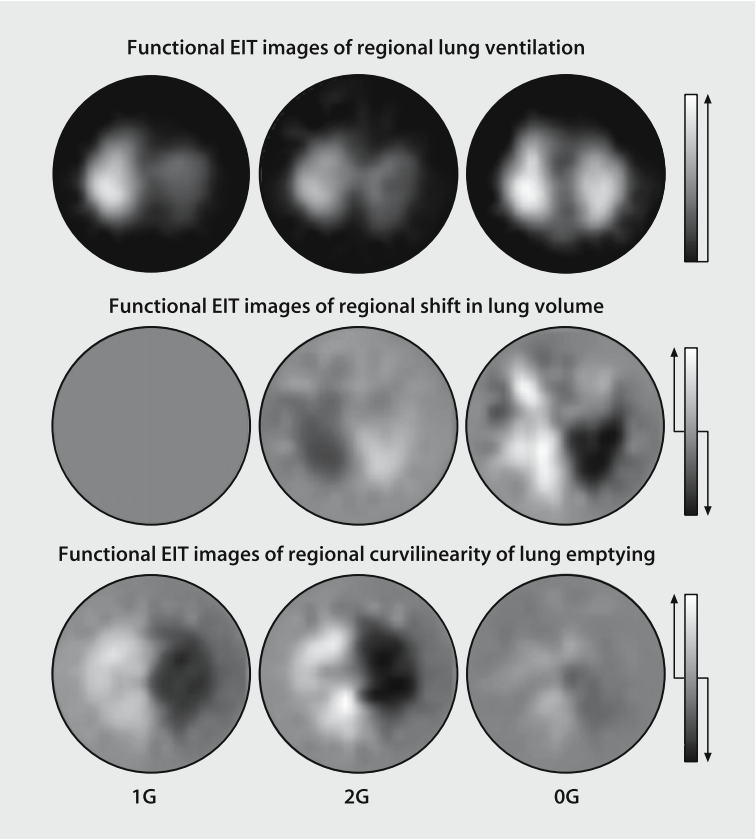

Fig. 3. Functional EIT images of regional lung ventilation (top), functional EIT images of regional shift in lung volume (middle), and functional EIT images of regional curvilinearity of lung emptying (bottom) obtained in a subject in the right lateral body position during spontaneous breathing in phases of different acceleration (1G, 2G, 0G) during a parabolic flight maneuver. Light regions in the top tomograms show the regional lung ventilation. Dark and light regions in the middle tomograms reveal a local fall and rise in lung volume in comparison with 1G, respectively. Light regions in the bottom tomograms are characteristic of areas with initially rapid and late slow lung emptying, whereas dark regions exhibit the opposite pattern of lung emptying. The image orientation is the following: ventral is at the bottom and the right side of the body is on the left of each image. From [36] with permission

Another type of functional EIT scan, characterizing the regional dynamic behavior of lungs, is also presented in Figure 3 (bottom). The generation of this type of scan is based on the following considerations: The differences in regional lung mechanics result in different patterns of regional lung filling and emptying during ventilation accompanied by regionally dissimilar changes in impedance with time. Thanks to the good time resolution of EIT scanning, the local changes in impedance can be sampled at a high rate and the local filling or emptying characteristics of the lung tissue mathematically described.

Thus, functional EIT in ventilation-oriented applications is able to assess different aspects of regional lung function, e.g., regional tidal volume distribution, shifts

in regional lung aeration or regional filling and emptying behavior. The positive side effect of functional EIT data evaluation is the elimination of the problem of interpreting a time series of hundreds or thousands of simple EIT images. We have presented three types of functional EIT scan here, however, we expect that new functional evaluation tools will be applied in the future to extract further information from EIT measurements and enhance the clinical relevance of the findings.

Findings Relevant for the Future Use of EIT in Intensive Care Patients

The validity of EIT with respect to its ability to correctly measure regional changes in lung air content is the crucial prerequisite for the clinical application of this technique as a monitoring tool of regional lung ventilation. Early validation studies used simple spirometry to establish the relationship between the global changes in lung volume and the EIT signal [10, 12, 13]. Later, more sophisticated reference techniques like CT [14], electron beam CT [15], single photon emission CT [16], ventilation scintigraphy [17] and nitrogen washout [18] were applied. Good correlation was found between the regional air content changes determined by EIT and the respective reference technique.

EIT will most probably be used in mechanically ventilated patients in the future. Changes in ventilator settings influence the regional distribution of ventilation and aeration and several EIT studies have demonstrated that these changes are discernible by EIT. Figure 4 shows the instantaneous changes in the EIT signal reflecting

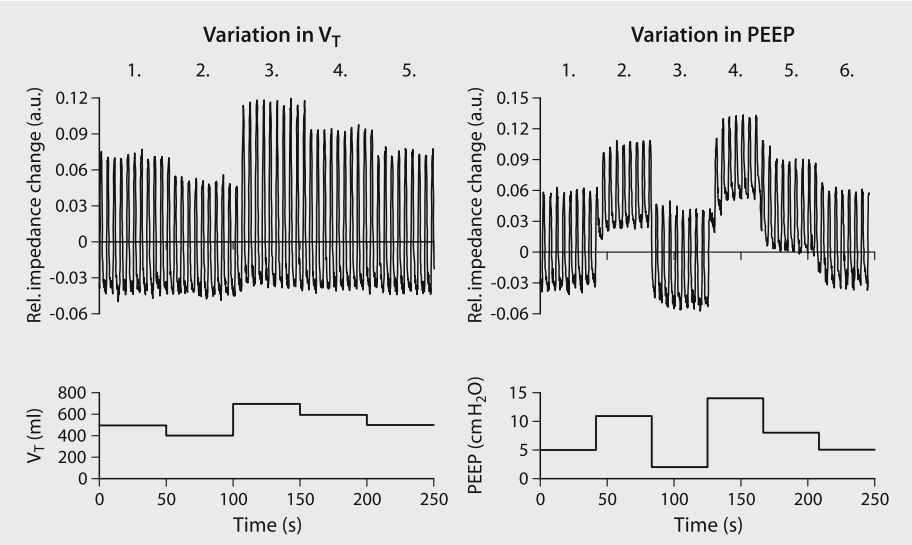

Fig. 4. EIT measurement performed during volume-controlled ventilation with different tidal volumes (V_T) at a constant positive end-expiratory pressure (PEEP) of 5 cm H_2O (left) and a constant V_T of 500 ml at varying PEEP levels (right). The breath-by-breath amplitudes of the EIT signal clearly reflect the stepwise changes in V_T (top left). The shifts in the end-expiratory values of the EIT signal are representative of the lung volume changes associated with the stepwise variation of PEEP (top right). From [38] with permission

the changes in two basic ventilator parameters: tidal volume (V_T) and positive end-expiratory pressure (PEEP). The data presented in the figure originate from an animal experimental study. The effects of various types of mechanical ventilation (e.g., intermittent and continuous positive pressure ventilation, synchronized intermittent mandatory ventilation, spontaneous breathing with continuous positive airway pressure, high frequency oscillatory ventilation) on regional lung ventilation have been established by EIT both in adult and neonatal intensive care patients [14, 19–22].

EIT has also been shown to be able to detect the changes in regional lung ventilation, aeration and lung mechanics associated with acute lung injury (ALI) or lung edema both in experimental [23–27] and clinical settings [28]. Regional pressure volume curves were generated by EIT in lung lavaged animals [24, 29, 30] clearly demonstrating the topographic heterogeneity of the relationship between the imposed airway pressure and regional lung volumes. Figure 5 shows the results of an EIT measurement performed after induction of experimental lung injury. Large dissimilarities in regional lung filling and emptying existed during the slow inflation and deflation maneuver. This figure additionally presents the beneficial effect of surfactant administration on regional lung mechanics detected by EIT. An improvement in regional lung ventilation distribution in response to surfactant treatment has also been documented by EIT examinations in preterm infants suffering from the infant respiratory distress syndrome [22].

There exist a few studies which indicate that EIT might be suitable not only for assessing regional lung ventilation but also lung perfusion [31–34]. Changes in regional electrical impedance associated with cardiac activity and pulmonary perfusion are much lower than those elicited by ventilation. However, filtering procedures or electrocardiogram (EKG)-gated EIT data acquisition make the impedance changes occurring synchronously with the heart rate accessible for analysis and interpretation. Promising results have also been achieved by the use of an EIT contrast agent (hypertonic saline solution) applied as an intravenous bolus for generation of regional pulmonary dilution curves and calculation of regional pulmonary blood flow [32].

Is There a Need for a New Lung Imaging Method?

It is obvious that EIT is able to detect highly interesting physiological and pathological phenomena in regional lung function. Is this fact sufficient to justify the use of this technique in a clinical setting? Do we really need a new lung imaging method in intensive care? Several well established medical techniques exist, which, along with the personal experience of physicians, are used to guide ventilator therapy at present. To assess the overall efficacy of pulmonary gas exchange, arterial, capillary and venous blood gas analysis, pulse oximetry, transcutaneous oxygen and carbon dioxide measurement, as well as end-tidal carbon dioxide measurement are used. However, these methods provide only global information on lung function and may mask regional differences in gas exchange. Morphological information on the lung structure may be obtained from chest X-ray, CT or MRI examinations. However, the use of these techniques is limited by several factors. Chest radiography and CT increase radiation exposure which is extremely unfavorable especially in critically ill neonate patients. Although simple chest X-ray examinations can be performed at the bedside, the use of other imaging techniques requires patient

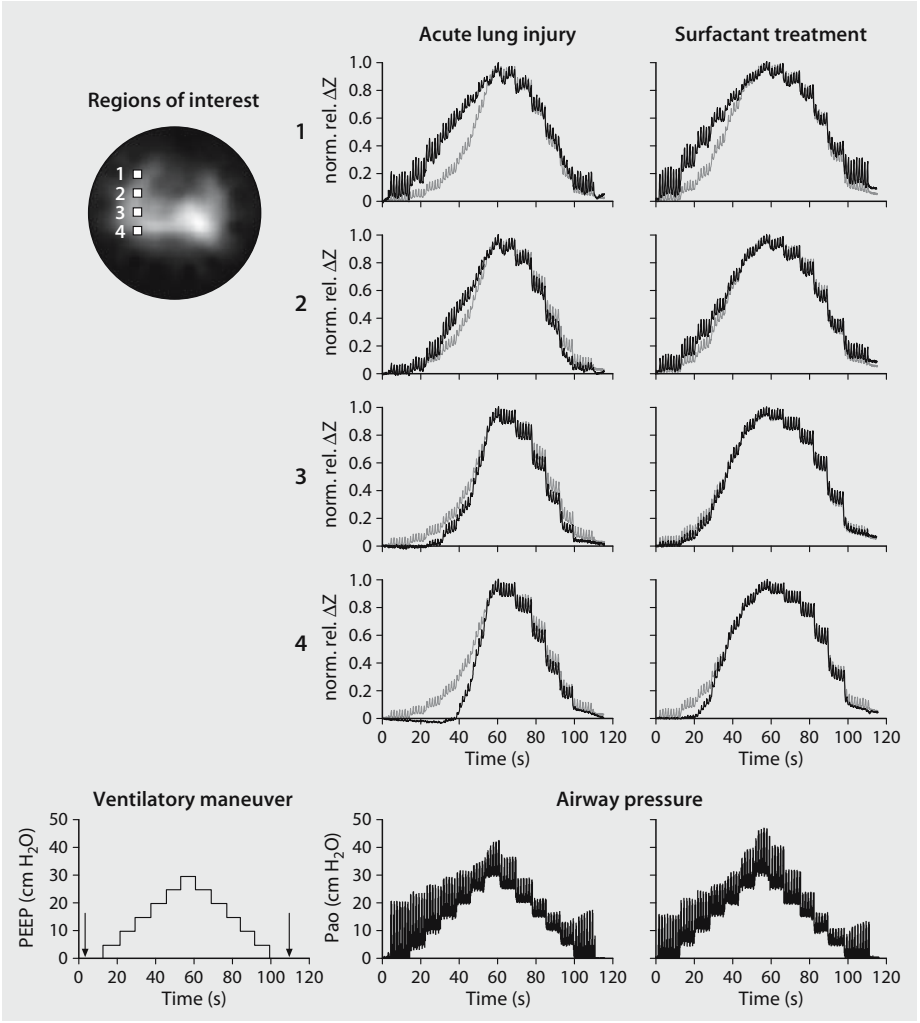

Fig. 5. Tracings of local relative impedance change (right top, dark thick lines) during volume controlled ventilation at different positive end-expiratory pressure (PEEP) levels in four regions of interest in the right lung (left top) before and after surfactant treatment. An increase in local aeration is accompanied by an increase in electrical impedance, the small fluctuations of the impedance signal represent the individual breaths. For better comparison and identification of instantaneous changes of end-expiratory lung volume and tidal volume with PEEP, the individual tracings were normalized and plotted together with the tracing of average relative impedance change in the whole thoracic cross-section (light thin lines). Timing of the PEEP maneuver (left bottom) and the tracings of airway pressure (right bottom) are shown. Z: impedance; Pao: pressure at the airway opening. From [24] with permission

transport to other areas of the hospital. Transport from the ICU is associated with risk because of the interruption and/or disturbance of therapy. Eestablished imaging techniques are not suitable for monitoring regional lung function as they primarily provide only a momentary anatomical image of lungs, although with excellent spatial resolution.

Thus, there remains a need for a new monitoring technique which would be able to 1) determine regional lung aeration and ventilation directly at the bedside, and 2) provide immediate feedback information on regional response of the lung tissue to changes in, particularly ventilator, therapy. Recently, clinical awareness of this deficit in lung function monitoring has become apparent. This was facilitated by the increasing bulk of knowledge on the heterogeneity of regional lung function. Even under physiological conditions, regional lung volumes, ventilation, perfusion and gas exchange are not homogeneously distributed within lungs. All lung diseases further increase this pulmonary functional heterogeneity mainly due to regionally dissimilar changes in lung mechanics. It is well known now that, e.g., ALI produces a marked heterogeneity of mechanical properties of lung tissue which make different lung regions more or less susceptible to different traumatic events elicited by mechanical ventilation. Overextended, atelectatic, cyclically opening and collapsing regions as well as normally ventilated regions may exist in lungs at the same time. Established techniques providing global information on lung function (blood gas analysis, spirometry, generation of pressure-volume curves) do not allow conclusions to be drawn on the regional behavior of the lung tissue and, consequently, optimum ventilator settings.

Perspectives and Limitations of EIT use in the ICU

EIT has the potential to become a new monitoring technique in the ICU mainly as a tool for optimizing ventilator therapy. EIT is able to assess changes in regional ventilation, aeration, perfusion and lung mechanics. Several experimental and clinical studies have provided proof of EIT's ability to determine several aspects of lung function at a regional level.

EIT has no known hazards, it is fully non-invasive and uses a radiation-free measuring principle. The technology is relatively cheap, the devices are small and further miniaturization is more than probable. Thus, EIT can easily be applied at the bedside. EIT examinations can be performed with an excellent time resolution (at present, scan rates up to about 40 scans per second are possible).

Despite of all these positive features and advantages, one has to be aware of the fact that the method has certain limitations which may limit its clinical use. First, the spatial resolution of EIT scans is low when compared with radiographic imaging techniques. For instance, the EIT scans shown in this review have a resolution of only 32×32 pixels. The resolution of EIT images can hardly be significantly improved even if higher numbers of electrodes are used [35]. Therefore, EIT can only be recommended for functional and not purely anatomical imaging. Second, the method requires the use of self-adhesive electrodes which have to be placed on the chest circumference. Lying patients have to be turned on both sides to allow the application of electrodes. The cables connecting the electrodes with the EIT device make nursing activities more difficult. Third, EIT is still an experimental method and the existing EIT data evaluation tools have usually been developed for specific

research projects and are not universally applicable. Therefore, complex and innovative evaluation of EIT data is only accessible to experts. Fourth, changes in regional lung impedance may result from different physiological and pathophysiological processes. For instance, an increase in regional fluid content and a decrease in air content both result in a fall in local lung impedance. Only the measurement of absolute values of electrical impedance and/or the EIT measurement using electrical currents of multiple frequencies may allow differentiation of such processes. Finally, EIT use in an electrically noisy environment like the ICU is challenging. Although modern EIT devices are rather robust and EIT measurements in a clinical setting are usually not problematic anymore, possible disturbances of the measurements caused by other electrical devices leading to decreased quality of the EIT data acquired should not be neglected.

Nevertheless, many of the limitations of existing EIT technology can be eliminated in the future. For instance, new electrode belts and software for data evaluation may be developed. Further development of EIT hardware may improve the quality of EIT measurements and even make the determination of absolute impedance and multifrequency measurements possible and reliable. Potential benefits and advantages of EIT prevail and make the further development of this technique worthwhile and its future use in the ICU possible.

Conclusion

EIT is a new, portable imaging technique which is increasingly being considered as a future tool for evaluation of the immediate effects of a change in ventilation or other therapeutic intervention in critically ill patients. The method is suitable for monitoring regional lung function directly at the bedside. Steady advances in the development of EIT technology over the past 20 years makes a routine application in a clinical setting in the next decade possible. Nevertheless, further development of both EIT hardware and software is necessary to increase the quality of data, user-friendliness, and clinical acceptance. Proof of clinical efficiency has to be provided. Results of several studies indicate that EIT might be of benefit in optimizing ventilator therapy and minimizing the incidence of ventilator-associated lung injury but this has to be proven in larger clinical studies.

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Electrical Impedance Tomography for Monitoring of Regional Ventilation in Critically Ill Patients

C. Putensen, J. Zinserling, and H. Wrigge

Introduction

Acute lung injury (ALI) is associated with an insult to endothelial and epithelial cells in the lung resulting in release of mediators, increased vascular- and alveolar permeability, interstitial edema formation, alveolar collapse, and thereby arterial hypoxemia [1-3]. Although acute respiratory distress syndrome (ARDS) was initially believed to be caused by a diffuse lung injury, computed tomography (CT) of patients with ARDS revealed radiographic densities corresponding to alveolar collapse localized primarily in the dependent lung regions, which correlate with intrapulmonary shunting and account entirely for the observed arterial hypoxemia. Thus, intrapulmonary gas is unhomogeneously distributed during ARDS due to uneven distribution of injury, regional surfactant dysfunction, pulmonary infiltrations and/or alveolar collapse. Positive pressure ventilation, commonly used to improve gas exchange, may further aggravate preexisting lung injury including pneumothorax, alveolar edema, and alveolar rupture [4, 5].

Mechanical ventilation with positive end-expiratory airway pressure (PEEP) titrated above the lower inflection pressure of a static pressure-volume curve and a low tidal volume (V_T) is thought to prevent tidal alveolar collapse at end-expiration and overdistension of lung units at end-inspiration in patients with ARDS [6]. This lung-protective strategy causes improvement in lung compliance, venous admixture, and PaO_2 without causing cardiovascular impairment in ARDS [6]. Mechanical ventilation using V_T of not more than 6 ml/kg ideal body weight has been shown to result in a better outcome when compared with a V_T of 12 ml/kg ideal body weight in patients with ARDS [6, 7]. Thus, cyclic opening and closing of lung units during tidal ventilation are considered major risk factors in the pathogenesis of ventilation-associated lung injury.

Arterial blood gas analysis and the recording of airway pressure–volume curves have been found to be useful only in the ventilator setting of some critically ill patients, while being misleading in patients with lobar atelectasis. Even if both techniques are available at the bedside, they reflect only overall lung function, and regional inspiratory overdistension of lung units or end-expiratory alveolar collapse may still be undetected. Regional ventilation and perfusion can be studied by isotope and magnetic resonance techniques while aeration of the lung can be imaged using CT. However, none of these techniques are available at the bedside.

Recently, electrical impedance tomography (EIT) has been introduced as a truly bedside technique which provides information on regional ventilation distribution.

Technique of Electrical Impedance Tomography

Principles of EIT

EIT was developed from experiments to create images from the distribution of the absolute value of the complex impedance, Z ($Z = Z_0 \times \exp(i\omega)$), within biologic material. The basic principle of image generation is based on the injection of small currents of about 5 mA via surface electrodes applied circumferentially to the thorax in one plane and measurement of potential differences with pairs of passive electrodes which are not used for current injection (Fig. 1). This is repeated for one 'cycle', a sequence of current injections around the thorax with potential difference measurement at pairs of passive electrodes (Fig. 2). Applications with 8 to 32 electrodes are currently used and in most systems only the specific resistance is used for image reconstruction, not the phase information. The application of the small currents used by EIT is generally considered to be completely non-hazardous and except for the need to apply electrodes in contact with the skin the method is non-invasive.

The use of more than one frequency is possible to differentiate between different tissue materials, but is rarely used in impedance tomography of the lung. The use of a wide range of current frequencies to gain information about tissue, bones and organs is a different field called impedance spectroscopy.

Technical Realization of EIT

There are currently four systems in use (Sheffield Mark 1, DAS 01-P, Goe MF II, and a prototype used by Amato et al. [8]). A comparison study shows that the Goe MF II system has the best signal-to-noise ratio of the three named devices which all use 16 electrodes [9]; the prototype system by Amato et al. makes use of 32 electrodes. The image reconstruction in these systems is done with the 'Sheffield Backprojection Algorithm', which does not reconstruct absolute impedance values, but impedance changes from a reference state to a new state later in time. These are linearly proportional if the impedance changes are small when compared to the ref-

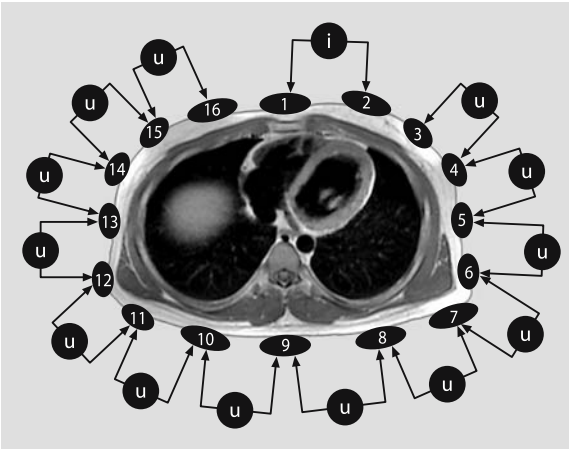

Fig. 1. Schematic drawing of the EIT electrodes (1–16) placed equally around the thorax in one plane and a set of measured potentials (u) with one position of current injection (i)

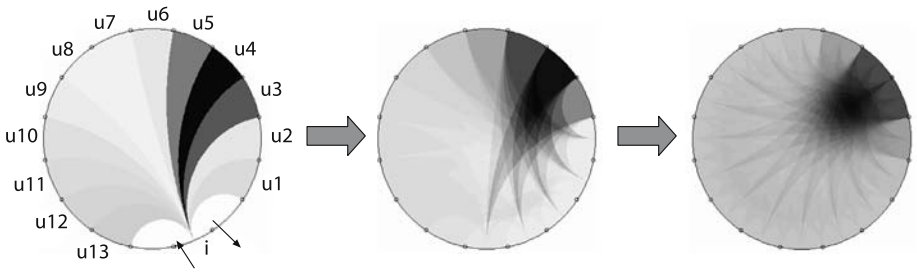

Fig. 2. Visualization of superposition of impedance information along the current pathways gained for an area (black) near one fixed measurement position (u4) for repeated current injections (i) at different positions (middle image) and all around the thorax (right image)

erence state [10]. With these restrictions it is possible to perform dynamic imaging of impedance changes which occur with the change of physiological parameters and the change of impedance within the lungs during the respiratory cycle results in a large signal compared to other impedance changes that would be suitable for dynamic imaging, like changes with cardiac activity or changes in the gastrointestinal tract. With the currently used hardware, impedance changes of less than 1% can be detected.

The underlying information for the reconstruction of the images is very limited when compared to the information used to reconstruct CT images and the reconstruction itself is a mathematically 'ill-posed' problem. The use of the Sheffield Backprojection Algorithm is a way to bypass this problem but imposes some limitations and assumptions on the reconstruction process. It is assumed that the electrodes build an exactly circular structure with equal spacing of the electrodes, with the consequence that the images reconstructed can be more or less skewed or distorted.

With EIT machines with 16 electrodes, an image of the electrode plane is defined by a 32×32 matrix and impedance changes from change of air content within the electrode plane can be followed regionally in one of the 1024 pixels or for larger regions of interest. Linear interpolation is used to increase the virtual resolution of the images to a 128×128 matrix, which enables the viewer to separate structures better even if the underlying resolution of the images is unchanged.

Data Processing

So called 'functional images' (Fig. 3) can be generated to compress the information of the time course into a color coded image which can show standard deviation or breath-by-breath tidal differences of impedance change for each pixel on a color scale [11]. 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. The time resolution of the EIT devices is currently between 1 and 44 images per second with a typical resolution of more than 10 Hz. This enables the recording of local and global time courses of impedance change which follow the air content of the cross section of the lung under the electrodes. It is important to remember that changes in fluid content or pulmonary blood volume also cause changes in thoracic impedance which might not be distinguishable from the changes of impedance by

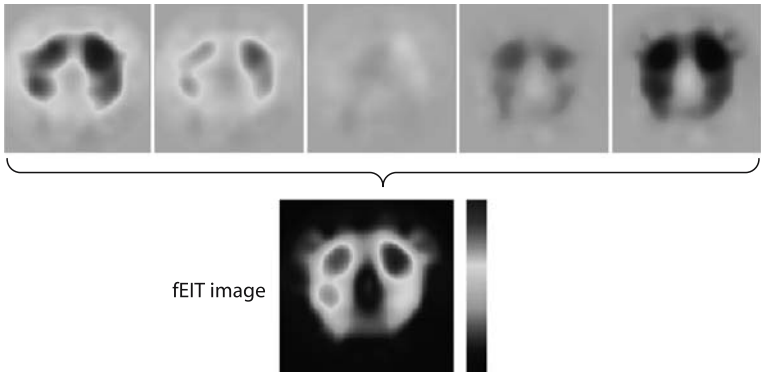

Fig. 3. Functional EIT images: Information about the time course of impedance changes (images on top of figure) is compressed by display of standard deviation of changes (lower image) or tidal changes on a color scale

ventilation (air content). Changes in air content produce much larger impedance changes than changes of fluid content. The impedance changes by physiologic mechanisms might be separated by using frequency filtering for cardiac or ventilator rate (frequencies). By linking electrocardiographic (EKG) signals to the EIT it may be possible to average signals over a period of time to gain information about perfusion or to subtract cardiac distortion from ventilation images.

Methodological Limitations of EIT

The spatial resolution of EIT systems is generally low compared to other imaging techniques like CT or magnetic resonance imaging (MRI). It is best in the area near the electrodes and gets worse in the interesting regions deeper in the thorax containing a large part of the lung volume. The spatial resolution 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 generally not possible to predict the spatial resolution within the thorax or even a region of interest because the pathways of the current flow depend on the changing properties of the tissue in those areas. Due to the current flow in three dimensions it can be assumed that the thickness of the volume slice seen by impedance tomography with one circular electrode ring is several centimeters deep in the thorax and decreases towards the surface near the electrode therefore defining a lens shaped volume.

Validation of EIT

Comparison of EIT and Electron Beam CT in Healthy Animals

Frerichs and coworkers [12] compared regional change in relative impedance using EIT and change in lung density using electron beam CT in animals with normal lungs. Local changes were compared with CT and EIT in ventral, middle, and dorsal lung areas on the left and on the right side of the lung while V_T was increased from

200 to 600 ml in 100 ml increments at PEEP levels of 2, 7, and 12 cmH_2O. The correlation between change in lung density and change in relative impedance was strongest in the dependent lung areas ($r^2 = 0.86$) and acceptable in non-dependent lung areas ($r^2 = 0.66$). The authors attributed the worsening correlation in non-dependent areas to movement artifacts during tidal ventilation that were expected to increase variability in the lung density measured by CT. Because time resolution of the EIT (13 scans/s) was higher than time resolution of CT (3.3 scans/s), movement artifacts should be minimized during EIT. Based on these data it was suggested that changes in relative impedance reflect regional ventilation.

Comparison of EIT and Single Photon Emission Computer Tomography (SPECT) in Experimental Lung Injury

Ventilation distribution was assessed in pigs with oleic acid-induced lung injury by comparing the change in relative impedance using EIT with simultaneous single SPECT scanning during inhalation of ^{99m}Tc-labeled carbon particles with a particle size of approximately 0.1 μm [13]. Distribution of these ^{99m}Tc-labeled carbon particles in the lung has been shown to be similar to that of radioactive gas. For both methods, evaluation of ventilation distribution was performed in the identical transverse slice that was approximately 4 cm in thickness. Then, the transverse slice was divided into 20 coronal segments. A highly significant linear correlation between regional ventilation measured by changes in relative impedance using EIT and SPECT scanning was found. However, the Bland-Altman analysis indicated that EIT tended to overestimate ventilation in regions that were poorly ventilated and to underestimate ventilation in well-ventilated regions. The difference between EIT and SPECT scanning was less than 10%. Thus, these data suggest that changes in relative impedance using EIT can quantify regional ventilation with sufficient accuracy in inhomogeneous ventilated lungs.

Comparison of EIT with a Multibreath Nitrogen-washout Technique in Patients with ALI

In mechanically ventilated patients with ALI, end-expiratory lung impedance changes assessed using EIT were compared with changes in the end-expiratory lung volume (EELV) measured by the multibreath nitrogen-washout technique during stepwise increases in PEEP [14]. End-expiratory impedance change was calculated by averaging the minimal lung impedance time course values of 14 consecutive breaths. Pooled data demonstrated a significant and linear correlation between end-expiratory lung impedance change measured by EIT and EELV (r^2=0.95). However, end-expiratory lung impedance change reflects relative impedance variations in one cross-section of the thorax, while EELV estimates the whole accessible lung volume. Therefore, regional heterogeneity of ventilation distribution should be expected to cause slightly different results between change in end-expiratory lung impedance and change in EELV depending on the lung region monitored with EIT (e.g., apical versus basal regions).

Comparison of EIT with Plethysmography in Experimental Induced Lung Injury

Changes in relative impedance using EIT and change in lung volume determined with strain gauge plethysmography were compared in pigs with lavage-induced lung injury. Pooled data showed a significant correlation between the increase in lung volume as measured by strain gauge plethysmography and impedance as measured by EIT ($r^2 = 0.76$). Based on this finding it was concluded that EIT can be used to measure global lung volumes non-invasively.

Comparison of EIT with CT in Patients with ALI

Recently, Victorino and coworkers [15] compared, in patients with ALI, regional impedance changes with lung density measurements by CT. In this study, a slow inflation maneuver was recorded with EIT in 10 mechanically ventilated patients with ALI. Later, an identical inflation maneuver was performed in the CT scanner. Both techniques detected inhomogeneous distribution of ventilation to dependent and non-dependent lung areas (upper/lower ratio 82%/18% and 75%/25% for EIT and CT, respectively). Regional relative impedance changes on the EIT image demonstrated an excellent correlation ($r^2 = 0.92$) with changes in lung density reflecting air content detected by CT. Compatible with observations by Frerichs and coworkers [12], the correlation between change in lung density and change in relative impedance was strongest in the dependent lung areas. In all patients with ALI changes in impedance showed good reproducibility (SD, 4.9%) between repeated measurements in the same patient. A major disadvantage of this investigation was that CT and EIT images were not obtained simultaneously due to electromagnetic interference of the EIT equipment in the CT scanner. Despite this methodological limitation these observations strongly support that regional relative impedance changes are closely correlated with regional lung volume changes detected by CT.

Comparison of EIT and Dynamic CT in Different Models of Lung Injury

In 10 anesthetized and mechanically ventilated pigs with direct lung injury induced with acid aspiration or indirect lung injury induced with abdominal hypertension and oleic acid injection, we performed a low flow inflation maneuver using constant inspiratory gas flow of 2 l/min until either a V_T of 1.2 l or an airway pressure of 60 cmH_2O was reached. Registrations were performed in parallel with an EIT device (Goe MF II, Göttingen, Germany) and a dynamic CT scan in the plane of the electrodes with a resolution of about 13 Hz. Differences between CT and EIT relative air contents calculated in four equivalent quadrants were in an acceptable range for all quadrants. Higher differences were observed in the ventral quadrants (bias $< 1\%$, $2 \times$SD-interval 12–14%), which also had higher air contents at the end of the maneuver than the dorsal quadrants, which showed good agreement with lower scatter ($2 \times$SD 6–10%) but a slightly higher bias (1–4%) as compared with the ventral half. Accuracy of methods was comparable for the different lung injury models studied.

Determination of Regional Ventilation

Interpretation of global lung function tests in patients with ALI is often misleading. This is in part due to the fact that global lung function tests like, e.g., a static pressure volume curve can only assess summarized overlapping information of several ventilatory units of different lung regions which differ in their mechanical behavior [16]. For example, the linear part of a static pressure-volume curve might include already overdistended lung units while other parts of the lung may still open up. Both phenomena could remain undetectable due to overlapping of regionally different mechanical behaviors. Regional EIT imaging might be helpful in separating different regional ventilation behaviors of the lung thereby helping to optimize ventilator setting. Figure 4 shows impedance changes during a low flow inflation maneuver. Note that amplitudes, shapes and offsets of regional impedance time curves differ in different quadrants indicating regional differences in ventilatory time con-

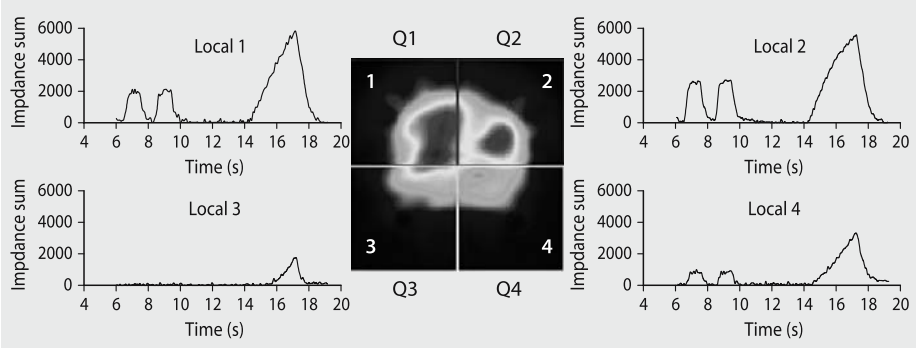

Fig. 4. Regional impedance curves by sum of impedance changes in four quadrants (Q1–Q4) of the functional EIT image (centre) with normal tidal breathing (up to 14 s) and a recruitment maneuver (starting at 14 s)

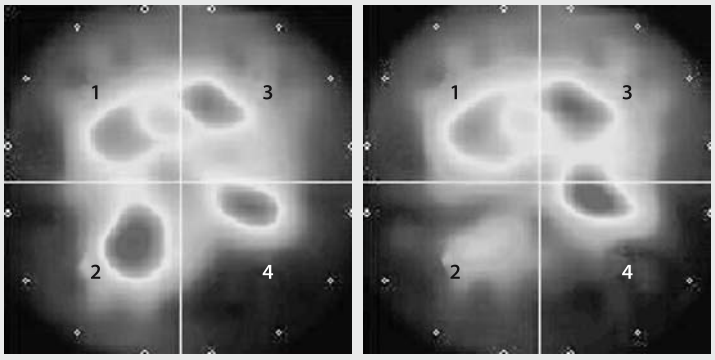

Fig. 5. Functional EIT images for a patient with acute respiratory failure ventilated in the APRV mode with (left image) and without (right image) spontaneous breathing

stants during such a maneuver. Preliminary data of comparisons with dynamic CT scanning suggest that the regional delay observed with EIT correlates well with tidal recruitment of previously non-ventilated ventilatory units.

Another example of EIT sensitivity to changes in regional ventilation distribution is shown in Figure 5. Figure 5, left, shows an EIT image of an intubated patient with acute respiratory failure breathing spontaneously in the airway pressure release ventilation (APRV) mode. When spontaneous breathing was abolished (Fig. 5, right), tidal changes in regional impedance nearly disappear in the right lower quadrant and moved ventrally in the left lower quadrant indicating redistribution of mechanical ventilation predominantly to ventral lung areas. This example fits well with previous observations in patients [17, 18] and experimental models [19] showing that spontaneous breathing results in more equal distribution of ventilation favoring dependent lung areas. EIT obviously allows bedside monitoring of these immediate changes in regional ventilation distribution.

Clinical Perspectives of EIT

EIT is a promising non-invasive monitoring tool which is almost ready for routine clinical use. It is sensitive to changes in lung volume, tidal recruitment and regional ventilation distribution. Further research is required, however, to distinguish between possible mechanisms of airlessness [20]. An interesting finding is that dependent lung regions inspire with a time delay during the slow inflation, with air going initially to upper regions. This could be explained by reopening of collapsed or flooded regions or by trapping air in distal alveoli [21]. Regional aeration curves can be of value in monitoring the amount of tidal recruitment or cyclic opening and collapse of ventilatory units. Cyclic opening and closing of airways and alveoli produce shear forces that can harm the lung [22]. Whereas these cyclic events can be detected by EIT, overdistension by excessive V_T and elevated airway pressure which might contribute to volotrauma and barotrauma will be difficult to detect with the presently used impedance technique. This is because the algorithms for calculating ventilation are based on changes in impedance, not on absolute values.

Further improvement of the EIT technique is warranted and might include increases in the number of electrodes, easier ways of fixing it around the chest, and improvements in hardware and software. Application of additional electrodes might provide more data for regional analysis [23]. The algorithms may also be modified [24]. The transverse area of the thorax was trapezoid in the studied patients, but the algorithms are based on a circular structure. Reliable recording of absolute air content would be valuable. EIT may also be used for the assessment of regional lung blood flow. By using the EKG signal for gating the impedance variation, regional lung blood flow can be determined. This has been tested to distinguish left and right lung blood flow [25] and might even result in ventilation/perfusion assessment at bedside.

EIT delivers complex regional information and its interpretation may be difficult. Thus, simple indices to describe tidal alveolar recruitment and/or changes in regional ventilation after changing ventilatory pattern are warranted. In addition, combination with other bedside monitoring methods such as pressure-volume curves, end-expiratory lung volume measurement, which will shortly be available for clinical routine use, as well as CO_2 elimination measurement may have synergistic effects in understanding impairment of pulmonary function and its response

to treatment efforts. This should result in clinical algorithms to optimize ventilatory settings. A bedside tool for the adjustment of mechanical ventilation with the capacity of measuring regional ventilation and perfusion would be worth waiting for. It seems like we are close to getting it.

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Volumetric Capnography for Monitoring Lung Function during Mechanical Ventilation

F. Suarez-Sipmann, G. Tusman, and S. H. Böhm

Introduction

Capnography has become standard of care in monitoring respiratory function during anesthesia [1] and together with pulse oximetry has contributed to a major improvement in safety and reduction in morbidity over the last three decades [2].

Carbon dioxide is an end product of the body's metabolism and is continuously produced in the cells. In a normal person, about 280 l of CO_2 is produced every day and after its transport by the systemic and pulmonary circulation is eliminated by the lungs via tidal ventilation. The amount of CO_2 reaching the alveoli depends on several factors including the rate of production, the equilibrium between the tissue stores, the venous return, cardiac output and pulmonary perfusion and finally alveolar ventilation.

In intensive care medicine, in contrast to anesthesiology, capnography has gained only limited acceptance as a monitoring tool. However, a better understanding of the pathophysiology of CO_2 kinetics as well as the introduction of new measurement techniques have increased the interest and the potential of capnography. In addition, the introduction of lung protective ventilation strategies [3, 4] in the clinical management of patients with acute lung injury (ALI) requires us to revisit our views of mechanical ventilation as a rather passive and supportive process and start to consider it instead as a highly dynamic and therapeutic intervention. This has increased the demand for improved bedside respiratory monitoring in order to facilitate its adequate and safe implementation.

Breath by breath volumetric capnography represents a very attractive monitoring option that provides the clinician with information not only about the amount of CO_2 eliminated but also about its elimination process, thus adding valuable information about the lung's physiological condition. In this chapter, we will review some of the theoretical and physiological principles behind volumetric capnography. Finally we will discuss some clinical applications that can be derived from a systematic use of this methodology especially in the context of lung protective ventilation strategies.

Time and Volume Capnography

The first capnographic measurement to be introduced and currently the most widely used is the time based capnogram that is obtained by plotting exhaled CO_2 against time. This measure provides continuous monitoring of end tidal CO_2 ($P_{ET-}CO_2$) and, more importantly, changes in the shape of its graphical display assist in

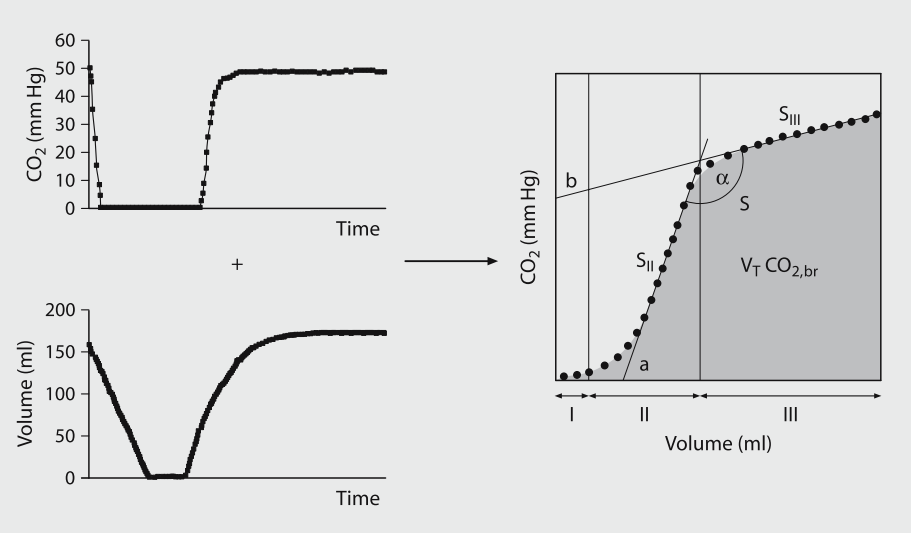

Fig. 1. The volume capnogram is obtained by integrating the CO_2 and the volume signals. The resulting graph, the single breath test of CO_2, can be divided into three main phases. Phase I: proximal airway gas, Phase II: expiratory upstroke, and Phase III: alveolar plateau. The slopes of phases II (S_{II}) and III (S_{III}), the angle between them (α) and the area under the curve define its shape. The area under the curve represents the volume of CO_2 expired in a single breath ($V_TCO_{2,br}$)

detecting a number of clinically relevant problems during mechanical ventilation such as: esophageal or bronchial intubation, circuit disconnections, spontaneous breathing, ventilator malfunctions, etc.

The synchronous measurement of both the CO_2 and the flow/volume signals measured at the airway opening allowed changes in CO_2 in the volume domain to be studied in real time, this way obtaining the volume capnogram also called expirogram or single breath test of CO_2 (SBT-CO_2). Figure 1 shows a normal volume capnogram with its components and phases.

The volume capnogram provides all the features of the time based capnogram, supplementing it, however, with important physiologic information related to the dynamics of CO_2 exhalation and the ability to analyze the sequence of tidal ventilation and dead spaces (V_D).

The Normal Capnogram: Definitions of Phases and Derived Variables

- Phase I begins with the start of expiration and ends when the concentration of CO_2 increases beyond 0.1% from baseline. The volume of gas in phase I comprises airway V_D ($V_D$aw) and represents part of the gas in the proximal airway.
- Phase II, or expiratory upstroke, starts at the end of phase I and ends at the intersection of the predictive slope lines of phases II and III. The midpoint of phase II (50% of the slope) is the limit between $V_D$aw and alveolar gas, and represents the 'interface' where gas transport by convection changes into transport by diffusion within the lung acini. Thus, phase II contains part of both $V_D$aw

and alveolar gas. Phase II is highly influenced by the time constant of emptying acini.

- Phase III, or the alveolar plateau, begins at the intersection of the predictive slopes lines of phase II and III and terminates at the end of expiration. This volume represents the gas inside the alveoli in contact with pulmonary capillary blood and is considered as the efficient part of the tidal volume (V_T).
- The area under the curve represents the volume of CO_2 expired in a single breath measured by flow integration, and represents the portion of alveolar gas that is in contact with the pulmonary capillary blood.
- The slope of phase II is derived from linear regression using data points collected commonly between 25-75% of phase II, and expressed as fraction/litre. Similar to the volume of phase II, the slope represents the spread of acini expiratory times. If all acini are empty at the same time ventilation is more homogeneous and the slope increases.
- The slope of phase III is derived from linear regression using data points collected between 25–75% of phase III, and expressed as fraction/litre. The slopes of phases II and III of individual breaths can be normalized by dividing the slope value by the corresponding mean alveolar fraction of CO_2 (expressed in %). The phase III slope is related to the ventilation/perfusion relationship (V/Q); when the V/Q ratio is more homogeneous, the phase III slope decreases whereas it increases when the V/Q ratio is more heterogeneous.
- Angle II–III or angle *alpha* is defined as the angle defined by the intersections of the slopes of phase II and III.

Finally, the slopes of phases II and III, their intercepts, angle alpha, the area under the curve, the volumes of phases I, II and III are the variables that can be determined non-invasively without having to use additional arterial blood gas determinations. They are representatives of the 'shape' of volume capnography and change with changing pulmonary states and diseases (Fig. 1).

Measurement of Dead Space and Efficiency of Ventilation

V_D is defined as 'wasted ventilation', i.e., the part of the V_T that does not reach perfused alveoli and therefore does not participate in gas exchange [5–7]. At the alveolar level it constitutes one extreme of the V/Q relationship ($V/Q = \infty$), while shunt represents the opposite phenomenon ($V/Q = 0$). Shunt can be considered a mirror concept of V_D. Therefore, it can be called 'wasted perfusion'.

The expired CO_2-volume curve has facilitated the bedside assessment of V_D as a measurement of the efficiency of ventilation that could previously be determined in the pulmonary function laboratory [5]. When adding the value of the arterial partial pressure of CO_2 ($PaCO_2$) to this curve, a complete V_D analysis can be performed at the bedside delivering the following parameters (Fig. 2):

- Instrumental V_D ($V_D$inst) consists of any additional V_D beyond the 'Y' piece causing a re-breathing of CO_2 with each breath.
- $V_D$aw, also known as anatomical or serial dead space, is the volume of gas in the upper and main airways down to the boundary between convective and diffusive gas transport at the bronchiolar level.
- Alveolar V_D ($V_D$alv) represents the alveolar gas within alveoli that are not perfused (West's zone I condition).

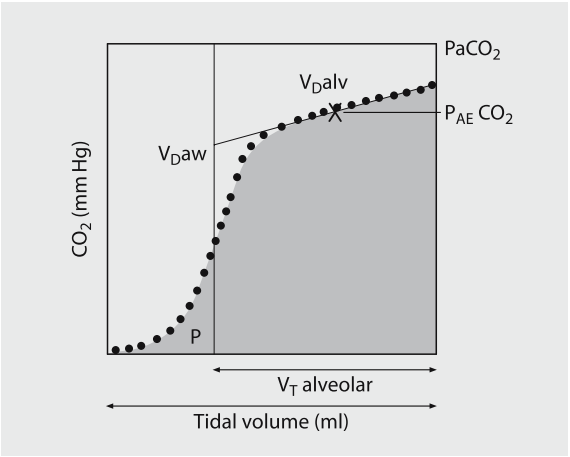

Fig. 2. Dead space analysis. Introducing the value of $PaCO_2$ allows for the calculation of dead space and its fractions. Physiological dead space is the total tidal ventilation dead space, i.e., the sum of airway ($V_D$aw) and alveolar ($V_D$alv) dead space. Fowler's equal area method uses a vertical line across the mid of phase II slope determining the areas p and q of equal size. The intersection of this line with phase II represents the limit between convective and diffusive gas transport within the lungs. $P_{AE}CO_2$: mean alveolar expiratory concentration of CO_2 – the value at the mid point of phase III must be taken to avoid an underestimation of V_D/V_T measurement

- Physiological V_D ($V_D$phys) comprises the total tidal V_D, thus the sum of $V_D$aw and $V_D$alv.
- Physiological dead space-to-tidal volume ratio (V_D/V_T) represents the relationship between the inefficient and efficient part of the V_T.
- $V_T$alv is the portion of V_T distal to the bronchial interface, in alveolar gas. This volume is constituted by the sum of alveolar CO_2 volume plus $V_D$alv. $V_T$alv is derived as V_T–$V_D$alv by Fowler's method.
- $V_D$alv-to-$V_T$alv ratio represents the relationship between the inefficient and efficient portion of the $V_T$alv.
- Bohr's dead space is defined as the sum of $V_D$aw and a portion of the $V_D$alv.
- Arterial to end-tidal difference of CO_2 (Pa-$ETCO_2$) is a clinically useful index that represents the magnitude of $V_D$alv.

Figure 2 represents the volume capnogram and its V_D subdivisions. $V_D$aw is commonly calculated by Fowler's equal area method, where a vertical line across the mid of phase II slope determines the areas p and q of equal size. This line represents the limit between convective and diffusive gas transport within the lungs and is called the 'transition zone' by some authors [6–8]. This border is dynamic, not fixed and changes with different physiological events, diseases or ventilatory settings.

Bohr was the first to describe the measurement of V_D/V_T using a Douglas bag:

$$V_D/V_T = FACO_2 - FECO_2/FACO_2$$

where $FACO_2$ is the alveolar gas and $FECO_2$ is the mixed CO_2 concentration in this expired gas. Later, Enghoff introduced a modification of Bohr's formula that simpli-

fied the measurement of V_D/V_T in patients [9, 10]. He replaced the alveolar CO_2 concentration (or partial pressure) by the $PaCO_2$ assuming that the partial pressure of CO_2 at the arterial side truly integrated the gas exchange function of each single alveolus within the lung:

$$V_D/V_T = PaCO_2 - ETCO_2/PaCO_2$$

Since the slope of phase III is almost always positive, the mean alveolar expiratory concentration of CO_2 ($PAECO_2$), i.e., the concentration of CO_2 at the mid point of this phase should replace the end-tidal concentration in order to avoid a systematic underestimation of V_D/V_T [7, 11].

$$V_D/V_T = PaCO_2 - PAECO_2/PaCO_2$$

$V_D$phys is calculated as:

$$V_D\text{phys} = V_D/V_T \times V_T$$

$V_D$alv is derived simply by subtracting $V_D$aw from $V_D$phys.

Theoretical Principles Related to the Kinetics of CO_2

The kinetics of exhaled gases are useful indicators of gas transport within the lung in healthy individuals and in patients with respiratory diseases [12–14].

Gas transport within the lungs occurs by two main mechanisms: convection and diffusion [8, 12, 15]. Convection, at the main airways, is the bulk flow created by the energy stored within the respiratory system at end-inspiration and the inertial forces created by the diffusive transport. Diffusion, which occurs at the alveolar level, is the passive movement of molecules following a concentration or partial pressure gradient that is given by Fick's law:

$$J = \text{Dmol A Dc/Dx}$$

where J is the instantaneous flux of CO_2, Dmol represents the gas-phase molecular diffusivity of CO_2 in air, A is the area of gas exchange, Dc the venous-alveolar gas concentration gradient for CO_2 and Dx is the width of the alveolar-capillary membrane. Diffusion also promotes the intra-acinar gas transport of CO_2 from the alveolus into the airways. For this movement, A is the cross-sectional area of the small airways and Dc the differential CO_2 partial pressure between the alveoli and the interface of convective-diffusive transport and Dx the distance between alveoli and the above interface. During normal physiology and in most of the pathological conditions, Dmol, Dc, and Dx are constant so that the area becomes the main variable affecting diffusion. A reduction of the area for gas exchange is observed in atelectatic lungs where the number of alveoli is reduced [16]. Here, diffusion is negatively affected and can be considered as an increment in the resistance to the removal of CO_2 from the blood. Similarly, any reduction in the cross-sectional area of the small airways increases the resistance to intrapulmonary gas transport by diffusion as observed in patients with chronic obstructive pulmonary disease (COPD) [17].

The slopes of phase II and III are closely related to the mechanism of transport of expiratory gases but their true genesis is still a matter of debate. The phase II slope represents the transition between alveolar and airway gas transport and depends on the spread of transit times of lung units with different time constants [6, 8, 12, 15, 18, 19]. An increase in the cross-sectional area of the bronchial tree in the lung periphery decreases the linear velocity of the bulk flow until a point where the two transport mechanisms within the lungs, convection and diffusion, are of equal magnitude.

The characteristic upward slope of phase III is explained by a number of mechanisms [6, 9, 20]: stratified inhomogeneity, continuous evolution of CO_2 from the blood and diffusive Pendelluft or sequential emptying of units with different CO_2 concentrations.

Changes in the acinar structure, that is, in pulmonary 3D-morphology, as in emphysema, bronchospasm, atelectasis, airway overdistension or embolism, directly affect gas kinetics. Therefore, any change in gas kinetics is reflected in a changing shape of the volume capnogram and in V_D [7, 11, 13, 14, 16, 17, 21]. These changes in the volume capnogram have been described for asthmatic [13, 14] and emphysema patients [17].

During mechanical ventilation, the acinar morphology is affected by different factors such as lung volume history, surfactant function, ventilator settings and pathologic lung condition. These changes can be dynamic, breath-by-breath and reversible (e.g., atelectasis, airway collapse, edema, etc.) or fixed and irreversible (e.g., fibrotic phase of acute respiratory distress syndrome [ARDS]).

Important factors influencing the phase III slope are the size of the V_T and the magnitude of pulmonary perfusion [19]. Schwartz et al. observed that pulmonary perfusion mainly affects the area under the curve. We have recently described that progressive increases in the amount of lung perfusion cause parallel increases in the phase III slope provided that all other ventilator settings are kept constant. On the other hand, this dependency of volume capnography on both V_T and perfusion can make distinguishing between changes brought about by changing ventilatory conditions and those due to hemodynamic alterations impossible. One proposed solution is to cancel out these effects by normalizing each slope by the mean exhaled alveolar concentration of CO_2 [22, 23]. In a study performed on patients during the weaning phase of cardiopulmonary bypass during cardiac surgery, we showed that this normalization eliminated the effects of a wide range of changes on the amount of pulmonary perfusion (e.g., cardiac output). Due to the revival of interest in capnography for monitoring purposes the clinical value of non-normalized and normalized phase III $slope_I$ and the use of other volume capnography-derived variables must be further investigated.

The Role of Volume Capnography for Monitoring Recruitment and Lung Protective Ventilation

The development of atelectasis is a constant phenomenon in ventilated patients with and without pulmonary disease. In addition to the impairment of gas exchange [24], lung collapse has been recognized as a direct cause of post-operative complications [25] and, together with lung overdistension, as a major contributing factor to the development of ventilation-induced lung injury in patients suffering

from respiratory insufficiency [26]. Recently, ventilation strategies aimed at protecting the lung from such damage by re-expanding the collapsed lung using recruitment maneuvers, stabilizing the lung with high levels of positive end-expiratory pressure (PEEP) and by limiting inspiratory overdistension, have been shown to reduce mortality of ventilated patients with severe respiratory insufficiency [3, 4].

Atelectasis causes a loss of functional units due to a decrease in alveolar, capillary and airway cross-sectional area. During lung collapse and recruitment, gas mixing and transport are affected causing a detectable change in the shape and variables of the volume capnogram and V_D fractions. Different lung conditions follow the same qualitative behavior although the magnitude of these changes is different. At similar lung volume history and ventilatory settings in a particular patient, the morphometric 'state' of the acini is the main determinant of gas mixing, gas exchange through the alveolar-capillary membrane and gas emptying. These phenomena, together, are responsible for the changes observed in the volume capnography curve. Figure 3 shows the changes in the shape of a volume capnogram due to recruitment. Table 1 lists the expected changes in volume capnogram variables after an effective lung recruitment.

In a recent study in anesthetized patients with perioperative atelectasis we showed that treating patients with a recruitment maneuver, in which inspiratory pressure was set to 40 cmH_2O [16] and PEEP to a level safely above the lung's closing pressure, led to a normalization of the shape of the volume capnogram and reduced V_D ventilation as compared to patients ventilated with the same level of PEEP but without a previous recruitment maneuver. There was a significant increase in the phase II slope and the area under the curve and a reduction in phase III slope and V_D/V_T. These changes were accompanied by an improvement in oxygenation, end-expiratory lung volume and lung compliance [16].

In a recent study it was shown that the increase in dead space was an independent predictor of mortality in patients with early ARDS [27]. Bedside monitoring using volume capnography has shown that V_D is not a static value but is strongly

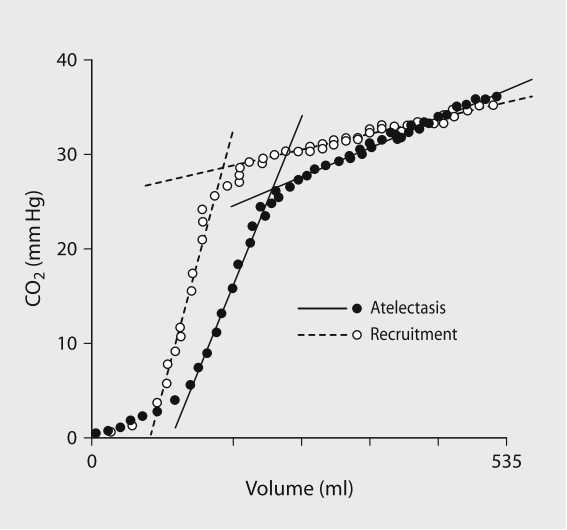

Fig. 3. Differences in the shape of the volume capnogram in an atelectatic and in a recruited lung. After recruitment the phase II slope increases, becoming steeper, and the phase III slope decreases

Table 1. Changes in the volume capnogram after an effective lung recruitment technique

Improved gas exchange	Improved intra-acinar gas transport
↓ Phase III slope	↓ Phase III slope
↓ $Angle_{II–III}$	↑ Phase II slope
↑ Area under the curve ($V_TCO_{2,br}$)	↓ Vol_{III}
↓ Dead-space	
↓ $Pa\text{-}_{ET}CO_2$	

$V_TCO_{2,br}$: Volume of CO_2 expired per breath; $Pa\text{-}_{ET}CO_2$: arterial to end-tidal CO_2 gradient; angle II–III: alpha angle between phase II and III: Vol_{III}: volume of phase III

influenced by ventilatory interventions such as recruitments and PEEP titration [16, 28].

Figure 4 shows changes in the V_D fractions in an experimental model of ALI where after an incremental PEEP trial, a recruitment maneuver was performed that was then followed by a decremental PEEP titration maintaining the other ventilatory settings unchanged. At each PEEP level a CT scan was performed to assess lung condition.

During the incremental PEEP steps, progressive recruitment resulting in a decrease in alveolar and physiologic dead space, was observed. During the decremental PEEP trial V_D fractions decreased initially until CT confirmed the beginning of collapse. At this point V_D fractions reached their minimum values before increasing again finally reaching pre-recruitment values at the lowest PEEP values. In contrast, $V_D$aw changed almost linearly with the changes in PEEP. This example shows how volume capnography could aid in implementing a lung protective ventilation strategy by identifying the settings at which V_D is minimal.

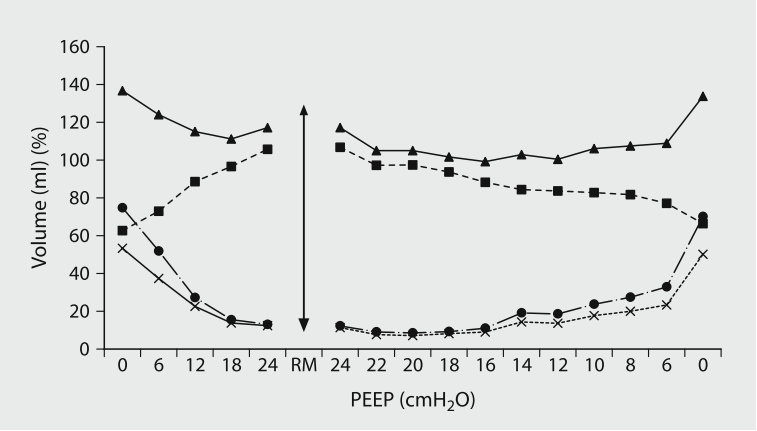

Fig. 4. Changes in dead space fractions during incremental PEEP steps and decremental PEEP steps after a lung recruitment maneuver while maintaining all other ventilator parameters unchanged. Triangles: physiological deadspace (ml); Squares: airway dead space in ml; Diamonds: Alveolar deadspace in ml; Crosses: Physiological dead space-to-tidal volume ratio (V_D/V_T) in %

Conclusion

Volume capnography is a promising non-invasive, inexpensive, breath by breath measurement that has the potential to become an indispensable bedside monitoring tool that can guide the therapeutic process of mechanically ventilated critically ill patients. The better understanding of the pathophysiology of the acutely injured lung on the one hand and the increasing knowledge about the kinetics of CO_2-exchange on the other has created a growing interest in this technology. Volume capnography provides information about the changes in the lung's condition and might therefore allow for improved monitoring of complex ventilatory interventions such as lung recruitment and PEEP titration. In addition, the bedside assessment of V_D helps to identify patients at risk with uneven modes of ventilation and to evaluate their response to different ventilatory strategies. Further studies will help define the true role of volumetric capnography in clinical decision making.

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Monitoring Respiratory Drive and Respiratory Muscle Unloading during Mechanical Ventilation

J. Beck and C. Sinderby

Introduction

Since Galen's description 2000 years ago that the lungs could be inflated artificially, mechanical ventilation has become a primary intervention for life support [1]. The common indication for mechanical ventilation is respiratory failure, defined as a major abnormality in gas exchange [2]. According to Esteban et al. [3], how the assist is delivered in terms of volume and respiratory rate varies widely among centers. Recent randomized clinical trials have suggested that limitation of tidal volume reduces the risk of ventilator-induced lung injury (VILI) [4].

Given that the positive pressure ventilator solely pumps air or medical gas into the lungs (negative pressure ventilation will not be discussed), one must assume that mechanical ventilation either substitutes or partially assists the respiratory muscles' function of inflating the lungs. If the patient is heavily sedated and/or paralyzed, the ventilator's delivery of positive pressure substitutes the respiratory muscles, and the settings for pressure, tidal volume and respiratory rate are decided upon by the caregiver.

New evidence indicates that the practice of deep sedation has a negative impact on care and increases both the duration of hospital stay and mortality [5] (for an excellent overview see Burchardi [6]). Algorithms for sedation, therefore, increasingly favor reduced levels and daily interruption of sedation [7].

If sedation is limited, patients will likely be breathing spontaneously, involving respiratory muscle activity, and allowing tidal volumes and breathing frequency to be controlled by the patient. Intuitively, one would anticipate that the ventilator should adapt to and follow the patient's breathing pattern. This is, however, not always the case. Poor interaction between the ventilator and the patient is commonly reported and has been suggested to increase the need for sedation [8, 9].

Respiratory Drive and Respiratory Muscle Unloading

To understand the underlying issues that cause poor patient-ventilator interaction, it is important to discuss how mechanical ventilation unloads the patient and controls respiratory drive. Unloading of the respiratory muscles is an indistinct term and several factors are involved in the process; these are discussed below.

The first aim during respiratory failure should be to reduce the underlying source that causes the increased load. For example, during acute bronchoconstriction, the aim would be to reduce airway resistance (reducing resistive load), which

would reduce dynamic hyperinflation (improves inspiratory muscle strength), and also increase compliance (reducing elastic load). Another manner in which the respiratory muscles can be unloaded is by reducing the respiratory drive with sedation and analgesia, which will reduce respiratory muscle pressure generation. To maintain adequate ventilation during sedation, an increase in the level of ventilatory assist may be necessary to compensate for the reduced patient work. Since mechanical ventilation is applied to increase ventilation, this in itself will of course reduce respiratory drive and pressure generation if it successfully reduces CO_2 levels. If the mechanical ventilator delivers the assist when the patient's inspiratory muscles actively try to inhale, the mechanical ventilator can be considered an artificial inspiratory muscle, aiding the inspiratory muscles to generate sufficient ventilation.

How Synchronous is Mechanical Ventilation?

In published clinical trials on outcome from mechanical ventilation, the differences between controlled ventilation vs partial ventilatory assist should be interpreted with caution, as it is not always stated what modes of ventilation are used or if the patient is spontaneously breathing. There have not yet been any clinical studies about the role of synchronized mechanical ventilation on patient outcome, where the assist is truly synchronized to patient effort. For example, the Cochrane review [10] on synchronized modes of mechanical ventilation does not include any quantitative index of patient ventilator interaction. In fact, the Cochrane reviewers call for evidence that modes that manufacturers refer to as 'synchronous' actually do provide synchronized assist.

In fact, reports on patient-ventilator interaction suggest that triggered modes of partial mechanical ventilation (e.g., pressure support ventilation) frequently are asynchronous to patient's efforts [11], especially when assist levels are high [12–14]. Patient-ventilator asynchrony may cause the patient to 'fight the ventilator' increasing both inspiratory and expiratory muscle activity (e.g., [15]), as seen in Figure 1. Since poor patient-ventilator interaction is inherent to the use of pneumatic triggering and cycling-off algorithms, intuitively, improved trigger and cycling-off could resolve issues related to patient's fighting the ventilator.

As asynchrony is usually manifested by the patient making an effort to inhale when the inhalation valve is closed or the patient is exhaling when the inhalation valve is open, it is interesting to observe how newer modes like bilevel positive airway pressure (BiPAP) and airway pressure release ventilation (APRV) overcome these shortcomings by simply not occluding the patient [16, 17]. These modes simply deliver time-cycled assist switching between two pressure levels. The patient can breathe freely during both the high and low pressure level such that one part of the minute ventilation is produced by the ventilator's pressure cycling and one part is obtained by the patient's spontaneous breathing [16, 17]. Evidently, the avoidance of occlusions reduces the load on the respiratory muscles. The delivery of assist with these modes is, however, not synchronized to patient effort and it is unclear how they differ from conventional (non-triggered, time-cycled) modes in terms of unloading.

Patient-ventilator asynchrony can also be manifested by the patient being passive and triggering with minimal use of the inspiratory muscles. Figure 2 shows an example of ventilator breaths that are triggered by small efforts, followed by a period of assist throughout which the diaphragm is not active. Inevitably, this pattern of

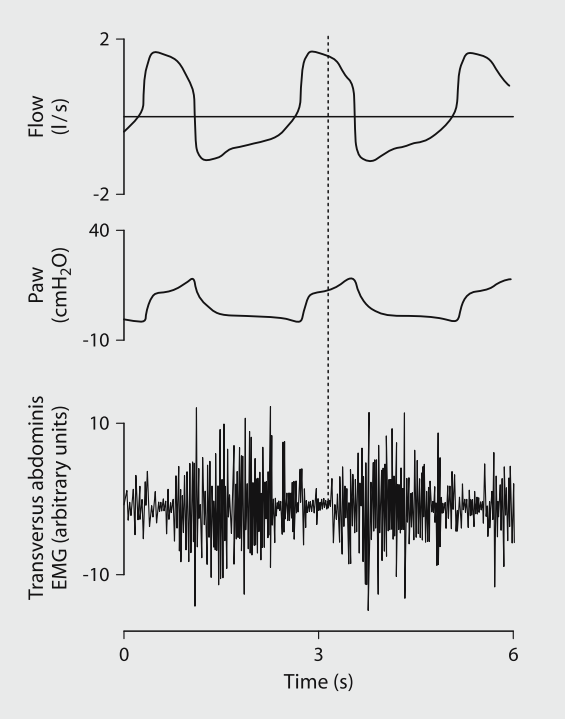

Fig. 1. Recordings of flow, airway pressure (Paw), and transversus abdominis electromyograph (EMG) in a critically ill patient with chronic obstructive pulmonary disease (COPD) receiving pressure support (PS) of 20 cmH_2O. The onset of expiratory muscle activity (vertical dotted line) occurred when mechanical inflation was only partly completed. From [15] with permission

breathing with small negative deflections in esophageal pressure and very brief bursts of diaphragm activation may result in inactivity-induced atrophy, a field that has recently gained interest [18]. Although it is obvious from the diaphragm electrical activity tracing in Figure 2 that changing the ventilator settings (i.e., reducing the rise time, reducing the level of assist, and/or earlier cycling-off) would improve patient-ventilator synchrony – and may increase activation – nothing in the airway pressure tracing suggests that the patient's breathing is over assisted.

To introduce further complexity on the issue of patient-ventilator asynchrony, Figure 3 shows an intubated rabbit with acute lung injury (ALI) spontaneously breathing on volume control (6 ml/kg) with no positive end-expiratory pressure (PEEP). Looking at the volume tracing there is no evidence of muscle activity. However, the diaphragm electrical activity tracing reveals that there is very high diaphragm activity during the expiration phase that is reduced during the ventilator breath. Hence, diaphragm activity is 100% asynchronous to the ventilator's assist and the high diaphragm activity indicates that the animal may well be actually 'under-assisted'. Actually, in the case presented in Figure 3 addition of PEEP reduced the diaphragm activity to low levels. Generally, by only having the flow, volume and airway pressure signals available, and no feedback about the respiratory drive, it is difficult to interpret whether the ventilator's assist is appropriate.

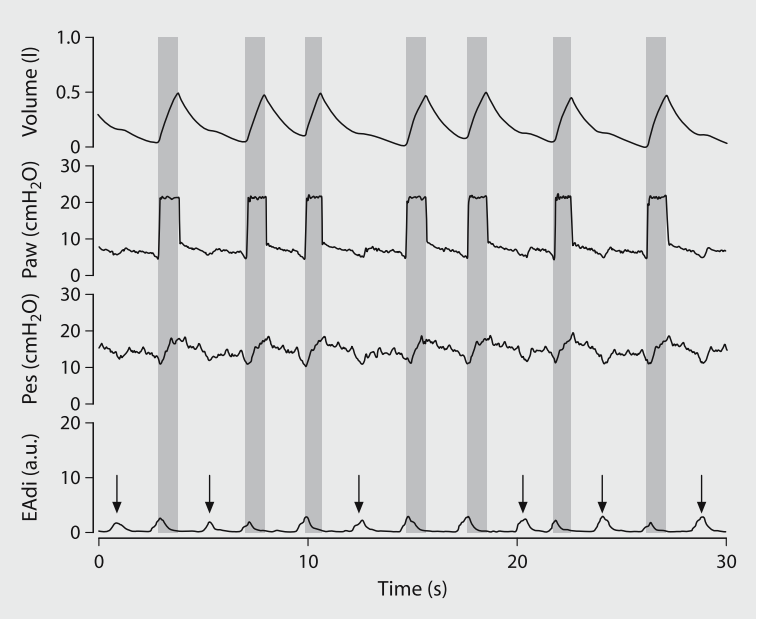

Fig. 2. Example of tracings obtained in a with chronic obstructive pulmonary disease patient, breathing on pressure support. Note that for the successfully triggered breaths, the diaphragm electrical activity (EAdi) and esophageal pressure (Pes) efforts cease immediately after the onset of the assist (Paw) and are characterized by inactivity of the diaphragm during the period of assist (grey bars). Also note the presence of wasted efforts (indicated by arrows) where the patient made a neural inspiratory effort, but failed to trigger the ventilator

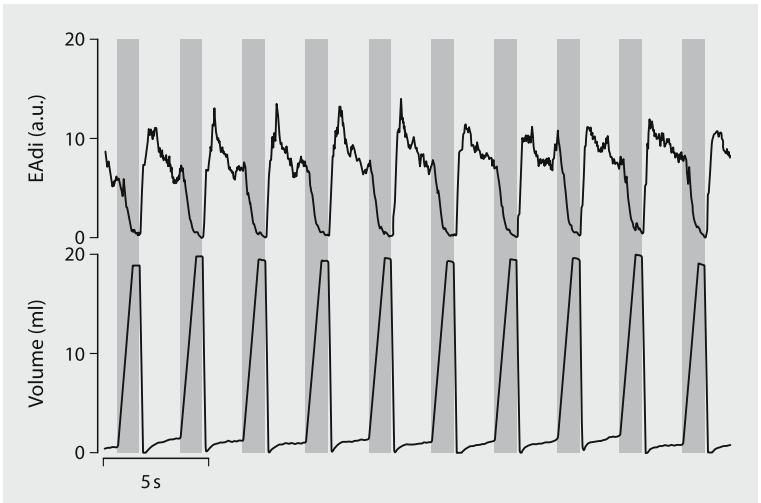

Fig. 3. Time tracing of diaphragm electrical activity (EAdi) and volume obtained in a non-vagotomized rabbit after HCl-induced acute lung injury in volume controlled mode. When inspiratory volume increases (grey shadowed bars) there is a suppression of the EAdi. Decreasing lung volume was related to an increase of the EAdi, producing severe asynchrony between rabbit and ventilator

How to Monitor Respiratory Muscle Efforts during Mechanical Ventilation

How do we know that the patient is using the inspiratory muscles during mechanical ventilation? Apart from the ventilator signaling that the patient is triggering, there are no clinical methods to ensure that the inspiratory muscles are active throughout the ventilator delivered breath. It should be noted that pneumatic triggers are today so sensitive that even flow oscillations induced by the heart may auto-trigger the assist without need for inspiratory muscles [19].

The recent introduction of adjustable off-cycling is aimed at matching the termination of ventilator assist to the end of the patient's inspiratory effort; however, without feedback about neural activation, determination of the 'optimal' off-cycling setting is difficult. Since there are no clinical methods available for determining the adequate physiological cycling-off criteria, it is impossible to determine when the patient's inspiration stops vis-á-vis the ventilator's assist.

Another enigma in the field of mechanical ventilation is the quantification of inspiratory muscle unloading. Conventionally, the only indications for successful ventilation are adequate blood gases and clinical inspection of the patient. There is no easy monitoring parameter available to quantify inspiratory muscle unloading in clinic. In other words, we know when and how much assist the ventilator delivers, and we know flow, tidal volume, and minute ventilation, however, we do not know if or how much the patient's effort was reduced by manipulating the assist.

Generally, two methods are available to monitor and quantify respiratory muscle unloading: 1) diaphragm electrical activity [20] and 2) esophageal pressure [21].

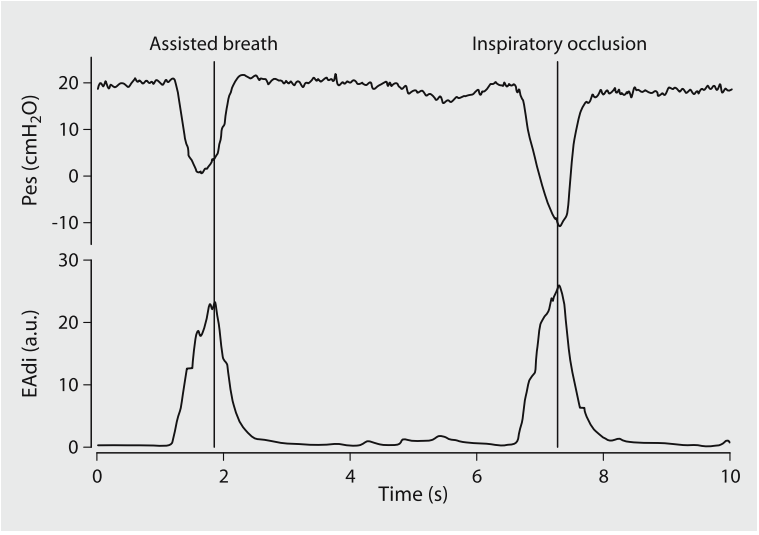

Fig. 4. Example in a mechanically ventilated patient of how delivery of assist affects the neuro-mechanical coupling of the diaphragm. During an inspiratory occlusion (right tracing), the nadir of the esophageal pressure (Pes) coincides with the peak of the diaphragm activation. When a ventilator breath is delivered (left tracing), the Pes becomes 'uncoupled' and the esophageal pressure nadir no longer coincides with the peak of diaphragm activation. EAdi: diaphragm electrical activity

Diaphragm electrical activity represents the temporal-spatial summation of the neural respiratory drive. The esophageal pressure mirrors the pleural pressure resulting from the muscle activation. A confounding factor during mechanical ventilation, however, is how it in itself affects the transformation of neural activity into pressure, the so called neuro-mechanical coupling [22, 23].

When using esophageal pressure, the nadir of the signal is typically used to indicate the end of the inspiratory effort. As depicted in Figure 4, the nadir of the esophageal pressure coincides with the peak of the diaphragm activation during an occluded inspiration (no assist). However, during assist delivery the esophageal pressure nadir no longer coincides with the peak of diaphragm activation. Thus, the ventilator's influence on the neuro-mechanical uncoupling must be taken into consideration when using the esophageal pressure signal to infer respiratory muscle unloading.

Conclusion

An important limitation with today's ventilatory management strategies is that while the timing and magnitude of assist is known, it is not possible to determine clinically the amount of unloading and reduction in respiratory drive. Moreover there are no methods to reveal if the ventilator's assist is delivered when the patient makes the inspiratory effort. To improve accuracy, new monitoring devices for determining respiratory drive and patient-ventilator synchrony are needed. Determination of respiratory drive using diaphragm electrical activity, especially in combination with esophageal pressures can readily help to determine the effect of assist delivery.

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Weaning from Mechanical Ventilation

Weaning from Mechanical Ventilation

R. P. Dellinger

Introduction

How does one predict which patient can be successfully extubated and removed from mechanical ventilation? This is usually simple in patients with known good pulmonary function prior to an acute, now reversed, event (e.g., drug overdose with central nervous system depression or surgery) in whom no new acute compromising event has occurred (e.g., aspiration, stroke). It is not simple in patients recovering from a prolonged ventilatory or respiratory illness that required mechanical ventilation or when a short acute ventilatory illness is superimposed on a chronic condition that may compromise respiratory reserve (Table 1). In this latter situation, knowledge and use of measures that allow some degree of prediction of readiness for withdrawal (liberation) from mechanical ventilation are often useful.

General Issues

Prior to consideration for extubation and removal from mechanical ventilation, the patient should be stable without acute processes such as new diagnosis of sepsis,

Table 1. Reasons for weaning failure

- Chronic hypercapnic state
- Decreased central nervous system drive
- Reduced respiratory pump capacity
  - Malnutrition
  - Electrolyte disturbances (calcium, potassium, magnesium)
  - Polyneuropathy of critical illness
  - Corticosteroid therapy
  - Prolonged neuromuscular blockade
- Inadequate rest of respiratory muscles
- Increased airways resistance
  - Bronchospasm
  - Endotracheal tube obstruction
- Decreased lung or chest wall compliance
- High ventilation requirements due to increased dead space
- Myocardial ischemia
- Anxiety

gastrointestinal hemorrhage, or acute myocardial ischemia. Hemodynamics should be stable. Any chest radiographic abnormality that led to initiation of mechanical ventilation should be significantly improved. When considering endotracheal tube removal, remember that not only must oxygenation and ventilation be considered but also airway protection (patients who otherwise meet weaning criteria but who have a depressed level of consciousness or bulbar cranial nerve palsies may still require the endotracheal tube for airway protection).

Oxygenation Issues

An acceptable PaO_2 ($\geq$65 mmHg) or achieving the baseline PaO_2 present prior to respiratory failure (with an FiO_2 of $\geq$0.40 and a positive end-expiratory pressure [PEEP] of $\leq$5 cm H_2O) is a generally used criterion to predict weaning for a 'need for mechanical ventilation as an oxygen source'. It must also be remembered that reduction in mean airway pressure may have some impact on oxygenation. In addition, following removal of mechanical ventilator support, atelectasis may develop in patients who cannot take a deep breath, especially in the presence of increased airway mucus production or decreased alertness.

Readily Available Measurements of Ability to Extubate and Remove Mechanical Ventilation

Vital Capacity

Normal vital capacity is between 65 and 75 ml/kg. A vital capacity of 10 ml/kg or more is considered a positive factor for successful weaning with $\geq$15 ml/kg ideal. Like maximal inspiratory pressure (PImax) the value is more likely to be falsely negative (predicted failure but actual success) than falsely positive (predicted success but actual failure). Values such as PImax and vital capacity that depend on patient effort are more helpful if they are in ranges that would predict weaning success than if they are in a range that might predict failure, since low values may be due to poor patient cooperation.

Minute Ventilation and Maximum Voluntary Ventilation

Normal minute ventilation for the adult is approximately 6 l/min. As long as $PaCO_2$ is adequate, a value below 10 l/min with minimal ventilator support (pressure support ventilation [PSV] 5 cmH_2O) is desirable in patients being weaned from mechanical ventilation support. Although high values (>15–20 l/min) may help predict weaning failure, values less than 10 l/min are not as reliable for predicting success. Maximum voluntary ventilation is a measure of respiratory reserve and measurements that are two times the resting minute ventilation are desirable. Maximum voluntary ventilation is derived by measuring spontaneous minute ventilation over 15 seconds of hyperventilation and multiplying by 4. Again, this is effort dependent.

Frequency/Tidal Volume Ratio or Rapid Shallow Breathing Index (RSBI)

One of the simplest but most useful indicators of weaning success is obtained by measurement of respiratory rate (frequency or f) and tidal volume (V_T) over one minute during a T-piece or T-piece-like (5 cmH_2O PSV, 5 cmH_2O continuous positive airway pressure [CPAP]) trial that has been in place for 3–60 mins [1, 2]. Values of f/V_T above 105 (breaths/minute/liter) suggest the likelihood of weaning failure. The RSBI produces excess false positives (patients with $f/V_T < 105$ who fail). Also, although very few patients with values >125 can be weaned from mechanical ventilation, many with values between 105–125 can be weaned. The test should only be performed after significant sedation/hypnotic effects have abated.

Thoracic Compliance

Normal thoracic static compliance for adults, measured on a mechanical ventilator, is 60 to 100 ml/cm H_2O. A value of 25 ml/cmH_2O or more has been proposed as a predictive value for successful weaning. Static compliance utilizes inspiratory plateau pressure minus total PEEP as the pressure measurement. Static compliance is, therefore, more reliable than using a dynamic characteristics measurement which utilizes peak airway pressure minus total PEEP, since peak airway pressure is affected by endotracheal tube size, as well as airways resistance and lung elastance; normal dynamic characteristics in the non-intubated patient is 50 to 80 ml/cm H_2O.

Measures of Respiratory Neuromuscular Function [3]

Respiratory Center Function

Airway Occlusion Pressure. The negative pressure generated by inspiratory muscle contraction against an occluded airway is directly related to neural input and is proportional to the diaphragmatic electromyographic (EMG) signal [3]. If an inspiratory occlusion is performed without warning, change in airway pressure at a point (typically 0.1 s), reflects the response of the patient without upper central nervous system integration. The increased $P_{0.1}$ value reflects respiratory distress and signifies the heightened response of the respiratory center to compromise pulmonary function. Occlusion is maintained for 0.25 to 0.30 s allowing the 0.1 s value to be captured as a useful index of respiratory center motor output. Although representing negative pressure, the value is reported as positive units. Normal $P_{0.1}$ is judged to be 0.93 ± 0.48 cmH_2O [4–6]. Patients with $P_{0.1}$ values greater than 4 to 6 cmH_2O of water are typically not weanable from mechanical ventilator support [7–9].

Respiratory Muscle Function

Maximum Inspiratory Pressure. PImax assesses the total strength of respiratory muscles. In the cooperative patient, measurement techniques for PImax may depend on patient effort so the measured value may not be the PImax [3]. PImax also does not take into account the demands placed on respiratory muscles so a good PImax in the presence of need for increased work of breathing due to poor lung compli-

ance may still lead to failure to successfully wean. In a patient who cannot cooperate, PImax can be measured by a 20 second occlusion that allows exhalation but not inhalation leading to increasing inspiratory efforts.

The PImax may be measured against an open or occluded airway. The latter has potential utility in patients that are not capable of following commands. Ideally the lung volume at which the measurement is made should be controlled and is typically measured after complete expiration (residual volume). An alternative is to measure the PImax at functional residual capacity as this measurement reflects no effect of respiratory system recoil. Normal men have PImax of approximately 111 ± 34 cmH_2O; values are lower in women (72 ± 26 cmH_2O). Values decrease with age, by 6% at age 31 to 35, 25% at age 40 to 60, and 32% at age 61 to 75. Values more negative than -30 cmH_2O are predictive of weaning success whereas values no lower than -20 cmH_2O predict weaning failure [10]. Some studies have reported a high rate of false-positive and false-negative results [11].

Transdiaphragmatic Pressure. While PImax is a good estimate of overall strength of the respiratory muscle, the diaphragm is the most important muscle of respiration and is responsible for approximately two-thirds of tidal volume [3]. Measurement of transdiaphragmatic pressure (Pdi) is the most direct method of assessing diaphragm strength. To accomplish this measurement, a two balloon catheter is inserted into the upper gastrointestinal tract with one balloon measuring esophageal pressure (Pes) and the other measuring gastric pressure (Pga). Pdi = Pga–Pes. This measurement is the optimal value for diagnosing severe weakness or paralysis of the diaphragm. Unfortunately, maximum transdiaphragmatic pressure (Pdi_{max}) shows considerable variability in normal subjects. A PImax more negative than -80 cmH_2O makes clinically imported diaphragmatic weakness extremely unlikely [12].

Role of Tracheostomy in Weaning From Mechanical Ventilation

Effect of Tracheostomy on Airway Resistance

Although the smaller radius of curvature of a tracheostomy tube increases turbulent flow and resistance, the shorter length of the tracheostomy tube more then compensates for this turbulence with a total of significant decrease in airway resistance [13–16]. This decrease in resistance is greater *in vivo* than *in vitro* due to increased airway turbulence from luminal secretions and tube angulation and deformation. Tracheotomy tubes are also less subject to progressive occlusion because of more ready and effective suctioning. Although airway resistance and work of breathing clearly decrease when a tracheostomy tube replaces an endotracheal tube, the clinical impact of this improvement has not been established. It makes sense, however, that in some patients the decrease in resistance and work of breathing would make a difference in whether or not mechanical ventilation support could be removed.

Effect of Tracheostomy on Dead Space

Dead space is reduced when a tracheostomy tube replaces an endotracheal tube [17]. It likely amounts to 10–20 ml of decrease in dead space [18]. This small difference is unlikely to have substantial impact, however.

Weaning: Methods and Prediction

General Concepts

Weaning may be accomplished by switching from assist-control to: (1) higher levels of PSV which are subsequently decreased over time as tolerated; (2) synchronized intermittent mandatory ventilation (SIMV) with an initial higher rate which is subsequently decreased over time; or (3) continuing full ventilatory support and performing periodic trails of low levels of CPAP and PSV (5 and 5 cmH_2O as part of a "T-piece-like" trial). With PSV, the pressure initially applied is that which gives a slightly lower V_T than assist control, and then the pressure is decreased over time while the patient is monitored for signs of weaning failure. When low levels of pressure support are achieved (5–8 cmH_2O based on tube size) and can be maintained over time, the patient is considered to be extubatable from a ventilatory standpoint. Weaning parameters may also be checked at this time. With SIMV, the mandatory breath rate is decreased over time with either technique. When a low SIMV rate has been reached, weaning parameters are checked and if supportive for successful weaning, the patient is given a trial of CPAP or extubated. During the weaning process, with both SIMV and PSV, it may be desirable to rest the patient at night with return to assist control, higher rates of SIMV, or high levels of pressure support. The patient is monitored for signs of weaning failure (high spontaneous rate, low spontaneous V_T, tachycardia, tachypnea, or distress).

The mode of mechanical ventilation obviously can have no effect on the ultimate success or failure of liberation from the ventilator since, following the moment of truth, the ventilator is no longer connected to the patient and of no benefit at that time. The patient succeeds or fails based on respiratory muscle function, central nervous system drive, and work of breathing required. However, the mode of ventilation used during the weaning process may determine the rapidity of weaning and allow assessment of likely success or failure. Literature has suggested that PSV leads to earlier successful discontinuance of mechanical ventilation compared to other methods. Another study suggested that going straight from assist-control to a daily trial of T-piece ventilation (or likely a 5 cmH_2O PSV, 5 cmH_2O CPAP trial) is more effective than either PSV or SIMV weaning.

Predicting Success in Weaning from Mechanical Ventilation

Esteban et al. performed a perspective randomized multicenter study with 546 patients receiving mechanical ventilation for a mean of 7.5 ± 6.1 days who were considered to be ready for weaning [19]. Patients were randomly assigned to undergo one of four weaning techniques: (a) SIMV with initial rate of 10 decreased if possible at least twice a day versus (b) PSV initially set at 18 ± 6.1 cmH_2O and reduced if possible at least twice a day versus (c) intermittent trials of spontaneous breathing conducted 2 or more times a day versus (d) once daily trial of spontaneous breathing. Duration of weaning to extubation, respectively, was 5 days versus 4 days versus 3 days versus 3 days. The rate of successful weaning was judged to be higher for daily trials of spontaneous breathing than with SIMV or PSV. There was no significant difference in the rate of success between once daily trials and multiple trials of spontaneous breathing. This approach was judged to simplify management without ventilatory need assessed only once a day. The remainder of the time, the patient could be receiving maintenance mechanical ventilation. It is also possible

that a once daily trial of spontaneous breathing with a prolonged period of rest in between daily trials may be the most effective method of a listing adaptive response. Intermittent trials of spontaneous breathing were gradually increased in length as tolerated. For the once daily trial group the patient was placed on a T-tube circuit for up to 2 hours. If no evidence of intolerance developed during the 2 hour period extubation occurred.

Brochard et al. conducted a randomized trial in three ICUs in which patients who could not sustain 2 hours of spontaneous breathing were randomly assigned to be weaned with T-piece trial versus SIMV versus PSV [20]. One hundred and nine patients entered the study. The success rate was 23% for PSV, 43% for T-piece, and 42% for SIMV. Initial PSV level was targeted to produce a patient rate of 20–30 breaths per minute. PSV was reduced, if tolerated, 2 times a day by 2 or 4 cmH_2O. SIMV was decreased as tolerated from an initial rate of 10 and T-piece technique included increase in duration of tolerated T-piece.

Meade et al. identified 65 observational studies of weaning predictors for analysis [21]. Populations of patients included COPD, cardiovascular ICU and heterogeneous. For trials of unassisted breathing, the best weaning predictors were: respiratory rate, RSBI, a product of rapid shallow breathing index and occlusion pressure <450 cmH_2O breaths/min/l, and PImax <20 cmH_2O. Summary data suggested a similar predictive power for respiratory rate and RSBI. A respiratory rate >38 breaths per min and a RSBI of >100 breaths/min/l appeared to reduce the probability of successful extubation. In general, tests to predict weaning success performed poorly.

A test result is classified as positive if it increases the likelihood of successful weaning and negative if it decreases the likelihood of successful weaning. When using likelihood ratios a likelihood ratio of 1 means the post-test probability is the same as pre-test probability and the test is unhelpful. Values of 1 to 2 change probability very little, whereas values of 2 to 5 or 0.5 to 0.2 lead to small changes in probability. Likelihood ratio values of 5 to 10 or 0.2 to 0.1 lead to moderate changes and probability and values of >10 or <0.1 lead to large changes in probability.

Meade et al found $P_{0.1}$/PImax ratio was the best predictor of trials of unassisted breathing and extubation with a pooled likelihood ratio of 16.3. Likelihood ratios of <0.2 for prediction of successful extubation were observed for RSBI >100 breaths/min/l and a respiratory rate >38 breaths/min. The best results achieved with any of these tests were moderate reductions in the probably of successful weaning in association with a negative test result. A positive RSBI result (breathing pattern is neither rapid nor shallow) is minimally helpful in increasing the probably of successful weaning. Likelihood ratios in this case are usually less than 2 meaning that the pre-test probably of 50% will rise no higher then 66%.

Use of PSV to Overcome Endotracheal Tube Resistance

When the endotracheal tube has been in place for significant periods of time, the resistive workload increases because of tube narrowing from kinking or secretions [22]. It is estimated that the pressure support level required to offset the imposed load of endotracheal tube varies from 3–14 cmH_2O [23]. Recent research, however, indicates that post-extubation work of breathing equals or exceeds that seen with the endotracheal tube in place. Under these conditions minimal pressure support to estimate work of breathing following extubation may be inappropriate and increase the likelihood of extubation failure. These findings might be used to support

the use of T-piece to best approximate post-extubation work of breathing. Several trials have found no difference in extubation failure when comparing trials of T-piece versus 7 cmH_2O PSV [24, 25].

Limitations of Weaning Studies

Factors limiting the interpretation of studies of weaning predictors include:

- weaning predictive variables used *a priori* to predict which patients will undergo spontaneous breathing trials;
- different measurement techniques among studies;
- large variability in measurements made by different investigators;
- investigators not blinded to analysis variables (bias); and
- lack of objective criteria for trial tolerance [26].

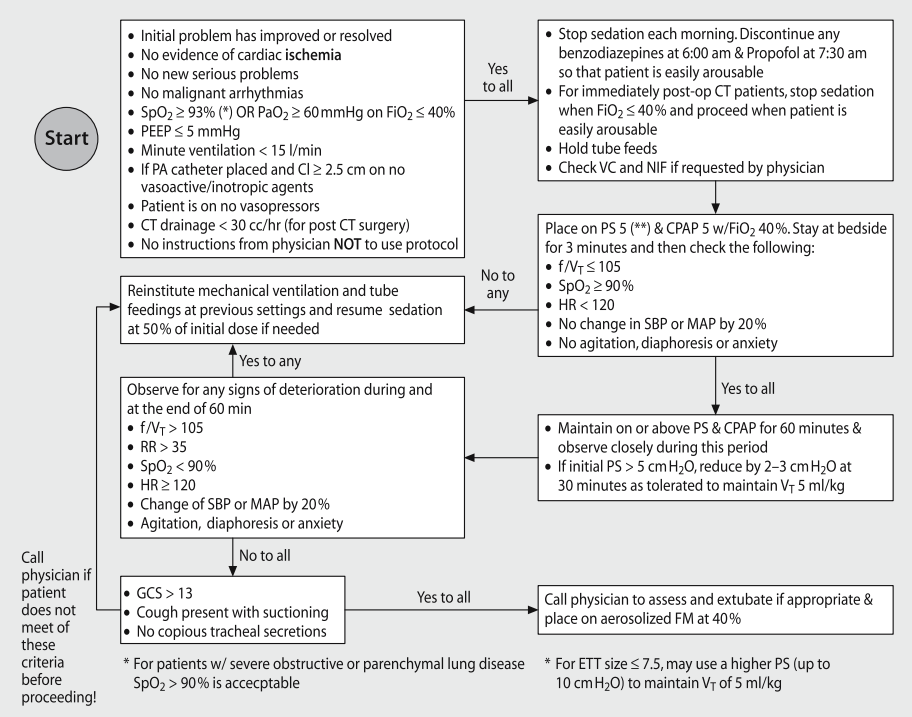

Fig. 1. Example of a weaning protocol. CI: cardiac index; RR: respiratory rate; SBP: systolic blood pressure; MAP: mean arterial pressure; GCS Glasgow Cona Scale; ETT: endotracheal tube; V_T: tidal volume; PS: pressure support; CPAP: continuous positive airway pressure; HR: heart rate; PEEP: positive end-expiratory pressure; VC: vital capacity

Weaning Protocols and Weaning Teams

Daily screening of the respiratory function of adults requiring mechanical ventilation which trigger trials of spontaneous breathing based on preset criteria can reduce the duration of mechanical ventilation [27–33]. Application of well defined protocols independent of specifics of protocol itself (such as mode) likely result in better outcomes in uncontrolled clinical practice. Collaborative weaning protocols among nurses, respiratory care practitioners, and physicians are optimal. Protocol-directed weaning may not be necessary in closed ICUs with physician staffed daily rounds. Weaning protocols driven by non-physician healthcare professionals using evidence based clinical practice guidelines may facilitate weaning where physician resources are scarce.

Conclusion

There is no perfect test or combination of tests that predict the ability to successfully extubate. However, many tests and measures are useful when combined with bedside clinical acumen and experience. Protocols are also very useful. An example of a multidisciplinary weaning protocol used at our institution is shown in Figure 1.

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Liberation from Mechanical Ventilation in Acutely Brain-injured Patients

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Introduction

Endotracheal intubation and mechanical ventilation are required for the majority of critically ill patients suffering from acute brain injury. While these patients certainly may develop cardiopulmonary complications, many of them receive mechanical ventilation because of a decreased level of consciousness and subsequent inability to protect the airway and clear secretions, rather than because of primary respiratory failure. Indeed in a large observational study of mechanical ventilation practices around the world, coma was the primary reason for initiation of ventilatory support in close to 20% of cases [1]. In addition, many brain-injured patients will need ventilatory support as part of the management of raised intracranial pressure (ICP) [2]. For example, severe stroke patients may not have underlying respiratory insufficiency but up to 25% of them require mechanical ventilation [3]. Like all patients receiving invasive ventilation, patients with acute brain injury are at risk of complications including airway injury and ventilator-associated pneumonia (VAP); as usual our goal is to discontinue ventilatory support as soon as it is safe to do so. However, the approach to weaning and discontinuation of mechanical ventilation in this patient population remains a challenge to clinicians as these patients have been understudied and because the usual cardiopulmonary markers of liberation readiness may not be applicable. In this chapter, we will discuss what is currently known about weaning and discontinuation from mechanical ventilation, as well as tracheostomy, in brain-injured patients.

Weaning and Discontinuation from Mechanical Ventilation

Although mechanical ventilation can be life-saving, it may also be associated with many deleterious effects including ventilator-induced lung injury (VILI), barotrauma, pneumonia, and airway complications [4]. In addition to a cumulative risk exposure, the daily risk of VAP increases over time [5]. It is therefore important to discontinue ventilatory support as soon as it is safe and possible to do so. On the other hand, premature discontinuation and subsequent reintubation may also have harmful effects; extubation failure has consistently been independently associated with poor outcomes in multiple observational studies [6–8]. The competing outcomes between premature disconnection and extubation delay make this area a prime candidate for clinical trials. Indeed the process of weaning and extubation has been extensively studied in general intensive care unit (ICU) patients [9–11].

In neurosurgical patients, the indications for intubation and mechanical ventilation are often different than in their medical-surgical counterparts. It is unknown whether or not the optimal process for discontinuing ventilation is different in this patient population. The decision-making around extubation is generally a two-step process. The first step is to determine whether the patient can breathe spontaneously without a ventilator. This weaning phase can take up more than 40% of the total duration of mechanical ventilation [1]. As part of this first step and similar to other ICU patients, patients with acute brain injury should undergo a formal assessment of weaning/discontinuation potential when there is reversal of the initial reason for intubation, adequate oxygenation, stable hemodynamics, and evidence of ability to initiate an inspiratory effort [9]. The second step, which may be more problematic in brain-injured patients, is to evaluate whether a patient can continue to breathe without an artificial airway. Clinicians are commonly faced with the scenario of a brain-injured patient who is able to breathe spontaneously with minimal or no ventilatory support but who has a persistent decrease in their level of consciousness. Can they be safely extubated? The optimal strategy in this situation remains controversial [12, 13].

Premature versus Delayed Extubation

Prolonged intubation in brain-injured patients can lead to similar deleterious effects as in other populations. In a prospective cohort study of 136 acutely brain-injured patients, Coplin et al. demonstrated that extubation delay was associated with a two-and-a-half-fold increase in the risk of pneumonia, and a doubling of the risk of mortality [14]. ICU and hospital lengths of stay were also increased by 5 and 7 days respectively in delayed-extubation patients [14]. Hence, there appears to be a significant advantage to extubate patients as early as possible.

On the other hand, premature extubation leading to reintubation may also have deleterious effects. Esteban et al. showed that when general ICU patients required reintubation within 48 hours of extubation, there was a significant increase in mortality [15]. Possible explanations for this increase include complications related to the reintubation procedure and development of other medical problems during the period when patients were not ventilated. These authors also reported that close to 20% of the reintubated patients had complications associated with the procedure, including pneumonia, bradycardia, cardiac arrest, ventricular tachycardia, gastric aspiration and atelectasis [15]. It is unclear, however, how they determined that these complications were directly related to the reintubation procedures. Another study by Epstein and Ciubotaru showed that those who failed extubation were 7 times more likely to die, 31 times more likely to spend 14 or more days in ICU, and 6 times more likely to be transferred to long term care facilities [7]. From a mechanistic viewpoint, Torres et al. found that reintubation increased the risk of pneumonia by 6 times [8]. Unfortunately there are limited data on the consequences of extubation failure on patients with acute brain injury. Until further studies are performed in these patients it seems sensible to extrapolate from the experience in general ICU patients and expect similar complications in acute brain injury.

Extubation Failure Rates and Predictors

In general ICU patients, evidence-based guidelines recommend the protocolized use of spontaneous breathing trials (SBTs) to identify patients who are likely to be able to breathe spontaneously, followed by prompt extubation when an SBT is successful [9]. The application of this strategy has been documented to lead to an extubation failure rate of 13–19% in these patients [10, 15–17]. Does this rate also apply to brain-injured patents? The data to address this question are few; however, it appears that extubation failure rates tend to be somewhat higher in the neurological patients. As displayed in Table 1, several studies have documented high rates of extubation failure in a variety of brain-injured cohorts. At the same time, there is clearly some variability in extubation failure rates between studies. This is at least partly explained by the inclusion of different sub-populations ranging from all comers in neurosurgical ICU [18], to carefully selected patients with isolated head injuries [14], to patients with isolated infratentorial lesions [19]. In addition, the single-center nature of these studies leads to the exaggeration of local practice differences that might impact outcomes.

Why do patients with acute brain injury have such a high reintubation rate? This may be related to the different indications for intubation, whereby these patients are more likely able to breathe spontaneously more quickly, but less likely to be able to maintain an adequate airway and pulmonary toilet following extubation. The traditional weaning parameters measure the patient's ability to breathe without assistance but not the ability to clear respiratory tract secretions or to protect their lower airways from aspiration. Indeed, Vallverdu et al. found that the most common reasons that patients with acute brain injury failed extubation were inability to clear secretions, fever with decreased level of consciousness, and hypoxemia, the first two of which are quite different from non-acute brain injury patients [20]. Similarly, in a retrospective review of practice at our center, we found that reintubation in neurosurgical patients was more likely to be due to airway obstruction, inability to clear secretions, or a decreased conscious level (in 69% of cases), compared with medical-surgical ICU patients who were more likely to be reintubated due to hypoxemia, hypercapnia, or increased work of breathing [21].

Table 1. Extubation failure rates in brain-injured patients

Author [ref]	Year	Number of Centers	Number of Patients	Top Diagnoses (%)	Extubation failure rate
Vallverdu [20]	1998	1	46	Trauma (39%) Stroke (24%)	35.7%
Coplin [14]	2000	1	136	Trauma (57%) Subarachnoid Hemorrhage (19%) Stroke (18%)	17.6%
Qureshi [19]	2000	1	69	Intracranial Hemorrhage (42%) Stroke (39%)	34.4%
Namen [18]	2001	1	100	Intracranial Hemorrhage (34%) Trauma (23%) Subarachnoid Hemorrhage (19%)	38.8%

What can we use to predict successful extubation in patients with acute brain injury? A few predictors have been studied in the past, but one of the most contentious is the level of consciousness. Namen et al. found that a Glascow Coma Score (GCS) ≥8 at the time of extubation had the greatest accuracy in predicting extubation success [18]. At the same time, in the study by Coplin et al. the GCS was not a predictor for extubation success; these investigators showed extubation success rates in excess of 90% in patients with very low GCS levels of 4 or less [14]. How can we reconcile such seemingly contradictory results? Closer inspection of the study by Namen et al. reveals that half of the patients who failed extubation were in fact not reintubated, a decision having been made to withdraw or withhold life-sustaining treatment. This suggests that many of these extubations were undertaken without high expectations of success, but in the spirit of giving the patient one last chance in the process of withdrawal of treatments. These patients are likely to have the lowest levels of consciousness and so may be falsely increasing the importance of this predictor variable. At the same time, the study by Coplin et al. took carefully selected brain-injured patients without any other organ failures that might interact with a decreased levels of consciousness – this may have resulted in higher than expected extubation success rates.

Other potential predictors of extubation failure include parameters that measure a patient's ability to cough and clear respiratory secretions. These have now been found to be important in medical ICU patients [22–24], but again available data in brain-injured patients are limited. Vallverdu et al., in their prospective observational study, showed that in patients with acute brain injury, maximum inspiratory pressure (PI_{max}), maximum expiratory pressure (PE_{max}), frequency-to-tidal volume ratio (f/V_T), and airway occlusion pressure were the best predictors for weaning and extubation outcome [20]. Conversely, Coplin et al. showed that a comprehensive 6-part semi-quantitative airway score (spontaneous cough, gag, sputum quantity, sputum viscosity, suctioning frequency and sputum character) was not predictive of extubation success in patients with acute brain injury. However, two individual components (spontaneous cough and suctioning frequency) were associated with successful extubation [14]. Similarly, Namen et al. showed that intact cough reflex and cough during suctioning had no bearing on extubation success [18]. All these studies are limited in the sense that decisions to proceed with extubation were not made independently of the predictor variables being tested. Overall, there are currently insufficient robust data to confidently identify the important predictors of extubation failure in these patients.

Tracheostomy

Prolonged translaryngeal intubation can potentially result in increased risk of oral, laryngeal, and tracheal complications (such as laryngeal ulceration, vocal cord paralysis, tracheal and/or glottic stenosis, and tracheomalacia) in brain-injured patients [25, 26]. It also carries the dangers of malpositioning of the endotracheal tube and of self-extubation. Other potential disadvantages include physical discomfort and need for increased sedation [27].

Tracheostomy reduces laryngeal ulceration and offers a relatively stable airway that is generally well tolerated because of reduction in airway resistance. It allows better communication, oral nutrition, and earlier ambulation [28]. Also, it provides an easier route for pulmonary toileting and thus easier nursing care. However, tra-

cheostomy is not without its risks. There are complications that are inherent with the procedure including stomal infections, stomal hemorrhage, pneumomediastinum, pneumothorax, and even rare deaths [29, 30]. Long term complications of tracheostomy include tracheomalacia, tracheal stenosis and granulation [31]. Finally, mechanically ventilated patients who receive a tracheostomy are more likely to be discharged to a long-term care facility [32]. These observational data do not imply causality – there is very likely to be a confounding interaction between the need for tracheostomy and the need for chronic care – they do, however, provide a note of caution as enthusiasm for tracheostomy increases.

Predictors for Tracheostomy

Who would benefit from tracheostomy? A few studies, both prospective and retrospective, have looked into clinical predictors for tracheostomy [19, 33, 34]. In 1999, Kollef et al., in a single-center prospective cohort study, demonstrated that VAP, aspiration, reintubation, and use of aerosol therapy were clinical predictors for tracheostomy [33]. In 2000, Qureshi et al., in a single-center retrospective study of 69 patients with infratentorial lesions, showed that a GCS of less than 7 and the presence of brainstem deficits were clinical predictors for tracheostomy [19]. Subsequently, Gurkin et al. retrospectively analyzed 246 patients with traumatic brain injury, finding a GCS of ≤ 8, an injury severity score ≥ 25, and duration of ventilation > 7 days were significant predictors for tracheostomy [34]. In summary, worse respiratory and neurological statuses have been found to be predictors of tracheostomy use, though whether these are optimal indications is unknown.

Timing of Tracheostomy

Despite tracheostomy being one of the most common surgical procedures performed in the intensive care setting, its exact timing remains controversial. The 1989 American Consensus Conference on Artificial Airways states that tracheostomy is preferred if the need for an artificial airway is anticipated to be greater than 21 days [35]. However, current evidence has been inconclusive in brain-injured patients and general ICU patients alike. Two randomized trials have shown improved outcomes with early tracheostomy [27, 36] while three others showed no change in outcomes [37–39]. In 1990, Rodriguez et al. demonstrated a reduction in duration of ventilation, length of ICU and hospital stays, and incidence of pneumonia in early tracheostomy group (≤ 7 days) compared to the late tracheostomy group (≥ 8 days) [36]. A recent study published by Rumbak et al. in 2004 randomized medical ICU patients who were projected to need ventilation for > 14 days to early (< 48 hours) or late (14^{th}–16^{th} day) tracheostomy [27]. They demonstrated a 50% relative reduction in mortality along with a shorter ICU length of stay in the early tracheostomy group. A major limitation of this study, however, was that the method for determining who would need prolonged ventilation (a key study inclusion criterion) was not standardized or explicated. Also, patients were allowed to be discharged to a step-down unit even though they were still being mechanically ventilated via tracheostomy. This would reduce the ICU length of stay but does not necessarily reflect improvement in clinical outcomes in these patients [27].

On the contrary, Sugerman et al. showed no difference in ICU length of stay, mortality, or incidence of pneumonia between the early (3^{rd}–5^{th} day) and late

(10^{th}–14^{th} day) or no tracheostomy groups. However, this study is potentially biased by significant losses to follow up [37]. In 2002, Saffle et al. randomized 44 burn patients to early (4 days) or late (14.8 days) tracheostomy [38]. They found no differences in length of ventilation, survival, or incidence of pneumonia. There were more patients being extubated in the control group by post-burn day 14 than the early tracheostomy group [38]. The limitation in this study is that the two groups of patients had important baseline differences (more full thickness burns and a lower PaO_2/FiO_2 ratio in the early tracheostomy group) which may have confounded the results [38]. Finally, in 2004, Bouderka et al. demonstrated in their randomized study that in patients with a GCS <8, cerebral contusion, and head injury, early (5^{th} or 6^{th} day) tracheostomy patients did not have a survival benefit compared to prolonged intubated patients [39]. There was also no difference in the incidence of pneumonia.

These studies were quantitatively summarized in a recent systematic review and meta-analysis. Griffiths and colleagues found that collectively these five studies did not demonstrate a reduction in mortality or risk of pneumonia, but they did find a reduction in both duration of ventilation and ICU length of stay in early tracheostomy patients [28]. As outlined above, however, many of the studies included in this review had significant methodological issues, and like any meta-analysis its validity is dependent on the quality of the summarized trials.

Because of the heterogeneity of the study populations and the inconsistency of the results, more studies are needed to determine who will likely benefit from tracheostomy and the ideal time for the transition. Also, other endpoints such as patient comfort and long-term outcomes, as well as costs should be investigated. At the current time, physicians must rely on their clinical acumen to individualize decisions regarding whether and when a tracheostomy is warranted.

Conclusion

Patients with acute brain injury are a unique group of ICU patients. Their indications for intubation and mechanical ventilation are often different from general ICU patients. Therefore, weaning predictors are unique and need to be refined in order to decrease the rate of reintubation while still avoiding prolonged intubation, as both can increase mortality and morbidity. Although tracheostomy may provide a theoretically attractive option for airway management in these patients, potential downsides do exist. Data are limited and the indications and optimal timing for tracheostomy are not well defined. We call for additional studies to investigate this common and important clinical problem.

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Non-invasive Ventilation for Respiratory Failure after Extubation

J. Sellares, M. Ferrer, and A. Torres

Introduction

Mechanical ventilation is a life-support procedure widely used in patients with severe respiratory failure. Despite its benefits, this technique is associated with various complications that can be classified into three categories [1, 2]:
1) those directly related to the process of intubation and mechanical ventilation;
2) those caused by the loss of airway defence mechanisms, mainly ventilator-associated pneumonia (VAP), with an important mortality rate [3, 4]; and
3) post-extubation complications.

When clinicians decide to proceed to withdraw mechanical ventilation, they have to assess different weaning parameters, trying to avoid unnecessary delays and to assure the success of this process [5]. Although a large number of patients who have recovered from the episode of acute respiratory failure may be successfully extubated, 6 to 23% of them will need re-intubation within 48 to 72 hours after extubation [6, 7], with remarkable consequences on their clinical outcomes [6, 8]. We will review the impact and the etiology of respiratory failure after extubation, as well as the use of non-invasive ventilation (NIV) in the management and prevention of respiratory failure after extubation.

Respiratory Failure after Extubation: Definition, Significance and Etiology

Respiratory failure after extubation is defined as the appearance of signs of respiratory distress within 48 to 72 hours after patients have undergone a planned extubation, requiring invasive or non-invasive ventilatory support [9]. It is important to differentiate this concept from weaning failure. When a ventilated patient is recovering from an episode of acute respiratory failure, the ability to tolerate spontaneous breathing may be assessed with the use of a Venturi oxygen source connected to the endotracheal tube through a T-piece or using low levels of pressure-support ventilation (PSV). If no signs of poor tolerance appear and weaning predictor parameters are favorable, patients are extubated. If there is no need of ventilatory support in the following 48 to 72 hours, it is considered as weaning success. Conversely, if patients develop signs of intolerance during the spontaneous breathing trial, namely weaning failure, they are reconnected to the ventilator and mechanical ventilation is gradually discontinued [5, 10].

Table 1. Causes and mortality of respiratory failure after extubation (adapted from [15])

	Patients No. (%)	Mortality* No. (%)
Respiratory failure	21 (28)	12 (57)
Congestive heart failure	17 (23)	8 (47)
Aspiration/excess secretions	12 (16)	2 (17)
Upper airway obstruction	11 (15)	2 (18)
Encephalopathy	7 (9)	3 (43)
Other	6 (8)	3 (50)

* Percentage of patients who died after re-intubation secondary to respiratory failure after extubation

Re-intubation due to extubation failure is an independent risk factor for nosocomial pneumonia [8], as well as for increased hospital mortality and length of stay [6]. Among others, neurological impairment, older age, severity of illness, cardiac failure, longer duration of ventilation prior to extubation, anemia, and the use of continuous sedation, have been identified as risk factors for extubation failure [6, 9, 11]. The pathophysiology of respiratory failure after extubation includes upper airway obstruction, inadequate cough, excess respiratory secretions, encephalopathy and cardiac dysfunction [9, 12–14].

In relation to the etiology of respiratory failure after extubation, we can differentiate airway and non-airway causes [15, 16]. Non-airway causes are defined as congestive heart failure, respiratory failure, encephalopathy and others, while airway causes include upper airway obstruction and aspiration or excess pulmonary secretions. Compared with the airway etiologies, the non-airway causes of re-intubation are more frequent, have a higher mortality, and patients tend to be re-intubated later compared with those who fail because of an airway cause [15] (Table 1). Hence, the authors hypothesized that the clinical deterioration after a longer period of time between extubation and re-intubation could be the reason for the worse outcome in these patients.

Recent studies have assessed the impact of cough strength, neurological function and endotracheal secretions in predicting extubation outcome [12–14]. Cough strength has been assessed qualitatively [12] or, more recently, quantitatively [13, 14], measuring the 'cough peak flow' (cough assessment using a peak flow meter placed in series with the endotracheal tube). In a prospective observational study with 88 patients who had passed a spontaneous breathing trial [14], patients with a cough peak flow of 60 l/min or less, endotracheal secretions of 2.5 ml/h or more and an inability to perform four simple tasks (opening eyes, following with eyes, grasping hand and sticking out tongue) had a failure rate of 100%. Conversely, only 3% of patients with none of these risk factors failed extubation.

Pathophysiologic Basis for the Use of Non-invasive Ventilation in Respiratory Failure after Extubation

In understanding the pathophysiology of respiratory failure after extubation, it is important to know the basic physiologic changes that occur after discontinuation of mechanical ventilation in patients who tolerate spontaneous breathing. In patients with chronic obstructive pulmonary disease (COPD) and compared with mechanical ventilation, there is an increase in the respiratory frequency and a decrease in the tidal volume during a spontaneous breathing trial, without changes in the minute ventilation. This rapid and shallow breathing pattern results in reduced alveolar ventilation and a worsening of the ventilation-perfusion (V/Q) mismatching [17]. At the same time there is a concomitant increase in cardiac output and mixed venous PO_2 that can explain in part why arterial oxygenation does not decrease during spontaneous breathing in COPD patients with successful weaning, despite V/Q mismatching [17]. Interestingly, patients who fail weaning attempts develop a progressive decrease in the mixed venous oxygen saturation (SvO_2) during a spontaneous breathing trial. This is caused by the combination of an increase in oxygen extraction by the tissues and the inability to increase the cardiac index and therefore the oxygen transport [18]. In this study, patients who tolerated the spontaneous breathing trial could increase their cardiac index, thus avoiding the failure of SvO_2 [18]. Other mechanisms, such as an imbalance between the respiratory muscle capacity and increased respiratory workload, are related to ventilator dependency and respiratory failure after extubation [9, 19]. So, similar pathophysiologic events that occur during weaning failure may also lead to failed extubation.

Acute hypercapnic respiratory failure in COPD patients is the most clear indication for NIV [2]. The work of breathing is decreased with inspiratory PSV, with an additional effect of positive end-expiratory pressure (PEEP) [20]. Moreover, in acute COPD patients treated with NIV, the improvement in hypercapnia and hypoxemia was due to an attainment of an efficient breathing pattern rather than an improvement of V/Q mismatching [21]. In another study in mechanically-ventilated COPD patients during ventilator weaning [22], PSV as a method of weaning was effective in avoiding V/Q worsening during the transition from positive-pressure ventilation to spontaneous breathing. In ventilator-dependent patients with chronic respiratory disorders, invasive and non-invasive PSV are equally effective in improving the work of breathing and the arterial blood gases, while the breathing pattern and the respiratory pump improved better with NIV [23].

In summary, if NIV and invasive PSV are effective in averting the development of a rapid and shallow breathing pattern, as often occurs during respiratory failure after extubation, we can expect that NIV could be effective in preventing respiratory failure and re-intubation after a successful spontaneous breathing trial.

Non-invasive Ventilation in Respiratory Failure after Extubation

NIV has shown consistent benefits in shortening weaning and facilitating early extubation in patients with difficult weaning from mechanical ventilation who are not ready to tolerate spontaneous breathing without ventilatory support [24-26]. In these patients, advancing extubation with non-invasive ventilatory support resulted in shorter periods of mechanical ventilation and ICU length of stay, less incidence of VAP and improved survival [24, 26].

The clinical benefits of NIV in the management of patients who have been extubated but have developed respiratory failure after extubation are less encouraging. NIV was considered a promising therapy after extubation failure in order to avoid re-intubation in an international consensus conference [27]. This information was based on the findings of physiological or non-controlled clinical studies. A physiological study in non-COPD patients who had persistent acute respiratory failure after early extubation showed that the use of NIV improved pulmonary gas exchange and breathing pattern, decreased the intrapulmonary shunt fraction, and decreased the work of breathing [28]. A clinical study compared 30 COPD patients in whom NIV was initiated when they developed extubation failure, if they had no criteria for reintubation, with a retrospective historical control group of 30 patients [29]. The group of patients treated with NIV had lower rates of re-intubation and shorter duration of mechanical ventilation with no differences in the mortality rates, compared with patients from the control group. The main limitation of this study was the use of historically matched controls, with the possible bias implicated. This trial emphasized the need for future randomized controlled trials assessing the application of NIV in post-extubation failure.

However, two randomized clinical trials have not shown benefits from NIV in avoiding re-intubation in patients who have developed respiratory failure after extubation [30, 31] (Table 2). The first trial assessed 358 patients who underwent a planned extubation and selected 81 patients who developed post-extubation respiratory distress [30]. Respiratory distress was defined as respiratory rate greater than 30/min, an increase in respiratory rate of greater than 50% from baseline, use of accessory muscles of respiration, or abdominal paradox. Patients were randomly allocated to be treated with NIV or a conventional approach. This trial failed to find significant differences in terms of rates of re-intubation, duration of mechanical ventilation, hospital and ICU stays and survival. It is remarkable that only 11% of patients included in this study had a diagnosis of COPD as, after the first year, patients with an acute exacerbation of COPD were excluded because the randomized trial evidence strongly supported the use of non-invasive positive pressure ventila-

Table 2. Summary of the randomized trials of non-invasive ventilation (NIV) therapy for respiratory failure after extubation

	Keenan et al. [30]			Esteban et al. [31]		
	NIV	Control	p	NIV	Control	p
Patients, n	39	42		114	107	
COPD patients, n (%)	3 (8)	6 (14)		14 (12)	9 (8)	
Re-intubation, n (%)	28 (72)	29 (69)	NS	55 (48)	51 (48)	NS
Delay of re-intubation, h:min*	–	–				
Median				12	2:30	0.02
Interquartile range				2:10–28:00	0:45–16:30	
ICU mortality, n (%)	6 (15)	10 (24)	NS	25 (25)	15 (14)	0.048

* Time elapsed from respiratory failure after extubation and re-intubation. COPD: chronic obstructive pulmonary disease

tion for these patients and because NIV was, therefore, applied when these patients developed respiratory distress.

A more recent multicenter international randomized controlled study confirmed the results of the previous study [31]. Among 980 patients from ICUs of different countries, 221 patients who developed respiratory failure after extubation were randomly allocated to receive NIV (114 patients) or standard medical therapy (107 patients). There were no statistical differences in rates of re-intubation (48% in both groups) and lengths of hospital stay. The striking point of this study was a higher ICU mortality in patients treated with NIV (25% vs 14%, $p = 0.048$), predominantly because the mortality rate among the patients who required re-intubation and received NIV was higher than that of the re-intubated patients from the control group. The time from extubation to re-intubation, an independent risk factor for increased mortality in re-intubated patients [15], was longer in patients who received NIV ($p = 0.02$) in this study [31]. Similar to the previous trial, only 10% of patients included in this study had a diagnosis of COPD. Another relevant feature of this trial is that patients from the standard medical therapy could be re-intubated or crossed over to NIV if they met the predefined criteria for re-intubation. Thus, 28 (26%) patients from this group were crossed over to receive NIV; this subset of patients had a lower rate of re-intubation (25%) compared with the overall population, as well as a lower mortality in the ICU (11%) compared with the remaining patients. Finally, a post-hoc analysis done in the 23 patients with a diagnosis of COPD showed that the rate of re-intubation was lower among those who received NIV, compared with the standard medical therapy group (50% vs 67%, $p = 0.67$), although the small size of this sample did not allow conclusions to be drawn.

Non-invasive Ventilation in the Prevention of Respiratory Failure after Extubation

Despite the disappointing results of the previous studies, there are some points to note. First, it is important to appreciate the small numbers of COPD patients enrolled in these studies [30, 31], taking into account that the most clear evidence on the efficacy of NIV is demonstrated in this population [2]. Second, the longer time from extubation to re-intubation is significantly associated with worse outcome [15]. Since NIV has not been demonstrated to avert re-intubation once patients have developed respiratory failure after extubation, and the delay in re-intubation is associated with poor outcome, a strategy based on the early use of NIV during the initial periods after extubation in order to avert respiratory failure after extubation in patients at risk for this complication seems more appropriate.

A randomized controlled trial assessed the role of the early application of bilevel positive airway pressure (BiPAP) on the outcome of extubation in ventilator weaning [32]. Niney-three extubated patients, 56 with planned and 37 with unplanned extubation, were randomly allocated to be extubated with early use of NIV just after extubation or conventional management. There were 24 COPD patients in the NIV group and 19 in the conventional therapy group. This study did not show any significant benefit of NIV in averting re-intubation. A possible reason for this lack of benefit of NIV could be that there was not a good selection of patients due to the high proportion of unplanned extubations, which was the main determinant of poor outcome.

Two recent randomized controlled studies assessed the efficacy of the early application of NIV after a planned extubation in selected patients at increased risk of developing respiratory failure after extubation. An Italian multicenter trial enrolled 97 consecutive patients mechanically ventilated for more than 48 hours considered at risk if they had one of the following criteria: hypercapnia, congestive heart failure, ineffective cough and excessive tracheobronchial secretions, more than one failure of a weaning trial, more than one co-morbid condition, and upper airway obstruction [33]. After a successful weaning trial, 48 patients were randomized to receive NIV for at least 8 hours a day for the first 48 hours, and 49 patients received standard medical therapy. Patients who received NIV had a lower rate of re-intubation, compared with those who received standard medical therapy (4 [8%] vs 12 [24%], respectively, $p = 0.027$). In this study, the need for re-intubation was associated with a higher risk of mortality, and the use of NIV resulted in a reduction in the risk of ICU mortality (–10%, $p > 0.01$). The authors concluded that NIV was more effective than standard medical therapy in preventing post-extubation respiratory failure in a population considered at risk for this complication.

Another randomized clinical trial was conducted by our group in 162 patients mechanically ventilated for at least 48 hours who tolerated a spontaneous breathing trial through a T-piece [34]. Patients were considered at risk for respiratory failure after extubation if they had at least one of the following criteria: age >65 years, cardiac failure as the cause of intubation or increased severity, assessed by an Acute Physiology And Chronic Health Evaluation (APACHE)-II score >12 on the day of extubation. Seventy-nine patients were randomly allocated after extubation to receive NIV continuously during 24 hours, and 83 patients received conventional management. Fifty-one percent of patients had underlying chronic respiratory disorders, mainly COPD or chronic bronchitis. Respiratory failure after extubation was less frequent in the NIV group (13 [16%] vs 27 [33%], $p = 0.029$). Patients from the two groups who developed respiratory failure after extubation but had no criteria for immediate re-intubation could receive NIV as rescue therapy in order to avoid re-intubation. Consequently, NIV as rescue therapy resulted in avoiding re-intubation in 4 out of 4 patients from the NIV group and 9 out of 19 patients from the control group. For this reason, the rate of re-intubation was not significantly lower in the NIV group. The ICU mortality (2 [3%] vs 12 [14%], $p = 0.015$) was lower in the NIV group, but the hospital and 90-day survival was similar among patients from the two groups. More interestingly, the authors did a separate analysis of patients with and without hypercapnia ($PaCO_2$ >45 mmHg) during the spontaneous breathing trial. This analysis showed that the ICU and hospital mortality, as well as the 90-day survival, improved significantly only in the subset of patients with hypercapnia during spontaneous breathing. Among hypercapnic patients, 48 out of 49 had underlying chronic respiratory disorders. The authors concluded that the beneficial effects of NIV in hypercapnic patients should be confirmed in a future clinical trial in this specific population.

In summary, these studies suggest that the early use of NIV in avoiding respiratory failure after extubation is useful in selected patients, and especially in patients with chronic respiratory disorders.

Conclusion

Respiratory failure after extubation is a frequent complication associated with an increased risk for nosocomial pneumonia, longer length of stay in the hospital, and mortality. Several randomized clinical trials in non-selected patients who have developed respiratory failure after extubation have not demonstrated the benefits of NIV in decreasing the incidence of re-intubation. NIV has even been associated with decreased survival because of a delay in re-intubation of patients treated with NIV. However, in selected patients at high risk, the early use of NIV after extubation seems useful in avoiding respiratory failure after extubation, although the benefits of NIV in improving survival appear restricted to patients with chronic respiratory disorders and hypercapnic respiratory failure during spontaneous breathing. The indication of the early application of NIV after extubation to all hypercapnic patients during spontaneous breathing should be confirmed with a new clinical trial in this specific population.

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Burn Injury

Importance of Airway Management in Burn and Smoke Inhalation-induced Acute Lung Injury

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Introduction

One of the detrimental complications of burns is the onset of acute lung injury (ALI). In patients with extensive cutaneous burns in which the burned area exceeds 30% of the total body surface area, capillary hyperpermeability occurs not only at the injured site but also in regions distant from the initial insult [1, 2]. The vascular hyperpermeability leads to a large amount of fluid flux from the circulating plasma to the interstitial spaces. This lung edema formation is even more severe when the thermal injury is associated with smoke inhalation eventually leading to acute respiratory distress syndrome (ARDS) [3]. Previously, we designed an ovine model of combined burn and smoke inhalation injury and described the pathophysiology of ALI [3]. The ALI in combined burn and smoke inhalation injury is characterized by severe deterioration of pulmonary gas exchange (decrease in PaO_2/FiO_2, and increase in pulmonary shunt fraction), pulmonary microvascular leakage with subsequent formation of interstitial edema which is evidenced by increased pulmonary transvascular fluid flux (increased lung lymph flow), increased lung water content (lung wet-to-dry weight ratio), and increased pulmonary vascular permeability to both fluid and protein. These pathological changes are associated with severe pulmonary hypertension, massive airway obstruction by obstructive cast material, and increased ventilatory (peak and pause airway) pressures. In previous studies, we have also evaluated factors that play a crucial role in pathogenesis of ALI. There are several pathogenic factors, which affect the pulmonary function. In the present chapter, we will focus on the role of airway impairment in the pathophysiology of ALI induced by combined burn and smoke inhalation and discuss the therapeutic approaches of airway management.

Bronchial Circulation

The bronchial circulation provides systemic arterial perfusion to the airway distal to the carina and their glands, nerves, visceral pleural surface, lymph nodes, pulmonary arteries and pulmonary veins. The return of venous blood from the capillaries of the bronchial circulation occurs either through true bronchial veins or through broncho-pulmonary veins. About two thirds of the total bronchial circulation is returned to the heart via the bronchopulmonary veins. There are anastomoses, or connections between some bronchial capillaries and pulmonary capillaries and between bronchial arteries and branches of the pulmonary artery. These inter-

related anatomical structures play a small role in normal conditions, but can cause potential alterations in pathological conditions.

Smoke inhalation is a potent hazardous irritant to airways. Depending on the fire source and smoke components, the extent of airway damage might vary. For instance, hot air flow and chemical compounds usually cause upper airway injury. Water-soluble materials such as acrolein and the other aldehydes damage the proximal airways and set off reactions that are inflammatory to the bronchi and parenchyma, whereas agents with lower water-solubility such as chlorine, phosgene, and nitrogen oxide are more likely to cause insidious injury. The airway damage in burn and smoke inhalation injury is characterized by an enhanced mucus secretion, destruction of airway epithelium, influx of inflammatory cells, and airway microvascular leakage [4–6]. Airways are swollen and edematous. Peribronchial cuffing and septal thickening are also present. Previously, the investigators in our laboratory reported that smoke inhalation increases airway (trachea) blood flow ~8-fold in sheep [7]. Recently, we have demonstrated that airway blood flow increases even greater – about 20-fold in sheep subjected to combined burn and smoke inhalation injury [8]. As mentioned earlier, drainage of this increased circulation in airways would occur through pulmonary veins causing pulmonary microcirculation flooding. There are a number of studies suggesting that this dramatic increase in bronchial blood flow during burn and smoke inhalation contributes to increases in pulmonary transvascular fluid flux eventually leading to pulmonary edema [7, 9, 10]. Investigators in our laboratory proved the importance of airway circulation in pulmonary edema formation following smoke inhalation using a bronchial artery ligation (mechanical occlusion of bronchial artery) technique [7, 11]. The sheep subjected to smoke inhalation without ligation showed a seven-fold increase in lung lymph flow 24 h after the insult whereas the sheep subjected to the same injury and ligation of the bronchial artery showed only a three-fold increase, suggesting an important role of increased bronchial circulation in pulmonary transvascular fluid flux. Wagner et al. reported that a 300% increase in bronchial blood flow by perfusion (55 ± 4 ml/min) increased lung lymph flow by 35% vs. baseline values in sheep 90 min after onset. In this series of experiments, injection of bradykinin, an inflammatory mediator known to cause vascular hyperpermeability, into the bronchial artery caused a 107% increase in lung lymph flow, whereas injection of the same dose of bradykinin into the pulmonary artery did not alter the lung lymph flow [12]. Efimova et al. reported a contribution of the bronchial circulation in the formation of lung edema in an acute study (4 h) where 120 breaths of cotton smoke was delivered to sheep [9]. The authors demonstrated that sheep with a ligated bronchial artery demonstrated significantly less lung lymph flow following smoke inhalation compared with those that had a sham ligation operation. Lung water content was much lower in the ligation group. Sakurai et al. demonstrated that while occlusion of the bronchial artery partially eliminated blood flow from the bronchial artery, sclerosis of the bronchial artery with ethanol, which apparently blocks all collaterals almost completely inhibited the increased lung lymph flow [11]. The ligation of the bronchial artery also resulted in significant improvement in pulmonary gas exchange, and reduction of lung water content. Thus, it is obvious that increased bronchial circulation during smoke inhalation contributes to parenchymal damage. Although no study has been performed, we cannot exclude the possibility that reduced bronchial blood flow protects the lung limiting the influx of toxic components from the injured airways to the parenchyma. Recently, we have demonstrated that occlusion of the bronchial artery attenuates ALI in sheep

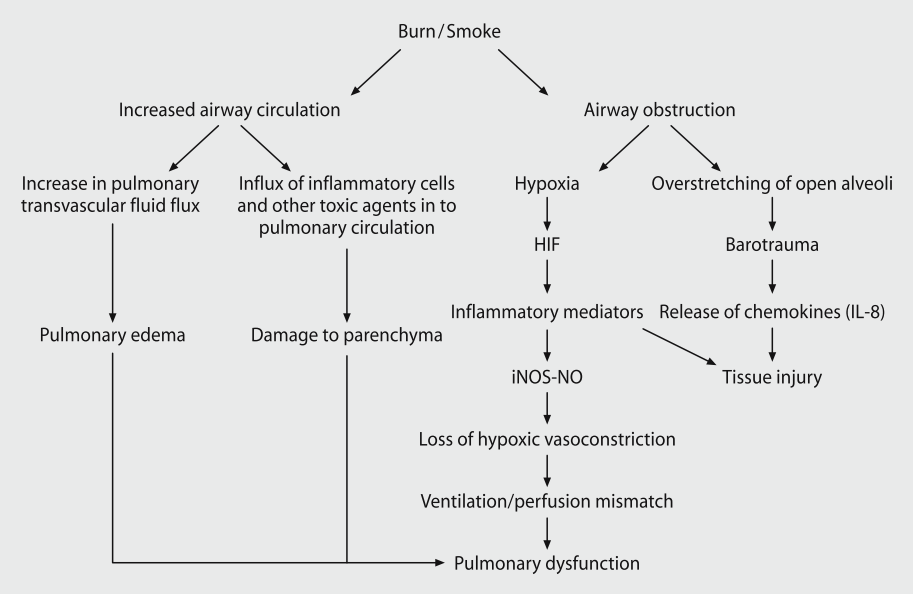

Fig. 1. Contribution of airway damage in thermal injury associated with smoke inhalation to pathogenesis of acute lung injury. HIF, hypoxia-induced factor; iNOS-NO, inducible nitric oxide synthase-derived nitric oxide

subjected to combined burn and smoke inhalation by reversing increased pulmonary microvascular permeability (unpublished data). Interestingly, the number of neutrophils and chemokines such as interleukin (IL)-8 were significantly lower in these animals compared to the injured control animals with a sham surgery. Thus, increased bronchial circulation caused by smoke inhalation may contribute to the pathogenesis of ALI as a direct cause of mechanical flooding of the pulmonary microvasculature and as a carrier of toxic substances from injured airways to the parenchyma that will result in tissue injury (Fig. 1).

Airway Obstruction

Airway obstruction has been shown to be potentially life threatening in humans. The removal of the obstructing cast has been shown to immediately improve oxygenation and the physical status of the patient [13]. Pruitt and Cioffi reviewed the importance of obstructive cast in the treatment of smoke inhalation injury and suggested that the presence of obstructive airway casts following smoke inhalation might promote atelectasis, pneumonia, and barotrauma [14]. However, in general, this potentially important pathologic process has received relatively little attention. In a previous study, we reported that there was significant airway obstruction with a mean reduction in cross-sectional area of about 29% in bronchi, 11% in bronchioles, and 1.2% in respiratory bronchioles in sheep 48 h after being subjected to combined burn and smoke inhalation injury [15]. The mean degree of bronchial obstruction peaked at 24 h after combined burn and smoke inhalation, whereas the mean degree of bronchiolar ob-

struction score continued to increase up to 72 h. We have also shown in the same group of animals that ~10% of the bronchi scored showed degrees of obstruction between 90–100% [15]. Figure 2 shows the macroscopic picture of the airway obstructive cast taken from one of the bronchi of sheep subjected to combined burn and smoke inhalation injury. The obstructive cast instills the airway wall narrowing the airway lumen. It spreads to the smaller airways maintaining their shape, and causing hypo-or non-ventilation of the alveoli. Near total obstruction of a few bronchi would prevent ventilation of individual lung segments [16], whereas partial obstruction would be expected to reduce ventilatory flow and produce hypoxia. The blood vessels in the under-ventilated areas fail to constrict normally (loss of hypoxic pulmonary vasoconstriction), causing a perfusion/ventilation mismatch. This transfer of blood from ventilated to non-ventilated areas results in poor oxygenation of arterial blood, which leads to hypoxemic changes in organs. Obstruction of part of the bronchial tree results in hyperventilation and over inflation of the non-occluded lungs, increasing airway pressure when volume-controlled mechanical ventilation is given [17]. The over-ventilation (overstretching) of alveoli supplied by airways without obstruction can cause so called ventilation-induced lung injury (VILI) or barotrauma, thus worsening oxygenation [17]. The over-stretching of the alveoli induces synthesis and secretion of pro-inflammatory chemokines such as IL-8, which attracts neutrophils to the site of injury causing additional tissue injury [18]. In addition, hypoxia itself can modulate the pro-inflammatory cytokines. Wood et al. reported that systemic hypoxia increases leukocyte emigration and vascular permeability in conscious rats [19]. Fischer et al. reported that hypoxia induces hyperpermeability in brain microvessel endothelial cells via vascular endothelial growth factor (VEGF) and nitric oxide (NO) [20]. Recently, Madjpour et al. have shown that acute hypoxia induces inflammatory changes in the lung representing a mild lung injury in rats [21]. In that study, the authors reported that pro-inflammatory activities such as DNA-binding activity of nuclear factor-kappa B (NF-κB) and expression of mRNA for hypoxia-induced factor-1 (HIF-1), tumor necrosis factor (TNF)-α, intercellular adhesion molecule-1 (ICAM-1), macrophage inflammatory protein-1 (MIP-1), and monocyte chemoattractant protein (MCP-1) were increased. Previously, we have designed an ALI model in sheep induced by combined burn and smoke inhalation injury and described the pathophysiology of ALI [3, 8]. The advantages of this model are:

- Continuous monitoring of cardiopulmonary variables;
- long-term study up to 96 h;
- sheep accept mechanical ventilation through a tracheostomy without requiring sedation; and
- most importantly, sheep have a bronchial mucus gland (which is present in humans) unlike other (mice, rat, rabbit etc.) laboratory animals.

The presence of bronchial submucosal glands in sheep allows us to study ALI associated with airway obstruction. As mentioned earlier, massive airway obstruction in these sheep was associated with the signs of ALI such as deterioration of pulmonary gas exchange (decreased PaO_2/FiO_2, increased pulmonary shunt fraction), increased pulmonary vascular permeability (increased lung lymph flow, increased lung water content, and extravasation of plasma protein), and increased ventilatory pressures (peak and pause airway pressures).

Thus, airway obstruction can contribute to lung injury by causing

- direct hypoxia, which may induce activation of the inflammatory cascade and increase microvascular permeability;

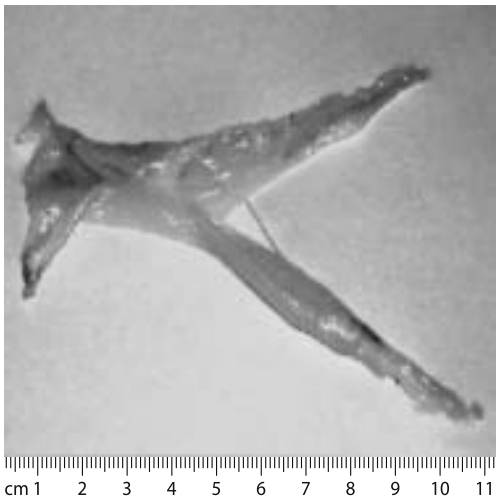

Fig. 2. A solid airway obstructive cast taken from a sheep subjected to combined burn and smoke inhalation injury. The cast was taken 48 h after the injury

- ventilation/perfusion mismatch resulting in poor gas exchange;
- barotrauma, which may lead to both mechanical stretching of alveolar wall and initiate release of inflammatory mediators such as IL-8.

The latter has been shown to mediate tissue injury (Fig. 1).

Therapeutic Approaches

The airway management in thermal injury associated with smoke inhalation should be directed considering the etiological and pathogenic factors (increased airway blood flow and airway obstruction associated with ~2–2.5-fold increase in plasma nitrogen oxides [NOx]) (Fig. 3). We have previously shown that the airway obstructive material is built from increased mucus secretion, airway epithelial cell debris, influx of inflammatory cells, airway microvascular leakage [4, 8]. In addition, we have reported an approximately 20-fold increase in airway blood flow [8], which ultimately contributes to the plasma leakage into the airways especially in conditions when microvascular permeability is increased and the airway epithelial barrier is destroyed. Previously, we and others showed that reduction of bronchial blood flow reduces the pulmonary transvascular fluid flux and ameliorates the ALI following smoke inhalation alone or combined burn and smoke inhalation. However, this approach is possible only in experimental models. There is no treatment method described that could be applied for the clinical use to limit enhanced flux from the airway circulation to parenchyma. Recently, we have described beneficial effects of a new and potent iNOS dimerization inhibitor, BBS-2, on combined burn and smoke inhalation-induced ALI [8]. The iNOS inhibitor markedly improved pulmonary function, reduced pulmonary microvascular permeability, and reduced the increased peak and pause airway pressures. An airway obstruction score developed by our pathology group to quantify the degree of airway obstruction was also markedly reduced by iNOS inhibition. Interestingly, all these beneficial effects of iNOS inhibition were accompanied by a sig-

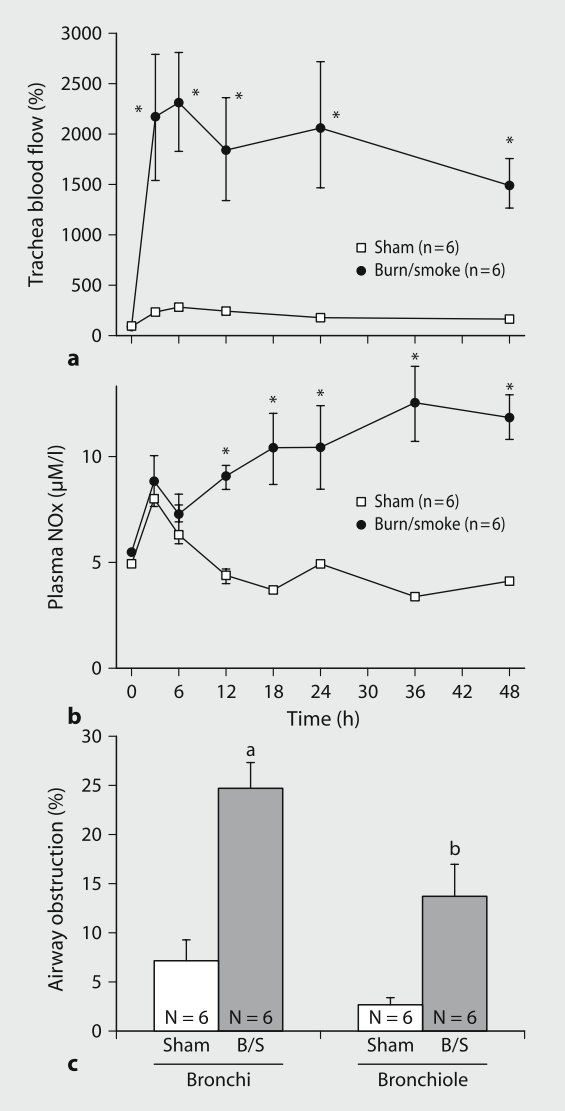

Fig. 3. Effect of combined burn and smoke inhalation on tracheal blood flow (**a**), plasma nitrite/nitrate (NOx) (**b**), and airway obstruction score (**c**). * $p < 0.05$ vs. Sham; [a] $p < 0.05$ vs. Sham (bronchi); [b] $p < 0.05$ vs. Sham (bronchiole). Airway obstruction score was evaluated 48 h after injury. Sham, normal sheep had no injury and no treatment; B/S, sheep subjected to combined burn and smoke inhalation

nificant reduction in tracheal blood flow in sheep treated with BBS-2. We do not exclude the fact that iNOS inhibition ameliorated ALI by several other mechanisms that we have described, however the results from our study that the increases in airway blood flow and plasma nitrite/nitrate were attenuated by the iNOS inhibitor may suggest that excessive NO plays an important role in enhanced bronchial circulation. The BBS-2 treated animals showed significantly less airway obstruction. One would as-

sume that lesser plasma leakage into the airway reduced the influx of procoagulants, consequently fewer fibrin deposits are in the airway, which is one of the major factors that builds an airway obstructive cast. Plasma exudate containing procoagulants in the alveoli and airway is activated with tissue factors from epithelial cells and pulmonary macrophages to cause the formation of fibrin in these areas [22]. Thus, reduction of enhanced bronchial circulation may benefit ALI by reducing lung edema formation and airway obstruction. We have detected large amounts of fibrin deposited in the airway as a part of airway obstructive casts. The presence of fibrin clots hardens the obstructive cast making it extremely difficult to remove even by aggressive airway toilet.

An approximate admission time of fire victims to burn units is 2 to 4 h. There are two ways to reduce the fibrin clots in airways. One way is to lyse already formed fibrin using fibrinolytic agents. This treatment strategy could be effective in burn patients, especially those with delayed admission to the burn unit. We have reported a beneficial effect of an aerosolized fibrinolytic agent, tissue plasminogen activator (tPA), which lyses already formed fibrin clot, in the same model of ALI [23]. Aerosolized tPA significantly reduced airway obstruction score and improved pulmonary gas exchange. Moreover, the fibrinolytic agent markedly reduced the size of an airway cast *in vitro*. These results strongly suggest an important role of fibrin clots in the formation of airway obstruction. The other way is to prevent fibrin clot formation using anticoagulants. The Shriner's Hospital of North America uses aerosolized heparin for airway management of patients with inhalation injury. The investigators in our laboratory demonstrated beneficial effects of aerosolization of various anticoagulants such as heparin [24] in a smoke/pneumonia model in sheep. Interestingly, high doses of heparin administered intravenously in the same sheep model failed to attenuate the degree of ALI [25]. The advantage of administering the anticoagulants as an aerosol is that a maximum local (airway) anticoagulant effect can be reached without significant systemic effects. Recently, we have reported that aerosolization of combined recombinant antithrombin and heparin markedly improved pulmonary gas exchange reducing the degree of airway obstruction in sheep following burn and smoke inhalation [26]. We believe that this combination might be a good therapeutic approach taking into consideration the fact that heparin and antithrombin exert potent anticoagulant effects only as an antithrombin/heparin complex. It has been shown that antithrombin inhibition of factor Xa and thrombin is a slow process. This slow rate dramatically increases in the presence of heparin. For instance, this rate is accelerated by heparin 2000-fold for the inhibition of thrombin and 600-fold for factor Xa [27, 28]. It is well known that antithrombin is a major regulator of blood coagulation. It inactivates a number of proteinases in the coagulation cascade, especially thrombin and factor Xa [29]. In addition, antithrombin has been shown to modulate the inflammatory cascade. It suppresses leukocyte activation, through inhibiting NF-κB [30]. Antithrombin is able to promote the release of prostacyclin from endothelial cells by interacting with heparin sulfate proteoglycans on the endothelial cell surface *in vivo* [31]. Antithrombin also affects neutrophil migration via its heparin-binding site interacting with cell surface syndecan-4 [32, 33], a transmembrane receptor expressed on multiple cell types including neutrophils. Thus, treatment with recombinant antithrombin would have beneficial effects against both hypercoagulability and inflammation, which are present in burn and smoke inhalation injury. Since endogenous antithrombin is depleted in about 50% of burn patients [34, 35], it might be more rational to use recombinant antithrombin therapy in clinical practice. Recently, we have reported that plasma antithrombin is reduced with time reaching its lowest level 12 h after combined burn and smoke inhalation and restoration of its concentration by intravenously ad-

ministered recombinant antithrombin in combination with aerosolized heparin markedly improved pulmonary function. Since co-infusion of heparin prevents antithrombin's anti-inflammatory effect, the later combination may be the most reliable combination, which will produce maximum local (airways) anticoagulant effect of both anticoagulants without interfering with antithrombin's anti-inflammatory effects.

Conclusion

In the present chapter, we have described an important role of airway damage in the pathogenesis of ALI in thermal trauma especially associated with smoke inhalation. Airway obstructive casts have been recognized as potentially life-threatening in humans. In patients with smoke inhalation injury, acute hypoxemia sufficient to produce cyanosis was attributed to airway obstruction [13]. However, this potentially important pathological process has received relatively little attention. As mentioned, heparin is clinically used for airway management of patients with thermal damage. We propose that local (airway) therapy with aerosolization of different anticoagulants, or anti-inflammatory agents, may help in a better management of burn patients, especially those cases associated with smoke inhalation.

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Metabolic Changes Following Major Burn Injury: How to Improve Outcome

W. B. Norbury, M. G. Jeschke, and D. N. Herndon

Introduction

The changes in patient metabolism following a major burn may be seen for more than 12 months after the initial injury. The ensuing period of hypermetabolism and catabolism post-burn leads to impaired immune function, decreased wound healing, erosion of lean body mass, and hinders rehabilitative efforts delaying reintegration into normal society. The typical changes in metabolism are the development of a hyperdynamic circulation [1], increased body temperature [2], increased protein catabolism with peripheral protein wasting [3], increased lipolysis leading to fatty infiltration of the liver [4], increased glycolysis and futile substrate cycling [5]. These changes are responsible for much of the morbidity and mortality seen with such an injury and as such are important targets for available treatments including: early excision and grafting; aggressive 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 erosion of lean body mass; these include anabolic agents such as recombinant human growth hormone, insulin, oxandrolone and beta-blockade with propranolol. This chapter will discuss the metabolic changes seen following a major burn and how different treatment options affect outcome.

Hypermetabolic Response Post-burn

The hypermetabolic and catabolic response after burn injury is similar to that seen following any major trauma; however, the severity of the response is greater than that for any other form of trauma or during sepsis [6]. The intensity of the response is dependent on the percentage total body surface area (TBSA) burned, body weight at admission, and time from injury to excision of eschar. Moreover, the magnitude of the ensuing catabolic process is dependent on the severity of the hypermetabolism and development of sepsis during the hospital stay [7]. The resting metabolic rate of patients with large burns increases in a curvilinear fashion from close to normal predicted levels for TBSA < 10% to twice that of normal predicted levels at 40% TBSA and above. In those patients with burns greater than 40% TBSA the resting metabolic rate at thermal neutrality (33 °C) reaches 180% of predicted basal rate during their acute admission, this reduces to 150% once the wounds are fully healed, 140% at 6 months after injury, 120% at 9 months post-burn and 110% after 12 months [8] (Fig. 1).

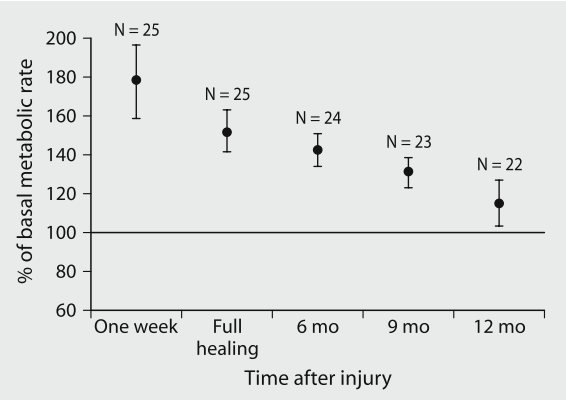

Fig. 1. Hypermetabolic response post-burn. (From [8] with permission)

During the initial hours following a severe burn the body enters an 'ebb' phase during which there is reduced glucose tolerance, oxygen consumption and cardiac output. These variables then increase together with respiratory rate, carbon dioxide production, protein, fat and glycogen breakdown, rising over the first 5 days to a plateau ('flow' phase) that lasts up to 12 months post-burn.

The cause of the hypermetabolic response is unclear; however endotoxin, platelet-activating factor (PAF), tumor necrosis factor (TNF), interleukins (IL) 1 and 6, arachadonic acid metabolites using the cyclooxygenase and lipooxygenase 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 [9].

The rise in oxygen consumption derives from increased total energy expenditure in the major visceral organs and tissues. This is especially prevalent in liver and skeletal muscle where rises of 1.6 and 1.4 times the normal oxygen consumption, respectively, are seen in a patient with a 50% TBSA burn [2].

Substrate Cycling and Glucose Consumption

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%; this, has been associated with an increased incidence of sepsis, however, no causative effect has been found [4]. A large proportion of the glucose produced by the liver is directed towards the burn wound where it is consumed by anaerobic metabolism of inflammatory cells, fibroblasts and endothelial cells; this in turn produces lactate which is recycled back to the liver and into glu-

coneogenic 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 [10]; patients in this situation have been shown to have an increased rate of muscle protein breakdown [11]. 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 [12]. 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 three times normal levels; however, in the presence of insulin resistance hyperglycemia remains a problem. Endogenous growth hormone levels also fall four to five 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.

Molecular Mechanisms Controlling Muscle Breakdown

The main pathway in muscle protein wasting has been shown to be the ubiquitin-proteasome pathway; this is true not only for burns but also cancer, fasting, diabetes, sepsis, metabolic acidosis, hyperthyroidism and excess glucocorticoids. Ubiquitin (7.6 kDa) is a small polypeptide that conjugates to protein substances via a sequence of ATP dependent reactions. The crucial enzymes in this process are the E3 ubiquitin ligases, these act as the substrate recognition component of the ubiquitin recognition machinery and stop proteins from undergoing non-specific modification. Three E3s are expressed in muscle alone: MAFbx/atrogin-1, MuRF1 and E3α-II [13–15]. 'Atrogenes' is a name proposed by Gomes et al. [14] to describe genes that are induced or suppressed in muscle during catabolic states. They represent a specific sequence of transcriptional changes potentially involved in muscle atrophy. IGF-I inhibits the expression of atrogin-1 and to a lesser extent MuRF-1 in muscle [16]. When IGF-I or insulin is given to streptozotocin-induced acute diabetic rats, attenuated muscle wasting and reduced levels of ubiquitin-proteasome system mRNA are seen [17]. Inhibition of phosphoinositide 3-kinase (PI3K) or of Akt inhibits the anabolic effects of IGF-I in cultured muscle cells [17]. One effect of activating PI3K is the Akt dependent phosphorylation of forkhead box O (FOXO) proteins which regulate transcription of metabolic genes and muscle mass. Hormones such as glucocorticoids released during the hypermetabolic response decrease this phosphorylation and increase expression of MAFbx/atrogin-1 and MuRF-1 [18–20], thereby, exerting their catabolic effect.

Evidence indicates that IGF-I and insulin inhibit muscle protein breakdown by mechanisms that are also independent of FOXO proteins. Cytochrome *c* is released from mitochondria soon after burn due to changes in mitochondria membrane potential. An important second messenger, ceramide, increases only in the caveolae

fraction of skeletal muscle; leading to activation of stress activated protein kinase which in turn leads to activation of caspases 1, 3 and 9. These changes occur in muscle distant from the burn wound. Further evidence suggests that insulin resistance following a burn is secondary to an impaired post receptor insulin signaling mechanism – it was also observed that PI3K activation was impaired [21]. Severe burns cause an increase in calcium availability in skeletal muscle; PI3K is suppressed by increased concentrations of calcium thus leading to reduced Akt and reduced anti-apoptotic action [22]. Therefore, the apoptosis seen in burns may be due to reduced growth factor signaling.

Another signaling system implicated in skeletal muscle mass regulation is the nuclear factor-kappa B (NF-κB) family of transcription factors. MyoD and cyclin D genes are targeted by NF-κB and are responsible for activating segments of the ubiquitin-proteasome pathway as well as muscle cell differentiation [23].

These studies suggest that via their action on the PI3k/Akt pathway, insulin and IGF-I are important regulators of muscle mass. Reductions in PI3K activity increases FOXO-dependent transcription of E3 ubiquitin ligases and caspase-dependent cleavage of myofibrillar proteins. It is probable that the proportionate role of each pathway involved depends on the mechanism of injury, distance from the burn wound and hormonal milieu.

Treatment Strategies to Reduce Hypermetabolism and Catabolism

Early Excision and Grafting

If a large burn wound (> 50% TBSA) is excised and covered with autograft, cadaver skin or bio-engineered skin substitute within the first 72 hours following injury, the patient's resting metabolic rate will be 40% less than a patient whose wound is not excised and covered until 7 days post-injury (Fig. 2) [7]. Cross leg nitrogen studies have shown an increase of 230% in net protein losses when comparing those patients who were excised and covered within 72 h and those who received delayed primary reconstruction at 10–21 days.

Early excision and prompt coverage is also associated with decreased mortality, reduced length of hospital stay [24], less operative blood loss [25], and fewer septic complications [26] in children and young adults when compared to conservative serial debridement.

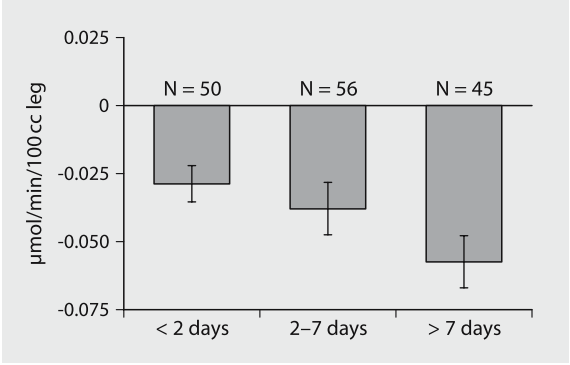

Fig. 2. Effect of delay to excision and grafting on protein catabolism. (From [7] with permission)

Table 1. Definitions of sepsis in burn patients. (From [7] with permission)

Burn sepsis	Modified AACP/SCCM sepsis
▮ At least 3 of the following:	▮ At least 2 of the following:
T > 38.5 or < 36.5 °C	T > 38.5 or < 36.5 °C
Progressive tachycardia	HR > 20% above NL for age
Progressive tachypnea	RR > 20% above NL for age or $PaCO_2$ < 32 torr
WBC > 12,000 or < 4000	WBC > 12,000 or < 400
Refractory hypotension	*and*
Thrombocytopenia	Bacteremia or fungemia
Hyperglycemia	Pathologic tissue source identified
Enteral feeding intolerance	
and	
Pathologic tissue source identified	

WBC: white blood cell; HR: heart rate; RR: respiratory rate; NL: normal

Effect of Sepsis on Metabolic Response

Sepsis raises the metabolic response to burn by 40% when compared to non-septic patients with burns of a comparable size. This increase in catabolism is seen throughout the acute stay and well into rehabilitation. Consequently, prevention and/or aggressive treatment of sepsis is important in reducing the ensuing mortality and morbidity of increased protein loss. Within the burns setting it is difficult to determine sepsis according to standard protocols. Table 1 shows modified scoring systems based upon the American Academy of Chest Physicians and the Society of Critical Care Medicine definition, together with an experienced burn clinician a diagnosis of sepsis can be reached at acted upon.

Effect of Ambient Temperature on Metabolic Response

A proportion of the energy generated during the hypermetabolic response is to offset heat losses secondary to evaporation through the burn eschar; this loss of body water can be up to 4000 ml/m^2 TBSA/day. The body tries to raise the core and skin temperature by 2 °C secondary to a hypothalamic reset mechanism similar to that seen in cold acclimatization. Therefore, the hypermetabolic response may be reduced by warming the ambient temperature to thermal neutrality (33 °C) at which point the heat for evaporation is derived from the environment, taking the burden away from the patient. Raising the immediate environmental temperature can reduce the magnitude of the hypermetabolic response from 2.0 to 1.4 times the resting energy expenditure in patients with burns > 40% TBSA [27].

Nutritional Support

Patients with burns greater than 40% TBSA can lose up to 25% of their body weight in the first three weeks following injury if sustained on an oral diet alone. Numerous formulae have been developed to calculate the requirements of each patient; the caloric requirement is based on size of burn, the total surface area of the patient, the presence or not of sepsis, and the body weight – 25 kcal/kg/day plus

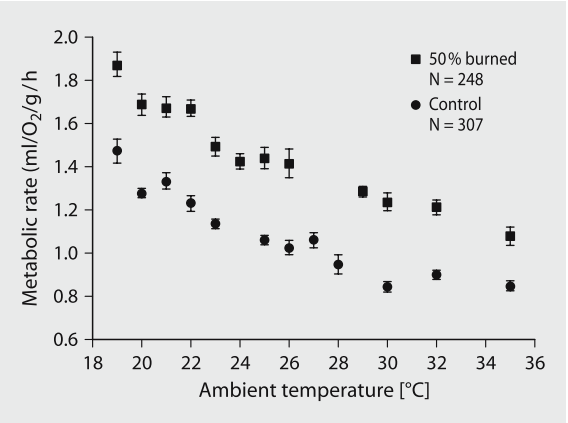

Fig. 3. Effect of ambient temperatue on metabolic rate. (From [50] with permission)

40 kcal/%TBSA/day would provide enough calories to maintain body weight in an adult patient. For children the requirement is 1800 kcal/day plus 2200 kcal/m^2burn/day. Ideally this calorific intake should be all via enteral feeding, with parenteral feeding held in reserve for those with prolonged ileus or intolerance of enteral feeding. Early initiation of enteral feeding can reduce the incidence of ileus and help to abate the hypermetabolic response. An increase in mortality, impairment of liver function and reduced immune response has been shown when using both enteral and parenteral feeding combined to reach caloric intake when compared with enteral feeding alone [28, 29]. Precise estimates of calorific requirements of each patient can be made with the resting energy expenditure (REE) measured by indirect calorimetry while the patient is in the intensive care environment. Portable calorimeters measure oxygen consumption and carbon dioxide production allowing the REE to be calculated using the Harris-Benedict equation. In pediatric burn patients receiving 1.4 times the REE (in kcal/m^2/day) body weight is maintained. A high carbohydrate diet (3% fat, 82% carbohydrate and 15% protein) stimulates protein synthesis secondary to increases in endogenous insulin production and improves lean body mass accretion, relative to an isocaloric-isoprotein but high fat enteral diet [30].

Sufficient protein intake is vital to maintain lean body mass as the rate of oxidation of most amino acids in burned patients can reach as high as 50% higher than rates seen in healthy individuals in a fasting state. Therefore, raising protein intake by more than 50% from 1 g/kg/day to between 1.5–2 g/kg/day will ensure adequate supply in adults, the same cannot be said for children, however, who may increase urea production without any beneficial anabolic effect.

Effect of Exercise on Rehabilitation

A progressive balanced physical therapy program is needed to maintain lean body mass, improve strength, reduce contractures, increase muscle strength, increase incorporation of amino acids into muscle protein and distance walked over time by about 50% [31]. Exercise related hyperpyrexia due to inability to dissipate heat is not a major problem in this group of patients as had been initially thought. A 12-week program of resistance exercise has been shown to greatly improve lean body

mass, muscle strength and power when compared to more standard rehabilitation programs without resistance exercise.

Pharmacotherapeutic Options for the Hypermetabolic Response

The hypermetabolic response to severe burns leads to a shift in protein kinetics such that there is a marked increase in protein breakdown and a concomitant decrease in protein synthesis. The drop in protein synthesis is sufficient to fall below the rate of protein degradation and so lead to a net loss of protein. The anabolic phase during rehabilitation following a burn injury is due to protein synthetic rates rising above protein degradation [32]. This is an important finding as it shows us that an anabolic state can be achieved during persistent protein catabolism. Analgesia and sedatives help to ameliorate the pain and anxiety associated with such injuries and hence prevent the rises in the metabolic rate that would otherwise be seen. Pharmacotherapy specific for the hypermetabolic response includes anabolic hormones such as recombinant human growth hormone (rHGH), insulin, IGF-I, and IGFBP-3 in combination; anabolic steroids such as testosterone or its synthetic analog, oxandrolone; and adrenergic antagonists such as propranolol or metoprolol.

Analgesia

The metabolic rate of the patient is adversely affected by activity, anxiety and pain secondary to increases in sympathetic nervous system activity. Day-to-day burn care including range of motion exercises, debridement, dressing changes and application of topical antimicrobials increase the almost unbearable pain levels still further. Therefore, prodigious quantities of narcotics and sedatives, as well as supportive psychotherapy are helpful in reducing these effects.

Anabolic Hormones

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 0.54 days/%TBSA [33] and led to improved quality of wound healing with no rise in scarring [34]. A favorable attenuation of the hepatic acute phase response [35] was also seen, with increased concentrations of IGF-I (the secondary mediator of rHGH) and increased albumin production [36]. 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 cessation of the treatment. Additionally, rHGH has a positive effect on immune function by reducing T-helper-2 and enhancing T-helper-1 cytokine production [37].

The benefits of rHGH are not without some side effects, most notably hyperglycemia during the acute admission [38]. An increased mortality rate seen in non-burned critical care patients [39] 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; on-going investigations are addressing these points along with trials incorporating beta-blocking agents.

IGF-I. 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 burns patients with significantly less hyperglycemia than rHGH alone [3]. Interestingly, there was no additional benefit seen with higher doses of the infusion; 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 [40, 41]. Side effects of IGF-I include episodic hypoglycemia; however, a recombinant product of IGF-I and IGFBP-3 has become available that shows a decreased incidence of hypoglycemia in phase I trials.

Insulin. Euglycemia maintained using insulin for non-burned critical care patients significantly reduced the incidence of infection and mortality [42]. 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 [43]. A continuous infusion used in burn patients prevents muscle catabolism and conserves lean body mass in the absence of increased hepatic triglyceride production [44]. 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 [45]. 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 increased lactate release [46], 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.

Anabolic Steroids

Oxandrolone is a synthetic testosterone analog that 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 [47], anabolic gene expression in muscle [48] and improves lean body mass by increasing net muscle protein synthesis [49]. Due to the ability of this treatment option to increase lean body mass in an outpatient setting together with the enteral route of administration it is 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 [47].

Catecholamine Antagonists

Propranolol has been used successfully to block the effects of endogenous catecholamines, which have been implicated as primary mediators of the hypermetabolic response. In the initial stages after burn, levels of catecholamines show a ten-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 of heart rate by 20% is seen to produce reduced

cardiac work load and fatty infiltration (secondary to reducing peripheral lipolysis). Propranolol has been shown to enhance intracellular recycling of free amino acids leading to reduced skeletal muscle wasting and increased lean body mass.

Gene Profiling for the Future

Ongoing studies to correlate genomic and proteomic changes induced by burn with phenotypic changes and clinical outcomes are being conducted by the 'Inflammation and the Host Response to Injury' Large-Scale Collaborative Research Program. Recent experiments demonstrate that dramatic, tissue-specific alterations in genomic expression patterns occur in a temporal fashion in blood leukocytes, skin, muscle, and fat. Further experiments will elucidate the genomic and proteomic differences in adults who survive a burn versus those who do not. Additional information (data and publications) can be found at *www.gluegrant.org*.

Conclusion

The hypermetabolic response that follows a severe burn cannot be halted or reversed, however with the use of prompt surgical intervention to remove the burn eschar, aggressive treatment of developing sepsis, early enteral feeding of high carbohydrate high protein diet together with a program of resistance exercises there are several ways that its effects can be limited. Adding to this an anabolic agent with or without an anticatabolic catecholamine antagonist we will be able to ameliorate the difficult sequelae to an already tragic event. Looking to the future with gene profiling, our understanding of this complex process can only increase and with it better treatment and care for our patients.

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Antibiotic Dosing in Burn Injury: Should We be Looking at the Tissues more Closely?

K. Ranasinghe, S.E. Cross, and B. Venkatesh

"We know everything about antibiotics except how much to give." – Maxwell Finland

Introduction

Multiple organ failure (MOF) and compromised immune function, which results in increased susceptibility to sepsis, remain major causes of burn morbidity and mortality [1]. The major frustration for the burns team is for the patient to survive the critical care period, only to succumb to infection, which is known to cause over 75% of burn deaths [2].

The clinical course of a burn is a dynamic cascade of pathological changes, which includes hypermetabolism, hypovolemia and decreased immune function. Understanding the pathophysiology of burn injury and the effects it will have on the pharmacokinetics and pharmacodynamics of drugs is central to developing appropriate antibiotic dosing in burns and potentially improving survival rates. It is important to recognize that the damage in burns extends beyond the skin. This in turn can influence a number of pharmacokinetic parameters such as bioavailability, clearance, volume of distribution, and protein binding. The purpose of this chapter is to review the pathophysiological changes in burns and their impact on antibiotic pharmacokinetics, critically review the evidence on antibiotic dosing in burns, and provide some emerging data on tissue antibiotic profiles in burns.

Extent of the Clinical Problem of Burn Sepsis

Infection in burn patients is common, with *Staphylococcus aureus* (Gram-positive) and *Pseudomonas aeruginosa* (Gram-negative) isolates occurring at frequencies of 38–75% and 22–25%, respectively [3, 4]. Alternative data suggest that 45–50% of burn patients become infected with *S. aureus*, of which methicillin-resistance is present in 45% of cases [5] a resistance statistic that has increased between the time periods studied in 1991–1996 and 1996–2001 [6]. Data from a number of other studies also document an increased incidence of nosocomial pneumonias [7] and systemic sepsis [8].

The problem of increased incidence of sepsis in burns is compounded by the emergence of resistant organisms. Methicillin resistant *S. aureus* (MRSA) is now a major cause of nosocomial infection in burns units specially in Europe [9]. Alongside MRSA, multiple drug resistant *Acinetobacter* sp, Pseudomonas and now vancomycin resistant enterococci (VRE) are increasingly being isolated. The increase

Table 1. Factors increasing the risk of burn wound infection in patients

Factor	Increasing Infection Rates
Age	Children most likely followed by elderly patients
Burn extent	Greater than 30% total body surface area
Burn type	Full-thickness injury
Pre-existing disease	Immune suppression, diabetes and vascular
Organism	Virulent or resistant organisms
Wound status	Prolonged open wounds, failed skin grafts or substandard wound care

in the number of reports about vancomycin resistant *S. aureus* [10] is also highly disconcerting.

Why is the Burn Patient Predisposed to Sepsis?

Multiple local and systemic factors predispose the burn patient to sepsis, as outlined in Table 1. In addition, at the site of the burn, loss of the skin barrier and the presence of devitalized tissue provide a conduit for entry of pathogens and medium for growth, respectively. This is compounded by the need for multiple vascular access catheters some of which are inserted by necessity through burnt tissue. Rapid edema formation occurs from hypoproteinemia and altered capillary permeability [11]. The increase in diffusion distances due to the edema leads to tissue hypoxia [12], which in turn results in reduced neutrophil kill effect and consequently an increased risk of sepsis. Thermal injury itself, without smoke inhalation, has been shown to produce significant lung changes in animal and human studies [13, 14]. This in turn adds to the risk of pulmonary sepsis.

Increased gastric secretion and ulcer incidence, gastrointestinal hemorrhage and local and general distribution of the blood flow with a decrease in gastrointestinal blood flow are among the effects of thermal injury on the gastrointestinal system [15, 16]. These factors compromise the integrity of the gut mucosal barrier [17] and result in bacterial translocation and endotoxemia [18]. Endotoxin has been shown to translocate across the gastrointestinal tract barrier within 1 h of thermal injury [19] and its concentrations reach a peak at 12 h and 4 days postburn [20].

Severe thermal injury induces an immunosuppressed state that predisposes patients to subsequent sepsis and MOF [21]. A growing body of evidence suggests that the activation of a pro-inflammatory cascade after burn injury is responsible for the development of immune dysfunction, susceptibility to sepsis, and MOF [22].

Pathophysiological Factors Contributing to Altered Drug Disposition in Burns and Burn Sepsis

Systemic

The changes that occur in burn patients can be broadly categorized into two phases: (i) the acute injury phase (generally the first 48 hr after injury) and (ii) the hypermetabolic phase. In the acute phase, protein-rich fluid is lost from the vascu-

lar system as a result of increased capillary permeability, and in large surface area burns, due to the release and systemic distribution of cytokines, altered capillary permeability can occur throughout the body [23]. These events lead to hypovolemia that causes a drop in cardiac output and tissue hypoperfusion, including reduced renal blood flow and a fall in glomerular filtration rate. The rapidity of fluid shifts between the various compartments can lead to a rapidly changing pharmacokinetic profile in the burn wound. Furthermore, until adequate skin cover is established, there is the potential for evaporative water losses from the skin, which might also contribute to altered tissue drug concentrations. After 48 hr, provided that resuscitation therapy has resulted in sufficient fluid replacement, a characteristic hypermetabolic change occurs resulting in increased cardiac output and increased blood flow to internal organs. The changes involved in this phase tend to evolve over several days and at variable rates. However, creatinine clearance may become elevated above normal as tubular function can become depressed during this phase despite increased renal perfusion. During these stages the effects on antibiotic tissue distribution and elimination can be significantly affected.

Local

Few studies have effectively addressed the physiological changes occurring at the wound site in burn patients. Waxman et al. examined the total loss of protein across burn wounds using a technique whereby samples of dressing applied to burn sites in patients for 1 hr were excised and wound fluid contained within them analyzed for protein content [24]. This work suggested that protein loss varied with wound type and was greater in the first 3 days following injury. However, the types of protein in this fluid were never analyzed.

An FDA guidance to industry in 1997 stated that clinical studies evaluating antimicrobials "should include the relating of the concentrations at the site of action to the in vitro susceptibility of the target microorganism". Therefore, the free drug concentrations in target tissues are the most appropriate pharmacokinetic index for the rational dosing of antibiotics, yet probably the area about which we know least in burn patients.

Considerable changes in plasma protein levels are associated with burn injury due to extravasation into burn wound sites (Fig. 1) which can potentially influence the need for changing loading doses of highly protein bound drugs. These effects have generally been thought to be relatively minor when designing maintenance dosing regimens for most antibiotics; however, data validating this assumption are lacking as changes in osmotic pressure may cause fluid shifts influencing even poorly bound antibiotic tissue distribution pharmacokinetics. A summary of some of the pathophysiological changes expected to occur in burn injury is shown in Table 2.

The Impact of Physiological Changes on Tissue Antibiotic Concentrations

To date, most of our knowledge of changes in drug pharmacokinetics in burns is based on plasma concentrations and renal excretion, as these parameters are more easily quantified. The greatest challenge lies in understanding what these changes

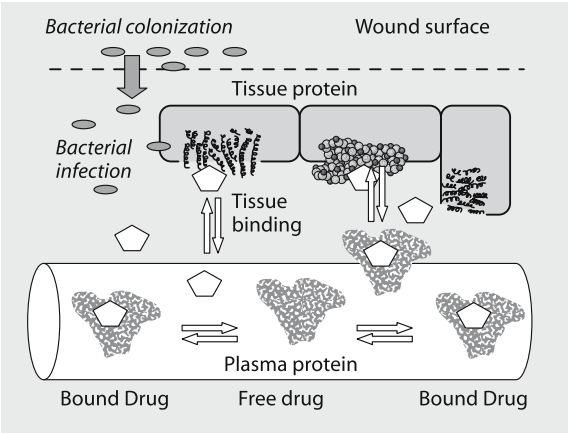

Fig. 1. Diagrammatic representation of plasma protein and tissue binding of antibiotics encountered during diffusion to superficial wound infection sites from the systemic circulation

Table 2. Pathophysiological changes in burn injury and their impact on antibiotic concentrations in the tissues

	Early phase	Late phase	Effect on drug disposition
Tissue circulation	Acute inflammatory response, oxidant induced cell damage, hyperemia and changes in tissue perfusion	Inflammatory phase can continue if local infection present, tissue perfusion and clearance may be variable	Drug clearance rates may be increased or loss of circulatory function may limit distribution and clearance
Effect of alterations in plasma albumin	Decreased plasma albumin (stress response)	Generally returning to normal after first 3 days	Plasma free levels of albumin-bound drugs may be increased, thus resulting in observed increased volume of distribution and clearance
Effect of alterations in AAG	Increased α_1-acid glycoprotein (AAG)	–	Plasma free levels of AAG-bound drugs (usually basic) may be decreased, thus resulting in observed decreased volume of distribution and clearance
Effect of edema	Peak 4–6 hours, tissue volume of distribution changes can be significant. Administration of resuscitation fluids can further increase tissue fluid levels	Lasts 2–3 days, tissue volume of distribution variable between patients. Fluid generally resorbed over 5–7 days	Increased tissue fluid volume causing changes in overall volume of distribution and possible increased tissue distribution into wound sites
pH	Metabolic acidosis likely at injury site	–	Acidosis may potentially change the ionized fraction of drugs and subsequent binding and membrane permeability. The magnitude of change will depend on the pKa of the drug

mean for actual tissue concentrations of antibiotic agents. The impact of the burn physiological changes on antibiotic pharmacokinetics are discussed in detail below:

Decreases in Circulating Albumin

The extravasation of plasma albumin into wound sites that causes the observed decrease in circulating levels can potentially alter the free fraction, distribution and clearance of drugs in the tissue space. As tissue albumin levels increase, the free fraction of albumin-binding drugs may be decreased, reducing availability, possibly tissue volume of distribution (V_d) and clearance. However, increased shifts of albumin from the plasma compartment may not cause significant changes in tissue compartment albumin concentrations (μg/mL), only the total amount of albumin in the tissue compartment, if they are accompanied by significant shifts in fluid volume. In this case, increased V_d may not be accompanied by any significant changes in the free fraction of drug.

Changes in Tissue pH

Local tissue acidosis has the potential to change the ionized fraction of drugs with a pKa in the physiological range. As pH decreases, basic ionizable groups will become more ionized and acidic groups less ionized. Changes in drug ionization have the potential to affect the degree and strength of protein binding interactions as well as the ability to partition across biological membranes, including both host tissue and bacterial cell walls.

Changes in Tissue Perfusion

Increases in tissue blood flow have two potential effects; first high blood flow may increase the clearance of drugs from the tissue into the circulating plasma, resulting in a faster elimination half-life from the tissue. Second, increased flow into areas of damaged vascular beds may rapidly increase the volume of material, both fluid and plasma protein, available for extravasation into the tissue compartment, resulting in a more rapid increase in tissue volume of distribution.

Protein and Fluid Extravasation

The loss of protein and fluid from the surface of injured tissue by evaporation, deposition or absorption into dressings may serve to establish a protein concentration gradient across the wound site from the capillary beds to the wound surface. At the present time, we know very little about the changes in injured tissue environments in relation to many highly significant determinants of drug tissue pharmacokinetics.

Preliminary data produced by Cross et al. [25] to support the concept of differential distribution of antibiotics into wound tissue of normal and diabetic rats, showed that significant retardation of the wound penetration of some antibiotics was observed in diabetic animals with altered wound perfusion and other physiological parameters (Fig. 2).

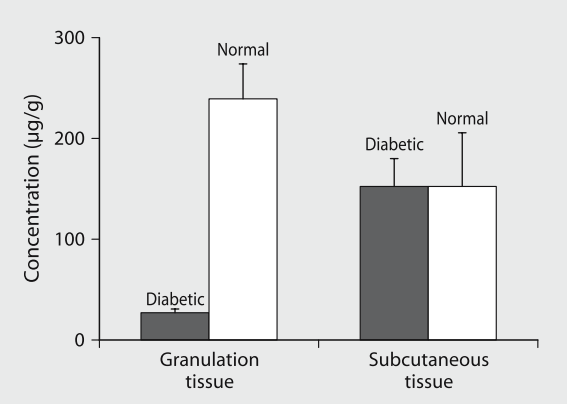

Fig. 2. Distribution of systemically administered ampicillin into the granulation tissue and underlying subcutaneous tissue of full-thickness wound sites in diabetic and normal rats (adapted from [25])

Potential Differences in Antibiotic Distribution Pharmacokinetics between Burn and Normal Tissue

Our own preliminary studies using microdialysis in burn patients to examine the differences in antibiotic pharmacokinetics in injured and normal tissue sites in the same patient (unpublished data) have shown trends for faster clearance from injured tissue sites (Fig. 3). Despite the need to establish how differences in microdialysis kinetics between the sites may have affected our overall antibiotic recovery at each site, the faster clearance of antibiotic from the burn tissue is quite marked and will be of significant importance for antibiotics where time above minimum inhibitory concentration (MIC) is the critical factor for their antibacterial effect.

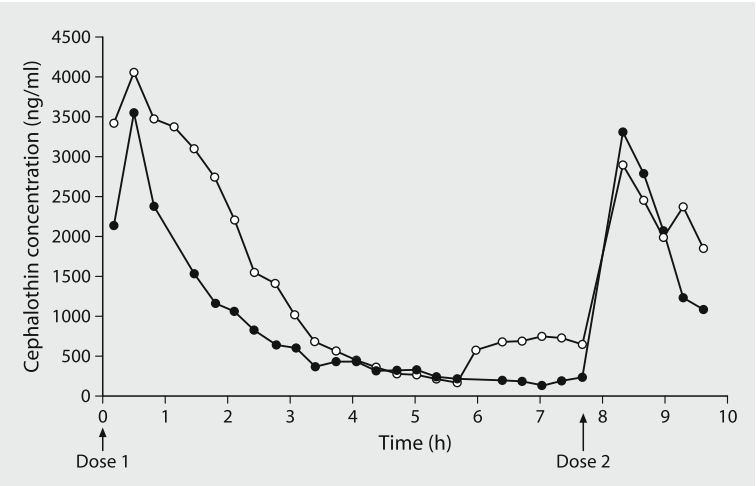

Fig. 3. Preliminary data from our own group showing concentrations of cephalothin recovered in microdialysis probe fluid in burn tissue (●) and normal tissue (○) of the same patient following two intravenous dosis of antibiotic at an eight hour interval (unpublished data)

Review of Published Data on Antibiotic Pharmacokinetics in Burns

Glycopeptides

Vancomycin. The traditional method of vancomycin administration, by short infusion two to four times per day, with measured peak and trough concentrations, has been challenged in both non-burn and burn patients. Bacterial killing by vancomycin is a function of time and the maintenance of antibiotic concentration at or above approximately four times the MIC. Theoretically administration of vancomycin by continuous infusion might better match the drug's characteristics, with cidal activity being maintained throughout the dosing interval. Studies in burns patients suggest that both creatinine and vancomycin clearance are significantly elevated in burns patients [26]. The study also examined vancomycin clearance in patients with burns and intravenous drug abuse and showed a higher clearance of the antibiotic in the former group. Similar findings were reported by Garrelts and Peterie who found that more frequent and higher dosing was required in burns patients [27]. The findings of Brater et al. [28] are also concordant with those of Rybak et al. [26] and Garrelts and Peterie [27]. Based on these observations, it has been suggested that burns patients often require higher and more frequent dosing of vancomycin. The unresolved issues are still what the optimum therapeutic plasma concentration of vancomycin is in burns patients and the lack of clear evidence demonstrating the relationship between serum vancomycin concentrations and resolution of infection.

Teicoplanin. Published data on teicoplanin clearance in burns are minimal [29, 30]. The cardinal finding of the studies was that there is a wide interpatient variation in burns patients and thus a need to monitor plasma concentrations especially in patients with high creatinine clearance [29, 30]. The studies also demonstrated that some patients with a low creatinine clearance may still be at risk of sub-therapeutic concentrations of teicoplanin as the correlation between excretion and creatinine clearance is not absolute. The additional noteworthy finding in the study by Steer et al. [29] was that the median concentration of teicoplanin in fluid from the burn wound was 60% of the serum antibiotic concentration.

Aminoglycosides. For gentamicin, amikacin and tobramycin shorter values for half-life were reported in burn patients [31–33]. A larger volume of distribution was also observed but there was no correlation between volume of distribution and burn surface area. Since the terminal elimination half-life decreased and volume of distribution increased, it suggests that the total clearance of aminoglycosides must be increased after burn injury. Hoey et al. have examined the validity of once daily aminoglycoside dosing in burns in 51 patients and demonstrated a marked variability in maximum concentration (Cmax) and duration of the aminoglycoside free period [34]. Based on their findings, they have cautioned against the use of once daily dosing of aminoglycosides in burns patients.

β-Lactams

Ticarcillin/Clavulanate. Only one published study has evaluated the pharmacokinetics of ticarcillin/clavulanate in burns patients. The pharmacokinetics of both components (5.2 g in total, administered 2–3 times/day) in 15 burn patients, seven with

burns ranging from 22–58% body surface area and eight with burns of 3–5%, showed wide intra- and inter-individual variations with an increase in volume of distribution of both drugs [35]. Despite increased terminal half-lives of ticarcillin and clavulanate, there was no evidence of accumulation, and in view of the serious nature of infection in burn patients, this study recommended the highest dosage of the drug.

Ceftazidime. In burns patients, the pharmacokinetics of ceftazidime has been reported to be significantly different to other patient groups and much inter-patient variation has been observed [36]. The apparent volume of distribution was substantially increased, as was the elimination half-life, changes having considerable implications for effective dosing. This study also found that tissue and burn blister fluid concentrations were above MIC. This is in contrast to teicoplanin, where subtherapeutic concentrations were reported in the tissue.

Carbapenems

With the emergence of *Acinetobacter* species the use of carbapenems in burns units has increased recently.

Imipenem. In a study of 11 burns patients (surface area 13–82%), pharmacokinetic parameters were comparable to those previously reported in normal volunteers [37]. However, as with other antibiotics, the authors noted a substantial interpatient variability and an adjustment in dosage or dose interval was suggested in those burn patients with abnormally high or low creatinine clearance.

Meropenem. There are limited studies on meropenem in burns. In experimentally burned rats, meropenem was demonstrated to achieve higher concentrations in burned skin as compared to normal skin [38]. Human data are limited.

Quinolones

Ciprofloxacin. A study on ciprofloxacin in burn patients by Garrelts et al. revealed that the renal clearance was increased compared with that in other acutely ill patients [39]. However, ciprofloxacin clearance was highly variable. At intravenous doses of 400 mg 8-hourly an area under the concentration-time curve/MIC >125 SIT (standard inhibitory titer) – an index shown to be associated with good clinical response – was achieved in the majority of patients.

Limitations of Using Plasma End-points for Titration of Antibiotic Therapy

A summary of the conventional measures of most importance in assessing antibiotic pharmacokinetic-pharmacodynamic relationships in patients with respect to known MIC data is shown in Table 3. Historically, the serum MIC has been used by the clinician to select antibiotic dosing for a particular infection. It is an *in vitro* value and several pathophysiological aspects may limit the ability of the plasma MIC to accurately reflect tissue concentrations in the burnt critically ill patient.

Table 3. Antibiotic groups and pharmacokinetic (PK)-pharmacodynamic (PD) parameters important in efficacy assessment

Antibiotic	Vital PK-PD Parameter
Fluoroquinolones/aminoglycosides	Cmax/MIC and AUC/MIC
β-Lactams	T > MIC
Glycopeptides	T > MIC

MIC: minimum inhibitory concentraton; Cmax: maximum concentration; AUC: area under the concentration-time curve

These include increases in body water, hypoproteinemia thus resulting in altered Donnan effect, which will impact on the tissue penetration of antibiotic, presence of cardiac, hepatic and renal dysfunction, altered tissue perfusion due to cardiovascular failure and sepsis related alterations in microvascular shunting of blood [40, 41]. Furthermore, the acidosis of burns and sepsis could potentially affect drug distribution.

Although traditionally, dose and drug selection in antimicrobial therapy is based on a single static *in vitro* parameter – the MIC, in practice an *in vivo* antimicrobial effect, will be the result of a dynamic exposure of the infective agent to the unbound antibiotic drug fraction at the relevant effect site.

As most infections occur in tissue sites (extracellular fluid), rather than in the plasma, the ability of antibiotics to reach the target site will be the most important determinant of clinical outcome especially in patients with burn injury.

Towards a Solution

Management of the septic burns patient remains a challenge. Drug pharmacokinetics are significantly altered in the burned patient but the interplay of a large number of variables is involved in deciding how an individual will deal with a drug. Consequently the burn patient population shows significant inter- and intrapatient variation. Current antibiotic dosing practice relies on the use of the MIC. As noted, the use of plasma concentrations frequently overestimates the target site concentrations and, therefore, clinical efficacy. Microdialysis, as shown earlier, is a new technique that allows direct measurement of unbound tissue concentrations. Preliminary data from microdialysis studies indicate that drug concentrations differ in healthy and burnt tissues. Furthermore, a better pharmacodynamic approach, bacterial time-kill curves, can offer more detailed information about the antibacterial activity as a function of time and antibiotic concentration than MICs. Kill curve approaches and subsequent pharmacokinetic-pharmacodynamic analysis may provide more meaningful information about the interaction between bacteria and antiinfectives because these approaches describe the dynamic integration of concentration and time and, hence, complete use of available information. It remains to be seen whether antibiotic dosing based on the above approaches will result in improved resolution of burn sepsis.

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Metabolic Support

Defining Relative Adrenal Insufficiency in the Critically Ill: The ACTH Test Revisited

M. F. C. de Jong, A. Beishuizen, and A. B. J. Groeneveld

Introduction

By elevating the blood cortisol level, the body attempts to adapt to stress associated with severe disease, trauma, surgery, and particularly sepsis [1–10]. Although primary insufficiency of the hypothalamus-pituitary-adrenal (HPA) axis is rare in the critically ill, relative adrenal insufficiency has been considered as a frequent complication of septic shock and other critical conditions [6, 9, 11–13]. In these conditions, the cortisol level, despite being normal or even elevated above normal, is considered inadequate for the degree of stress, as manifested by a subnormal rise of the cortisol level in response to additional stimuli [3, 5, 6, 9, 10–31]. The latter could indicate a transiently diminished adrenal reserve following adrenal exhaustion, in the absence of structural defects [6, 19, 20]. In contrast, primary adrenal insufficiency is caused by destruction or failure of the adrenal cortex. Conversely, various terms have been used in the critically ill, including adrenocortical deficiency, hypocortisolemia, functional or occult hypoadrenalism, adequate and inadequate cortisol production, and non- or hyporesponsiveness (to adrenocorticotropic hormone [ACTH]). We will lump these together as relative adrenal insufficiency (and non-responsiveness to ACTH), as opposed to primary or secondary adrenal insufficiency with failure of adrenal or hypothalamus/pituitary, respectively [4, 6–9].

Although vasopressor insensitivity or resistance has been considered as a cardinal clinical sign of relative adrenal insufficiency and arterial blood pressure of some patients rapidly increases after hydrocortisone boluses, the modulation of vascular reactivity by corticosteroids does not necessarily indicate relative adrenal insufficiency. In other words, there are no clinical indicators, including eosinophilia, with proven sensitivity and specificity for relative adrenal insufficiency [27], partly because of the lack of a reference standard. The most commonly applied diagnostic tool for primary adrenal insufficiency is the short corticotropin (ACTH) stimulation test [4, 7–9], first introduced in the 1960s to evaluate adrenocortical function by intravenously injecting 250 μg of synthetic ACTH and measuring serum cortisol levels before and 30 and 60 minutes afterwards [32]. The consensus is that, in non-critically ill patients, a baseline cortisol level exceeding 500 nmol/l or a post-ACTH peak (t = 30 or 60 min) level of at least 415–550 nmol/l indicates normal adrenal function (500 nmol/l = 18 μg/dl) [4, 7–10]. The test has been widely used in the intensive care unit (ICU) as well, particularly in patients with vasopressor-insensitive (septic) shock, in whom adrenal insufficiency may be suspected [5]. There is an ongoing debate, however, concerning the appropriate cortisol levels in response to the ACTH test, which can be considered as indicative of relative adrenal insufficiency in critically ill patients. In the absence of uniformly accepted indications for ACTH testing and criteria to define abnormal

Table 1. Relative adrenal insufficiency (RAI) in the critically ill

First author [reference]	Cut-off (nmol/l)	Prevalence RAI (%)	Prediction steroid treatment response H, M (y/n)	Mortality without RAI/ responders (%)	Mortality RAI/non-responders (%)	Association disease severity score (y/n)
Random/baseline cortisol						
Schein [34]	≤550	5	–, –	71	100	–
Moran [17]	<500	32	–, –	–	–	–
Braams [11]	<550	2	–, –	19	0	n
Rydvall [12]	< 400 or < 500	36–47	–, –	–	–	n
Marik [6, 28]	<690	61	y, –	–	–	–
Offner [35]	<500	60	–, –	11	8	n
Hamrahian [29]	<415	38	n, –	–	–	–
Widmer [10]	<415 or < 690	4–84	–, –	4	0	–
Peak levels on ACTH test						
Jurney [15]	≤500	3	n, y	38	0	y
Patel [36]	≤600	–	–, –	–	–	–
Bouachour [18]	≤500	6	–, –	–	–	n
Soni [19]	<500	24	y, n	44	80	n
Manglik [27]	<550	9	n, n	–	33	n
Hamrahian [29]	<510	21	–, –	25	43	n
Widmer [10]	<690	0–4	–, –	4	0	–
Cortisol increases on ACTH test						
Sibbald [14]	<125	19	–, –	59	80	n
Rothwell [16]	<250	41	–, –	32	100*	n
Moran [17]	<200	67	–, –	–	–	–
Bouachour [18]	<250	75	–, –	–	–	n
Bollaert [37]	<165	29	y, n	45	50	–
Rydvall [12]	<200	56	–, –	–	–	y
Annane [26]	≤250	77	y, y	57	58	n
Hoen [13]	<250	47	–, –	6	25	n
Bollaert [25]	<200–250	34–38	–, –	39	68	–
Widmer [10]	<250	2–39	–, –	–	–	–
Baseline (b) and increase (i)/peak (p) on ACTH test						
Span [3]	p<500 and/or i<200	17	–, –	23	0	y
Barquist [21]	b< 415, 415< b<550 +p< 690	0.66–37	–, –	43	57	–
Annane [22]	b≤940+ i≤250	70	–, –	30	72*	y

Table 1 (continued)

First author [reference]	Cut-off (nmol/l)	Prevalence RAI (%)	Prediction steroid treatment response H, M (y/n)	Mortality without RAI/ responders (%)	Mortality RAI/non-responders (%)	Association disease severity score (y/n)
Oppert [23]	≤1000, b≤500 + i≤200	55	y, n	44	45	n
Rydvall [12]	b<400 and/or i<200	69	–, –	–	–	n
Rivers [24]	b<830, b<550 + i≤250	33	y, n	46	29	–
Bollaert [25]	b>550, i<250	–	–, n	61	82	n
Parikshak [30]	b<415, 415<b<940 + i<250	30–67	y, –	14	17	–

500 nmol/l = 18 μg/dl; b = baseline; p = peak; i = increase; H = hemodynamic; M = mortality; * $p < 0.05$ for mortality among RAI groups.

ACTH/cortisol patterns and so called non-responders, the prevalence and significance of abnormal ACTH/cortisol patterns, predicting a hemodynamic response to corticosteroid administration and a contribution to mortality, independent of disease severity, varies widely among studies [2, 3, 5, 6, 10–19, 21–31]. Cortisol levels are influenced by binding proteins, so that only the free form is biologically active. Cortisol-binding globulin and albumin, the major binding proteins for circulating cortisol are often decreased in critical illness thereby lowering total but not free cortisol values [29, 33]. Of note, the increase in total circulating cortisol upon ACTH may be greater, for the same increase in free cortisol concentration, when binding is high than when binding is low [29]. It is unclear, however, how this affects the interpretation and therapeutic implications of the ACTH test, based on total cortisol determinations, in the critically ill [29, 33]. Moreover, measuring free cortisol is laborious and expensive and there is considerable interassay variation of cortisol determinations. Finally, a 1 μg ACTH test may be a better test for adrenal secretion than the supraphysiologic 250 μg ACTH test. Comparisons in the critically ill are scarce [5, 10, 21, 28] and it is unclear, therefore, if the former carries higher sensitivity and specificity for relative adrenal insufficiency than the latter.

In this chapter, we will give an overview of the criteria of abnormal 250 μg ACTH-induced cortisol patterns described in the literature and used to define relative adrenal insufficiency/non-responsiveness in the critically ill (Table 1). We will also evaluate the value of these patterns in predicting hemodynamic responses to treatment with corticosteroids and mortality rates, in order to better define relative adrenal insufficiency. The results of this exercise suggests that, currently, relative adrenal insufficiency is not a clear and definable disease entity or syndrome.

Relative Adrenal Insufficiency in the Critically Ill: Cut-off Values and Prevalence

Circulating cortisol levels could be too low for the severity of disease and might respond to ACTH less than normally. We will summarize the various cut-off levels considered to indicate these abnormalities and, thereby, relative adrenal insufficiency, and its prevalence, in a variety of conditions [5] (Table 1). This will show that relative adrenal insufficiency has not been defined unequivocally.

Random/baseline Cortisol Levels

Several authors have used a random cortisol level (without or prior to ACTH testing) to diagnose relative adrenal insufficiency, although the random cortisol level is determined by the activity of the entire HPA-axis. Marik et al. stated that a random level <500 nmol/l used as a criterion of primary adrenal insufficiency in non-critical illness is inappropriate to define relative adrenal insufficiency in stressed critically ill patients, and used a cut-off of 690 nmol/l [6, 28]. The prevalence of relative adrenal insufficiency (defined by a cortisol <690 nmol/l) in their septic shock patients was 61%, whereas Sam et al. found (at a cut-off of 550 nmol/l) a prevalence of 30% [31]. In the septic shock patients studied by Moran et al. [17], a random cortisol level <500 nmol/l also occurred in about 30% of patients. When using 550 nmol/l as a threshold, the incidences of relative adrenal insufficiency were 5% and 2% in patients with septic shock and ruptured abdominal aneurysms, respectively [11, 34]. However, a random cortisol <500 nmol/l was much more common in the studies by Offner et al. [35] and Rydvall et al. [12], occurring in 60% and 47% of surgical and critically ill patients, respectively, while 36% had values below 400 nmol/l [12].

Peak Cortisol Values on ACTH Testing

The peak cortisol values (after ACTH testing) used to define relative adrenal insufficiency range from 500 [3, 15, 18, 19], 510 [29], 550 [11, 27] to 600 nmol/l [36] and prevalences from 2 to 77% in these studies. The wide variety may be only partly attributable to differences in case-mix. Patel et al. [36] assessed cortisol values in acute hospital admissions. All patients had peak levels >600 nmol/l and baseline cortisol levels >250 nmol/l, and peak and baseline values correlated highly.

Cortisol Increases on ACTH Testing

The increment from baseline to peak cortisol level after ACTH is often used to denote relative adrenal insufficiency. The cut-off increases used to separate low from normal responses range from 125 [14], 165 [37], 200 [3, 12, 17, 25] to 250 [10, 13, 16, 18, 26, 25] nmol/l, and prevalences of relative adrenal insufficiency (with increments less than the cut-off values) varied between 17 and 77%, depending, perhaps only in part, on the patient population studied. While peak values on ACTH testing may relate to baseline cortisol values, increases may not [2, 6, 10–12, 16–18, 20, 36, 38]. Almost 13% of healthy, unstressed controls do not reach an increase in cortisol >250 nmol/l, and the significance of using only increases to define relative adrenal

insufficiency can thus be criticized [6, 10]. Indeed, a maximally stressed patient may adequately produce most of the ACTH that the pituitary can secrete and most of the cortisol that the adrenals can synthesize, so that adrenal reserve upon (supraphysiologic doses of) ACTH may be exhausted, resulting in relatively low cortisol increases [6, 23]. One could thus seriously question relative adrenal insufficiency in these cases [23]. The 2 out of 159 chronic critically ill patients in the study of Span et al. [3], who had relative adrenal insufficiency on the basis of peak levels <500 nmol/l and/or increases <200 nmol/l, had been treated with corticosteroids prior to the ACTH test.

Baseline and Increases on ACTH Testing

Although low cortisol baseline values may denote more severe or different relative adrenal insufficiency than high baseline values with low increments, some authors take the patterns together to define relative adrenal insufficiency [12, 21–24, 30]. In acute medical illness (n=40), relative adrenal insufficiency was not found by Drucker and Shandling [1], observing minimum baseline cortisol values of 212 nmol/l and peaks of 550 nmol/l. In a study by Barquist and Kirton [21], the overall prevalence of relative adrenal insufficiency, defined by a baseline cortisol level <415 nmol/l (together with a baseline between 414–550 and a peak <690 nmol/l) in surgical (trauma) patients was 0.66–37%. In the surgical patients studied by Rivers et al. [24], the prevalence of relative adrenal insufficiency (baseline/peak cortisol <830 or increase ≤ 250 nmol/l, or baseline/peak cortisol <550 nmol/l and an increase ≤ 250 nmol/l) was 33%. Parikshak et al. [30] defined relative adrenal insufficiency as a baseline cortisol level <415 or between 415–690 nmol/l with an increase upon ACTH of <250 nmol/l, and the prevalence was up to 67% in their vascular surgery patients. Sixty-nine percent of critically ill patients tested by Rydvall et al. [12] had a baseline <400 nmol/l and/or an increase <200 nmol/l. Annane et al. defined abnormal ACTH/cortisol patterns on the basis of their relatively independent predictive value for 28-day mortality in 189 septic shock patients [22]. ACTH-responders had a baseline cortisol level <940 with an increase >250 nmol/l, and non-responders with higher baseline values and/or smaller increases amounted to 70% of patients. Oppert et al. [23] used either a high baseline cortisol value >1000 nmol/l as a cut-off level or a baseline value >500 nmol/l with an increase of >200 nmol/l to define normal responses in septic shock patients: 55% did not fulfill these criteria and could be considered as having relative adrenal insufficiency. According to these authors, a high baseline cortisol above 1000 nmol/l could also reflect a decreased hepatic cortisol clearance [18], rather than adrenal (hyper)function adapted to severity of disease.

Relative Adrenal Insufficiency and the Hemodynamic Response to Corticosteroids

Another argument favoring the concept of relative adrenal insufficiency is its potential capacity to predict the response to corticosteroids in vasopressor-resistant shock, allowing rapid tapering or discontinuation of dopamine or norepinephrine while maintaining mean arterial pressure. Nevertheless, insensitivity of the vascular wall to vasopressors may relate to corticosteroid-sensitive mechanisms in vascular

smooth muscle, which may have nothing to do with relative adrenal insufficiency. Conversely, the literature is heterogeneous as to the predictive value of ACTH/cortisol patterns for steroid-responsiveness.

Random/baseline Cortisol Levels

All 59 septic shock patients studied by Marik et al. [28] received stress doses of hydrocortisone. Corticosteroid responsiveness was defined as the cessation of the need for norepinephrine to maintain a mean arterial pressure of >65 mmHg within 24 hrs of the first dose of hydrocortisone. While 95% of the 22 corticosteroid-responsive patients had a baseline (prior to ACTH) cortisol level <690 nmol/l, only 22% had a peak <500 nmol/l upon 250 μg of ACTH, so that the former was a better predictor of corticosteroid-responsiveness than the latter. The optimal baseline concentration as cut-off value to predict a positive response to corticosteroids was estimated as 658 nmol/l.

Peak Cortisol Levels on ACTH Testing

The three of five patients with relative adrenal insufficiency (peak cortisol <500 nmol/l) in the study by Soni et al. [19] on septic shock receiving corticosteroids were weaned off pressors within several hours after starting the drugs.

Cortisol Increases on ACTH Testing

In the Annane et al. study [26], time to vasopressor discontinuation was, on average, 10 days in the placebo- and 7 days in the corticosteroid-treated group of non-responders to ACTH with cortisol increases <250 nmol/l. Among responders, time to shock reversal was 9 days in the corticosteroid- and 7 in the placebo-treated group. Hence, corticosteroid treatment appeared particularly effective in non-responders. In contrast, there was no clear ACTH/cortisol pattern (increase > or <165 nmol/l) that predicted favorable hemodynamic responses to stress doses of hydrocortisone in the septic shock patients studied by Bollaert et al., after excluding those with a peak cortisol <500 nmol/l [37].

Baseline and Increases on ACTH Testing

Oppert et al. [23] studied catecholamine dependency among 20 septic shock patients treated with hydrocortisone. The 11 ACTH non-responders (having a baseline cortisol <1000 nmol/l or <500 nmol/l and an increase of <200 nmol/l) were free of vasopressor support earlier than responders. Therefore, the non-responding ACTH/cortisol pattern suggestive of relative adrenal insufficiency seemed to predict a beneficial response to corticosteroid administration. Rivers et al. [24] also observed, in surgical patients with relative adrenal insufficiency (baseline/peak cortisol <830 nmol/l or a cortisol increase ≤250 nmol/l, or baseline/peak cortisol <550 nmol/l and an increase ≤250 nmol/l), that vasopressors could be more easily tapered after treatment with hydrocortisone than in patients with a normal response. Also, hydrocortisone-treated responders who could be successfully weaned from vasopressor therapy within 24 h had significantly lower baseline cortisol values as compared with those who continued requiring therapy, even though ACTH re-

sponses were similar. Vasopressor dose tapering was denoted as rapid among the non-responders to ACTH receiving hydrocortisone in the Parikshak et al. study [30], but this was not compared to that in responders.

Relative Adrenal Insufficiency and Outcome

The clinical relevance of various cut-off levels to define relative adrenal insufficiency can also be evaluated by their prognostic significance, and particularly when corticosteroid (hydrocortisone) administration (in stress doses) lowers mortality in so defined non-responders to ACTH.

Random/baseline Cortisol Levels

Mortality increased with an increase in baseline cortisol in septic (shock) patients [14, 17, 31]. In the septic shock patients studied by Schein et al. [34] and Rothwell et al. [16], however, baseline cortisol levels did not have any prognostic significance. Bouachour et al. [18] noted that only increases in the levels over the first 72 hours were associated with non-survival. Cortisol levels among non-survivors did not significantly differ from those among survivors in the study by Hamrahian et al. [29] in critically ill patients or in the study by Offner et al. [35] in trauma patients, but only two (one responder and one non-responder with a random cortisol <500 nmol/l) of the 22 patients in the latter study died. In contrast, a study on patients with a ruptured aneurysm of the abdominal aorta showed lower baseline cortisol levels in survivors than in non-survivors [11]. In other studies on ICU patients [15, 38, 39], non-survivors also had higher baseline cortisol levels than survivors.

Peak Cortisol Levels on ACTH Testing

Mortality rates among critically ill patients non-responding to ACTH did not seem to increase when using a relatively low peak level (<500 nmol/l) as cut-off [3, 15]. Non-surviving ICU patients had similar peak cortisol levels to survivors; the two non-responding patients (with peak ≤ 500 nmol/l) who survived were treated with hydrocortisone [15]. The two patients with relative adrenal insufficiency according to criteria by Span et al. [3] (peak ≤ 500 nmol/l and/or increases <200 nmol/l) survived without need for corticosteroids and their low cortisol values were thought to reflect a milder disease severity compared to those with high levels. Peak cortisol values were higher in non-survivors than survivors, in critically ill and vascular surgery patients, independent of treatment with corticosteroids [11, 38]. The peak values in non-surviving (non-steroid treated) vascular surgery patients were even reported to exceed 550 nmol/l [11]. When using a cut-off of 510 nmol/l, Hamrahian et al. [29] showed no significant difference in mortality among responding and non-responding critically ill patients. The levels did not differ among septic shock outcome groups in the study by Bouachour et al. [18]. On the other hand, in the study by Soni et al. [19], the 28-day mortality rate of septic shock was almost doubled in non-responders (peak cortisol <500 nmol/l, 80%) compared to responders (44%), despite treatment with corticosteroids in three of five non-responding patients, but statistical significance was not reached. In the study by Manglik et al.

[27] four of nine septic non-responders were treated with corticosteroids and one of them died, and two of the five untreated non-responders died. Mortality in responders was not reported.

Cortisol Increases on ACTH Testing

The cortisol increase was lower among non-survivors than among survivors in studies on sepsis and shock by Sibbald et al. [14] (not significantly) and Rothwell et al. [16]. Moreover, mortality could be predicted by a low cortisol increase in other studies [17, 25], regardless of the threshold value of 200 or 250 nmol/l. Treatment with hydrocortisone did not affect this association, because mortality rates did not differ from those in non-treated patients, both in responders and in non-responders [25]. In contrast, cortisol increases did not differ between survivors and non-survivors in the study by Bouachour et al. [18] on septic shock patients. Using a low cut-off value of <165 nmol/l increases in circulating cortisol, Bollaert et al. [37] observed no significant difference in mortality rates between responders and non-responders. In this study, 41 septic patients were randomized to treatment with placebo or hydrocortisone: there were four non-responders in the treatment group and eight in the placebo group. Twenty-eight-day mortality appeared to be lower in all non-responders together, treated and non-treated, versus the responders (ns), but there were no differences between the treated or non-treated patients when only responders or only non-responders were analyzed. Annane et al. [26] investigated the effect of low doses of hydrocortisone combined with fludrocortisone on mortality in patients with septic shock. Corticosteroids reduced mortality among non-responders (cortisol increase <250 nmol/l) but failed to do so in responders. Among non-responders, 73 patients (63%) treated with placebo died while 60 (53%) of corticosteroid-treated patients died. Among responders, the 28-day mortality was 53% and 61% (not statistically significant), respectively. In the study by Jarek et al. [38] on ICU patients, the ACTH-induced increase in cortisol did not have prognostic value, independent of treatment with corticosteroids. In the Hoen et al. study [13] on trauma patients with hemorrhagic shock, mortality did not differ between responders and non-responders to ACTH (<250 nmol/l cortisol increase).

Baseline and Increases on ACTH Testing

In acute medical disease like sepsis, non-survivors had higher baseline and post-ACTH cortisol values than survivors [1, 14]. In the study by Barquist and Kirton on surgical patients [21], a higher percentage of non-responding relative adrenal insufficiency patients (baseline cortisol <414 nmol/l, or between 414 and 550 nmol/l with a post-ACTH peak <690 nmol/l) than responders died, in spite of treatment by corticosteroids, but numbers were small while only 2 deaths among the 4 of 7 non-responding patients who died were attributable to sepsis and critical illness. In the 34 non-responders of the study by Rivers et al. on surgical patients [24], mortality was lower in the hydrocortisone-treated group than in the untreated group, but the predictive value of ACTH responsiveness was not studied. Treatment with hydrocortisone in surgical ICU patients improved survival among patients with relative adrenal insufficiency [24], so that relative adrenal insufficiency could be regarded as a mediator for death, although the study was not designed with mortality as the primary outcome measurement. ACTH test results apparently did not predict the lack of effect on outcome of vascular surgery patients by treatment with corticosteroids [30].

Moran et al. [17] concluded that an increasing baseline and a decreasing cortisol increase in septic shock augmented the risk of dying. Annane et al. [22] distinguished three groups with a combination of baseline and increases in cortisol in 189 septic shock patients and indicated three levels of patient prognosis with these groups. As in other studies [15, 25], non-survivors had higher baseline cortisol levels and lower increments after ACTH administration, while treated more often with hydrocortisone than survivors [22]. In some smaller studies [23], however, mortality did not differ between responders and non-responders, all receiving hydrocortisone, while the pretreatment cortisol status between survivors and non-survivors did not differ either. Taken together, the literature on the predictive value of ACTH/cortisol patterns for (steroid-induced modification of) mortality is highly heterogeneous. Moreover, the predictive value should be independent of accepted predictors and organ failures, if supporting the concept of relative adrenal insufficiency, and we will now address the available evidence thereof.

Relative Adrenal Insufficiency as a Marker or as a Mediator of Mortality?

Even if relative adrenal insufficiency may have some prognostic significance, the question remains whether relative adrenal insufficiency is a marker or mediator of severe illness contributing to death in the ICU. Indeed, an association between relative adrenal insufficiency and mortality that largely depends on severity of underlying disease and organ failure, could argue in favor of a marker rather than a mediator role. We will now briefly review the relation between ACTH/cortisol patterns and severity of disease, as expressed by scoring systems. Again, the severity of disease-dependency of the relation between ACTH/cortisol patterns and mortality appears highly heterogeneous.

Random/baseline Cortisol

In critical illness, the HPA axis is activated, directly relating to severity of disease, so that cortisol levels relate to acute physiology and chronic health evaluation (APACHE) II scores, among others [2, 3, 15]. Higher APACHE II scores correlated to baseline (prior to ACTH) cortisol levels also in a study among 159 chronic critically ill patients [3] of whom 23% died; these patients had higher APACHE scores and higher baseline values, suggesting adaptation of adrenal function to severe stress. This was also suggested by Jarek et al. [38] and Jurney et al. [15] in critically ill patients, who showed a correlation of baseline cortisol levels with outcome, somewhat independent of severity of disease [38]. However, Rothwell and Lawler [39] analyzed cortisol levels of 260 ICU patients in a multiple logistic regression model and concluded that the baseline levels were direct predictors for outcome, independent of APACHE II scores. Several authors did not find a direct correlation between baseline cortisol levels and disease severity during septic shock [18, 31, 34]. Sam et al. [31] evaluated 100 septic patients and observed increasing mortality with higher cortisol levels, independent of APACHE II scores, suggesting a contribution of high (!) cortisol levels to mortality, independent of disease severity.

Peak Cortisol on ACTH Testing

Several authors [18, 19, 27] did not find any relationship between non-responding septic patients with peak levels < 500 or 550 nmol/l or responders on the one hand and APACHE II scores or simplified acute physiology scores (SAPS) on the other. In critically ill patients, however, the post-ACTH peak may be higher when the APACHE II score is high than when it is low [2, 15].

Cortisol Increases on ACTH Testing

Increases were significantly lower in non-survivors than in survivors among 32 septic patients, while the APACHE II scores were only somewhat (not statistically significantly) higher in non-survivors [16]. An inverse relationship between cortisol increases and disease severity (APACHE II score) was shown by Briegel et al. [20], Manglik et al. [27], and Rydvall et al. [12], in septic or critically ill patients, but not by Wade et al. [2]. We have found a similar pattern among 520 ACTH-tested, hemodynamically unstable patients with vasopressor-insensitivity, mostly in the course of sepsis, in our unit (Fig. 1), showing lower increments in patients with higher sequential organ failure assessment (SOFA) scores indicating greater disease severity, but there was no additive predictive value for mortality based on the SOFA scores, in multivariate analysis (unpublished data). Nevertheless, the inverse relation with disease severity, independent of baseline values, suggests a continuum, so that abnormality and cut-off values are hard to define and relative adrenal insufficiency is only poorly quantifiable. Nevertheless, the explanation for this inverse relation is lacking, unless increasing severity of disease results in a progressive decline in cortisol binding proteins and thus a blunted increase in total cortisol levels, for a given adrenal secretion and free cortisol level [29]. In contrast, in the study by Annane et al. [26], the ACTH non-responders had similar disease severity to the responders (increases > 250 nmol/l) and yet had higher survival rates when treated with hydrocortisone. Hoen et al. [13] also did not observe differences in the SAPS II scores or in mortality rates between responders and non-responders

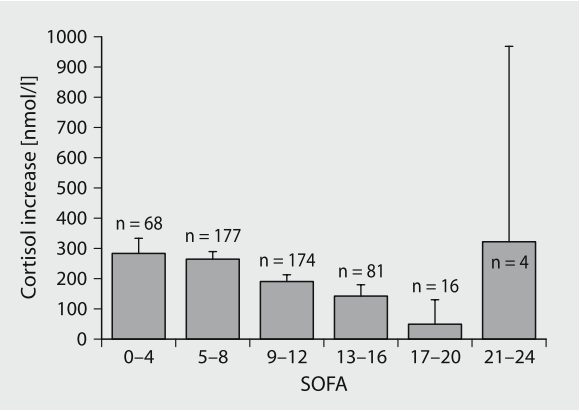

Fig. 1. Association between cortisol increase and stratified sequential organ failure assessment (SOFA) score on day of ACTH testing among 520 critically ill patients in our unit (unpublished data). ($p < 0.0001$, Kruskal-Wallis test)

(<250 nmol/l cortisol increase) among 34 trauma patients, although shock and organ failure were more severe in non-responders than in responders.

Baseline and Increases on ACTH Testing

In a study of 30 ICU patients [2], two groups were created according to the patients' APACHE II scores. One group had an APACHE II score ≤10 and one an APACHE II score ≤25. Patients with the higher scores had a higher mortality rate and higher cortisol levels, but increases on ACTH testing were similar. Hence, the increased cortisol levels related to severity of disease and no impairment of adrenal cortisol secretion was suggested. In the study by Oppert et al. [23] all septic shock patients were treated with hydrocortisone but those with relative adrenal insufficiency, as defined by a baseline cortisol <500 nmol/l and an increase <200 nmol/l, did not differ in APACHE II scores or mortality from those without relative adrenal insufficiency. Annane et al. [22] developed a multivariate regression model and a high baseline/low increment (see above) was predictive for mortality, together with (and thus independently of) an ultimately or rapidly fatal underlying disease and more than two organ system failures. The relative independency of the severity of disease could either imply insufficient characterization of the severity of disease or, indeed, maladaptation of (even baseline) cortisol secretion for the level of stress and thus relative adrenal insufficiency. Similarly, a baseline cortisol >550 nmol/l and an increase <250 nmol/l were independent predictors of mortality in multivariate analysis in a retrospective study on 82 septic patients, whereas organ failure was not [25].

Conclusion

We suggest, on the basis of the presented material, that relative adrenal insufficiency is a hardly definable disease entity or syndrome, even though the concept is appealing. Abnormal ACTH/cortisol patterns could reflect severity of underlying disease and remote organ failure rather than relative adrenal insufficiency. Further research into this matter should include objective evaluation of the ACTH/(free) cortisol pattern with the highest and independent predictive value for mortality, if there is any, of the pattern with the greatest predictive value for corticosteroid responsiveness, and of the outcome after administering stress doses of corticosteroids guided by ACTH tests indicative of a poor outcome. Some of these studies are underway, and until their publication one should hesitate to diagnose relative adrenal insufficiency and use the results of ACTH testing to guide therapy by corticosteroids in vasopressor-insensitive shock. Conversely, (stress) doses of hydrocortisone can be safely used in attempts to increase vascular wall sensitivity to vasopressors and survival in (septic) shock, even without ACTH testing [37].

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Enteral Nutrition in the Critically Ill: Should We Feed into the Small Bowel?

A. R. Davies, N. Orford, and S. Morrison

Introduction

In critically ill patients, artificial nutritional support is now considered to be the standard of care, as it improves wound healing [1], reduces complication rates [2], and improves clinical outcomes [3, 4]. A multicenter, cluster-randomized clinical trial investigating the dissemination and practice of evidence-based algorithms for nutritional support in intensive care unit (ICU) patients demonstrated that the use of evidence-based algorithms (as opposed to standard clinician-driven management of nutritional support) increased the amount of nutritional support delivered (8.5 versus 6.9 days per 10 patient days; $p = 0.02$) and this led to reduced hospital length of stay (25 versus 35 days; $p = 0.003$) and a trend toward reduced mortality (27 v. 37%; $p = 0.058$) [4].

Published guidelines regarding nutritional support in the critically ill recommend the use of enteral nutrition rather than parenteral nutrition [5–7] based on evidence that enteral nutrition is associated with better clinical outcomes than parenteral nutrition [2, 7, 8]. Although the advantages of enteral nutrition over parenteral nutrition have been challenged [9–11], there is no doubt that enteral nutrition is less expensive than parenteral nutrition [9, 12].

The Problems Associated with Gastric Feeding

Enteral nutrition is typically delivered via a nasogastric tube into the stomach [13–15] and in many cases this leads to satisfactory delivery. However, gastric motility (particularly gastric emptying) and absorption are impaired in critical illness [16–18], particularly in the presence of commonly administered medications such as opiates and catecholamines [17, 19].

The manifestations of impaired gastric function include large gastric residual volumes and vomiting [13, 19, 20]. The syndrome of 'upper gastrointestinal tract (GIT) intolerance of enteral nutrition' (which includes the clinical features of large gastric residual volumes and vomiting) has been reported to occur in as many as 46% of patients who receive gastric feeding [19] and the syndrome appears to be linked to important sequelae.

Upper GIT intolerance of enteral nutrition is one of the main reasons that ICU patients receive inadequate amounts of enteral nutrition (compared to predicted requirements), as has been repeatedly demonstrated in prospective observational studies in many different countries over the last decade [13, 19–22]. Depending on the definition used, as few as 25% of patients achieve 'tolerance' of their enteral nu-

trition [21], and this can take as long as 6 days to occur [13]. An Australian study found that over a 7 day period, patients received only 51% of their predicted energy requirements overall [21]. Although upper GIT intolerance is not the only reason to explain this inadequate delivery of enteral nutrition (other reasons include temporary cessation for medical and surgical procedures, and mechanical enteral tube problems), it is the most common reason in many published series [13, 20–22].

Patients who receive gastric feeding and develop upper GIT intolerance have been shown to have increased pneumonia (43 versus 24%; $p = 0.01$) and mortality rates (41 versus 25%; $p = 0.03$) as well as longer durations of ICU stay (23 versus 15 days; $p = 0.01$) than those who tolerate gastric feeding [19]. Whilst these data are from an observational study, they certainly give rise to concerns that gastric feeding may not always be as safe as it is sometimes considered. Pneumonia, particularly ventilator-associated pneumonia (VAP) has been shown to prolong ICU stay by about 4 days and is associated with a strong trend towards increased mortality [23].

ICU clinicians therefore face a dilemma in attempting to optimize patient outcomes whilst minimizing the risk of the interventions that are used. Recent study results [3, 4] and evidence-based guidelines [7] recommend the early commencement of nutritional support (predominantly as enteral nutrition) to improve clinical outcomes. Yet current evidence also suggests that patients who receive gastric feeding have an appreciable risk of inadequate enteral nutrition delivery occurring (compared to predicted requirements) and pneumonia, both of which may lead to inferior clinical outcomes.

This dilemma is exemplified by a recent clinical trial in ICU patients where early enteral nutrition (using a standard dose via a nasogastric tube) was compared with delayed enteral nutrition (using a dose reduced to 20% of standard via a nasogastric tube over the first 4 days) [24]. Patients in the early enteral nutrition group received four times as much enteral nutrition as those with delayed enteral nutrition; however, the early enteral nutrition group had a higher rate of pneumonia and an increased hospital length of stay.

Small Bowel Feeding

Rationale

There is a reasonable biological rationale that delivery of enteral nutrition into the small bowel may be superior to delivery into the stomach. Several possible advantages of the small bowel include: (1) improved absorptive capacity [25]; (2) less impairment of motility [26]; and (3) a greater distance between the delivery site and the pharynx and respiratory tree. By delivering enteral nutrition into the small bowel (and particularly the proximal jejunum rather than the duodenum), it seems reasonable to assume that the volume of gastric residual fluid will be less, leading to less frequent interruption of enteral nutrition administration and consequently larger amounts of enteral nutrition delivered to the patient. In addition, there should be less regurgitation of stomach contents into the esophagus and pharynx, as well as less aspiration into the trachea, bronchi and lungs, thereby leading to reduced risk of the development of pneumonia.

Clinical Studies

There have now been a number of studies comparing small bowel and gastric delivery of enteral nutrition in critically ill patients [27–38] and it is well worth reviewing their results.

When compared to gastric feeding, small bowel feeding affects several outcomes that are considered important clinical practice aspects when enteral nutrition is being administered to patients in the ICU. Small bowel feeding has been shown to reduce gastric residual volumes, particularly in the first 2 days after enteral nutrition is commenced [36]. Whilst the importance of gastric residual volume remains controversial [39], a large gastric residual volume is the most common reason accounting for inadequate enteral nutrition delivery in observational studies [13, 19–22]. In addition, a large gastric residual volume has been shown to increase the risk of development of pneumonia [19]. Small bowel feeding has also been shown to reduce total gastrointestinal complications of enteral nutrition when compared to gastric feeding, although this is largely because of the reduction in the number of episodes of large gastric residual volume [37].

If enteral nutrition delivered into the small bowel leads to a lower gastric residual volume, then this should lead to less frequent interruption of enteral nutrition and an improved adequacy of enteral nutrition. However, mixed results have been found with regard to nutritional outcomes. Two studies comparing small bowel and gastric feeding found that small bowel feeding increased the proportion of predicted energy requirements delivered [27, 30]. One of the explanations for improved adequacy of enteral nutrition is that patients receiving small bowel feeding are more likely to receive their target nutrition rate sooner [28]. However, improved adequacy of enteral nutrition has not been demonstrated in all studies comparing the two sites of delivery [32, 36–38], possibly because delays in placement of a nasojejunal tube of around 12 to 24 hours have led to small bowel feeding being commenced later than gastric feeding [36–38] and this is not always 'caught up' despite the less frequent interruptions due to the reduced gastric residual volume. Nevertheless, none of the studies comparing the small bowel and gastric routes found the small bowel feeding to be inferior to gastric feeding and a meta-analysis showed a strong trend towards small bowel feeding increasing total energy intake when the five studies which reported on this outcome were aggregated (weighted mean difference of +169 calories, 95% CI −34 to +320, $p = 0.09$) [40].

The recognition of gastric dysmotility in the critically ill has also prompted the use and investigation of promotility drugs for this problem. Metoclopramide [41] and erythromycin [42] have both been shown to improve gastric emptying. Erythromycin would appear to be the superior agent as it has also been shown to improve short-term tolerance in patients with upper GIT intolerance of enteral nutrition (as evidenced by a large gastric residual volume) [43, 44] and also when administered routinely with gastric feeding [45]. Given these results, a study was designed to compare the use of a promotility drug (erythromycin) in patients receiving gastric feeding with small bowel feeding (without erythromycin). This small study (of 80 patients) did not investigate pneumonia rates, but found no differences between the 2 groups in the adequacy of enteral nutrition, mortality and duration of ICU stay [35].

Because each of the individual studies comparing small bowel and gastric feeding were relatively small, they have been underpowered to detect differences in pneumonia (or specifically VAP) rates. The largest study, which was multicenter and enrolled 101 patients, found a nosocomial pneumonia rate of 40% in the gas-

tric group and 32% in the small bowel group [37]. Whilst this difference was not statistically significant, a much larger sample size would have been required to clearly demonstrate the effect of small bowel feeding on pneumonia.

A meta-analysis, which aggregated the results of 9 randomized, controlled trials comparing the small bowel and gastric routes, found a significant reduction in the rate of pneumonia in favor of small bowel feeding (RR 0.77; 95% CI 0.60–1.00; $p = 0.05$) [7]. A subsequent meta-analysis found a non-significant difference in the rate of pneumonia, also in favor of small bowel feeding (odds ratio for gastric feeding of 1.44; 95% CI 0.84–2.46; $p = 0.19$) [40]. These meta-analyses should not be seen as discordant as they both infer that there is at least a moderate likelihood that small bowel feeding is associated with a reduction in the rate of pneumonia.

The mechanism by which small bowel feeding reduces the risk of pneumonia is most likely that gastroesophageal regurgitation is reduced, leading to less microaspiration into the lung [33]. In addition, the more distally the tube is positioned in the small bowel (i.e., jejunum rather than duodenum), the less likely that gastroesophageal regurgitation and microaspiration occur.

Most studies of small bowel delivery of enteral nutrition have placed enteral tubes in the duodenum (or 'post-pyloric') rather than in the jejunum. It is worthwhile, therefore, to focus on the three studies which specifically compared jejunal to gastric feeding. Two of these studies demonstrated significant reductions in gastric residual volume in favor of jejunal feeding [36, 37], whilst the other reported a non-significant reduction in the number of individual gastric residual volumes > 250 ml [27]. One study demonstrated that jejunal feeding led to a greater proportion of predicted energy requirements being given than gastric feeding [27]; one study also detected such a difference in a post-hoc analysis of those patients from ICUs with previous experience in jejunal feeding (yet not in the entire study group) [37], whilst the other study did not detect a difference [36].

Nasojejunal Feeding Tubes

Small bowel enteral tubes are placed in only 7% of patients in Australia and New Zealand [46]. In other countries, rates have varied between 1% [20] and 16% [47]. These low utilization rates are contrary to clinical practice guidelines as a Canadian multidisciplinary committee recently recommended that small bowel feeding should be routinely used, in units where obtaining small bowel access is feasible [7], based on a meta-analysis which showed small bowel feeding reduced the incidence of pneumonia.

The most likely reasons for these low utilization rates in clinical practice are the lack of a large randomized controlled trial and the logistical, technical and expense aspects of using nasojejunal tubes. Because they can be difficult to place, numerous techniques for nasojejunal tube insertion have been described. 'Blind' placement at the bedside is certainly the simplest method logistically, but when compared to placement of standard nasogastric tubes, blind placement is both more time-consuming and less successful, with more attempts required to achieve adequate position [38]. Several techniques using various mechanical maneuvers have, therefore, been described to improve success rates of blind nasojejunal tube placement. Whilst reported results in particular institutions have been very good [48], these may not always be able to be replicated in clinical practice.

Promotility drugs have also been used to enhance the success of tube passage into the small bowel; however, results of clinical studies have not been consistent.

Metoclopramide has been shown to increase the success rate of tube insertion in one study [49] but not in two others [50, 51], whilst erythromycin appeared to be useful in 3 studies [52–54] but not in another [51]. A recent systematic review concluded that erythromycin should be administered when blindly placing a small bowel tube [55].

Fluoroscopy allows the advantage of real-time imaging to ensure correct placement and appears to be more successful than blind techniques with reports of 90% success rates into the small bowel and more than 50% into the jejunum [56]. Whilst fluoroscopy can be brought to the bedside, many institutions still require the ICU patient to be transported to the radiology department. This has obvious logistical difficulties, but can also compromise patient safety as deterioration of respiratory function [57] and an increased incidence of VAP [58] have been reported when ICU patients are subjected to intrahospital transport.

Endoscopically-placed tubes appear to have the highest success rates and we have shown a success rate of 98% for placement into the jejunum [36]. Nevertheless, the logistical and cost considerations of arranging for an endoscopist to perform the procedure remain a strong deterrent which most likely explains the low usage of nasojejunal tubes in clinical practice.

The recent development of the frictional nasojejunal tube, ('Tiger tube', Frictional Nasal Jejunal Feeding Tube, Cook Critical Care, Bloomington, IN, USA) appears to provide the combination of simplicity and high insertion success rates. This 'self-migrating' tube features innovative soft flaps on the sides of the distal half of the tube (Fig. 1) which work like a soft form of a barb on a fishhook, thereby gently lodging in the mucosa of the stomach and small bowel so that peristalsis can gently drag the catheter into the jejunum.

Bedside clinicians can pass the tube into the stomach (which takes around 5–10 minutes) and then wait for the tube to migrate into the jejunum over the next 6–12 hours. Two recent case series [59, 60] (one of which we reported) suggest that these tubes are associated with success rates above 90% (even in patients intolerant of enteral nutrition despite promotility agents) with few adverse effects. As they obviate the need for additional equipment and personnel, they therefore offer the possibility of nasojejunal tube placement in a timely and cost effective manner. Additionally, it is common for the tube's distal tip to migrate itself a significant distance into the small bowel (Fig. 2), which may be important in reducing the risk of pneumonia.

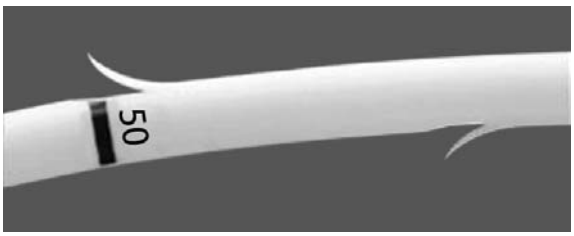

Fig. 1. The frictional nasojejunal tube ('Tiger tube', Cook Critical Care) showing the innovative flaps which allow the tube to migrate into the small bowel by peristalsis

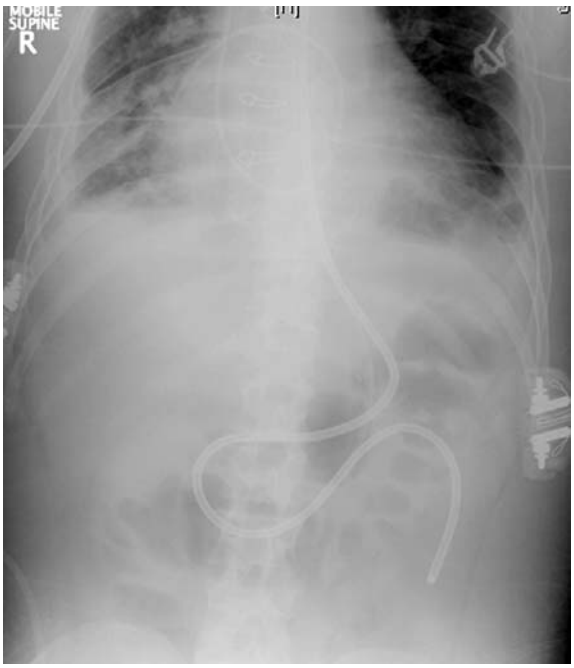

Fig. 2. A radiograph of a frictional nasojejunal tube which has migrated well into the jejunum

Conclusion

In critically ill patients the delivery of nutritional support (largely in the form of enteral nutrition) using evidence-based algorithms is associated with improved outcomes. Delivery of enteral nutrition into the stomach is relatively straightforward, however the high incidence of upper GIT intolerance due largely to poor gastric motility may result in inferior outcomes due to inadequate delivery of enteral nutrition and increased risk of VAP.

The delivery of enteral nutrition directly into the small bowel is a logical alternative as a means of reducing these complications. Although not conclusive, current evidence suggests that small bowel feeding is at least equivalent and may be superior to gastric feeding in terms of nutritional delivery and VAP rates. Unfortunately the clinical practice of small bowel feeding has been hampered by the logistic, technical, and time difficulties associated with placement of nasojejunal tubes.

The question of where best to deliver enteral nutrition in critically ill patients to provide optimal nutritional support and reduce feeding related complications remains unresolved. There may also be subgroups of the critically ill population who truly benefit from the early placement of a nasojejunal tube and further clinical research comparing gastric and small bowel delivery is awaited. Because of the logistical difficulties associated with nasojejunal tube placement, the development of newer tubes which have the potential to overcome these problems, such as the frictional nasojejunal tube, may offer this possibility.

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Tight Energy Balance Control for Preventing Complications in the ICU

P. Singer, J. Singer, and J. Cohen

Introduction

Following injury, stress, infection or surgery, most critically ill patients have evidence of an increased metabolic response and protein catabolism, a result of the release of cytokines and other inflammatory mediators [1, 2]. Early enteral feeding has been the recommended method of artificial feeding in these patients but is frequently associated with inadequate delivery of calories and nutrients [3]. In addition, feeding generally receives a lower priority when compared to hemodynamic resuscitation, modes of ventilation or control of septic shock. In this chapter, the deleterious effects of underfeeding will be emphasized, new tools for tight energy control will be proposed and specific conditions like severe obesity will be discussed.

Deleterious Effects of Underfeeding

The role of the hospital in initiating and aggravating underfeeding has recently been emphasized by a declaration and resolution of the European Council [4]. Malnutrition has been associated with increased infectious morbidity, prolonged hospital stay and increased mortality [5]. Weight variations, nutritional scores, plasma albumin or lymphocyte counts are not reliable measures of nutritional status since they are altered by critical illness. Kondrup et al. proposed that the patient's physical aspect, nutritional history, and the presence of acute illness on admission to the intensive care unit (ICU) are the best tools to assess nutritional status [6]. Mid arm circumference [7], body composition using bio electrical impedance [8] and resting energy expenditure guided by indirect calorimetry complement the nutritional assessment.

It was commonly believed that daily energy expenditure of critically ill patients exceeded resting energy expenditure by 50%, reaching 36 kcal/kg [9]; however, when this amount was provided, adverse outcomes due to overfeeding occurred, with an increased rate of infections and ventilatory requirements [10]. These harmful effects have been attributed to hyperglycemia, which induces numerous adverse metabolic effects. The achievement of euglycemia has been shown to improve the clinical outcome of patients in surgical and general intensive care departments [11]. In fact, most critically ill patients, who are moderately stressed and inactive, have an average resting energy expenditure close to their total daily energy expenditure (22–25 kcal/kg/day) [10, 12]. However, no ICU meeting goes by without at least one abstract pointing out the disparity between nutritional requirements and

the nutritional support provided, with resultant negative energy balance. Resting energy expenditure was measured by indirect calorimetry that enabled a fairly accurate estimation of the 24-hour resting energy expenditure [13]. This disparity between measured resting energy expenditure and calorie intake has been demonstrated in numerous studies [14–17].

The reasons for this disparity are numerous and include:

- Lack of accuracy of predicted resting energy expenditure (e.g., Harris Benedict equation) when compared to measured resting energy expenditure (by indirect calorimetry);
- While indirect calorimetry is ideal, it is limited by technical and practical problems [18];
- Delays in commencing and advancing nutritional support. It has been demonstrated that the nutritional goal is reached in only 22% of ICU patients receiving enteral nutrition compared with 75% with parenteral nutrition [19];
- Gastric emptying disorders. McClave et al. showed the poor sensitivity of residual gastric volume as a predictor of gastric emptying in ICU patients [20];
- Instability related to illness;
- Frequent interruption of enteral nutrition, e.g., related to patient transport;
- Underestimating total energy expenditure by not measuring the activity energy expenditure. In trauma and sepsis, total energy expenditure is significantly higher in the second week than during the first week (3257 ± 370 kcal/d vs. 1927 ± 370 kcal/d, $p<0.05$ in sepsis, 4123 ± 518 vs. 2380 ± 422 kcal/d, $p < 0.05$, in trauma) [21].

On a clinical basis, it is impossible to predict if a patient is hypo-, normo- or hypermetabolic. However, increasing evidence demonstrates the relationship between hypocaloric feeding and poor outcome in the intensive care. In a retrospective study, Barlett et al. [22] showed that a negative energy balance $> -10{,}000$ kcal during hospitalization was associated with a significant increase in mortality. Mault prospectively demonstrated that length of stay and length of ventilation were increased with negative energy balance [23]. Rubinson et al. [24] showed that patients who did not receive food by mouth for more than 96 hours after ICU admission and thereafter received less than 25% of their nutritional requirements had a significantly higher rate of positive blood cultures. Using a computerized bedside information system and daily indirect calorimetry energy expenditure measurement, we recently showed that the greater the negative energy balance, in particular negative cumulative energy balance, the more the complications [25]. Interestingly, the largest negative energy balance occurred at the beginning of hospitalization in the ICU (Fig. 1) and the occurrence of complications was also higher during the first 7 days (Fig. 2). These results were recently confirmed by Villet et al. [26] who studied the negative impact of hypocaloric feeding and energy balance on the complication rate of ICU patients. In a prospective study of 48 patients, they found a strong correlation between a negative energy balance (–1279 kcal/day during the first week) and the number of infectious complications, length of stay and length of ventilation. Berger et al. [27] in a study of ICU patients with circulatory failure, found that while most received artificial nutrition, only 70% of their energy target was provided and enteral nutrition was possible in the majority.

Since relation does not mean causality, prospective studies have been conducted to demonstrate a decrease in complication rate when tight energy control is achieved.

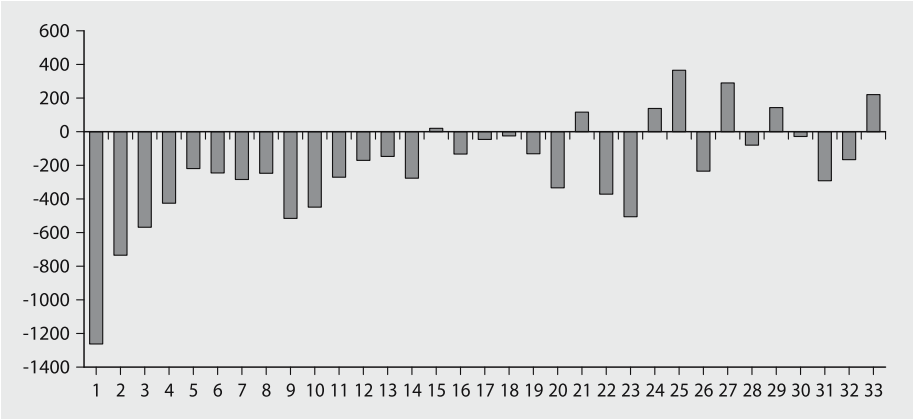

Fig. 1. Mean daily energy balance in 50 critically ill patients fed enterally. The x axis is days and the y axis mean daily energy balance in kcal/day. From [25] with permission

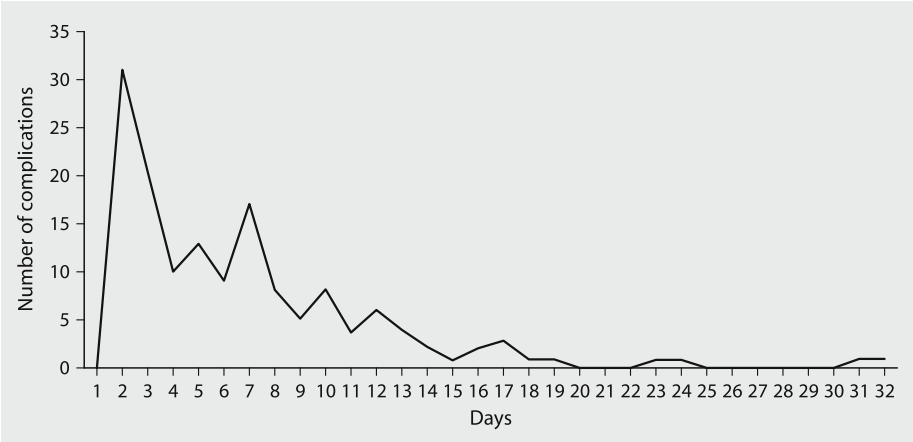

Fig. 2. Number of complications related to time (in days). From [25] with permission

New Tools for Tight Energy Control

Recent clinical practice guidelines from Canada [28] and the European Society for Clinical Nutrition and Metabolism (ESPEN) [29] do not recommend the general use of indirect calorimetry or specific strategies regarding the amount of calories to be administered to critically ill patients. There is a general agreement that hyperalimentation may be harmful and even 25–30 kcal/kg/day may be excessive during the acute phase of critical illness (Level C of evidence). During the anabolic recovery flow phase, target values should be 25 to 30 kcal/kg/day for men and 20 to 25 kcal/kg/day for women (Level C of evidence). Expert opinion also favors the view that critically ill patients who are hemodynamically stable and have a functioning gastrointestinal tract, should wherever possible receive early, i.e., <24 h,

enteral feeding, using an appropriate amount of enteral formula (Level C of evidence).

There are tremendous difficulties in measuring energy balance in critically ill patients [30]. While prescribed energy intake may be recorded, administered energy intake should also be accurately noted. This includes infusions of dextrose-containing solutions, carbohydrates as components of colloid solutions and mannitol, and lipid emulsions as components of propofol. Computerized bedside information systems are increasingly used in the intensive care setting and may aid in the collation of total of energy intake. Bedside indirect calorimetry can be used once a day [30] or continuously, but with decreased accuracy related to the technique [31]. Plank and Hill [32] determined energy balance over periods of 5–10 days by measuring changes in fat, protein and carbohydrate stores in the body. Changes in total body fat were positively correlated with energy balance over the 5-day study periods in patients with severe sepsis ($n = 24$, $r = 0.56$, $p = 0.004$) or major trauma ($n = 24$, $r = 0.70$, $p < 0.0001$). Fat oxidation occurred in patients whose energy intake was insufficient to achieve energy balance. There was no correlation between energy balance and changes in body protein. The authors concluded that since achievement of positive non-protein energy balance or total energy balance did not prevent negative nitrogen balance, nutritional therapy should focus more on glycemic control with insulin and specialized nutriments. Scheinkestel et al. [33] assessed the energy balance of anuric, ventilated patients treated by continuous renal replacement therapy in 50 consecutive patients who received 99% of their prescribed energy requirements either according to the Schofield equations corrected by a stress factor, or based on the metabolic cart readings of energy expenditure. They found that fed patients had a lower mortality than predicted, enteral feeding conferred a significant benefit to patient outcome ($p = 0.04$), and a protein intake of 2.5 g/kg/day increased the likelihood of achieving a positive nitrogen balance and improved survival. Kan et al. [34], in a study of 54 patients, noted that 15 were underfed (receiving 68.3% of their energy requirement), 20 were appropriately fed and 19 were overfed (receiving 136.5% of their energy requirements). Adequate feeding was associated with an improvement in nutritional status as assessed by the Nutritional Risk Index at day 7.

In another study involving 60 patients receiving enteral feeding for at least 10 days, 46% failed to reach 80% of their target resting energy expenditure. The main reasons for this were abdominal trauma, gastrointestinal intolerance, problems with the feeding tube, additional surgical interventions and the use of fentanyl. Gastrointestinal complications were the cause of more than 50% of inadequate enteral delivery [15].

Our group [35] prospectively compared 45 critically ill patients randomized to tight calorie balance intake or standard nutritional support. The patients with tight calorie balance had a significant decrease in sequential organ failure assessment (SOFA) score on the first days of hospitalization and a decrease in complications, especially the requirement for renal replacement therapy. Length of ventilation and length of stay were decreased if head trauma patients and patients with post-coronary artery bypass graft (CABG) complications were excluded (hospitalization longer than 28 days).

The suggested solutions:

- Implementation of protocols. It has been shown that implementation of a protocol for enteral nutrition delivery increased the percentage of patients receiving 80% of their estimated energy requirements from 20 to 60% ($p < 0.001$). Time to

initiation of enteral nutrition between groups was 1.76 days pre-protocol and 1.44 days post-protocol implementation [36].
- Rapid introduction of duodenal tube when low delivery is related to impaired gastric emptying [37].
- Prescription of over nutrition [38]. Higher calorie prescription has been demonstrated to improve nutrient delivery during the first 5 days of enteral nutrition, reaching faster isocaloric balance.
- Prescription of early total parenteral nutrition (TPN) from admission to bridge the energy gap [39].

Hypocaloric Support: For Whom?

The provision of excessive calories, mainly with carbohydrates, to ICU patients who have an increased resting energy expenditure, accelerated whole body proteolysis and lipolysis, is associated with hyperglycemia, additional metabolic stress, hyperdynamic cardiorespiratory response, increased CO_2 production and hepatic steatosis [40]. The use of hypocaloric TPN, using 100 to 200 g of glucose, decreased this stress.

Hypocaloric feeding may be adequate for obese critically ill patients. It has been demonstrated that obesity was not an independent risk factor for mortality in obese patients admitted to the ICU [41]. Defining the optimal caloric requirements for obese ICU patients remains a subject of discussion. The goals are to minimize catabolic losses and to avoid overfeeding and hyperglycemia with their complications such as infection [42]. Conversely, semi-starvation should not be induced. While equations and energy requirement recommendations are numerous, it is difficult to estimate the actual expenditure of these patients and unintentional overfeeding may be the result. This is related to which body weight (BW) to use: actual BW, ideal BW or adjusted BW (Ideal BW + 0.25 (Actual BW–Ideal BW)). As ideal BW does not correlate significantly with basic metabolic needs in obese patients, both actual [43] and adjusted BW have been proposed for the use in the various predictive equations of energy expenditure.

The use of hypocaloric high protein feeding in obese patients has been used, mainly with TPN [44]. When given half of the measured resting energy expenditure as dextrose (881 kcal/d) and 2.1 g/kg ideal body weight per day of protein, patients lost weight (1.7 kg per week) but achieved net protein anabolism, complete tissue healing and closure of fistulae [45]. Choban et al. designed two prospective, randomized, double blind trials [46, 47] comparing a hypocaloric with a normocaloric diet (in the form of TPN and high protein diet) in obese ICU patients. Positive nitrogen balance was achieved and the hypocaloric group required less insulin.

Our group [48] prospectively compared a hypocaloric, low-protein diet to normo-caloric, normo-protein enteral diet in 30 obese patients. Nutritional support was provided according to indirect calorimetry measurements (control group 100% resting energy expenditure and study group 50% resting energy expenditure). There were no significant differences in age, sex, body mass index, APACHE II or SOFA scores between the two groups. Prealbumin levels decreased in the hypocaloric group during the ICU stay. This group also had a lower requirement for insulin to maintain tight glucose control, while the control group required twice the amount of daily insulin. No difference was found in complication rates, length of ventilation, length of stay and mortality in the ICU (3/16 in the control group and

2/14 in the study group) or 6 months after discharge. We concluded that a hypocaloric diet in obese, critically ill patients is as safe as a normocaloric diet and could improve glucose tolerance without impairing outcome. However, a hypocaloric, high-protein diet should be compared to the diet used in our study.

Most authors support the use of indirect calorimetry as the only validated method to measure the energy expenditure of obese (>30% above ideal BW) as well as non obese patients [49]. If this is not available, an intake of 11–14 kg/adjusted BW/day with a protein intake of 1.5–2.1 g/adjusted BW/day should be recommended.

Conclusion

Targeting energy delivery according to resting energy expenditure and achieving this goal using protocols, aggressive, early enteral feeding through duodenal tubes, or supplementary parenteral nutrition appears most likely to reduce the negative energy balance observed in most ICU patients, especially in the first days after admission. The resultant energy control may decrease the number of complications and improve morbidity. In obese patients, lower energy goals can be provided with similar results.

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Bacteriological Problems

Acute Pneumonia and Importance of Atypical Bacteria

I. Boyadjiev, M. Léone, and C. Martin

Introduction

The term and concept of atypical pneumonia appeared in the 1940s following observations of penicillin-resistant pneumonia [1]. Despite the identification of a large number of microorganisms, the challenge of isolating so-called 'atypical' bacteria is the principal cause of failure of the etiologic diagnosis of pneumonia. These pathogenic agents in the tracheobronchial tree include a large variety of bacteria, viruses

Table 1. Advantages and disadvantages of the different microbiologic diagnostic examinations

Examination	Advantages	Disadvantages
Chest x-ray	Distinction pneumonia/bronchitis	Cost
Blood culture	Good specificity for *S. pneumoniae*	Poor sensitivity
Direct Gram stain	Rapidity; isolation of the predominant pathogen	Does not provide the type of microorganism
Cell culture	Specificity; isolation and identification of the strain; antibiotic profile	24 to 72 hours, variable sensitivity
Antigen tests		
– immunofluorescence detection – ELISA and related	Rapidity, very good specificity automation	Subjective reading, Sensitivity = 80% Specificity = 95% Confirmation test necessary
Antigen detection Urinary *Legionella*	Very good specificity	Specific to *Legionella pneumophila*
Antigen detection Urinary *Histoplasma*	Very good specificity	Rare pathogen
Molecular tests		
Simple hybridization PCR	Automation, sensitivity and specificity	Sensitivity and specificity = ELISA Sensitivity to enzyme inhibitors (false negatives) Contamination problems, Cumbersome technique
Suicide PCR	Very good sensitivity, specificity = 100%	Cost, Cumbersome technique

and even protozoa. Among atypical bacteria, *Chlamydia pneumoniae, Mycoplasma pneumoniae, Legionella pneumoniae, Bordetella pertussis,* and *Coxiella burnetii* are the most widespread. Numerous other bacteria are emerging pathogenic species whose virulence is currently being evaluated. Clinical examination only provides a diagnostic orientation in a restricted number of cases. The availability of rapid and specific microbiologic examination improves the diagnostic performance for this type of pneumonia (Table 1) [2]. Since most of these bacteria are intracellular, diagnosis is based principally on serology.

Microbiologic Techniques for Specific Diagnosis

Sampling

The contribution of bronchoalveolar lavage (BAL) in the diagnosis of pulmonary infection has been clearly demonstrated [3]. However, this technique is reserved for the diagnosis of pneumonia in ventilated patients.

Direct Diagnosis

Direct Examination. Gram-staining has not been assessed for pneumonia caused by atypical bacteria. No direct technique is adequate for direct examination of strict or facultative Gram-negative intracellular bacteria. The weakness of this examination is its very low sensitivity and specificity. Moreover, there is no kit available to detect atypical bacteria, nor those bacteria whose pathogenic roles are beginning to be understood.

Sample Culture. Culturing the pathogenic bacteria remains the method of choice since it provides a microbiologic diagnosis with certainty. However, its relevance depends on the nature of the samples (tracheal aspiration, BAL, pleural liquid, lung biopsy) and the moment they are harvested (immediately following the onset of symptoms and before any antibiotic). Since its isolation is difficult, an atypical bacteria requires specific isolation media. *Legionella* require BCYE agar, which is sometimes made selective by the addition of antibiotics and/or antifungals. If these antibiotics are ineffective on common bacteria, the samples are treated by acidification (pH 2) and thermal shock before being cultured. The specificity of a culture performed in this manner is 100% and its sensitivity ranges from 50 to 80%. However, the results are not available before two to three days, and they can be delayed until two to three weeks for some other organisms such as *M. pneumoniae*. Identification and differentiation of colonies depends on their cultural, biochemical, and enzymatic characteristics as well as their antigenic characteristics in direct immunofluorescence or by agglutination with specific immunserums. The association of these different elements is sometimes not sufficiently discriminating to provide identification of the species. Molecular biology is an innovative tool [4]. Identification of the etiologic agent is particularly important in the perspective of an epidemiologic investigation.

Examination of Samples by Direct Immunofluorescence. Observation by direct immunofluorescence with polyvalent or monovalent conjugates provides a rapid diagnosis. Strict or facultative Gram-negative intracellular bacteria appear as small bacilli.

The sensitivity of direct immunofluorescence is still unknown and varies from 25 to 70% [5]. Although there are cross reactions, its specificity is approximately 95%.

Detection of Soluble Antigens. Another method for the rapid diagnosis of atypical pneumonia is the detection of soluble antigens in urine, like for *L. pneumophila* serogroup 1. The immunoenzymatic (enzyme-like immunosorbent assay [ELISA]) or radioimmunologic methods use polyclonal antibodies. The sensitivity of this test for the detection of *L. pneumophila* serogroup 1 ranges from 60 to 80% with a 100% specificity [6, 7]. On the other hand, there is no test that can detect all of the *L. pneumophila* serogroups or all *Legionella* species.

Indirect Diagnosis

Serologic Diagnosis. Indirect immunofluorescence is still the method of reference. While it is not useful in the acute phase, indirect immunofluorescence provides a retrospective diagnosis. A serum sequence is essential to record the increase in antibodies. This usually appears one week after the beginning of the illness, but can sometimes be much later (1 to 9 weeks). A variation of two dilutions between early serum and late serum with a count of ≥1/128 for the late serum is sufficient for a diagnosis in the case of *L. pneumophila* serogroup 1 antibodies. Likewise, the association for *M. pneumoniae* of an antibody titer of ≥1/64 and cold agglutinins of ≥1/64 provides a diagnosis. According to the literature, the sensitivity of indirect immunofluorescence ranges from 67 to 90% [8]. Numerous crossed reactions have been described with different species (mycobacteria, leptospires, *Chlamydia, Mycoplasma, Citrobacter, Campylobacter, Coxiella burnetii*) [8] as well as between the different serogroups and species of *Legionella* [8]. However, the specificity of immunofluorescence remains good since it ranges from 75 to 99% [8].

Atypical Bacteria with Known Pathogenic Properties

Mycoplasma pneumoniae and *Chlamydia pneumoniae*

Mycoplasmas are bacteria without walls. This characteristic gives them an extreme plasticity and fragility in an external medium. They are resistant to beta-lactams and their size (inferior to 300 nm) allows them to go through antibacterial filters.

The prevalence of *M. pneumoniae* pneumonia in adults ranges from 1.9 to 30% in the form of necrotizing pneumonia or pleural effusions [9]. Beginning in 1938, a series of cases described atypical pneumonia as characterized by pneumonia with a prolonged duration, no reaction to treatment with penicillin, and non-specific radiological images [10]. The chest X-ray shows either a non-systematized alveolar syndrome or systematized opacities. The inferior lobes are affected more often than the superior. The patient's immunological condition plays a role in the acquisition of a *M. pneumoniae* pneumonia. Thus, an examination of the BAL fluid of a group of immunosuppressed patients with pleural effusions in the ICU led to the isolation of this bacterium in 5% of the cases [11]. A serologic examination of two sera sampled at a 10-to-14-day interval is the most common diagnostic method used. However, the diagnosis can be hampered by cross reactions between *M. pneumoniae* and other commensal mycoplasms among the oropharyngeal florae. There are different opinions on this issue in the literature: a cause of error for some authors

[12], a negligible parameter for others [13]. At present, the IgA ELISA tests have a sensitivity of 86 to 100% and a specificity of 100% for the serologic diagnosis of acute *M. pneumoniae* infection [14].

Chlamydiae are limited by a cytoplasmic membrane and a wall that is comparable to that of Gram-negative bacteria, made up of both an internal and external membrane containing lipopolysaccharides (LPSs) and protein-linked penicillins (PLPs) [15]. They are strict intracellular organisms whose development cycle can be divided into several stages (Fig. 1).

Under certain conditions, the development cycle is altered. There is a delay in the maturation of the reticulate body and an inhibition of infectious elementary body differentiation which is expressed by a persistence of aberrant forms in the host cell. The bacterium is viable but not cultivatable. A parallel can be made with viral latency. *C. pneumonia* is transmitted through the air. It is responsible for both upper and lower respiratory infections but no symptom is specific. The radiological findings are minor but show extensive bilateral pneumonia with, sometimes, pleural effusion. In adults with chronic bronchitis, immunosuppressed patients, or the elderly, *C. pneumoniae* can cause severe infections. However, the pathogenic role of this bacteria is now challenged [16].

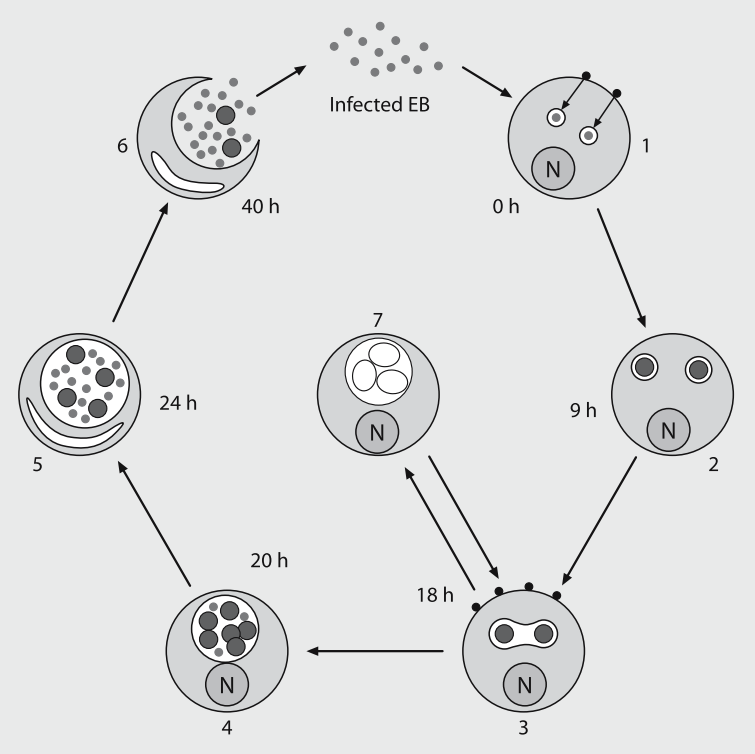

Fig. 1. Development cycle of *Chlamydia* [21].
1 Attachment and ingestion of elementary bodies (EB) in a vacuole; **2** transformation of EB into reticulated bodies (RB); **3** fusion of vacuoles; **4** appearance of neoformed EB; **5** maturation of EB; **6** cell lysis; productive infection; **7** aberrant bodies: persistent infection, antibiotics, nutriment depletion, interferon-γ

Rapid and precise diagnostic tests for *M. pneumoniae* and *C. pneumoniae* are not currently available: culture isolation of these bacteria requires the collection of cells by scraping the mucous membranes, then rapid transport at a stable temperature (+4 °C). Amplification techniques have been improved [17]. The most studied and widely used is the polymerase chain reaction (PCR) [18]. Values superior to 98% have been described in terms of specificity. On the other hand, these tests do not improve sensitivity vis-à-vis culture. Serodiagnosis of *M. pneumoniae* and *C. pneumoniae* is difficult to interpret because seroprevalence is high in the population. Approximately 75% of subjects have anti-*C. pneumoniae* antibodies and there are cross reactions with *C. trachomatis* and *C. psittasi* in 50% of the cases [19]. Thus, when the etiologic and epidemiologic data and the seroconversion threshold are available, they help in the careful interpretation of the microbiologic results [20].

Coxiella burnetii

Coxiella burnetii is a strict intracellular microorganism with a wall that is similar to Gram-negative bacteria but with particular properties. *C. burnetii* proliferates in low pH phagolysosomes. It appears in two forms, one of which is a resistant form resembling a spore with considerable virulence. In aerosol form, this bacteria can be pathogenic at a concentration of one colony forming unit (cfu). Contamination is essentially respiratory and its incidence is probably underestimated. A retrospective study that included 22 s496 sera was performed between 1982 and 1990 at the Centre National Français de Référence des Rickettsies (French Rickettsia Reference Center). Serologic observation by indirect immunofluorescence was also performed in 1988 on 924 blood-donor sera. The seroprevalence was 4%. The incidence recorded during this 9-year period was 0.58 per 100 000 inhabitants [21]. *C. burnetii* causes Q fever, a polymorphic disease (Q from the word 'query') [22, 23]. Infections in man are essentially contracted through the air and by inhaling aerosols in contact with mammals. Current major epidemics are principally linked with livestock [24, 25]. Dissemination by the wind of infected dust is possible making it difficult to confirm animal contact. Q fever is a disease that is more frequently diagnosed, and more severe, in men than in women [26]. Indeed, the sex ratio of 323 patients admitted to hospitals in France with a diagnosis of Q fever between 1982 and 1990 was 2.5/1 [21]. However, systematic research for antibodies directed against *C. burnetii* in the sera of 942 blood donors in the same country revealed a sex ratio of 1/1. Sex hormones, especially estradiol, are said to play a protective role in the development of the disease [27].

From a clinical standpoint, Q fever is extremely polymorphic – this disease can appear in both chronic and acute forms. Diagnosis can only be made by systematic serology. The mean duration of incubation is 20 days but can reach two months, thereby confusing the epidemiologic data [24]. Schematically, there are three forms of acute Q fever which are shown in Figure 2: self-limited flu-like syndrome, hepatitis, and pneumonia. Pulmonary symptoms were the origin of the description of the disease [28]. Most cases are asymptomatic or benign, characterized by a nonproductive cough, fever, and minimal auscultatory abnormalities. In certain cases, pulmonary symptoms can be severe with major hypoxemia or acute respiratory distress syndrome (ARDS) [29]. Hemoptysis can be observed. Chest X-ray reveals nonspecific images of interstitial pneumonia, or, sometimes, an acute lobular pneumonia. The images initially appear in the hilus and are most often located in the inferior lobes [30]. The duration of symptoms ranges from 10 to 90 days and the

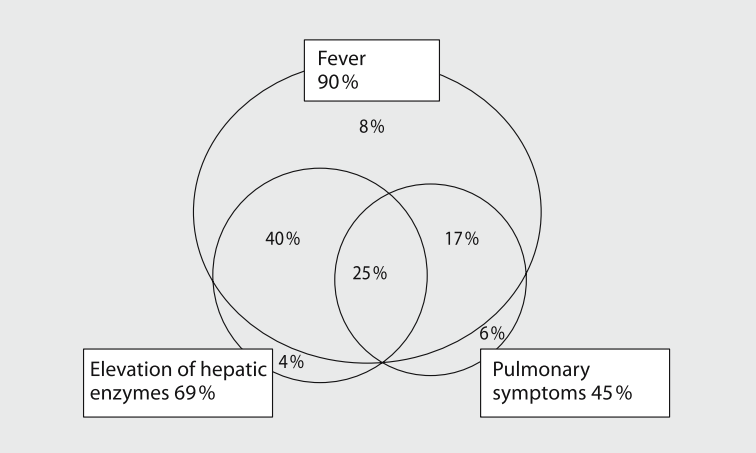

Fig. 2. Relative importance of the three principal clinical forms of Q fever. From [30] with permission

mortality rate is from 0.5 to 1.5% [21]. Standard biological examinations are nonspecific: the absence of hyperleukocytosis and thrombopenia is of interest. The specific diagnosis is principally based on serology by indirect immunofluorescence.

Treatment requires the use of antibiotics that are active in the specific conditions of growth of this microorganism. Indeed, the proliferation of *C. burnetii* in an acid pH phagolysosome can explain the potential resistance to antibiotics [31, 32]. Betalactams cannot concentrate in such cells, aminoglycosides are inactive in acid pH and macrolids are effective in basic pH. Moreover, none of the antibiotics that are active against *C. burnetii* (rifampicin, cotrimoxazole, tetracyclines, clarithromycin and fluoroquinolones) is bactericidal [33]. The prognosis of acute Q fever is usually spontaneously favorable with these bacteriostatic antibiotics [34]. On the other hand, for chronic forms such as endocarditis, bacteriostatic antibiotics control the disease but cannot cure it [35]. Maurin et al. [36] hypothesized that the absence of bactericidal activity of antibiotics against *C. burnetii* was due to their inactivation by the acid pH in phagolysosomes. By adding a lysosomotropic alkalinizing molecule, hydroxychloroquine, to fluoroquinolones and doxycycline, the authors succeeded in restoring the bactericidal activity of these molecules [36, 37].

Legionella and Legionellosis

Bacteriology. There are 43 different species in the *Legionellaceae* family including 19 with pathogenic powers. *Legionella* are facultative intracellular bacteria. After penetrating the cell hosts, they block phagosome activity and phagolysosomal fusion. This mechanism enables the proliferation of *Legionella* in the phagosome until cellular lysis is triggered.

Legionella are aquatic bacteria that can be found in numerous tanks and freshwater sources that are warm or hot (40 to 60 °C). *Legionella* are not often found in cold-water pipes (temperature below 20 °C). They are abundant in water-air interfaces such as siphons, ventilators, and especially mixing faucets and shower heads [38]. In buildings, hotels, and hospitals, 60 to 70% of the samples taken from taps

are positive [39]. Another essential element in the development of *Legionella* is their presence in the biofilms of water distribution systems [38]. Finally, the relationships of *Legionella* with other microorganisms in aquatic media are an essential element of their survival and cast light on the particular epidemiology of legionellosis. The host-parasite relationships with protozoa observed by Rowbotham [40] beginning in 1980 now appear to be fundamental in the pathogenesis and epidemiology of *Legionella* [41]. *Legionella* are capable of entering and proliferating in numerous amoebic species. They are found in the phagolysosomal vacuoles according to a process that is in every respect comparable with that observed in infected human macrophages [42]. They survive in amoeba *in vitro* between 0 and 45 °C. The actively proliferate at 35 °C in vacuoles and trigger lysis in amoeba cells. The result is bacterial culturing of the environmental medium. *Legionella* survive also in amoebic cysts. Cysts are extremely resistant entities which protect amoebas when environmental conditions are unfavorable. The *Legionella* obtained in co-culture preserve their virulence for the experimental subject.

Epidemiology. Legionellosis is a disease linked to our urban civilization. The risk factors are age (> 50 years), male, smoker, alcohol, diabetes, and immunosuppression [5]. The mode of transmission is usually inhalation of infectious aerosols due to air conditioning systems, contaminated shower heads, and respiratory device nebulizers and humidifiers [43]. Cases of legionellosis have also been reported after drowning [44] and after the use of bronchoscopes rinsed with tap water [45]. The extrapulmonary mode of transmission for legionellosis (especially endocarditis on a prosthetic valve) is the contamination of surgical wound by infected water [4]. Interhuman contamination has not yet been reported. The incidence and prevalence of legionellosis, including nosocomial cases, are certainly underestimated. *Legionella* are the origin of 1 to 37% of hospitalized cases of pneumonia [46]. The incidence of *Legionella* (15% vs 7%, $p < 0.05$) is significantly larger in intubated patients. *Legionella* cause 0 to 47% of nosocomial pneumonia, particularly in immunosuppressed patients [47].

Clinical Signs (Table 2). The clinical signs are not specific to the disease. *Legionella* is frequently responsible for severe community-acquired pneumonia [46], requiring intensive care. After two to ten days of incubation, patients present with high fever (> 39 °C) and bradycardia. In addition, there are symptoms such as watery diarrhea (20 to 40% of the cases) and neurological signs with confusion. The neurological forms [48] are the signs of encephalic involvement, probably due to a toxin. Cardiac injury is usually located in the pericardium. Endocarditis on valve prostheses has been confirmed by culture [49]. Direct involvement of the digestive tube is in the form of peritonitis or necrotizing colitis. Classic liver lesions are due to the toxin. Renal involvement, observed in 50% of legionellosis cases, is shown by proteinuria and transitory hematuria. In 13% of the patients, acute renal failure secondary to rhabdomyolysis can appear. Similarly, signs on chest X ray are observed in 90% of the cases, and do not distinguish *Legionella* pneumonia except for their rapid aggravation, especially under inappropriate antibiotics. Nonspecific biological anomalies are observed during systemic involvement. Biological alteration that is more characteristic of legionellosis is hypophosphatemia. However, neither hypophosphatemia, nor hyponatremia, nor elevated transaminases alone constitute a specific diagnostic criterion. Their association with other symptoms could cause one to suspect legionellosis.

Diagnosis. In its classic form, legionellosis is characterized by a febrile pneumonia, watery bloodless diarrhea and confusion (Table 2). Resistance to treatment by beta-lactams and bradycardia are also good orientation elements [50]. The existence of five to 10% mixed infections makes clinical diagnosis difficult and requires a bacteriological diagnosis. Bacteriological diagnosis of legionellosis is difficult. The culture has a specificity of 100% but a sensitivity of 50 to 80% after a growth period of at least two or three days. Observation by direct immunofluorescence with conjugates provides a rapid diagnosis – *Legionella* appears as small bacilli. The sensitivity of this examination is poorly known: 25 to 75% of the positive cultures have a positive direct immunofluorescence [8]. Detection of the urinary antigens of *Legionella pneumophila* serogroup 1 is considered as a specific, rapid and confirmed diagnosis. Urinary antigens are detected from the first days of the infection and up to 60 days later, as after appropriate antibiotics [9]. However, serology remains the most frequent means to diagnose legionellosis even though it is often late or even retrospective.

Even though it is a major cause of pneumonia, legionellosis is still underestimated. The combined use of urinary antigen test and cultures improves the diagnosis. PCR is a promising tool but standardized test kits are not available. The development of both antigen urinary tests that detect a larger number of *Legionella* species and standardized PCR methods could make up this current shortcoming.

The bactericidal antibiotics are rifampicin, macrolides, fluoroquinolones, and aminoglycosides. Antibiotic treatment of legionellosis produced by infected aerosols in experimental subjects has confirmed the excellent *in vivo* efficacy of these antibiotics [51]. The duration of treatment must be 15 days [51].

Table 2. Frequency (in percentage) of clinical and biological signs during legionellosis [52]

Clinical and physical signs		Biological signs	
▮ Fever			
> 39 °C	90	Leukocytes >10000/mm^3	45
> 40 °C	50		
▮ Cough	80–100	Hyponatremia	68
▮ Chills	75		
▮ Expectoration	50	Hypophosphoremia	51
▮ Dyspnea	54–94	**Increase in enzymes**	
▮ Thoracic pain	25–33	LDH	45
▮ Diarrhea	50	ASAT	65
▮ Nausea, vomiting	25	Alcaline phosphatase	62
▮ Abdominal pain	15–23	Hyperbilirubinemia	15
▮ Cephalgia	41–71	Proteinuria	50
▮ Confusion	30–40	Hematuria	50
▮ Myalgia	14–83		
▮ Bradycardia	60		
▮ Râles	80		
▮ Dullness on percussion	80–100		

Conclusion

The diagnosis of pulmonary infection caused by *Mycoplasma* and *Chlamydia pneumoniae*, *Coxiella burnetii*, and different species of *Legionella*, is often long and challenging although they are the major etiologic agents of pneumonia. For this reason, the treatment of these infections remains probabilistic. Advances in new diagnostic techniques, such as PCR sequencing, show the relative predominance of atypical organisms and serves to identify emerging pathogenic agents. Moreover, these techniques should clarify the correlation between common and atypical pathogens.

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Antibiodic Resistance in the Intensive Care Unit

L. del Sorbo and J.C. Marshall

Introduction

Antibiotic resistance is an increasingly common problem in the contemporary health care system, and in particular, in the intensive care unit (ICU) [1, 2]. Critically ill patients are five to ten times more likely to develop a hospital-acquired infection than patients on a general hospital ward, and antibiotic-resistant pathogens are responsible for more than half of these infections [3, 4]. A better understanding of the factors responsible for the emergence of resistant pathogens in hospitalized patients is fundamental to the control, or reversal of this trend.

Definition and Mechanisms of Antibiotic Resistance

Penicillin and streptomycin, introduced into clinical practice approximately 65 years ago, were the first antibiotics available to treat bacterial infections. The phenomenon of antibiotic resistance emerged at the same time. In 1940, Abraham and Chain [5] described a bacterial enzyme called beta-lactamase that could inactivate penicillin. Since then, almost all strains of bacteria responsible for human illness have developed resistance to one or more classes of antimicrobial agents.

However, the origins of antimicrobial resistance extend much farther back, and are not entirely related to the introduction of antibiotics. It would be teleologically improbable that the energetic and metabolic disadvantage, and the biological complexity of the processes necessary to express factors providing antibiotic resistance, would have emerged over the three billion year history of bacterial evolution, unless resistance genes served other roles that supported bacterial survival. It is known, for example, that antibiotic-producing microorganisms that are not agents of human disease also express genes encoding antibiotic resistance factors [6] that provide an adaptive advantage in limiting the growth of other microbial competitors. Thus it is most probable that innate and conserved mechanisms of antibiotic resistance in bacteria have become more prevalent under the powerful selective pressure provided by widespread antimicrobial exposure.

Antibiotic resistance at the cellular level is mediated through several biologic mechanisms, and may be intrinsic or acquired. Some strains of bacteria are naturally resistant to certain antibiotics (for example, enterococci are resistant to fluorquinolones and cephalosporins), because they do not express the appropriate molecular target of the antibiotic, or because the cell wall is impermeable to penetration by the agent. These organisms behave clinically as opportunistic pathogens,

since they selectively proliferate in the presence of antibiotics that suppress other components of the normal flora. In addition to enterococci, coagulase-negative staphylococci, *Acinetobacter baumannii* and *Stenotrophomonas maltophilia* are frequently resistant to multiple antimicrobials and emerge as common causes of ICU-acquired infections, particularly in the chronic critically ill patient who has received multiple courses of broad spectrum antibiotics [6].

Antibiotic resistance is acquired through one of two principle mechanisms: spontaneous mutation and DNA transfer [3, 6, 7]. Genetic mutations can result in structural modification of proteins, and in the up- or down-regulation of proteins relevant to the antibiotic effect, for example, porins, active transporters, and gene promoters or repressors. If mutations occur in the genes that regulate DNA replication or repair, the mutants can become 'hypermutators' – bacteria characterized by a mutation rate 200 times higher than the usual. This phenomenon, triggered in part by a stress response system, increases the capacity of the microorganism to adapt to the surrounding environment, and thus to develop resistance to antibiotics that are present [8].

Antibiotic resistance can also be acquired through DNA transfer. Genes encoding antibiotic resistance factors can reside in chromosomes or in plasmids, circular structures of nucleic acid that are transferable from one bacterium to another. DNA transfer is responsible for the spread of antibiotic resistance from one species of bacteria to another [6, 7].

Most Gram-negative, and some Gram-positive bacteria (for example, *Staphylococcus aureus*), are able to inactivate antibiotics through the synthesis of specific enzymes, such as beta-lactamases that hydrolyze the beta-lactam ring present in penicillins and cephalosporins. There are a number of beta-lactamases, with differing affinity for different types of beta-lactam antibiotics. Moreover, over the past few decades, antibiotic exposure has resulted in a much broader spectrum of beta-lactamase enzymatic activity including activity against third generation cephalosporins. More than 150 extended spectrum beta-lactamases (ESBL) have been identified; some are highly resistant to third generation cephalosporins and to beta-lactamase inhibitors (clavulanic acid, tazobactam) [9].

Another mechanism of bacterial antibiotic resistance derives from modification of the antibiotic target, altering the affinity for the antimicrobial drug. For example, *S. aureus*, streptococci and enterococci, synthesize a modified penicillin-binding protein that has a low affinity for beta-lactam antibiotics and so renders them inactive [3].

Modification of porin channels in the bacterial cell wall can prevent the movement of antibiotic across the bacterial outer membrane, a phenomenon that has been documented in some Gram-negative bacteria, such as *Pseudomonas aeruginosa*. *P. aeruginosa* and other enterobacteriaceae possess an energy dependent system that can actively remove drugs or other undesirable substances from the organism before they exert their biologic effects. Some of these pumps are drug specific; others are active across a broad spectrum of agents [7].

In addition to intrinsic cellular mechanisms of resistance, multiple other factors are relevant to the emergence of antibiotic resistance, including the site of the infection, the pharmacodynamic properties of the antibiotic used and its concentration in the infectious focus, and the immunocompetence of the human host. Bacteria that are sensitive *in vitro* to a certain dose of antibiotic may be completely resistant *in vivo* because of the particular distribution of the drug in the body, or the inaccessibility of the drug to the infected tissue. Sub-therapeutic doses of anti-

biotics can also increase the rate of emergence of resistant microorganisms. Finally, an antibiotic that is effective in an immunocompetent patient may fail in an immunocompromised host whose innate immune system is incapable of clearing the micro-organism [7, 10].

Prevalence of Antibiotic Resistance in the ICU

The prevalence of antimicrobial resistance has been increasing over time around the world. NNIS data from the United States have shown, for example, that methicillin resistance in *S. aureus* increased by 29% between 1995 and 2000 [11]. However, rates of increase differ by geographic area and microbial species. Certain geographic trends in resistance have been noted. In Europe there is a north-south gradient in the prevalence of resistance, with a higher prevalence of resistant bacteria in more southerly countries. According to the 2003 report of the European Antimicrobial Resistance Surveillance System, the proportion of methicillin resistant *S. aureus* (MRSA) was < 1% in Iceland, increasing to 51% in Greece (www.earss.rivm.nl). The report also noted an increase in methicillin resistance in the Scandinavian countries, and a stabilization of rates in the United Kingdom and Ireland. Similar patterns are also evident in North America, where rates of resistance for the most common pathogens are lower in Canada than in the United States [6].

Several studies have addressed the prevalence of resistance in the ICU [1, 12]. Archibald et al. found that the prevalence of MRSA, vancomycin-resistant enterococci (VRE), and *P. aeruginosa* resistant to ceftazidime or imipenem to be twice as high in ICU patients compared to patients in other hospital wards or to outpatients [13]. The EPIC study (European Prevalence of Infection) was a one day point prevalence study, undertaken in 1417 European ICUs, to determine the prevalence of hospital-acquired infections in ICU patients. The study showed that 45% of ICU patients had an infection, and that 21% had an ICU-acquired infection, the prevalence varying by country and being higher in the south of Europe. *S. aureus* was the most common infecting organism, present in 30% of cultures; more than half of these yielded MRSA. In addition, 65% of isolates of *P. aeruginosa* and 73% of isolates of coagulase-negative staphylococci were resistant to one or more antibiotics [14].

Jones et al. analyzed the *in vitro* antibiotic susceptibility of the most common bacterial isolates from ICU patients in five different countries – France, Germany, Italy, Canada and the USA – over the period from 2000 to 2002 [12]. They reported that the most frequently isolated pathogens were *S. aureus* in ICUs in the USA, Canada and France, coagulase-negative staphylococci in Germany, and *P. aeruginosa* in Italy. *S. aureus* was the most common Gram-positive pathogen overall (13.6–20.2%); among Gram-negatives, the most prevalent species were *Escherichia coli* (7.7–15.5%) and *P. aeruginosa* (10.8%–22.3%). Analysis of the susceptibility of the microorganisms to the most commonly used antibiotics showed that antibiotic resistance varied for each bacterium across countries (Fig. 1). The prevalence of oxacillin resistance in *S. aureus* was 59.4% in Italy, 52.3% in the USA, 40.6% in France, 21% in Germany and 19.7% in Canada. The proportion of *Enterococcus faecium* resistant to vancomycin was 76.8% in the USA, 14.5% in Canada, 24.2% in Italy, 4.8% in Germany and 0.8% in France. The prevalence of vancomycin resistant *Enterococcus faecalis* was low in all countries (< 5%). The prevalence of ESBL production was generally low, but resistance to ceftazidime was higher, particularly in *Klebsiel-*

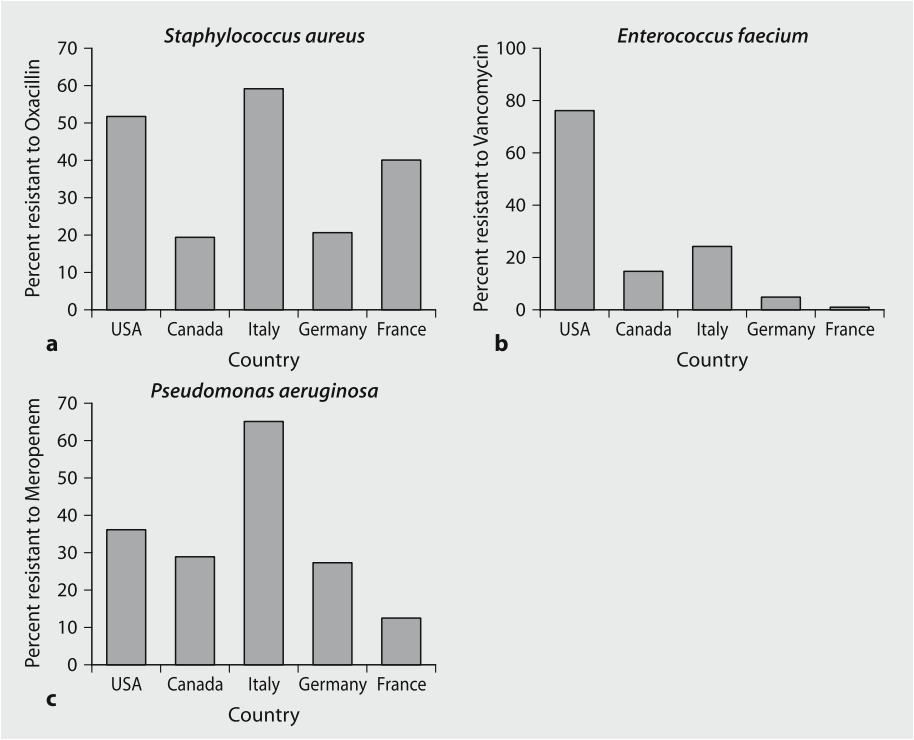

Fig. 1. Rates of resistance of common nosocomial ICU pathogens to commonly used antibiotic agents vary significantly by country. Data for five different countries are shown for resistance of *S. aureus* to oxacillin (**a**), *Enterococcus faecium* to vancomycin (**b**), and *Pseudomonas aeruginosa* to meropenem (**c**). (Data from [12])

la pneumoniae, with rates ranging between 2.2% (Canada) and 28.5% (Italy), and *M. morganii* (7.7% in Germany, 17.3% in USA). Italian isolates of *K. pneumoniae* and *Proteus mirabilis* had a significantly higher rate of resistance to most of the antibiotics except cefepime and imipenem, and enterobacteriaceae had a slightly higher rate of resistance to fluorquinolones (ciprofloxacin, levofloxacin) than to cephalosporin across all five countries screened.

This work showed that even though the predominant pathogens are similar from one country to the next, patterns of antibiotic resistance vary considerably, and so programs for surveillance and empiric treatment of infections need to be implemented at the local level, based on local profiles of antimicrobial susceptibility. Because infections in the critically ill are caused by a relatively small number of bacteria, it is valuable to look at the more important of these more closely.

Staphylococcus aureus

S. aureus is the most frequently isolated pathogen in the ICU [12]; the incidence of infections caused by MRSA is increasing [3, 15]. The prevalence of MRSA amongst strains of *S. aureus* in North American and European ICUs ranges from 20% to

55%. MRSA has also been isolated outside the hospital environment, and is increasingly seen as a community-acquired pathogen. Risk factors for infection with MRSA are nasal colonization, previous hospitalization, prior or prolonged antibiotic therapy, chronic underlying conditions, use of intravascular devices, advanced age, exposure to other MRSA colonized or infected patients and prolonged ICU stay [15]. The standard treatment for MRSA is vancomycin, however strains of MRSA with only intermediate sensitive to vancomycin or other glycopeptides have been identified, including a fully resistant strain of MRSA in a patient treated with long term vancomycin. This particular strain carries the same gene for vancomycin resistance expressed by VRE (vanA) [16]. Linezolid and quinupristin/dalfopristin are currently the treatments of choice for patients infected with VRSA.

Coagulase-negative Staphylococci

Coagulase-negative staphylococci are the cause of 15 to 20% of ICU-acquired infections, and the most frequent isolates in intravenous catheter-related bacteremias. *S. epidermidis* is the species most commonly isolated in humans. Coagulase-negative staphylococci are typically resistant to antibiotics, such as methicillin (90%), cotrimoxazole and erythromycin, and display variable sensitivity to cephalosporins and aminoglycosides. Vancomycin is the drug of choice for this organism, and must be used judiciously, since vancomycin resistance in coaqulase-negative staphylococci has been described, although it is uncommon [3, 15].

Enterococcus faecalis and Enterococcus faecium

Enterococcus faecalis and *Enterococcus faecium* are also common isolates in ICU patients [15]. *Enterococci* are intrinsically resistant to a number of antibiotics, including cephalosporins and fluoroquinolones, and can develop acquired resistance to other vancomycin, aminoglycosides, and beta-lactam antibiotics. VRE have emerged as a significant threat over the past ten to 15 years. The NNIS reported an increase in prevalence of VRE from 0.4 to 37.5% among enterococci isolated in USA ICUs from 1990 through 2002. Enterococci have a low pathogenic potential, and thus rates of VRE colonization are significantly higher than rates of VRE infection, except in critically ill and immunocompromised patients. The most important independent risk factor for VRE colonization or infection is prior exposure to antibiotics, in particular vancomycin, cephalosporins, and agents with anti-anaerobic activity. Other risk factors include the presence of a feeding tube, exposure to other patients with VRE colonization or infection, contamination of the environment or equipment, and a prolonged hospital stay. The antibiotics of choice for VRE are linezolid and quinupristin/dalfopristin; the latter is active only against *E. faecium*, whereas linezolid is active against both species. Linezolid resistance in VRE has been reported [17].

Pseudomonas aeruginosa

P. aeruginosa is a common pathogen in hospital- and ICU-acquired infections, but rarely causes disease in healthy people. Rates of colonization increase following hospital admission; within 7 days of admission, 23% of patients are colonized and the percentage increases to about 60% within 14 days. *P. aeruginosa* is the most

common cause of ventilator-associated pneumonia (VAP). Acute respiratory distress syndrome (ARDS), antibiotic exposure, and prolonged mechanical ventilation are the major risk factors for *P. aeruginosa* pneumonia. *P. aeruginosa* has intrinsic or acquired resistance to many antibiotics. The prevalence of resistant strains is strongly associated with prior antibiotic use, and is higher in ICUs [18]. The organism has an ability to develop antimicrobial resistance rapidly, and often in patients who are receiving adequate treatment. Multi-drug resistant strains have been described. In the USA, 16% of pseudomonads are resistant to at least three commonly-used anti-pseudomonal antibiotics, and 1% of isolates are resistant to six antibiotics. Combination therapy with two or three of the most active agents against *P. aeruginosa* (carbapenems, piperacillin, cefepime, ceftazidime, ciprofloxacin, amikacin and tobramycin) is commonly used for infections caused by these highly-resistant organisms. However, the efficacy of such an approach is unknown.

Clinical Impact of Antimicrobial Resistance

Several clinical studies show that infections caused by antibiotic-resistant bacteria are associated with higher in-hospital morbidity, increased mortality, longer hospitalization, and higher costs [19–21]. Antibiotic choices are more limited, with the result that inadequate empiric therapy is common. Indeed the increased prevalence of antibiotic-resistant organisms, rather than the inadequacy of a therapeutic decision, may well explain the elevated risk of death described when initial empiric therapy is judged to be inadequate [22–25], and administering a more appropriate antibiotic when drug sensitivities become available does not appear to affect outcome [26].

However, whether infection with resistant bacteria is causally related to an increased mortality is still unclear. For example, Gonzalez et al. failed to demonstrate a difference in mortality rates when infections caused by MRSA or methicillin-sensitive *S. aureus* (MSSA) were compared [27]. Intriguingly, these authors found a significantly higher mortality rate in patients with MSSA infection who were treated with vancomycin when compared to MSSA-infected patients treated with cloxacillin, raising the possibility that, despite adequate antimicrobial activity, antibiotics may also induce harm.

It is uncertain whether infection is the cause or the consequence of adverse outcome in critical illness, and whether infection with resistant microorganisms is simply a marker of illness severity, and a consequence of longer exposure to risk factors for infection from resistant bacteria. For example, Peres-Bota and colleagues studied 949 patients admitted to the ICU; of these, 30% had infection at the time of ICU admission, and of 439 patients remaining in the ICU for longer than 48 hours, 186 (42%) developed an ICU-acquired infection, and 42% of these were caused by resistant microorganisms [28]. The authors found that the maximum degree of organ failure during the ICU stay and the presence of respiratory dysfunction, but not infection with a resistant pathogen, were independent predictors for death. Moreover, the multivariable logistic regression analysis showed that prior use of multiple antibiotics, hospitalization prior to infection, and the degree of liver failure were independent factors for development of infection with resistant bacteria [28].

In addition to being associated with a higher mortality rate, antibiotic resistance significantly affects the cost of care and increases the length of hospitalization [29–31], prompting physicians to choose more potent and expensive antibiotics, such as vancomycin and carbapenems, as first line therapy.

Risk Factors for Antimicrobial Resistance

Prior antimicrobial exposure has been identified as an independent risk factor for the development of resistance in Gram-positive and Gram-negative bacteria, especially in ICUs, where the antibiotic selective pressure is higher [32, 33], and exposure to broad spectrum antibiotics, such as carbapenems, vancomycin, intravenous fluoroquinolones and third generation cephalosporins, is more common [34].

Several studies have demonstrated that reducing antibiotic exposure is effective and safe. Fagon et al. reported that diagnosing VAP using quantitative cultures of distal airway samples reduces the use of antibiotics, the length of hospital stay and costs [35]. Moreover, Chastre et al. showed that for the treatment of VAP, an eight day course of antibiotics is as effective as a fifteen day course, and results in a reduction in antibiotic exposure and cost [36].

As pointed out in the Surviving Sepsis Campaign guidelines, any use of useless antibiotic in the ICU, as well as any non-usage of the useful ones, should be considered as a non-quality indicator, for the severe consequences associated [37]. This observation suggests that antibiotic resistance can be considered, at least in part, iatrogenic!

Other risk factors promoting antibiotic resistance in ICU are cross-transmission and impairment of innate host defenses. A lack of compliance in handwashing, in application of asepsis during invasive procedures, and in the training of health care providers are associated with an increased likelihood of disseminating pathogens and their antibiotic resistance plasmids from patient to patient. Transfer of patients to other facilities has also been identified as a risk factor for spreading antibiotic resistance. Invasive devices that breach normal physical barriers are risk factors for infection with resistant species, as are co-morbid conditions that alter the immune status of the patient, for example, immunosuppressive drugs, malnutrition, and diabetes [2].

Interventions to Prevent Antibiotic Resistance in the ICU

A number of strategies to prevent the occurrence of antibiotic resistance in the ICU setting have been studied. Some have proven effective, for others the evidence is less clear.

Reducing the length of hospital stay can reduce the duration of exposure to risk factors for infection, thus decreasing the rate of nosocomial infections, including those caused by resistant pathogens. For example, reducing the length of mechanical ventilation using formalized weaning protocols has been demonstrated to reduce the rate of VAP, the duration of mechanical ventilation and the length of ICU stay [38].

Minimizing the use of central venous catheters may also aid in reducing ICU-acquired infection with resistant organisms; strict implementation of hygiene precautions during the insertion and maintenance of these catheters also lowers rates of infectious complications [33].

One of the simplest and most effective maneuvers to prevent horizontal transmission of pathogens is handwashing. Alcohol solutions have been introduced to reduce the lack of compliance resulting from the inaccessibility of soap and sink, or excessive workload. The use of gloves and gowns also reduces horizontal transmission, especially in combination with controlled antibiotic use [10, 33].

Protocols and guidelines to standardize the management of antibiotics can be effective in minimizing inappropriate or inadequate therapy, and so in controlling and reducing the occurrence of resistant pathogens. The use of guidelines can reduce the overall use of antibiotics, enhance the selection of adequate treatment, and minimize adverse drug effects, and is associated with more stable susceptibility patterns [39–41]. Similar effects have been shown when infectious disease specialists were responsible for the management of antimicrobial treatments. Guidelines should be developed and implemented at the local level, with input from all the relevant stakeholders, to increase acceptance and compliance in clinical practice, and to accommodate local patterns of antimicrobial resistance [1, 10, 33].

Another strategy to reduce the prevalence of antibiotic resistance is to restrict the hospital formulary, with the intent of reducing the use of particular antibiotics. Similarly, antibiotic cycling can potentially change the pattern of resistance in microorganisms. The utility of both these approaches is controversial, though they have been shown to be particularly successful during specific outbreaks with resistant pathogens [42, 43].

The selective use of narrow spectrum antibiotics can also reduce rates of antibiotic resistance [44]. Broad spectrum empiric antimicrobial therapy is commonly employed in the ICU, because of the challenges of establishing an infectious diagnosis and the prevalence of antimicrobial resistance among ICU isolates. Conversely, however, since the clinical course can be closely monitored in the ICU, a more restrictive approach to empiric therapy is intuitively appealing, and the focus of an ongoing clinical trial.

Mathematical models suggest that therapy with combinations of antibiotics can reduce rates of resistance [45], although whether this strategy is effective in clinical practice is unknown.

Selective decontamination of the digestive tract (SDD) is a prophylactic strategy whose objective is to lower the endogenous load of potentially pathogen organisms, including antibiotic-resistant Gram-negative bacteria, and so to reduce rates of infection and the need for antibiotics. SDD reduces mortality and Gram-negative antibiotic resistance in ICUs with low prevalence of MRSA and VRE; however, the protocol can lead to selection of resistant strains of Gram-positive pathogens [46].

Finally, treatment of antibiotic resistant organisms is at least partly dependent on the development and introduction of new antimicrobial agents [47]. The prospect is challenging. Minor structural alterations are unlikely to provide prolonged efficacy, and so entirely new classes of agents are needed. However, these agents are only effective if their use is tightly controlled, and so the development costs may not be covered if the resulting agents are used optimally.

Conclusion

Escalating rates of antimicrobial resistance among Gram-positive and Gram-negative bacteria provide particular challenges for the treatment of critically ill patients.

Widespread empiric antibiotic therapy in the ICU has fostered the emergence and dissemination of resistant bacteria. However, strategies aimed at controlling antibiotic use have been only partially successful. Normal host-microbial interactions are complex, and inappropriate use of antimicrobials can disrupt this fine balance, with deleterious consequences for both host and micro-organism: the line be-

tween benefit and harm is a fine one. More rational administration of antimicrobial agents and more reliable and rapid diagnostic technologies, are key priorities for future development.

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Fungal Infections

Systemic *Candida* Infection in the ICU

P. Svoboda and I. Kantorová

Introduction

Invasive fungal infections are a growing problem in the intensive care unit (ICU), with *Candida* species being the most common cause of these infections. *Candida* is now the forth or fifth most frequent pathogen isolated from bloodstream infections [1, 2]. In 2004, in order to summarize current knowledge about treatment of the different forms of invasive candidiasis, new guidelines for the treatment of candidiasis were published by experts on behalf of the Infectious Diseases Society of America (IDSA) [3]. The application of these guidelines to the treatment of systemic candidiasis in the ICU will be discussed, as well as new combination approaches to therapy, involving use of more than one antifungal agent.

Candida species and Candidiasis

Candida can cause not only usually mild, mucocutaneous infection, but also much more dangerous invasive candidiasis, also known as systemic or deep-seated candidiasis. Invasive candidiasis is associated with significant morbidity and high mortality [4–7]. It is a life-threatening infection, usually involving multiple organs, the *Candida* having disseminated via the bloodstream to organs such as the liver, spleen and kidney ('disseminated candidiasis'). Acute disseminated candidiasis is characterized by the rapid onset of fever, sometimes shock, and other signs of sepsis. Occasionally clinical signs such as the presence of retinal deposits or cutaneous nodules provide clues to the correct diagnosis but much more commonly the diagnosis is made on the basis of general signs or symptoms of active infection plus growth of *Candida* from the blood or another specimen taken from a normally sterile site. In immunocompromised patients, a presumptive diagnosis may be made based on multiple positive cultures from non-sterile sites. Because culture-confirmation is relatively slow compared to that achieved with bacterial infections, and may be negative even in the presence of subsequent proven fungal sepsis [8], antifungal therapy is sometimes given on an empiric basis, because early, aggressive administration has been associated with a lower mortality [9, 10].

Candida albicans is the commonest species associated with deep infection but other ("non-*albicans*") species are becoming increasingly common, of which *Candida glabrata* now dominates [11, 12]. This shift in the epidemiology of *Candida* species is probably a direct result of a significant increase in the use of fluconazole over the last decade, both as prophylaxis or long term suppressive therapy in high-risk patients and as first-line and empiric therapy in non-neutropenic patients. Flu-

conazole is used because it is less toxic than amphotericin B, but some non-*albicans* species, such as *C. krusei* and some strains of *C. glabrata,* are intrinsically resistant [11, 13]. Consequently, while fluconazole may reduce the incidence of candidiasis, its use has been associated with the emergence of fluconazole-resistance among strains of *C. albicans* [14, 15] and an increase generally in the incidence of azole-resistant infections [16]. Multiple *Candida* infections (with more than one species of *Candida*) may also now be more common. In a survey conducted in the USA among patients with candidemia between 1995–97 [12] multiple fungal infections occurred with a frequency of 5%. These had the highest *Candida*-attributable mortality (15%).

Because patients who succumb to this infection are already critically ill, the overall mortality is inevitably high. However, methods for distinguishing deaths due to *Candida* from other causes of death are well described in the published literature. Thus while, for example, in a large prospective survey of candidemia involving 1447 patients in the USA, the overall mortality was 47%, the *Candida*-attributable mortality was 10–15% [3]. The species associated with the highest *Candida*-attributable mortality was *C. albicans* (14%), rising to 15% when the infection was due to more than one species [12]. Most deaths from invasive candidiasis occur within 10–14 days of cultures from the blood or other clinically significant site becoming positive. The duration of a course of treatment in adults with candidemia, recommended by the IDSA guidelines, is 14 days after the last positive blood culture and resolution of signs and symptoms [3, 17, 18].

Existing Treatments

A number of antifungal products are available but the mortality and morbidity of invasive candidiasis is still high despite current best therapy. The frequency of persistent candidemia is 12–17%, while *Candida*-attributable mortality is 10–19% [3, 19, 20]. Antifungal drugs currently recommended for the treatment of invasive candidiasis by the IDSA are amphotericin B, fluconazole or caspofungin [3]. In each case these are given as monotherapy.

The IDSA makes an important point: extensive data from randomized trials are only available for acute blood-culture positive candidiasis and well-controlled trial-based data are lacking for other forms of invasive candidiasis (including intra-abdominal and urinary candidiasis, which are common in ICU patients).

Amphotericin B deoxycholate (Fungizone) has been the mainstay of treatment for systemic fungal infections since its introduction into clinical practice in 1959 [21]. It is a fungicidal drug with a broad spectrum of activity. However, clinical benefit can be limited by infusion-related toxic reactions (such as fever, rigors, chills and hypotension) and dose-limiting toxicity, especially to the kidney and liver. This toxicity limits the intravenous dose of amphotericin B deoxycholate, with the usual dose being 0.6 mg/kg, but doses can be increased to a maximum of 1 mg/kg of body weight per day if tolerated. Resistance to amphotericin B is rare (it is seen in some *C. lusitaniae* isolates), but some isolates of *C. glabrata* or *C. krusei* show reduced susceptibility, suggesting a need for the higher dose.

The toxicity of amphotericin B has been considerably reduced by the development of lipid-associated formulations, of which the most commonly used are Ambisome and Abelcet. The manufacturer's recommended dosage of these lipid-asso-

ciated formulations is 3 mg/kg for Ambisome and 5 mg/kg for Abelcet per day. Their superior safety profile has made them the new 'gold standard' for clinical practice and research [21]. This is particularly important for comparative trials being conducted in ICU patients, because the use of a lipid-associated formulation in place of amphotericin B deoxycholate avoids treatment-failures due to toxicity, rather than lack of efficacy, so that the actual baseline efficacy of amphotericin B can be determined. For the same reason, these agents are used in clinical practice in patients with actual or incipient organ failure, particularly renal failure, as is often the case in patients on ICU. In patients in whom organ failure is less prevalent, such as patients with cryptococcal meningitis, conventional amphotericin B is more commonly used.

An alternative means of reducing the toxicity of amphotericin B deoxycholate, is to give it as a 24-hour infusion [22, 23]. This provides a less costly alternative to lipid-associated formulations.

Fluconazole is an alternative treatment for candidemia in non-neutropenic patients, provided the species is not *C. krusei* or *C. glabrata,* these species being intrinsically resistant, though *C. glabrata* may respond to high doses [3, 11, 19]. Increasingly, strains of *C. albicans* are emerging which are resistant to fluconazole [14]. Fluconazole is largely used in non-neutropenic patients because it is not fungicidal, preventing the yeast from growing rather than killing it. Fluconazole is widely used in the treatment of superficial candidiasis and as empiric treatment in suspected cases of invasive candidiasis; but treatment is often subsequently switched to amphotericin B if the patient fails to respond clinically. The exponential rise in the use of fluconazole over the last decade, often on an empiric basis rather than for the treatment of documented infections, is probably responsible for keeping down the incidence of candidiasis but increasing the frequency of more azole-resistant fungi, such as *C. glabrata* [16].

Voriconazole is a new second-generation triazole, with a wider spectrum of activity than fluconazole. It has market approval for treatment of serious invasive *Candida* infections (including *C. krusei*). Its mechanism of action is similar to that of all azole agents, namely inhibition of cytochrome P450 14α-demethylase, and consequently carries the risk of cross-resistance with fluconazole-resistant strains [13]. Activity is fungistatic rather than fungicidal against yeasts [24]. Side-effects include visual disturbances, rashes, and hepatitis. There is marked interpatient variability in serum levels secondary to variation in the P450 genotype affecting metabolism. The potential for drug interactions is high because of its metabolism by cytochrome P450 isoenzymes. Dosage adjustment is necessary in patients with liver dysfunction and intravenous preparations should be avoided in patients with renal insufficiency [24]. The duration of therapy required is often long (>12 weeks).

Caspofungin is a member of the echinocandin family of antifungals. It inhibits synthesis of β (1,3)–D–glucan, an integral component of the fungal cell wall, and, like amphotericin B, is fungicidal against a wide range of *Candida* species. Efficacy against invasive candidiasis was comparable to amphotericin B deoxycholate in a comparative trial, although the amphotericin B treatment arm was at a disadvantage because of treatment failures due to toxicity [18, 25]. The *Candida*-attributable mortality rates in both treatment arms of this trial were lower (4–7%) than in other studies [12, 20] probably because the trial excluded patients who had failed on prior antifungal therapy and had very few patients with abdominal or thoracic infections (both carrying a poor prognosis) and a high proportion (18%) of pa-

tients with *C. parapsilosis,* which is associated with a low *Candida*-attributable mortality 2% [12]. The minimum inhibitory concentration (MIC) to caspofungin of *C. parapsilosis* is relatively high and this appears to be clinically significant, since 5 of the patients with candidemia due to *C. parapsilosis* remained persistently blood culture positive, compared to none in the amphotericin B-treatment arm [18, 25]. Caspofungin currently provides a useful alternative antifungal drug to amphotericin B. A concern with caspofungin is the occurrence of spontaneous mutations in the glucan synthase enzyme, *in vitro,* which have been associated with echinocandin-resistance in *C. albicans* [26]. In addition, caspofungin has potentially significant drug interactions with tacrolimus and cyclosporin, while cytochrome P450 inducers may decrease serum concentrations, necessitating dose increases [27].

In conclusion, none of these antifungals is of proven superiority in the treatment of systemic candidiasis in ICU patients. Apart from limited efficacy, there are issues regarding toxicity (amphotericin B, voriconazole), resistance/limited spectrum of activity (caspofungin, fluconazole), drug interactions (caspofungin, voriconazole), and interpatient variability in serum concentrations (voriconazole). Therefore combination therapy is increasingly being considered as a possible superior method of treatment, provided suitable combinations of antifungal drugs can be identified. An overview of a strategy for the treatment of invasive candidiasis is shown in Table 1.

Table 1. A strategy for the treatment of invasive candidiasis

First line therapy	**Rationale**
Abelcet or Ambisome	▮ Widely used when species and sensitivity unknown because of broad spectrum of activity ▮ Preferable to amphotericin B deoxycholate in ICU patients because much less nephrotoxic
Alternatives:	
Amphotericin B deoxycholate	▮ Less expensive but more toxic ▮ Continuous infusion may reduce toxicity
Caspofungin	▮ As effective as amphotericin B deoxycholate but better tolerated (more expensive) ▮ Some *C. parapsilosis* may be relatively resistant
Subsequent therapy	
Fluconazole	▮ Often used as first line therapy but if species and sensitivity unknown there is an increasing risk of fluconazole-resistance (*C. krusei* and some *C. glabrata* and *C. albicans*) ▮ Valuable as follow-up therapy, once the patient has stabilized and the organism has been shown to be fluconazole-sensitive in the laboratory – particularly in patients who have developed toxicity or to reduce cost
Future first-line therapy	
Synergistic combinations of antifungal drugs?	▮ More effective, shorter courses of treatment ▮ Greatly reduced risk of resistance developing

Combination Therapy

Combination therapy is now routinely used in the treatment of metastatic cancer and life-threatening infections with bacteria (tuberculosis, endocarditis, Gram-negative septicemia), viruses (human immunodeficiency virus [HIV], cytomegalovirus [CMV]) and drug-resistant malaria. The use of a combination of antifungal drugs was first introduced into clinical practice for the treatment of cryptococcal meningitis. This followed a study in non-acquired immunodeficiency syndrome (AIDS) patients, which showed that 6 weeks combined therapy with flucytosine and amphotericin B was associated with a more rapid clinical response than 10 weeks of monotherapy with amphotericin B [28]. A subsequent study in patients with AIDS showed that, at two weeks, this drug combination produced an enhanced rate of cerebrospinal fluid (CSF) sterilization and reduced mortality compared to amphotericin B alone [29].

Renewed interest in the use of combination therapy for the treatment of other fungal infections has been fuelled by the availability of new antifungal drugs and the failure to improve on the efficacy of amphotericin B [30–32]. However, not all drug combinations are advantageous. In the absence of definitive data from controlled clinical trials, laboratory data (*in vitro* sensitivity testing and animal models) have been used to predict the added clinical benefit from using combinations of the currently marketed antifungal drugs. The most obvious advantage would be if the enhanced antifungal activity of the combination exceeded the activity of the agents used in isolation (synergy). This is not necessarily the case. Some antifungal combinations may exhibit no added benefit (indifference) or may actually be less active than the single drug alone (antagonism). The bulk of the data on the pharmacodynamics of combinations of currently available antifungal drugs, based on *in vitro* testing and animal models of infection, suggest that there is little if any benefit to be derived from using them in combination to treat invasive candidiasis [13, 30]. A large, placebo-controlled study comparing high dose fluconazole (800 mg/day) with or without a 5–6 day course of amphotericin B deoxycholate in patients with candidemia failed to show a statistically significant increase in success rate [19].

A serious disadvantage of combination therapy would be if it increased the risk of toxic side-effects, either due to amphotericin B or one of the other many concomitant medications given to critically ill patients. Triazoles are generally safe and well tolerated but itraconazole and voriconazole are associated with a greater risk of hepatotoxicity than fluconazole and have the potential for a broader range of severe drug interactions due to their potent inhibition of cytochrome P450 3A4 [25, 33]. Caspofungin is generally well tolerated, but many of the mild, causally related adverse events, such as fever, phlebitis and nausea, experienced with monotherapy are also shared by amphotericin B [18].

New Possibilities in Combination Therapy

A new approach to the treatment of serious, culture-confirmed cases of invasive candidiasis, would be to combine an antifungal antibody with a conventional antifungal chemotherapeutic drug. Antibodies rapidly bind to and neutralize their target, are highly antigen-specific, are not dependent on liver metabolism or renal excretion, and are not influenced by concomitant medications. This makes antibody-based therapeutics highly attractive for the treatment of acute, life-threatening infections in ICU patients.

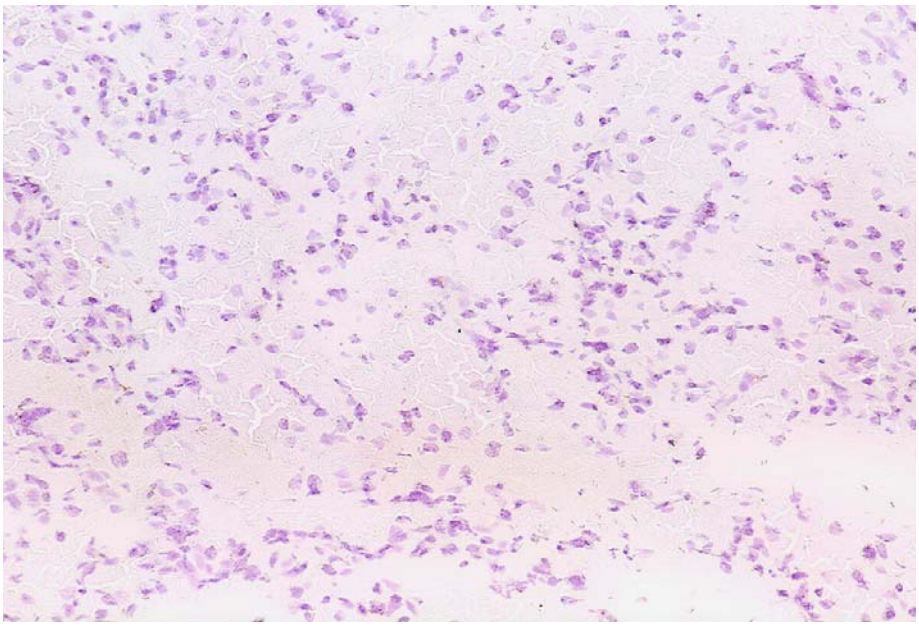

Fig. 1. Negative control: Infected mouse kidney without Mycograb (figure prepared by Inveresk Research, Tranent, Scotland)

The discovery of an antibody with antifungal activity began with the clinical observation that in patients with culture-confirmed systemic candidiasis there was a close correlation between the presence of antibody to the 47 kDa component of heat shock protein 90 (HSP90) and recovery, whereas patients who failed to produce this antibody invariably died [34]. Ultimately, this discovery led to the development of Mycograb, a human recombinant monoclonal antibody with the same epitope-specificity as the endogenous human antibody. Mycograb differs from other available antifungal drugs in having been particularly designed for use in combination with amphotericin B for the treatment of invasive candidiasis. This is because of the synergy shown against *Candida* when Mycograb is used in combination with amphotericin B both *in vitro* and *in vivo* [35]. Mycograb alone shows antifungal activity but the levels required are far higher than those which could be achieved systemically in patients.

In *Candida*, HSP90 is located in the cell wall (Figs. 1 and 2) where it probably plays a key role as a molecular chaperone in the folding of cell wall kinases. Hence, inhibition of this process weakens the cell wall, producing synergy with antifungal drugs active at the cell wall or cell membrane such as amphotericin B. In addition, Mycograb binds to extracellular HSP90 (released from lysed yeast cells or damaged human tissues) and may reverse HSP90-induced hypotension and inflammation.

In an initial report of a double-blind placebo-controlled trial in which patients with culture-confirmed invasive candidiasis received a lipid-associated formulation of amphotericin B with or without 5 days Mycograb [36], it was found that those on combination therapy were much more likely to have achieved clinical and culture-confirmed resolution of the infection by day 10 (47 out of 56 patients, 84%,

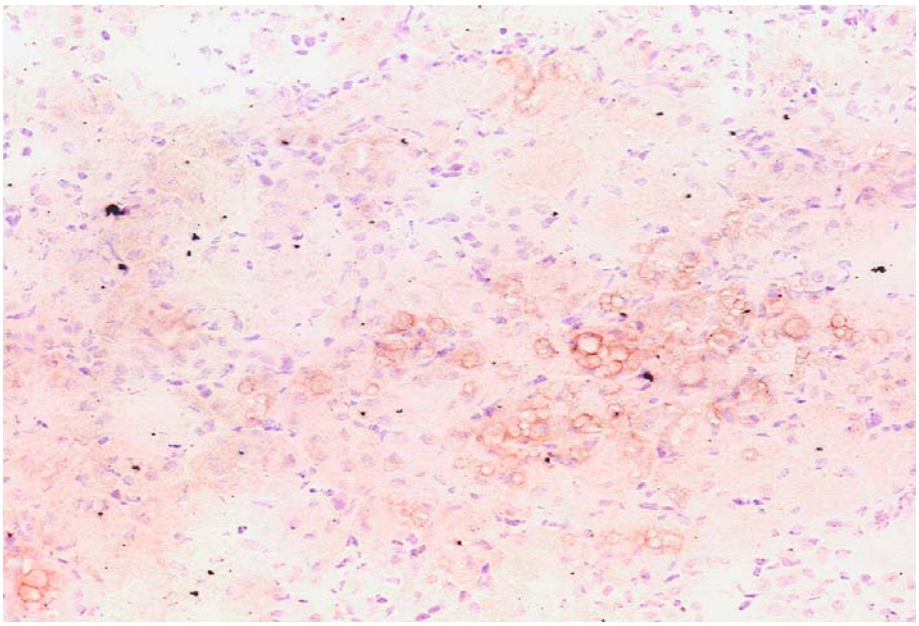

Fig. 2. Positive control: Infected mouse kidney probed with Mycograb (100 μg/ml) showing foci of yeast highlighted by heat shock protein 90 in the yeast cell wall and surrounding tissue (figure prepared by Inveresk Research, Tranent, Scotland)

compared to 29 out of 61, 48% on monotherapy, $p < 0.001$), and obtained culture-confirmed clearance of their infection over twice as quickly. Comparable synergy with amphotericin B against *Candida* has not been demonstrated for other new antifungal drugs, either in the laboratory or in clinical trials. Mycograb is also the first antifungal agent to have shown a highly statistically significant improvement in efficacy using monotherapy with lipid preparations of amphotericin B as comparator, rather than conventional amphotericin B.

Conclusion

The incidence of systemic *Candida* infection in the intensive care setting has been increasing over the last two decades. While *C. albicans* continues to be the commonest species, and has the highest associated mortality, there has been an increase in other species of *Candida,* such as *C. glabrata* and *C. krusei,* which are more likely to be intrinsically resistant to fluconazole. Therefore, while current IDSA guidelines recommend amphotericin B deoxycholate, caspofungin or fluconazole as primary therapy for adult non-neutropenic patients with candidemia, it is prudent to avoid using fluconazole unless the isolate is known to be sensitive to this drug. Antifungal trials comparing these drugs have focused on patients with candidemia and as a result there is a lack of data on their comparative efficacy in other forms of invasive candidiasis, such as intra-abdominal candidiasis, which can be particularly difficult to treat. There appears to be little to choose between these

drugs in terms of efficacy. Caspofungin and fluconazole are less toxic than amphotericin deoxycholate, but the toxicity of amphotericin B is much reduced if a lipid-associated formulation is used.

Combination therapy may provide a much needed means of reducing the morbidity and mortality of invasive candidiasis and reducing the emergence and spread of drug-resistant strains of *Candida,* which are much more likely to occur on prolonged courses of suboptimal monotherapy. A potential combination is amphotericin B with the antifungal antibody Mycograb. The added cost of such drug combinations will need to be evaluated in the context of the cost of the disease itself, as well as the savings which result from reduced reliance on widespread antifungal prophylaxis and empiric treatment as more effective therapy for proven cases becomes available, and shortened courses of antifungal treatment are possible due to faster resolution of the infection.

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Candida Colonization Index in the Management of Critically Ill Patients

P. Eggimann and D. Pittet

Introduction

Invasive candidiasis, which includes candidemia and severe *Candida* infections, remains a dreadful complication in hospitalized patients with a prognosis comparable to septic shock [1–3]. With incidences around 5 to 10 per 1000 intensive care unit (ICU) admissions, invasive candidiasis represents 5 to 10% of all nosocomial infections [4]. Difficult to diagnose, except for candidemia, which manifests only late in the course of the disease, early pre-emptive or empirical antifungal treatment has been shown to improve prognosis [5].

The high proportion of bone marrow transplant recipients developing invasive candidiasis has stimulated extensive clinical research, which has established the value of systematic prophylaxis with triazole compounds and early empirical or pre-emptive antifungal therapy for persisting fever of unknown origin. These aggressive strategies have progressively been imposed as a standard of care in severely neutropenic patients [6], as well as in solid organ transplant recipients [5]. However, they have been repeatedly implicated in the epidemiological shift, from *Candida albicans* to increasing proportions of non-albicans *Candida* in many cancer centers [7, 8]. This has generated considerable debate, and guidelines have been modified accordingly [9].

The situation is, fortunately, not the case in immunocompetent patients, in whom international surveillance programs have shown that *Candida albicans* remains the predominant strain in most countries [10]. More specifically, this is also the case in all recent series on candidiasis in ICU patients [11–16]. Many ICU patients present risk factors for invasive candidiasis, and a large proportion of them become colonized with *Candida* species during their stay, but only a minority will develop an invasive candidiasis [11, 12, 15, 17–19]. However, it is difficulty to identify subgroups of patients likely to benefit from prophylaxis and a majority of clinicians systematically treat all colonized patients [20, 21]. With the experience acquired from immunocompromised patients, such practice may have a strong negative impact on the ecology of *Candida* species by selecting resistant strains, and it should be avoided [22].

The majority of risk factors for invasive candidiasis are directly linked to an underlying disease or to its treatments and it is not possible to target them for prevention [4]. However, a progressive increase in colonization almost invariably predicts the development of invasive candidiasis [11–13, 18, 23–26]. This dynamic may be taken into account by determination of the colonization index, and we proposed to integrate this index in a clinical pathway to help in the early identification of critically ill patients likely to benefit from early empirical antifungal treatment [27]. This chapter reviews the rationale for using the colonization index.

Pathophysiology of Invasive Candidiasis

Candida species are part of the endogenous flora and may be found in the digestive tract of nearly half the population. Colonization, which is a prerequisite for the development of candidiasis, develops following alterations of the endogenous flora promoting *Candida* overgrowth on mucosal and skin surfaces [18]. Repetitive and/or continuous exposure to risk factors promotes further invasion (Fig. 1). Carefully-designed studies using genotyping techniques for the identification of strains of *Candida* confirmed that colonization from an endogenous source is responsible for a large majority of cases of severe candidiasis [28]. Nosocomial exogenous cross-transmission of *Candida* species has also been described, but infection control measures should control this source [29].

Many risk factors predicting the development of a candidiasis have been identified over the last three decades (Table 1) [18, 25, 30, 31]. Colonization by *Candida* species independently predicts candidiasis [17, 18, 24, 32, 33]. Sequential spread from the abdominal cavity to other body sites before candidemia occurs was demonstrated early in the 1980s, and heavy and/or increasing growth of *Candida* species in fluids obtained from the peritoneal cavity is predictive of subsequent invasive candidiasis [11, 14, 17, 32, 34].

In critically ill patients, it may, however, be difficult to differentiate colonization from invasive candidiasis. At ICU entry, only 5 to 15% of patients are colonized by *Candida* species, but this proportion may increase up to 50 to 80% with time and exposure to risk factors [11, 14, 33, 34]. However, only 5 to 30% of these patients will develop true invasive candidiasis [11–13]. Thus, the clinical significance of surveillance cultures positive for *Candida* species is difficult to assess.

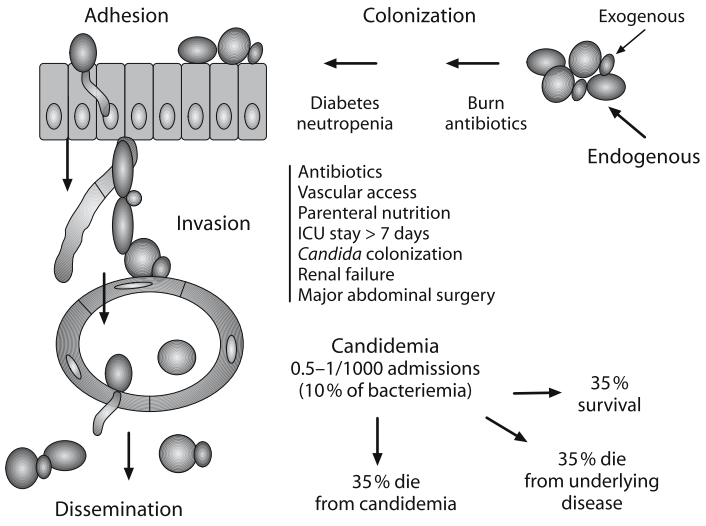

Fig. 1. Pathophysiology of invasive candidiasis. From [4] with permission

Table 1. Risk factors predisposing to the development of severe candidiasis

High risk factors	Non-specific risk factors
▮ Colonization of several body sites	▮ Young and old ages
▮ Broad-spectrum antibiotics	▮ Diabetes
▮ Immunosuppression	▮ Renal failure
▮ Neutropenia	▮ Recent surgery
▮ Burns (> 50%)	▮ Urinary catheter
▮ Perforation of the digestive tract	▮ Vascular access
▮ Major abdominal surgery	▮ Prolonged ICU stay (> 7 days)
▮ Surgery on the urinary tract (if candiduria)	▮ Multiple transfusion
▮ Major trauma (ISS > 20)	
▮ Parenteral nutrition	
▮ Hemodialysis	
▮ APACHE Score II > 20	
▮ Central venous catheter	
▮ Candiduria > 10^5 cfu/ml	

▮ Diagnosis of Candidiasis in Critically Ill Patients

Except for candidemia, invasive candidiasis is difficult to diagnose. Clinical signs are identical to those of any other nosocomial infection, and more specific manifestations, such as retinal emboli or hepato-splenic involvement are rare [35]. Cultures other than blood, or from normally sterile body sites, are nonspecific and may become positive only late in the course of infection. In contrast to *Aspergillus* species, and despite recent advances in experience with mannan antigens and anti-mannan antibodies, biological tools have not helped with the diagnosis of candidiasis [36]. In addition, using clinical and microbiological criteria currently available, the threshold between colonization and infection may be difficult to distinguish [4]. In contrast, this is not the case for colonization by *Candida* species. As assessed by the colonization index proposed by Pittet et al. in 1994 and recently confirmed by others, increasing growth of *Candida* species from multiple body sites is predictive of subsequent invasive candidiasis [12–15, 18, 37]. By restricting this evaluation of the dynamics of colonization to the subset of critically ill patients with persistence of other risk factor for invasive candidiasis, this approach may help to select critically ill patients likely to benefit from early empirical antifungal treatment. This may also avoid overexposure of ICU patients to antifungal agents [27].

▮ Colonization Index in Critically Ill Patients

Some experts have suggested that in cases of clinical suspicion of candidiasis, the presence of *Candida* species in more than two body sites may be sufficient to justify the initiation of antifungal therapy [32, 38]. In a prospective cohort study of critically-ill, surgical patients, Pittet et al. showed that the dynamics of *Candida* colonization are better evaluated by a colonization index [18]. This index was deter-

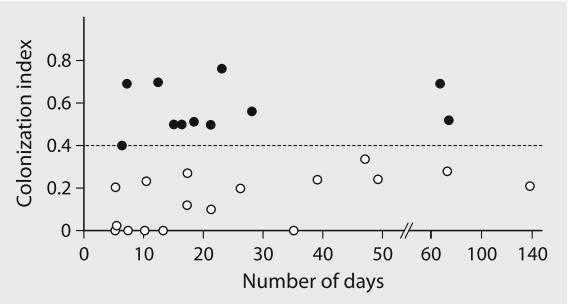

Fig. 2. The colonization index is defined as the ratio of the number of non-blood distinct body sites colonized by *Candida* species to the total number of distinct body sites tested. It was recorded for each patient from the first day of colonization until discharge from the ICU among non-infected patients and until time of severe candidiasis among infected patients. Black circles: patients who developed severe candidiasis. White circles: patients who remained colonized. From [18] with permission

mined daily as the ratio of the number of distinct body sites colonized with genotypically identical strains of *Candida* species over the total number of sites tested (Fig. 2) [39]. Twenty-nine of 650 patients admitted in the surgical ICU were colonized at several distinct body sites. Eleven of 29 patients developed severe *Candida* infection, including candidemia in eight. The other 18 patients remained colonized but did not develop candidiasis. The severity of illness and the degree of colonization independently predicted the development of invasive candidiasis among colonized patients. The average *Candida* colonization index was 0.47 in colonized vs. 0.70 in infected patients, respectively ($p < 0.01$). A threshold of ≥ 0.5 correctly identified all infected patients, and this value was reached at an average of 6 days before documented candidiasis. This delay may open the door for early empirical antifungal treatment.

The predictive value of this index has never been tested in a large prospective clinical trial, but at least seven studies suggest that it may be clinically useful. Dubau et al. determined the colonization index in 89 of 669 consecutive patients staying for more than 7 days in a surgical ICU, or in whom the protein C level was greater than 100 mg/ml [37]. Of the 35 patients with an index above 0.5 empirically treated with antifungals, only one developed candidiasis and the degree of colonization rapidly decreased in the 34 other patients. In a survey on candiduria performed in French ICUs, Chabasse found a correlation between quantitative cultures above 10^4 cfu/ml and a colonization index ≥ 0.5 [40]. Garbino et al. prospectively determined the colonization index in all patients included in a double-blind, placebo-controlled study on antifungal prophylaxis in critically ill patients mechanically ventilated for at least 5 days [13]. *Candida* colonization developed in 53% (29/55) of patients free of colonization at study entry in the fluconazole group compared to 78% (40/51) of patients in the placebo group. Colonization increased over time in the latter group but decreased in the former. *Candida* infection occurred less frequently in patients in the fluconazole group and 90% of candidemias developed in patients in the placebo group. Moreover, candidemia developed only in patients heavily colonized with *Candida* species. In a ten-year retrospective cohort study on 51 cases of candidemia in the ICU, Charles et al. reported a prior high-density of colonization by *Candida* species with a colonization index ≥ 0.5 in 21 (45.6%) of

the 46 assessed patients (0.56 ± 0.31). At the onset of candidemia, the colonization index was significantly higher in medical patients compared to surgical patients, (0.74 ± 0.31 versus 0.45 ± 0.40, $p = 0.01$) [41]. In a further prospective study on 92 non-neutropenic patients consecutively admitted on a medical ICU for more than 7 days, the same group evaluated the dynamics of colonization by performing the colonization index weekly [42]. The colonization index increased significantly by 0.10 over the ICU stay ($p = 0.016$) and the threshold of 0.5 was reached in 36 patients (39.1%). Invasive candidiasis developed in six patients, in whom the colonization index was ≥ 0.5, compared to three in whom it was < 0.5 (p value non significant). However, significantly more patients with a colonization index ≥ 0.5 received antifungal therapy for more than 2 days: 14/36 (61.1%) versus 7/56 (12.5%), respectively. Hematological malignancy, duration of exposure to broad-spectrum antibiotics, fungal colonization at entry and candiduria predicted an increase in the colonization index. In contrast, the duration of exposure to antifungals was significantly associated with a decrease in the index. In a before/after trial, Piarroux et al. prospectively screened 478 surgical ICU patients for *Candida* species colonization. These patients received preemptive antifungal treatment if the corrected colonization index was > 0.4 [15]. Compared to a historical cohort of 455 controls, invasive candidiasis decreased from 7.0 to 3.6%, respectively. Moreover, this strategy completely prevented the development of ICU-acquired invasive candidiasis. Finally, in an open-label study, 98 patients mechanically ventilated for more than 48 hours were randomized by Normand et al. to receive prophylaxis with oral nystatin or a placebo. No invasive candidiasis developed in these low-risk patients, but prophylaxis significantly reduced the colonization index and prevented colonization [43].

Pre-emptive Empirical Antifungal Treatment in Critically Ill Patients

Pre-emptive therapy is early antifungal treatment given to patients with risk factors for infection and significant *Candida* species colonization. However, overexposure of critically ill patients to antifungals may promote the emergence of resistant strains and empirical treatment must be strictly limited to patients likely to benefit from it [7, 8]. As discussed in the previous section, periodic determination of the colonization index is currently the best way to adequately select these patients [22].

Management Strategy for Immunocompetent Patients

A large proportion of critically ill patients have several risk factors for *Candida* infection. A high proportion of such patients may become colonized with *Candida* species during their hospital stay, but only a minority will develop severe candidiasis.

Review of the indications for antifungal prophylaxis is beyond the scope of this paper. The reader can find extensive reviews elsewhere [27, 44, 45]. In brief, despite a series of at least 11 clinical studies suggesting a benefit of antifungal prophylaxis in critically ill immunocompetent patients, none of them reached sufficient power and this approach is considered as insufficiently validated. Accordingly, antifungal prophylaxis is not included in most published guidelines [5, 38, 46]. However, antifungal prophylaxis should nevertheless be considered for selected groups of patients

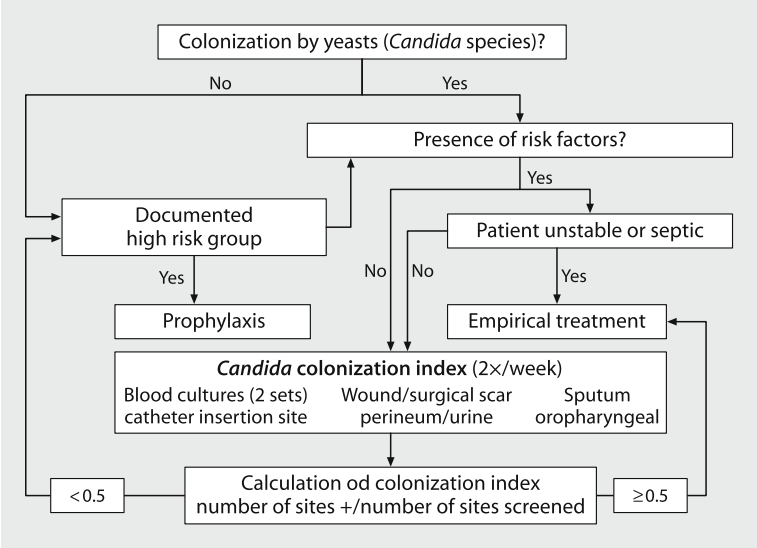

Fig. 3. *Candida* colonization index in the management of clinical suspicion of candidiasis. Adapted from [27] with permission

in whom the incidence of candidemia is sufficiently high to be beneficial [11–13, 47–50]. In any case, prospective surveillance of the proportion of strains of non-albicans *Candida* should be organized.

Apart from a positive fundoscopic examination, which is relatively infrequent during candidemia, the clinical manifestations of invasive candidiasis are non-specific, and, the diagnosis is made only late in the course of the disease [7]. Nevertheless, pre-emptive antifungal therapy should be restricted to unstable critically ill patients with well-established risk factors, such as those with a septic shock of intra-abdominal origin [49]. For patients in whom the immediate start of antifungal treatment is not justified by their severe clinical condition, considering the dynamics of colonization using the colonization index will allow early detection of those patients who might also benefit from pre-emptive antifungal treatment. Although not tested in prospective multicenter trials, we propose a strategy for the clinical diagnosis and practical management of critically ill patients suspected, or at risk, of severe candidiasis, which distinguishes prophylactic from pre-emptive therapy (Fig. 3) [27].

Conclusion

In conclusion, invasive candidiasis is a severe complication with mortality rates comparable to those of septic shock (40% to 60%), and represents 5 to 10% of all nosocomial infections. Many ICU patients cumulate risk factors for invasive candidiasis, and a large proportion become colonized with *Candida* species, although only a minority will develop invasive candidiasis [11, 12, 15, 18, 19]. However, it is difficulty to identify subgroups of patients likely to benefit from preemptive treat-

ment and a majority of clinicians will systematically treat all colonized patients [21]. Following lessons leaned in immunocompromised patients, such practice should be avoided. As biological tools are currently unavailable at the bedside, empirical treatment relies on clinical strategies aimed at identifying the subgroup of patients likely to benefit from it. A progressive increase in *Candida* colonization predicts the development of an invasive infection and the colonization index takes these dynamics into account. Starting empirical antifungals only when the colonization index reaches a threshold value that predicts subsequent candidiasis will avoid overexposure of critically ill patients to antifungal drugs.

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Antifungal Therapy in Surgical ICU Patients

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Introduction

The incidence of fungal infections has increased dramatically over a 20-year-period by 207% [1]. Fungi are the fourth leading pathogen in nosocomial infections in the USA. Five percent of all cases of sepsis are caused by fungal infections. The incidence of candidemia, which constitutes the majority of fungal nosocomial pathogens, in non-neutropenic surgical patients is 9.8/10,000 intensive care unit (ICU) days [2]. The mortality associated with systemic fungal infections remains high (20–60%). New therapeutic options, like modern triazole derivates (e.g., voriconazole) and the new echinocandin agents (e.g., caspofungin) or lipid-formulations of amphotericin B provide new options for the antifungal treatment of surgical ICU patients. In the light of limited diagnostic options, considerable costs, and the high mortality of fungal infections, therapeutic strategies should be clearly defined in appropriate guidelines.

Epidemiology of Fungal Infections

In the past two decades, fungal infections have become increasingly apparent in critically ill patients. Yeasts like *Candida* species are the predominant fungal pathogens in intensive care units (ICUs) both in Europe and the United States, with reported rates ranging from 2.1 to 20.0 per 1000 admissions. In non-neutropenic surgical patients, the majority of fungal nosocomial pathogens are *Candida* species [3]. *Candida albicans* accounts for about 59% of these and important additional *Candida* species include *Candida krusei* and *Candida glabrata*. The number of these non-albicans candidemias has increased constantly to about 44%, which is called a 'shifting epidemiology'. *C. krusei* has primary resistance against fluconazole and *C. glabrata* frequently develops resistance to fluconazole and itraconazole [4–6]. Some authors have attributed this increased emergence of non-albicans species to the use of fluconazole, whereas other reports have failed to find a clear epidemiological association [5–7]. Antifungal prophylaxis has proved effective in certain high-risk patients, such as bone marrow transplant and liver transplant patients [8, 9]. Due to the lack of reliable diagnostic tests and limited therapeutic options, fungal infections are associated with a high overall mortality (35–80%) [10].

The major risk factors for invasive fungal infections are colonization of at least two sites with *Candida*, previous broadspectrum antibiotic therapy, gastrointestinal surgery or gastrointestinal perforation, acute renal and liver failure, invasive devices such as central venous catheters, immunosuppression, parenteral nutrition, high

APACHE II score, and length of ICU stay (LOS) [2, 6, 11]. In the NEMIS study, most cases of candidemia (76%) occurred within the first 3 weeks of admission to a surgical ICU [2].

Molds, like *Aspergillus* species, are of particular interest to immunocompromised patients. The currently growing number of patients receiving systemic corticoid therapy for internal disease, or immunosuppression after solid-organ transplantation paves the way for a significant rise in these fungal infections in surgical ICUs. Nevertheless, patients without drugs for immunosuppression can also acquire *Aspergillus* infections, especially after severe peritonitis.

Symptoms and Diagnosis of Fungal Infections

The diagnosis of a systemic fungal infection is very difficult, particularly in non-neutropenic surgical patients. In case of a septic syndrome which shows no response to a broadspectrum antibiotic therapy, a disseminated fungal infection must be taken into consideration [12].

Histologically proven invasive fungal growth in a biopsy of sterile tissues ensures the diagnosis. Positive blood cultures confirm the diagnosis of a fungal sepsis, however, their sensitivity is just 70%. Intraoperative samples, percutanous punctures, drainage fluids and urine cultures could lead to the diagnosis of fungal infection, but do not really prove an infection.

Candida species belong to the habitual microflora, especially in the gastrointestinal tract. In most cases this normal colonization serves as an endogenous source for infection [13]. Candidal colonization in critically ill patients precedes, and can lead to, infection. If multiple body sites are colonized, there is an increased risk of a severe infection in high-risk patients, and the chance of invasion can be predicted by the extent of preexisting colonization [14–17]. However, the therapeutic relevance of candidal colonization is still under discussion. *Candida* infections range from candidemia, often associated with *Candida* catheter infections, to invasive candidiasis in multiple organs. A primary *Candida* pneumonia is a rare event.

Aspergillus spores are ubiquitous in the surroundings, especially in soil and water. Unfiltered air of ventilation systems, dust during construction work, carpeting, food, plants, and water supply systems constitute the reservoirs in hospitals. *Aspergillus* species are acquired exogenously, most by inhalation, and less frequently through damaged mucocutaneous surfaces (e.g., following surgery, contaminated equipment). Invasive pulmonary aspergillosis is the most common *Aspergillus* infection in surgical ICU patients, however, a hematogenous dissemination to other organs, e.g., brain or liver is possible [18].

Therapeutic Options in Fungal Infections

The various antifungal agents have different targets of effectiveness, including molecules of the cytoplasma wall, particularly ergosterol, cytoplasmic enzymes, and nucleic acid synthesis. Because of these different targets a combination of various antifungal drugs could make sense to achieve synergistic effects. In principle, antifungal agents can be divided into drugs with fungistatic or fungicidal activity. Pharmacological data and susceptibility patterns for the different antimycotics are shown in Tables 1 and 2.

Table 1. Pharmacology of antifungal agents

	Loading dose	Parenteral dose	Enteral dose	Plasma half-time (hours)	Elimination	Protein binding	Cerebral penetration
Amphotericin B deoxycholate	No	0.5–1.5 mg/kg in 1–6 h	–	15–24	renal	95%	< 10%
Liposomal amphotericin B	No	3–6 mg/kg in 0.5–1 h	-	174	?	90%	< 10%
Flucytosine	No	100 mg/kg every 6 h	–	3–6	renal	minimal	> 75%
Caspofungin	70 mg/day 1	50 mg/day > 80 kg: 70 mg/day	–	9–11	hepatic	97%	10%
Fluconazole	10–12 mg/kg day 1	400–(800) mg per day or 5–6 mg/kg	100–400 mg per day	24–30	hepatic	10%	80%
Itraconazole	200 mg twice a day for 2 days	200–(400) mg per day	200 mg per day	19–22	hepatic	99%	< 10%
Voriconazole	Parenteral: 6 mg/kg twice a day oral: ≥ 40 kg 2×400 mg < 40 kg 2×200 mg	4 mg/kg twice a day	≥ 40 kg: 2×200 mg < 40 kg: 2×100 mg	6–9	hepatic	60%	60%
Posaconazole	No	–	800 mg	25–31	hepatic	90%	< 10%

Polyenes like amphotericin B form an irreversible complex with ergosterol, which alters membrane fluidity and makes the fungal cell wall permeable for the loss of cytoplasmic elements. Therefore, the lipophilic polyene shows fungicidal activity to almost all fungi, both yeasts and molds. They have no bioavailability for enteral absorption, display a long half-life, and have important renal toxicity.

In the absence of other alternatives, amphotericin B deoxycholate has long been the standard therapy for life-threatening systemic fungal infections, although it has many adverse effects. The acute renal failure induced by amphotericin B therapy is the most relevant for ICU patients because it is often dose-limiting, is sometimes irreversible, and can prolong length of stay and increase costs [19, 20]. The development of three lipid formulations of amphotericin B (amphotericin B lipid complex, amphotericin B colloidal dispersion, and liposomal amphotericin B) offers the possibility to use preparations with reduced toxicity. Many studies show strong evidence that lipid formulations of amphotericin B may be equal or more effective, and are consistently less toxic than amphotericin B deoxycholate. Given their supe-

rior safety profiles, these preparations can be considered suitable replacements for conventional amphotericin B [21]. However, the available alternative agents, which show a reduced frequency of drug-related toxicity, may be more cost-effective, especially among patients at high risk of developing renal failure. Thus, we conclude that, at least, the use of amphotericin B deoxycholate in ICU patients with organ dysfunction must be limited to indication where less toxic agents are not effective. Flucytosine is used for combination therapy with amphotericin B in candidiasis. Currently, this anti-metabolite of cytosine does not play a role in the therapy of surgical ICU patients.

Azoles are the second group of antifungal agents which develop their activity through the inhibition of ergosterol biosynthesis by blockade of the fungal cytochrome P450 enzyme, lanosterol 14-desmethylase. All azoles are fungistatic and effective against *Candida* species and *Cryptococcus*. Additionally, modern azoles, such as voriconazole, show fungicidal activity against *Aspergillus* species and *Fusarium* species. However, azole antifungals interfere with a number of human hepatic and intestinal cytochrome P450 subtypes that are responsible for the metabolism of a plethora of other therapeutic agents. The P450 isoenzymes which are involved in the metabolism of azole antifungal agents include CYP 3A4 (ketoconazole, fluconazole, itraconazole, voriconazole and posaconazole), CYP 2C9 (ketoconazole, fluconazole and voriconazole) and CYP 2C19 (ketoconazole, fluconazole and voriconazole). Thus, azoles produce drug interactions with a variety of drug classes, including H1-antihistamines, antineoplastics, steroids, antimicrobials, antiretrovirals, opioids, cardiovascular agents, psychotropics and oral contraceptives [22].

Fluconazole is a triazol-derivate with a high bioavailability (>90%), and oral as well as parenteral application are possible. Fluconazole is (fungistatic) active against yeasts including *Candida* species and *Cryptococcus neoformans*, *Coccidioides* and *Histoplasma* species. However, 9% of *C. albicans* are already resistant to fluconazole [4]. Fluconazole has a dose dependent activity against *C. glabrata*, whereas *C. krusei* is completely unsusceptible to fluconazole. Nearly one-third of all non-albicans species is not affected by fluconazole. Fluconazole has no activity against any molds. Because of the lack of other alternatives, fluconazole was the traditional first-line antifungal agent for therapy and prophylaxis. But the newer epidemiological shift to resistant *Candida* species and the importance of aspergillosis in immunocompromised patients limits the use of fluconazole in patients with acute life-threatening fungal infections in surgical ICUs.

Itraconazole is a well known triazole with a broader spectrum than fluconazole including *in vitro* activity against *Aspergillus* and is usually used for oral therapy of mucosal forms of candidiasis. However, its variable bioavailablity makes itraconazole unattractive for life threatening invasive fungal infections. An intravenous preparation of itraconazole solubilized with cyclodextrin has been licensed recently. However, studies of intravenous itraconazole for ICU patients with invasive fungal infections are not yet available, thus, there are no recommendation for its therapeutic administration in this setting [23].

Voriconazole is a second generation triazole with high structural similarities to fluconazole but with a broader antifungal spectrum including resistant *Candida* species and molds exclusive of *Mucor* species. Its excellent bioavailability enables oral and parenteral application. Voriconazole exhibits non-linear pharmacokinetics due to saturation of its metabolism. A greater than proportional increase in exposure is observed with increasing intravenous or oral doses. Because of the non-linear pharmacokinetics, the apparent terminal half-life is dose dependent and is not

predictive of the accumulation or elimination of voriconazole. No dosage reduction for voriconazole is needed in acute or chronic renal failure or acute hepatic failure. Voriconazole for intravenous administration is solubilized in a cyclodextrin excipient that accumulates in renal failure, which limits its utility in this setting [24]. For oral therapy a liquid formulation is available, which may be an alternative for ICU patients. In case of moderate chronic liver cirrhosis (Child A, B) the dosage should be reduced by half after a normal initial loading dose. In general, voriconazole is well tolerated. Important infusion-related adverse side effects are reversible abnormal vision, abnormal liver function tests, and worsening renal function. Polymorphisms in hepatic elimination capacity by isoenzymes CYP 2C19 and chronic liver disease influence plasma concentration of voriconazole, which is why some authors recommend drug monitoring [25].

Posaconazole and ravuconazole are new investigational azoles with promising results in clinical studies. Posaconazole has structural similarities with itraconazole. However, in contrast to this and other azoles, first clinical studies for salvage therapy showed that posaconazole possess a wider activity spectrum in molds including *Mucor* species, *Fusarium* species, and *Rhizopus* species [26]. Clinical studies are only available for oral administration of posaconazole. It is a light inhibitor of CYP 3A4 and only few drug interactions have been noted so far. The most common treatment-related adverse events are gastrointestinal dysfunction.

Echinocandins are a new group of antifungal agents which inhibit the synthesis of cell wall glucans. All echinocandins possess fungicidal activity against yeasts and are fungistatic against *Aspergillus* species, but not against *Cryptococcus* and other molds like *Zygomycetes* or *Fusarium* species. They are hydrophilic, but have no good bioavailability which limits their use to the intravenous route. Adverse events are generally mild, including local phlebitis, fever, mild hemolysis, and abnormal liver function tests. Echinocandins are metabolized by the liver and a dose reduction is recommended for patients with liver failure. No important renal toxicity has been during therapy with echinocandins. Echinocandins are poor substrates for the cytochrome P450 enzymes, so fewer drug interactions are described for these agents than for the azoles [27].

Caspofungin is the first licensed echinocandin drug. Slight increases in caspofungin clearance have been seen with powerful inducers or inhibitors of hepatic metabolism, such as phenytoin, carbamazepine, and dexamethasone, so a slight increase in daily caspofungin dose (70 mg) is appropriate. A slightly reduced exposure to tacrolimus (20%) was seen with co-administration of caspofungin, and monitoring of tacrolimus concentrations was recommended. Caspofungin and cyclosporin do seem to interact, resulting in raised caspofungin plasma concentrations due to a limited uptake of caspofungin into the liver; no change in the amount of cyclosporin in whole blood is seen [27]. Due to its good activity against all *Candida* and *Aspergillus* species and its low toxicity, caspofungin is an attractive antifungal agent for critically ill patients with organ dysfunction.

Micafungin is another echinocandin, which will be licensed in Europe soon. No substantial differences are expected regarding its antimycotic activity compared to caspofungin. Drug interactions seem not to be a problem; in particular, no effects are seen in combinations with cyclosporin or tacrolimus [27].

Anidulafungin is another investigational echinocandin but only limited data are available.

Table 2. Patterns of fungal susceptibility

	Fluconazole	Itraconazole	Voriconazole	Posaconazole	Caspofungin	Micafungin	Anidulafungin	Flucytosine	Amphotericin B
Yeasts									
Candida Species									
C. albicans	S	S	S	S	S	S	S	S	S
C. glabrata	SDD-R	SDD-R	S-I	S-I	S	S	S	S	S-I
C. parapsilosis	S	S	S	S	S-I	?	?	S	S
C. tropicalis	S	S	S	S	S	S	S	S	S
C. krusei	R	SDD-R	S-I	S-I	S	S	S	I-R	S-I
C. lusitaniae	S	S	S	S	S	S	S	S	S-R
Cryptococcus neoformans	S	S	S	S	R	R	R	S	S
Molds									
Aspergillus Species									
A. flavus	R	S	S	S	S	S	S	I-R	SDD-R
A. fumigatus	R	S	S	S	S	S	S	I-R	S
A. terreus	R	S	S	S	S	S	S	I-R	SDD-R
Zygomycetes									
Mucor spp.	R	R	R	S	R	R	R	R	S
Rhizopus spp.	R	R	R	S	R	R	R	R	S
Fusarium spp.	R	R	S	S	R	R	R	R	SDD-R
Scedosporium	R	R	S	S	R	R	R	R	SDD-R
Pneumocystis jiroveci	R	R	R	R	S	?	?	R	R

I: intermediately resistant; R: resistant; S: susceptible; S-DD: susceptible-dose/delivery dependent

Therapeutic Strategies

For treatment of fungal infections, there are two main questions:
1) when should antifungal therapy be applied, and
2) which agent is appropriate?

Four fundamental strategies for the use of antifungal prophylaxis/therapy exist:
1) prophylactic application to reduce infection rates of patients at risk without any symptoms of infection
2) empiric therapy of symptomatic patients deemed to be at high risk for invasive fungal infection
3) pre-emptive therapy of patients at risk who possess some surrogate clinical, radiological, or laboratory markers of an invasive fungal infections that suggest the presence of an invasive fungal infection, but without any reliable evidence, and finally
4) therapy of a proven invasive mycotic infection.

Prophylaxis

In most clinical trials, fluconazole has been used for antimycotic prophylaxis. It has been shown to be effective against *Candida* mycoses in certain high-risk patients, such as bone marrow transplant and liver transplant patients [8, 9]. Shorr et al. [28] published a metaanalysis of fluconazole prophylaxis in critically ill surgical patients, which showed that prophylactic fluconazole therapy might decrease the rate of fungal infections, but this strategy did not improve the survival of surgical ICU patients [29, 30]. Due to the lack of a survival advantage, routine fluconazole prophylaxis in surgical ICU patients has to be evaluated in light of drug side effects, costs, and the potential for development of resistance and selection of non-albicans isolates. It is currently unclear whether prophylactic antifungal treatment has beneficial effects when broadly used in surgical ICUs.

Empiric and Pre-emptive Therapy

The high morbidity and mortality of disseminated fungal infections necessitate early therapy starting when further complications can still be reduced. However, inadequate diagnostic opportunities make the indications for antifungal therapy difficult to determine. Widespread use of inappropriate antimycotic therapy may have deleterious epidemiological consequences, including selection of resistant organisms. In the guidelines for the treatment of candidiasis [23], empirical therapy is recommended for patients with

1) *Candida* species colonization (preferably at multiple sites [15]),
2) multiple other risk factors like prolonged use of antibiotics, presence of central venous catheters, hyperalimentation, surgery (especially surgery that transects the gut wall), and prolonged ICU stay, and
3) absence of any other uncorrected causes of fever.

However, colonization of at least two sterile sites usually should not be treated because a reduction in mortality rates has not been demonstrated. Even the recovery of *Candida* species from urine samples is not associated with a significant increase of subsequent candidemia [2]. The absence of colonization by *Candida* species indicates a lower risk for invasive candidiasis and warrants delaying empirical therapy.

As treatment options, amphotericin B and, for a limited spectrum of *Candida* species, fluconazole, are mentioned in the guidelines [23]. The various studies published regarding currently available alternative antifungal drugs have been performed for the empirical therapy of persistent fever in patients with neutropenia. Caspofungin [31] and voriconazole [32] were as efficacious as liposomal amphotericin B with fewer therapy related adverse side effects and a tendency to lower rates of breakthrough infections. For empirical antimycotic treatment of surgical patients, caspofungin and voriconazole both appear to be suitable, and perhaps preferable, alternatives to conventional and liposomal amphotericin B. The important issue now facing clinicians is to determine which patients are most likely to benefit from empirical antifungal therapy [33]. So far, empiric antifungal therapy is not part of the therapy of non-neutropenic septic patients [14, 34].

If empirical therapy is started with a modern broad-spectrum antifungal agent, and the fungal pathogen is verified as being sensitive to a narrower spectrum antimycotic, the therapy regime could be de-escalated, e.g., to fluconazole for *C. albicans*.

Candidiasis

Guideline therapy for candidemia in non-neutropenic patients [23] is amphotercin B, or fluconazole, or caspofungin. Caspofungin was at least as effective, and less toxic, as amphotericin B for the treatment of candidemia in the randomized, double-blind study by Mora-Duarte et al. [35]. In addition, voriconazole has promise as a salvage agent for the treatment of invasive candidiasis, even in the setting of previous azole therapy [36, 37], and was as effective as amphotericin B followed by fluconazole in the primary therapy of invasive candidiasis [38]. Thus, both seem to be favorable alternatives for critically ill patients. As combination therapy, amphotercin B plus fluconazole for 4 to 7 days, followed by single use of fluconazole [39] could be a further alternative, but drug toxicity of amphotericin B has to be taken in account. All therapies should be given until 14 days after the last positive blood culture and resolution of infectious symptoms. Intravascular catheters should be removed or exchanged [23].

Large randomized, double-blind studies are available for the therapy of mucocutaneous candidiasis, like candidal esophagitis, with caspofungin that appeared to be as effective as amphotericin B [40, 41] or fluconazole [42] and is generally a well tolerated therapy. Voriconazole showed at least the same activity as fluconazole in the treatment of candidal esophagitis [43].

Candida pneumonia is very rare in non-neutropenic patients. Most commonly, hematogenous dissemination, rather than primary invasion is the reason for pulmonary lesions, in combination with multiple additional organs and therapy is directed at disseminated candidiasis. Candida pneumonia requires histopathological confirmation because benign colonization of the airway with *Candida* species and contamination of the respiratory secretions with oropharyngeal material is much more common than invasive candidiasis. Nevertheless, when a patient does not respond to antibiotics and *Candida* is the predominant or sole pathogen in bronchoalveolar lavage (BAL) fluid, therapy has to be given. Furthermore, laryngeal candidiasis is associated with severe morbidity and potential mortality, so that rapid clinical diagnosis and prompt initiation of therapy are important and outweigh any adverse effects of antifungal therapy [23].

For surgical ICU patients *Candida* peritonitis may develop in association with recurrent gastrointestinal perforations or anastomotic leakage after surgical intervention. *Candida* species are isolated in 20% of all patients with peritonitis. If these patients are improving under antibiotics, there is no need for antifungal treatment. However, patients who received chemotherapy for neoplasm or immunosuppressive therapy for transplantation or other inflammatory diseases are at high risk and have to be treated [14]. *Candida* species are isolated in intraoperative samples, percutanous punctures, and drainage fluids as part of a complex polymicrobiological infection [44]. If these patients do not improve despite adequate broadspectrum antibiotics, antifungal therapy must be started after surgical repair and drainage [14].

Aspergillosis

Amphotericin B has been the standard therapy for invasive aspergillosis, although the response rate of less than 40% is suboptimal [18, 45]. In addition, amphotericin B is associated with multiple adverse side effects, which are mentioned above. Furthermore, diagnosis of invasive aspergillosis is very difficult and delayed diagnosis contributes to the high mortality rate of more than 50%. Thus, even if the di-

agnosis is not proven, therapy against *Aspergillus* species is often initiated empirically. Voriconazole [37, 46] and caspofungin [47] were as effective as amphotericin B in salvage therapy of refractory invasive aspergillosis. However, we have to mention that caspofungin is not effective against other molds which are an emerging problem for patients after bone marrow or solid organ transplantation. In a comparitive study for primary therapy of invasive aspergillosis, proven or empiric, in neutropenic patients, voriconazole showed better response rates and an improved survival, accompanied with fewer severe side effects than standard therapy with amphotericin B [48]. Because of its excellent cerebral penetration and fungicidal activity, voriconazole may be the best choice for treatment of cerebral aspergillosis [37]. Because of the high mortality of invasive aspergillosis infections, combination therapies of voriconazole plus caspofungin, caspofungin and liposomal amphotericin B, or voriconazole and amphotericin B seem attractive. However, no relevant clinical data are yet available for these combinations.

Conclusion

Although invasive candidiasis is a widespread problem, prophylaxis is not yet established. Large, multicenter, randomized clinical trials are lacking. Furthermore, the epidemiologic shift to non-albicans *Candida* species, with their relevant fluconazole resistance, moves other agents into the field of interest for further studies. Otherwise, empirical and/or preemptive therapy, triggered by validated risk assessment procedures may be more valuable as prophylaxis. Emerging fungal infections in surgical ICU patients – caused by yeasts or molds – force us to establish new elaborate therapeutic strategies. We will see whether the outcome of our patients can be improved by implementation of these treatment protocols.

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Hepatosplanchnic Failure

Splanchnic Perfusion and Oxygenation in Critical Illness

L. A. Schwarte, M. F. Stevens, and C. Ince

Introduction

A central task in critical care medicine is the continuous maintenance of adequate tissue oxygenation. However, impairment of tissue perfusion and, thus, oxygenation is a common issue in critical care medicine, e.g., caused by anemia, cardiac failure or sepsis. If systemic oxygen delivery is reduced or maldistributed, certain organs may be impaired in oxygenation even before systemic markers of tissue dysoxia occur. Herein, the splanchnic region is particularly vulnerable in critical illness. Impaired splanchnic tissue perfusion and oxygenation play a crucial role in the development and maintenance of critical illnesses, e.g., the gastrointestinal tract may become the motor of sepsis and the multiple organ dysfunction syndrome. Thereby, the splanchnic region plays a role both as a target (e.g., through ischemia/reperfusion phenomena), but also as a source of the disease process (e.g., translocation of gastrointestinal endoluminal bacteria and toxins). Regarding the latter, continuous adequate microcirculatory oxygenation appears important to maintain the integrity of the gastrointestinal barrier function. The splanchnic region is not only affected by the disease process, but also by numerous therapeutic interventions, e.g., ventilation or drugs.

Monitoring of splanchnic perfusion and oxygenation may identify patients at risk of developing critical hypoperfusion or hypoxygenation early, and guide therapy targeted at restoring splanchnic perfusion and oxygenation [1]. Therefore, the splanchnic region has been regarded as the "canary of the body" [2]. However, this concept is hampered by the fact that direct, regional measurement of splanchnic perfusion and oxygenation is not routinely possible, and available systemic markers of splanchnic impairment (e.g., increased blood levels of liver enzymes) are regarded as only late and less sensitive signs.

Furthermore, the splanchnic region cannot be regarded as a homogeneous region, as it shows marked differences in the distribution of perfusion and oxygenation from organ to organ (e.g., higher perfusion of small intestines vs. stomach), but also within organs (e.g., higher PO_2 of serosa vs. mucosa). Thus, findings at one site cannot be extrapolated to other parts within the splanchnic region. The pathophysiology of splanchnic perfusion is further complicated by the unique features of blood supply, both at the macroscopic level (e.g., serial arrangement of the gastrointestinal tract and the liver via the portal vein; dual blood supply of the liver and its buffering capacity), but also at the microscopic level, e.g. countercurrent architecture of intestinal villi arteries and veins.

This chapter highlights aspects of the impairment of splanchnic perfusion and oxygenation in critical care settings. The first section introduces methods allowing direct or indirect measurement of splanchnic perfusion and oxygenation, followed by a second section on the impact on the splanchnic region of commonly used interventions in critical care medicine, especially on splanchnic tissue perfusion and oxygenation. We focus on respiratory therapy, renal replacement therapy, nutrition, volume resuscitation, vasoactive drugs and epidural anesthesia, because these therapies are most commonly used in the critically ill and may alter splanchnic perfusion.

Methods to Measure Splanchnic Oxygenation and Perfusion

Assessment of the efficacy of perfusion and oxygenation requires adequate monitoring tools. Microcirculation and tissue oxygenation can be determined by various techniques. Each technique has its limitations in terms of specificity and sensitivity and it is important to be aware of the aspects of various techniques when interpreting and comparing results of different studies.

Tonometry

Hollow organ tonometry primarily measures intraluminal CO_2 [3], using a CO_2-permeable balloon probe introduced into the organ of interest, mainly the stomach. The sample within the balloon is aspirated after a sufficient equilibration time and analyzed for PCO_2. Under the assumption that intraluminal PCO_2 reflects the balance between tissue CO_2 production and tissue CO_2 removal, the tonometrically determined $PiCO_2$ is regarded as an estimate of the adequacy of tissue perfusion and oxygenation. The measured $PiCO_2$ may be used to calculate the intramucosal pH (pHi), or more commonly the PCO_2-gap (PCO_2-gap = $PiCO_2$-$PaCO_2$). Of all measures to assess splanchnic perfusion/oxygenation clinically, tonometry remains the most widely distributed, although a number of problems in the interpretation of the findings still need to be resolved. The main problems are that it has a rather long equilibrium time, preventing the detection of rapid changes of gastrointestinal perfusion and that it cannot distinguish between tissue dysoxygenation and hypoperfusion, since both lead to an increased regional PCO_2.

Perfusion Measurements

Blood flow and its distribution have been estimated by laser-Doppler and electromagnetic flow probes, indicator dye techniques, and administration of labeled microspheres. Although once regarded the gold-standard, microsphere techniques determine the distribution of labeled microspheres in different tissues only post-mortem at a single point in time. Furthermore, microspheres occlude at least a part of the capillary bed and can thereby acutely increase vascular resistances and lead to tissue hypoxia [4]. A more advanced method to measure perfusion is orthogonal polarization spectral imaging (OPS): The OPS technique is a further development of intravital microscopy [5], particularly developed for the measurement of surface-near microvascular anatomy and perfusion. Here polarized green light illuminates the tissue surface. Filtering of surface reflection by cross polarization allows intravital flow of blood cells to be observed in the microcirculation with great detail on

organ surfaces at the bedside and during surgery [5]. Recently an improvement in the OPS technique has been introduced called sidestream dark field (SDF) imaging, which uses side illumination, eliminating surface reflections producing sharper, more detailed images than the OPS technique [6]. These sharper images enabled us to develop software to automatically analyze and quantify microcirculatory flow patterns.

An advantage of the OPS and SDF techniques is that in contrast to other intravital microscopic methods, the application of contrast enhancing dyes is not required and the usually occurring, quality limiting reflections from the tissue surface are largely eliminated. The quantification of microcirculatory perfusion from the OPS-video recordings is computerized and results in parameters, such as the functional capillary density (FCD), reflecting the cumulative length of perfused capillaries per view area. In septic patients with ileostoma we applied and validated a scoring system to analyze the flow patterns in the mucosal villi [7].

Oxygenation Measurements

Tissue oxygenation can be measured directly by polarographic oxygen electrodes. Polarographic O_2 electrodes have been widely used in different models of hemorrhaghic shock and resuscitation, but are limited by their rather superficial penetration depth (10–20 μm) and their falsly high measurements in the vicinity of arteries [8]. Therefore, hypoxic tissue pockets may be overseen by this technique.

Reflection Spectrophotometry

Reflection spectrophotometry using the Erlangen Microlightguide spectrophotometer (EMPHO II) has been used as a measure of tissue oxygenation in a number of our own studies presented in the second part of this chapter. It serves to measure the microvascular hemoglobin oxygenation (μHbO_2), based on the principle that oxygenated and deoxygenated hemoglobin differ in their absorption spectra. A major advantage of this method is, that by using highly flexible light guides, spectrophotometry is able to measure luminal surface oxygenation minimaly invasively, e.g., the microvascular hemoglobin oxygenation of the gastric mucosa in patients or animals, by introducing the flexible lightguide via an orogastric tube.

Quenching of Palladium Porphyrin Phosphorescence

This method has been used to determine the microcirculatory PO_2 (μPO_2) of various splanchnic tissues, e.g., the intestinal mucosa and serosa. This method is based on the principle that certain dyes elicit phosphorescence depending on the local PO_2 [9]. After infusion of the O_2-sensitive dye (Pd-meso-tetra [4 carboxy-phenyl] porphine) the O_2-dependent phosphorescence is stimulated by a defined pulsed light, non-invasively detected and analyzed. The decay time constant of the phosphorescence signal (quenching) after stimulation is quantitatively related to the present PO_2 by the Stern Volmer relation and allows quantitative measurement of μPO_2. Since the O_2-sensitive chromophore is complexed with high molecular compounds, the dye remains within the blood vessels, thereby allowing the measurement of intravascular PO_2. The PO_2-signal derives from both the capillaries and first order venules and the measured PO_2 is therefore termed *microvascular* PO_2.

Hepatic Vein Oximetry and Blood Analysis

The placement of catheters into the liver veins allows blood sampling for the determination of oxygenation (e.g., hepatic venous oxygen saturation) and of metabolites (e.g., hepatic venous lactate). Although the interpretation of these data is sometimes difficult (e.g., an increased hepatic venous lactate may be due to an increased splanchnic lactate production and/or a decreased hepatic lactate clearance), the hepatic venous catheter yields integrative markers of overall splanchnic perfusion and oxygenation.

Indocyanine Green (ICG) Extraction

The extraction of ICG, selectively metabolized by the liver, from plasma is used to estimate splanchnic blood flow. Splanchnic blood flow is hereby determined by combining measurement of ICG extraction and the Fick principle, requiring the placement of hepatic venous catheters. A non-invasive technology using ICG clearance is also available (Limon, Pulsion); however, it provides only surrogate markers of splanchnic perfusion and/or hepatic function (e.g., the plasma disappearance rate of injected ICG).

Beside this, there are a number of other methods, not routinely used, to assess the splanchnic region (e.g., tests of intestinal mucosal permeability). Since they are not discussed in the second part of this chapter, they are omitted here.

Summarizing the above methods, a single method to determine splanchnic perfusion or oxygenation clinically on a routine basis is still not available. However, the studies performed in animals and patients clearly show that regional splanchnic effects cannot be derived from systemic markers of perfusion and oxygenation, thus prompting the need for combined approaches to assess the splanchnic region. The following section of this chapter reviews how disease processes and interventions occurring in critical care medicine affect splanchnic perfusion and oxygenation. The section serves both to describe how common diseases and interventions affect the splanchnic region, and to show the effect of therapies aimed at improving splanchnic oxygenation and perfusion.

Alteration of Splanchnic Perfusion and Oxygenation in Intensive Care Medicine

Circulatory Shock

Under conditions of hemorrhagic or cardiogenic shock there is a generalized vasoconstriction throughout the body. This response is accentuated in the splanchnic region leading to a compromised mesenteric circulation. Such a stress reaction activates the sympathetic nervous system, which redistributes perfusion from the splanchnic region to more vital organs and thereby decreases splanchnic oxygenation [10]. The liver and the gut are highly efficient in redistributing perfusion to systemic circulation under stress, irrespective of what caused the cardiovascular stress. This vasoconstriction can even override blood flow autoregulation of the enteric system leading to an increased oxygen extraction rate. Beside the effect of increasing systemic vascular resistance (SVR), sympathetically induced vasoconstriction also empties veins in the splanchnic region causing autotransfusion. This effect

can increase the circulating blood volume up to one third leading to increased cardiac filling pressures, thereby supporting cardiac output by means of the Starling mechanism. The vasoconstriction is not only induced sympathetically, but also by other circulating vasoconstrictors (e.g., angiotensin, vasopressin, endothelin, vasoactive intestinal protein). These mediators are responsible for the disproportional increase in splanchnic vascular resistance compared to SVR. Gastric tonometry during short-term hypovolemia in healthy volunteers revealed a reduced pHi which was restored after volume resuscitation. This effect of hemorrhage on gastric pHi occurred despite stable heart rate, blood pressure and peripheral perfusion [11]. Furthermore, if hypovolemia is successfully treated, the splanchnic hypoperfusion markedly exceeds the time of systemic hemodynamic compromise [12]. Furthermore, the effects of circulatory shock on splanchnic perfusion are highly dependent on the extent and duration of the comprised circulation.

In conclusion, the initially reduced splanchnic perfusion in shock is teleologically advantageous, but it may become the motor of critical illness if reduced to a critical extent.

Sepsis

Sepsis is a disease of the microcirculation, caused by many pathogens over a myriad of mechanisms [6]. These pathogenic mechanisms unleashed during sepsis target many aspects of the function of the microcirculation, leading to tissue distress and ultimately to organ dysfunction and multiple organ failure (MOF). Hence, the responses of the mesenteric vasculature in sepsis are more variable than in circulatory shock. Human hepatosplanchnic blood flow in sepsis is usually increased, mediated by a direct vasodilatory effect of bacterial endotoxins on the vascular smooth muscles. Nevertheless, this increased perfusion cannot balance the increased oxygen demands in sepsis. The increased oxygen consumption is caused by an elevated metabolism and uncoupling of the ATP-generating phosphorylation also leading to the generation of reactive oxygen species further fueling the toxic cascades of sepsis. However, a decrease in splanchnic circulation can be caused by a decreased perfusion pressure, especially during relative hypovolemia. Therefore, later in sepsis, oxygen delivery and consumption decrease, especially when therapy does not include sufficient volume resuscitation. Therefore, the main problem to building an overall picture of splanchnic pathophysiology during sepsis is the variety of models used to simulate a sepsis-like response in laboratory animals. Many endotoxin-mediated models of sepsis show decreased mesenteric perfusion, in contrast to models in which live bacterial inoculation results in a hyperdynamic response. Nevertheless, all models produce abnormal blood flow distribution with decreased capillary perfusion and zones of impaired regional tissue oxygenation, while mesenteric oxygen delivery is preserved. Furthermore, an increased capillary hemoglobin concentration and edema formation occur suggesting capillary leakage and postcapillary vasoconstriction. Clinically, it is, therefore, almost impossible to judge splanchnic pathophysiology by looking exclusively at systemic hemodynamics. This is where the new tools to evaluate splanchnic perfusion may help in prognostic judgment and guiding therapy.

Attempts to monitor gastric pHi to predict outcome were promising in critically ill patients who still had preserved splanchnic perfusion ($pHi \geq 7.35$), since only these patients had an improved outcome after increasing systemic oxygen delivery [13]. Unfortunately, tonometry was of no value in patients who already showed

signs of altered splanchnic perfusion (pHi < 7.35), since they did not benefit from intensified therapy. Similarly, in a recent study, the sublingual microcirculation was monitored using OPS imaging in septic patients. At the onset of shock, survivors and non-survivors had similarly perfused small vessels, but microvascular perfusion only increased in the survivors [14]. Therefore, OPS, and its extension SDF, imaging are promising new tools to evaluate the function of the microcirculation, but their final predictive and therapy-guiding value has to be confirmed.

Therapies Altering Splanchnic Perfusion and Oxygenation

Respiratory Therapy

The aim of respiratory therapy in critical ill patients is to increase oxygenation and tissue perfusion while minimally altering lung function. This is often necessary in septic patients, since acute respiratory distress syndrome (ARDS) is usually a component of the MOF seen in severe sepsis. The main therapeutic options to achieve these goals are to increase inspiratory oxygen fraction and mean airway pressure. The latter is most commonly achieved by positive end-expiratory pressure (PEEP). In order to avoid high pressure or volume ventilation, the concept of permissive hypercapnia was introduced decades ago. Therefore, the effects of each of the above therapies on gastrointestinal oxygenation and perfusion will be discussed:

Inspiratory Oxygen Fraction. Increasing the inspiratory oxygen fraction from 0.3 to 1.0 in pigs more than doubled the intramural PO_2 of the large intestine and increased the oxygenation of the small intestine by 45% [15]. Since the risk of infection is inversely related to the subcutaneous PO_2, supplemental oxygen has been shown to reduce the rate of wound infections in patients [16].

In contrast to these results, hyperoxia did not induce changes in mucosal, serosal and mesenteric PO_2 in isovolemic pigs, whereas it reversed the deleterious effects of severe isovolemic hemodilution on serosal, mucosal and mesenteric venous oxygenation [17].

PEEP. Positive end-expiratory pressure in healthy anesthetized dogs leads to a decrease in mucosal μHbO_2 in a pressure dependent manner. These effects are not due to altered systemic hemodynamics, since restoring cardiac output with volume resuscitation increased the mucosal oxygenation only partially [18]. Similarly in healthy volunteers, PEEP decreased gastric mucosal oxygen saturation in a pressure-dependent manner [19]. One possible mechanism is that PEEP increases intraabdominal pressure and splanchnic venous pressure, which may alter splanchnic perfusion. Thus, in patients undergoing laparoscopy the intraabdominal pressure decreased gastric mucosal oxygenation [20]. This mechanism might explain why an increased intraabdominal pressure *per se* is an independent predictive factor of survival and a major component in the development of multiple organ dysfunction syndrome [21].

Permissive Hypercapnia. The concept of permissive hypercapnia in critical illness, especially in ARDS, was introduced decades ago and proved useful clinically. Nevertheless, only recently an experimental study evaluated the beneficial effect of permissive hypercapnia on subcutaneous and intestinal intramural oxygenation [15].

We investigated the effect of permissive hypercapnia on mucosal oxygenation in anesthetized chronically instrumented dogs. Permissive hypercapnia ($etCO_2 = 70$ mmHg) significantly increased mucosal oxygenation (μHbO_2) in a PCO_2-dependent manner. During conditions of hemorrhagic shock it maintained μHbO_2 within the physiologic range, whereas normocapnic controls developed severe mucosal hypooxygenation (unpublished data). In conclusion, permissive hypercapnia may help to maintain adequate splanchnic oxygenation during hypovolemia.

Renal Replacement Therapy

Extracorporeal renal replacement modalities, e.g., hemodialysis, have become an established component in the treatment of critically ill patients with MOF; however, only few studies have focused on their regional effects. For non-splanchnic organs, a reduction in tissue oxygenation has been established: For example, Jensen et al. found a marked depression in subcutaneous oxygen tension from 52 to 28 mmHg in non-ICU patients, postulating a link to the increased risk of impaired wound healing and infections in dialyzed patients [22]. In respect to the splanchnic region, van der Schueren et al. studied the systemic and splanchnic responses (tonometry derived PCO_2-gap) to intermittent hemodialysis in ICU-patients [23]. The authors found a significant increase in inotrope requirements to maintain systemic hemodynamics (e.g., arterial blood pressure, cardiac output) together with a significant increase in the PCO_2-gap and intramucosal acidosis. Although the few available studies on this topic do not permit a final conclusion, the given data suggest that renal replacement therapies potentially impair the splanchnic region.

Volume Resuscitation

Intact autoregulatory mechanisms ensure that resuscitation from hypovolemic shock through volume resuscitation is effective in recruiting vulnerable microcirculatory beds. Volume provision also restores intestinal barrier function and promotes splanchnic microcirculatory oxygen transport. However, severe isovolemic hemodilution can cause a redistribution of oxygen delivery within the splanchnic compartment, preserving minimal perfusion of the mucosa [24]. The significance of such redistribution of oxygen supply and its role in the pathophysiology of volume resuscitation, however, has yet to be established. Blood is a much better oxygen carrier than colloid or crystalloid fluids and transfusion indeed improves oxygen delivery to the microcirculation more so than fluids. Thus, early volume resuscitation with either fluids or blood has been shown to increase survival in early goal directed therapy [25], and aggressive fluid resuscitation is highly recommended in the surviving sepsis campaign [26]. The study of the effects of volume therapy on splanchnic oxygenation and perfusion will certainly increase our pathophysiologic knowledge, both in the timing of volume resuscitation and the type of the administered volume.

Nutrition

Enteral feeding increases splanchnic blood flow depending on caloric load, food volume and type [27]. The increased metabolic demands of active absorption are greater than the increases in splanchnic blood flow, suggesting that the active gut may incur an oxygen debt. In the clinical setting the increased metabolic demand

of an absorbing gut coupled with inadequate perfusion due to relative hypovolemia or vascular insufficiency may lead to an imbalance in oxygen requirements and demand. This effect can be deleterious especially to the oxygen countercurrent exchange of the intestinal villi and may produce hypoxia at the tip of the villi compared to its base. The importance of such an imbalance in the critically ill patient is highlighted by case reports of patients who developed mesenteric infarction soon after the initiation of enteral nutrition. Nevertheless, it is current consensus that early enteral nutrition is beneficial.

Cardiovascular Drugs

The class of preferentially cardiovascular active drugs may be grossly divided into the adrenergic drugs (including dopaminergic drugs) and the so-called non-adrenergic drugs, e.g., including phophodiesterase inhibitors, vasopressin analogs and the newly developed calcium sensitizer, levosimendan.

Adrenergic Drugs. There is a larger number of studies on the splanchnic effects of adrenergic agents, however, a full discussion of this topic would by far exceed the extent of this chapter. Unfortunately, the results of these studies are difficult to summarize, because various experimental or clinical designs caused contradictory results.

In a review on the therapeutic options for the treatment of impaired gut function, Meier-Hellmann et al. concluded that epinephrine should be avoided because it seems to redistribute blood flow away from the splanchnic region, a conclusion also valid for dopamine [28]. In contrast, they concluded that for norepinephrine, no negative effects on gut perfusion have been demonstrated. Particularly in septic states, the excessive splanchnic vasoconstriction caused by norepinephrine may be blunted by preexisting vasodilation and/or reduced α-adrenoceptor response.

The synthetic catecholamine, dobutamine, is reported to more consistently exert beneficial splanchnic effects. It increased mucosal perfusion/oxygenation in experimental endotoxemia [29] and mesenteric ischemia [30]. Additionally, dobutamine elicited beneficial splanchnic effects also under several clinical conditions, like an increased pHi in sepsis. Only few studies have failed to demonstrate beneficial splanchnic effects of dobutamine [31]. However, there are no clinical data supporting a selective increase in splanchnic perfusion by dobutamine.

The role of dopexamine in the protection of hepatosplanchnic perfusion in high-risk surgical and critically ill patients has recently been reviewed by Renton and Snowden, analyzing 21 randomized, controlled clinical trials [32]. The authors conclude that there is insufficient evidence to recommend the general clinical use of dopexamine for the protection of hepatosplanchnic perfusion both in high-risk surgical patients and in critically ill patients. However, for distinct surgical subpopulations they report positive splanchnic effects such as reduced colonic mucosal damage and improved gastric mucosal pHi. In contrast, they found no study demonstrating a beneficial effect of dopexamine on hepatosplanchnic perfusion in critically ill patients.

Non-adrenergic Drugs. Phosphodiesterase type-III inhibitors are known as inodilators, because they have vasodilating and positive inotropic effects. They have been shown to be clinically effective in increasing splanchnic perfusion and oxygenation. Enoximone applied during and after cardiopulmonary bypass reduced endotoxin levels

in suprahepatic venous blood, indicating preservation of gastrointestinal barrier function [33]. Comparing the effects of a phosphodiesterase inhibitor with the catecholamine dobutamine, Kern et al. showed an increased lignocaine metabolism and a reduced hepatic tumor necrosis factor (TNF)-α release after 12 hours of treatment in fluid-optimized patients in septic shock [34]. However, hepatosplanchnic oxygen consumption was increased during enoximone treatment. Additionally, hepatic blood flow was increased, but the fractional hepatic blood flow (hepatic blood flow/cardiac index) remained unaltered. In patients undergoing cardiopulmonary bypass one study observed a slight (9.3%) increase in hepatosplanchnic blood flow, whereas another study could not detect an alteration in gastric perfusion [35].

Vasopressin Analogs. Vasopressin and vasopressin analogs have been advocated to support systemic perfusion pressure in catecholamine refractory septic hypotension. However, despite their desired systemic effects, they may have detrimental effects on splanchnic perfusion and tissue oxygenation. An example of this is given in Figure 1, depicting the systemic hemodynamic and regional effects of vasopressin in dogs [Schwarte et al., unpublished data]. Similar detrimental effects of vasopressin on splanchnic mucosal perfusion and oxygenation have also been demonstrated in clinical studies. Van Haren et al. demonstrated that, while vasopressin

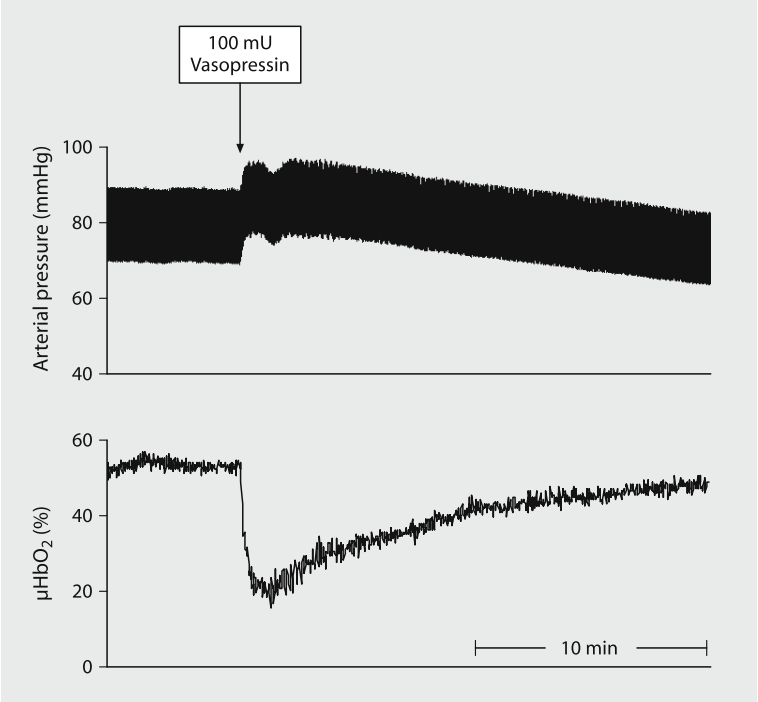

Fig. 1. Effect of vasopressin (100 mU) on arterial blood pressure (upper trace) and microcirculatory gastric mucosal hemoglobin oxygenation (μHbO_2, lower trace). Original recording of a sedated, healthy dog. Although the selected vasopressin dose only induced a minor increase in mean arterial blood pressure of about 5 mmHg, it dramatically reduced the microcirculatory gastric mucosal hemoglobin oxygenation from ~55% to ~20% , with only slow recovery over time

was able to increase arterial blood pressure in catecholamine dependent septic patients, it markedly increased the gastric mucosal PCO_2-gap from ~5 mmHg to ~20 mmHg [36]. Sub-lingual OPS imaging in a meningococcal septic patient confirmed the deleterious effects of terlipressin, a vasopressin analog, on the microcirculation [37].

Levosimendan. Levosimendan is a novel inodilator, acting by calcium sensitization of cardiac myofilaments (positive inotropic effect) and K_{ATP} channel activation of vascular smooth muscle cells (vasodilation). There is only a limited number of studies reporting the splanchnic effects of levosimendan. In anesthetized dogs, levosimendan increased blood flow, as measured by the microsphere technique, to the small intestine and the liver and reduced vascular resistance in these organs [38]. In accordance, in a recent study we demonstrated that levosimendan was superior to milrinone and dobutamine in selectively increasing microvascular gastric mucosal oxygenation in dogs [39]. In endotoxic pigs, levosimendan improved portal venous blood flow and gut oxygen delivery, however, without concomitant reduction in endotoxin-induced intestinal mucosal acidosis [40]. In a recent randomized controlled clinical trial, Morelli et al. studied the systemic and splanchnic (i.e., gastric mucosal laser Doppler flowmetry, and gastric air-tonometry) effects of levosimendan in 28 patients with septic shock related myocardial dysfunction [41]. The authors found beneficial systemic (increased cardiac output) and splanchnic effects (increased mucosal perfusion and reduced PCO_2-gap) of levosimendan. Taken together, levosimendan improved splanchnic perfusion and oxygenation both under unstressed conditions and in diseased conditions.

Thoracic Epidural Anesthesia

During recent years thoracic epidural anesthesia has been used increasingly in critical care medicine to treat severe pain and to reduce the requirement of sedatives and systemic analgesics, and thus the need for respiratory support. In particular, it has been shown to facilitate early extubation and to shorten the period on the intensive care unit (ICU) in perioperative fast-track cases.

Thoracic epidural anesthesia may be a concept to selectively overcome splanchnic hypoperfusion. Epidural anesthesia blocks regional sympathetic outflow thereby increasing perfusion of the splanchnic region. Furthermore, it depresses the release of catecholamines from the adrenal glands, depresses the renin-angiotensin system and blocks nociception leading to a reduced stress response. All these effects are theoretically beneficial for intestinal oxygenation, but there are other effects possibly reducing splanchnic perfusion. Epidural anesthesia reduces systemic blood pressure and cardiac index and increases venous pooling. The systemic hypotension then leads to a compensatory release of vasopressin, a vasoconstrictor with potent and selective action on the splanchnic vasculature. Therefore, it is difficult to predict whether gastrointestinal tract perfusion benefits from epidural anesthesia.

In anesthetized pigs, thoracic epidural anesthesia did not reduce mesenteric blood flow, intestinal PO_2, $PiCO_2$ or intestinal oxygen uptake, although systemic hemodynamics were severely depressed by the epidural block [42]. Similarly, in a rodent model of hemorrhagic shock, intravital microscopy revealed that epidural block ameliorates the deleterious effect of hemorrhage on capillary density and erythrocyte velocity [43]. In contrast, in anesthetized dogs, epidural anesthesia ag-

gravated the effect of increased airway pressure on systemic circulation and μHbO_2 [44]. These contradictory results are mirrored in clinical studies. In patients undergoing major abdominal surgery, thoracic epidural anesthesia preserved pHi and $PiCO_2$ better than in controls, although mean arterial pressure was unaltered. Interestingly these differences only became apparent after 3 to 4 hours of surgery [45]. Contradictory to this finding, during aortic surgery peridural block could not ameliorate the decreases in gastric and sigmoid pHi intra- or postoperatively [46]. Similarly, studies in patients undergoing abdominal surgery investigating recovery of bowel function clinically (return of peristalsis or bowel movements) could not demonstrate a beneficial effect in all cases, possibly because the effect may depend on the extent of epidural block or the type and duration of surgery. Thus, although epidural anesthesia has been advocated to enhance gastrointestinal recovery, especially in fast-track concepts, its beneficial effect is not unequivocal.

Vasodilators

In sepsis or shock, shunting of blood in weak microcirculatory units leads to hypoxic pockets without any signs in the systemic circulation. This phenomenon has been demonstrated by us and others in a number of clinically relevant pig models of shock and sepsis. Fluid resuscitation following endotoxemia results in preferential oxygen transport to the mucosa leaving the serosa shunted and thus ischemic [47]. Based on the idea that recruitment of these microcirculatory units is mandatory for adequate resuscitation during sepsis, we hypothesized that vasodilator therapy can homogenize – and thus improve – the microcirculation [6]. We found that nitric oxide donors improved microcirculatory oxygenation, oxygen extraction and also gastric PCO_2 in a fluid resuscitated porcine sepsis model [48]. Although there is ample evidence that vasodilators improve tissue oxygenation in animal models of sepsis, surprisingly little is known about the clinical use of vasodilators in sepsis [49]. In fluid resuscitated septic patients, we could demonstrate that nitroglycerin, a nitric oxide donor, opened microcirculatory beds, especially in small microvessels (10–20 μm) [50]. Whether resuscitation procedures aimed at correcting microcirculatory distress will result in improved outcome has to be determined in future investigation.

Conclusion

Since splanchnic impairment of perfusion and oxygenation triggers and perpetuates critical illness, including sepsis and MOF, it is crucial to elucidate the splanchnic effects of common clinical interventions applied in intensive care medicine. Herein, findings of experimental studies may serve to reduce the complexity of splanchnic pathophysiology and generate promising concepts to be tested in the clinical setting. Maybe we have to become familiar with the thought that there is not a single variable guiding our therapy of splanchnic hypoperfusion. Just as we have learned not to judge systemic hemodynamics by a single variable, future splanchnic monitoring tools will enable us to extend our ability to recognize patterns indicative of splanchnic hypoperfusion. Despite major advances in splanchnic monitoring techniques, a combination of easy-to-use and minimally-invasive metabolic and perfusion measurements allowing us to recognize pathophysiologic patterns in splanch-

nic perfusion and metabolism is not in sight. Ultimately, this concept should enable us to base our therapy on systemic *and* splanchnic circulatory variables to improve outcomes for the critically ill.

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Liver Failure: Diagnostic Assessment and Therapeutic Options

A. Kortgen and M. Bauer

Introduction

Liver failure is formally defined by the triad jaundice, coagulopathy, and encephalopathy. It can develop on a background of chronic liver disease as a result of acute decompensation ('acute-on-chronic' liver failure) as well as in the absence of pre-existing liver disease (acute liver failure *sui generis*). Due to the central role of the liver in metabolism, acute liver failure, regardless of the underlying cause, often culminates in multiple organ dysfunction and is still associated with an extremely high mortality [1]. These extrahepatic complications, including hepatic encephalopathy, hepatorenal syndrome (HRS), and susceptibility to infections are major causes of death in patients with liver failure. Thus, prevention and therapy of extrahepatic complications may help to improve outcome, as the liver itself bears the potential to regenerate. In this light, extracorporeal liver support to retard progression of multiple organ failure (MOF) seems an attractive option. Monitoring of liver function is then crucial to predict recovery and ultimate outcome and to identify those patients requiring liver transplantation.

Diagnostic Assessment of Hepatocellular Dysfunction: Conventional 'Static' And Functional 'Dynamic' Tests

In the clinical setting, estimation of liver function and injury is frequently based on a one-point assessment of the serum concentration of an enzyme, reactant or metabolite. These tests are referred to as 'static' tests. However, serum concentration of liver enzymes, such as transaminases, indicating hepatocellular damage, and cholestatic parameters, as well as bilirubin, have only limited diagnostic, therapeutic, and prognostic value [2], and more or less reflect processes that have taken place in the last couple of hours or even days. Thus, these tests are helpful in the long term assessment of chronic liver diseases, but lack sensitivity regarding kinetics of a disease process in critical care medicine. Monitoring of proteins synthesized by the liver may be more helpful; e.g., coagulation factors are included in prognostic indices like the King's College Hospital and Clichy criteria, which have been elaborated to predict unfavorable outcome in acute and subacute liver failure as an indication for liver transplantation. However, negative predictive value is low [3]. Lactate concentration – a complex dynamic rather than a static assessment due to impaired lactate clearance in the liver – early and after initial resuscitation in paracetamol-induced acute liver failure revealed an equal or even slightly better

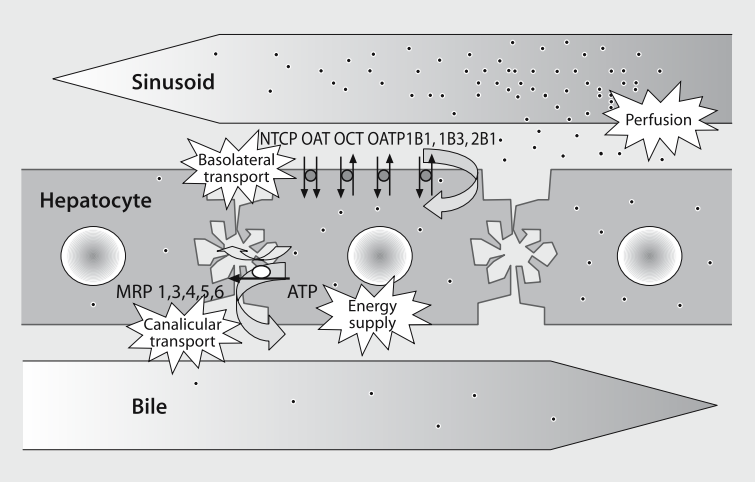

Fig. 1. Estimation of plasma disappearance rate of indocyanine green (ICG) as a parameter for global liver function. Diagnostics of excretory function as a good overall test to assess a critical partial function can be achieved by bedside estimation of the plasma disappearance rate of the anionic dye ICG. The figure summarizes the key determinants for this compound measure, i.e., perfusion, the basolateral and canalicular transport proteins and hepatocellular high-energy phosphates

predictive value [4]. Other 'dynamic' assessments of complex liver functions, i.e., parameters that depend on active processes in the hepatocyte at the time of measurement, such as hepatic clearance of organic substances (e.g., the anionic dye indocyanine green [ICG] (Fig. 1)) and/or formation of metabolites (e.g., monoethylglycinxylidid [MEGX] from lidocaine), can reveal otherwise hidden hepatocellular dysfunction [5]. Dynamic tests depend on the investigated partial liver function as well as on liver perfusion (Fig. 1). As a result, a normal value rules out both significant hepatocellular dysfunction and impairment of flow, while pathologic values always require differential diagnostics regarding perfusion deficit versus metabolic dysfunction. The MEGX-test correlates well with survival in chronic liver failure and in critically ill patients [6, 7]. However, it is affected by the problem of toxicity as well as being influenced by many frequently used drugs that use the cytochrome P450-pathway. The plasma disappearance rate of ICG can be easily monitored at the bedside by techniques using the optical properties of the dye, e.g., pulse-densitometry or hemoreflectometry, and also correlates well with outcome in critically ill patients [8, 9]. It has been used successfully to define resectability in pre-existing liver disease as well as to direct and control the effect of different resource-consuming treatment modalities, such as extracorporeal liver support systems [10, 11]. Nevertheless, thorough validation in terms of improvement of outcome is still pending.

Conservative Therapy of the Patient with Incipient Liver Failure

In most patients presenting with acute liver failure, intensive care therapy is mandatory. Specific therapeutic options exist only for a few disease entities, such as high-dose N-acetyl cysteine (NAC) for paracetamol-induced liver failure, silibinin

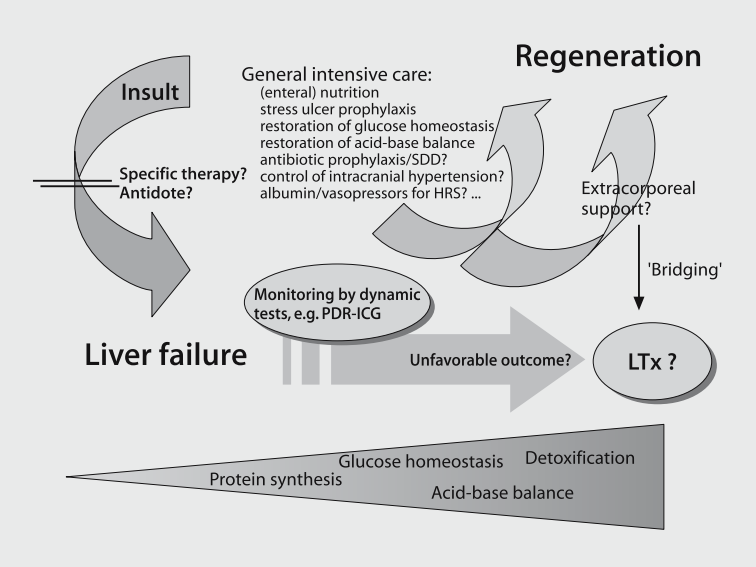

Fig. 2. Treatment algorithm in the context of failing partial functions. Monitoring recovery/regeneration of the failing liver under the described standard care to identify patients with a poor prognosis is required to assess the indication for extracorporeal support and/or liver transplantation. SDD: selective digestive decontamination; HRS: hepatorenal syndrome; LTx: liver transplantation; PDR-ICG: plasma disappearance rate of indocyanine green

and penicillamine in amanitotoxin intoxication, antiviral therapy for some types of fulminant hepatitis, or delivery for pregnancy-associated liver failure within the context of hepatopathy of pregnancy, HELLP (hemolysis, elevated liver enzymes, and low platelets) syndrome or eclampsia. Therefore, in many patients general intensive care therapy with prevention and treatment of complications is the sole therapeutic option in order to gain time to allow the liver to recover or regenerate (Fig. 2). Prophylaxis of stress ulcers is recommended because of increased risk of bleeding due to coagulopathy as well as hypertensive gastropathy in chronic liver disease. Feeding, preferentially via the enteral route, is desirable as energy turnover is increased by 20–30% in acute liver failure, and malnutrition is common in patients presenting with acute-on-chronic liver failure. In cirrhotic patients with gastrointestinal bleeding, antibiotic prophylaxis is recommended to reduce rate of infections and mortality [12]. Likewise, selective digestive decontamination intuitively seems promising, as it reduces infection rates with pathogens of the intestinal flora in acute liver failure [13] and onset of sepsis worsens the prognosis of acute liver failure. While supportive treatment modalities like application of NAC, for causes of liver failure other than paracetamol intoxication, or prostaglandin E1 or iloprost infusion may improve surrogate parameters, evidence for better outcomes is still missing. Coagulopathy is of particular concern in the surgical patient. Administration of vitamin K is of limited effect in patients with a failing liver. However, in acute liver failure not only coagulation factors but also their physiological inhibitors are less efficiently produced. As a result, coagulation disorders not only reflect reduced production but also increased turnover up to overt disseminated intravascu-

lar coagulopathy (DIC). Thus, liberal substitution is neither required nor consistent with the pathophysiology of coagulation disorders in acute liver failure. Currently activities in the range of 20% of normal are considered to be sufficient, although controlled studies do not exist. Recombinant factor VIIa has been increasingly reported as a potential treatment option although the risk-benefit ratio remains uncertain [14].

Nevertheless, development of remote organ injury as a result of impaired liver function or toxic liver syndrome typically determines course and prognosis of these high risk patients and requires sophisticated critical care therapy.

Extrahepatic Complications and Management of Liver Failure

Remote organ dysfunction frequently occurs in liver failure and often culminates in multiple organ dysfunction. All organ systems can be impaired but cerebral and renal function are the most important with respect to survival.

Hepatic Encephalopathy

Hepatic encephalopathy is the hallmark of and discriminates acute liver failure from other acute liver diseases, such as hepatitis with jaundice and impaired synthesis. In hepatic coma, increases in intracranial pressure (ICP) are common and frequently predict the outcome. Both accumulation of toxic metabolites, most notably ammonia and glutamine, as well as increases in cerebral blood flow (CBF) may contribute to rises in ICP ultimately leading to herniation and death of the patient with acute or fulminant liver failure [15, 16]. In addition to evaluation of patients fulfilling criteria for poor prognosis according to established scores for liver transplantation and consideration of extracorporeal support in experienced centers, conventional measures to control increases in ICP, such as mechanical ventilation and mannitol administration reflect the standard of care. Furthermore, mild therapeutic hypothermia can be helpful as it leads to a decrease in ICP and, thereby, increases cerebral perfusion pressure [17], and continuous infusion of hypertonic saline reduces ICP in acute liver failure and pronounced hepatic encephalopathy [18]. Special caution is, however, required in patients presenting with increased ICP and impaired renal function; vasopressin and its analogs which might be useful to restore urine output (see below) can increase CBF and, thus, ICP [19].

Hepatorenal Syndrome

HRS is of key significance among the extrahepatic complications of liver failure in patients with chronic liver disease. While liver transplantation remains the most effective treatment with five year survival rates exceeding 70%, supportive therapy with plasma expansion and vasopressin analogs or artificial liver support systems such as albumin dialysis can reverse ‘acute-on-chronic’ liver failure in general, and specifically HRS, and, thus, offer effective treatment when liver transplantation is not an option [20, 21].

HRS is characterized by impaired renal function in the presence of advanced liver disease. From a pathogenetic point of view, the syndrome primarily affects the arterial circulation and the activity of several endogenous vasoactive mediator sys-

tems leading to arteriolar underfilling with parallel congestion of the hepatosplanchnic bed [22]. Based on the kinetics of onset of renal dysfunction, two forms of HRS can be discriminated with implications for prognosis and therapy: HRS type 1 is characterized by a doubling of initial serum creatinine to greater than 2.5 mg% or a 50% reduction in the initial creatinine clearance to a level lower than 20 ml/minute in less than 2 weeks. In contrast, HRS type 2 is characterized by a more insidious increase in serum creatinine or reduction in creatinine clearance [23]. HRS is a prototypical prerenal cause of impaired kidney function. Thus, potentially reversible alternative causes of prerenal kidney failure, in particular in the course of septic complications or secondary to administration of substances (e.g., nonsteroidal antiinflammatory drugs or aminoglycosides) must be ruled out. HRS occurs with an incidence of 7–15% in patients with chronic liver disease and is characterized by sodium and water retention (reflected by a urinary sodium of less than 5 mmol/l and dilutional hyponatremia) and reduced glomerular filtration rate (GFR) [24]. Diagnostic criteria for HRS according to the international ascites club are a creatinine of 1.5 mg% or creatinine clearance <40 ml/min in the absence of prerenal causes such as circulatory insufficiency/fluid loss or infection. By definition, neither withdrawal of diuretics nor plasma expansion (by 1.5 l of plasma expanders) will improve renal function after renal parenchymal or obstructive disease have been ruled out [25]. The kinetics of development of criteria for HRS are important for clinical management as HRS type 1 carries a high mortality, exceeding 60% within 2 weeks after diagnosis. Administration of vasoconstrictors to patients with HRS has been advocated since the early 1950s. The pathophysiological concept underlying this treatment modality is based on the observation that renal vasoconstriction leading to HRS is ultimately the result of profound and persistent vasodilation primarily in the splanchnic circulation [26]. Vasodilation, along with relative hypovolemia, impairs effective arterial blood volume and induces substantial activation of various endogenous vasoconstrictor systems such as the endothelin system [22, 27]. Consistent with this pathophysiological concept, phase II pharmacological studies indicate that vasoconstrictors, such as vasopressin analogs and α-agonists, such as norepinephrine, in particular when combined with plasma expansion, can reverse hemodynamic sequelae culminating in HRS [20, 28]. However, most patients fail to recover completely, indicating that either hemodynamic sequelae are not completely reversed by these treatment modalities or factors other than hemodynamic alterations are responsible, at least in part [29]. Although the beneficial effects of placement of transjugular intrahepatic portosystemic shunts (TIPS) add to the beneficial effects of pharmacological therapy of circulatory dysfunction, the mechanisms are far from clear [29, 30]. A possible explanation could relate to the reduction in portal pressure due to TIPS-placement exerting a direct beneficial effect on renal function indicative of suppression of a putative 'hepatorenal reflex'. This latter concept is supported by the observation that patients with refractory ascites are less likely to develop HRS if a TIPS is in place [30]. In addition, data obtained with extracorporeal support systems, which are able to reverse HRS type 1 to some extent, support the notion that endogenous accumulation of toxins which can no longer be removed by the liver accumulate to mediate toxic effects on the kidneys [21]. Basic monitoring and support of the patient with HRS includes repeated assessment of urine output, weight, hemodynamics (at least arterial pressures), plasma and urine electrolytes and initiation of dialysis with or without specific liver support. A small, prospective randomized trial assessing the impact of dialysis of blood against an albumin-enriched dialysate using the 'molecular adsor-

bent recirculating system' (MARS) to remove albumin-bound toxins/compounds such as bilirubin, bile acids, indols and phenols in addition to small, water-soluble substances such as NH_4^+, confirmed that all patients in the control group were dead by day 7, while 2 out of 8 patients subjected to supportive MARS therapy survived the observation period of 30 days [31]. Based on current understanding of the pathophysiology of HRS, the combination of potent vasoconstrictors, most notably the long-acting vasopressin-analog terlipressin, along with plasma expansion primarily using albumin, consideration of TIPS, and extracorporeal support, e.g., using MARS in experienced centers is advocated as conservative therapy, although controlled clinical studies assessing comparative and additive effects are clearly warranted. Although published data point toward a superiority of albumin as the plasma substitute it remains uncertain whether or not equipotent amounts of either crystalloid or artificial colloid solutions were given in these studies. Despite these promising short term results, survival rates from HRS remain poor and transplantation should be considered to improve the prognosis in eligible patients.

Other Organ Systems Affected

Extrahepatic manifestations of impaired liver function or toxic liver syndrome might involve almost every organ system and seem to critically depend on impaired detoxifying capacity of the failing liver with accumulation of cytotoxic but also vasoactive substances. Examples involve cirrhotic cardiomyopathy, hepatopulmonary and portopulmonary syndromes, and also less obvious remote dysfunctions such as thyroid or adrenal insufficiency ('hepatoadrenal syndrome'). From these pathophysiological considerations it seems obvious that supporting the critical detoxifying capacity of the liver should be able to disrupt the vicious circle of toxic liver syndrome leading to multiple organ dysfunction.

Extracorporeal Liver Support

There is an ever-increasing need for alternatives to liver transplantation in liver failure with the shortage of donor organs and a substantial share of patients developing liver failure as part of the multiple organ dysfunction syndrome in severe sepsis where liver transplantation is not a feasible option. Among the various partial functions of parenchymal and non-parenchymal liver cells, failure of their detoxifying capacity may give rise to further decompensation of the liver function, promoting remote organ failure. Whether plasma concentrations of bilirubin typically observed in these patients are toxic or even beneficial remains unclear and additional parameters, such as ICG clearance, should be used to identify and monitor patients for extracorporeal support [32], since a decrease in bilirubin level only indicates effectiveness of the support system but not recovery of the liver. While development of an 'artificial' liver system ultimately requires bio-engineering and use of liver or pluripotent stem cells [33], recompensation of 'acute-on-chronic' liver failure and 'bridging' to liver transplantation could be achieved potentially by mechanical extracorporeal support systems, such as albumin dialysis, using e.g., MARS or similar devices, such as the recently introduced fractioned plasma separation and adsorption system 'Prometheus'. However, although several thousand MARS treatments have been performed so far worldwide, data from sufficiently

powered studies are missing. A recent meta-analysis of 206 articles screened revealed only 4 randomized controlled trials of 67 patients. Analysis of the data documented no overall benefit regarding survival for acute or acute-on-chronic liver failure (relative risk: 0.56; confidence interval 0.28–1.14 [34]). Data obtained from a prospective, randomized, multicenter, controlled trial of an extracorporeal porcine hepatocyte-based bioartificial liver including a total of 171 patients presenting with fulminant/subfulminant hepatic failure and primary non-function following liver transplantation were reported in 2004 [35]. For the entire patient population, 30-day survival was 71% for the bioartificial liver compared to 62% for control. The trial suggests a potential benefit of the bioartificial liver (risk ratio: 0.67; $p = 0.13$) in particular in the subgroup with fulminant/subfulminant liver failure (risk ratio: 0.56; $p = 0.048$). This observation, along with our own experience, suggests that recompensation can be best achieved in a subset of patients without substantial hepatocellular impairment prior to onset of liver failure.

Conclusion

Liver failure is a rare but life-threatening condition affecting a multitude of other organ systems, most notably the brain and kidneys, following severe hepatocellular injury. While quality of life is best if recovery/regeneration can be achieved without an allograft, liver transplantation remains the gold-standard in liver failure with predicted unfavorable outcome. Extracorporeal support should be considered in patients where liver transplantation is either not an option or to 'bridge' time to transplantation in experienced centers (Fig. 2). Recent evidence would suggest that fractionated plasma separation and adsorption might be more efficient at removing albumin-bound substances than classical albumin dialysis [36], however, their potential to delay regeneration requires further investigation [37]. Dynamic tests, most notably ICG-clearance, have the potential to substantially improve monitoring and to identify patients at risk as well as those recovering. Although the mortality of acute liver failure remains high, substantial progress has been made in monitoring and support of the patient, not only regarding support of the failing liver but also regarding symptomatic therapy of remote organ dysfunction, e.g., in HRS.

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Immunoparalysis in Liver Disease

C. G. Antoniades, P. A. Berry, and J. Wendon

Introduction

Although acute liver failure and acute on chronic liver failure are distinct clinical conditions, they commonly result in progressive and refractory organ dysfunction and death. Activation of the systemic inflammatory response is central to the pathogenesis of vasodilatory shock and multiple organ failure (MOF) encountered in both these conditions. Acute liver failure is characterized as a syndrome of circulatory failure that in many ways is similar to septic shock. It is inherently difficult to discern the relative contribution of hepatocellular necrosis and sepsis to the systemic inflammatory reaction encountered in these patients. Irrespective of the inciting event, the end product is that of MOF and death. Therefore, it is important to elucidate the components of the inflammatory cascade 'downstream' to the inciting event.

The concept of uncontrolled intravascular inflammation occurs due to various types of insult such as infection, trauma, major surgery and, as previously mentioned, acute liver failure. Both innate and adaptive immune responses result in activation of polymorphonuclear (PMN) cells, mononuclear cells that in turn release a complex array of mediators. Phylogenetically, this response aims to eliminate the original insult. However, the loss of a 'homeostatic' immune response will lead to the immunopathology encountered clinically as refractory MOF.

Over the past few years, there has been an increased appreciation of the functional significance of monocytes in the conditions characterized by the systemic inflammatory response. Monocytes are pivotal mediators of the systemic inflammatory response, secreting large amounts of both pro-inflammatory (tumor necrosis factor-alpha [TNF-α], interleukin [IL]-1, IL-6) and anti-inflammatory cytokines (IL-10) thereby recruiting other inflammatory effector cells such as PMN cells and lymphocytes. Furthermore, monocytes possess antigen presentation skills by virtue of the surface expression of histocompatibility (HLA) antigens.

Monocyte HLA-DR Expression in Conditions Characterized by the Systemic Inflammatory Response

Reduced monocyte HLA-DR expression was initially described more than a decade ago in patients who had sustained major trauma [1]. In these patients, low monocyte HLA-DR expression was associated with the development of severe sepsis. Numerous subsequent observational trials have reproduced the finding of low mono-

cyte HLA-DR expression in both trauma and postoperative patients [2–6]. Interestingly, the reduction in monocyte HLA-DR expression is present at the point of admission in both patient groups. In those with an uneventful recovery, HLA-DR expression returned to normal within 2 weeks of admission whereas in those who developed septic complications or died, HLA-DR expression remained persistently low [1, 2]. This led to the hypothesis that reduced HLA-DR expression is of true pathogenic significance.

The functional significance of decreased monocyte HLA-DR expression was initially coined by Docke and co-workers who noted that patients with septic shock would usually succumb to repeated bacterial and opportunistic infections. The monocytes in these patients were found to have a profound decrease in monocyte HLA-DR expression and a reduced ability to secrete TNF-α following stimulation with lipopolysaccharide (LPS) *in vitro* [7]. Furthermore, exogenous administration of interferon–gamma (IFN-γ) increased HLA-DR levels to normal levels and concomitantly restored LPS induced monocyte TNF-α production capacity [7]. The administration of exogenous IFN-γ resulted in resolution of sepsis in eight out of the nine patients included in this small study. Although no mortality benefit was detected, due to low patient numbers, this study elegantly identified monocyte 'dysfunction' in septic shock. Given that sepsis-induced 'monocytic deactivation' gives rise to the clinical manifestations of a secondary immunodeficiency, Docke and coworkers termed this phase immunoparalysis.

On the basis that the levels of monocyte HLA-DR can be modulated *in vitro* [8] and *in vivo* [5–7], small phase I and II studies were performed evaluating the effect of exogenous granulocyte/macrophage-colony stimulating factor (GM-CSF) in patients with severe sepsis [5, 6]. Once again, exogenous administration of these cytokines resulted in a significant increase in monocyte HLA-DR levels and *ex vivo* TNF-α production capacity. Although preliminary, these immunomodulatory strategies do reverse sepsis-induced 'monocytic deactivation' but have not improved overall outcome.

Pathogenesis of Low Monocyte HLA-DR Expression

Both pro-(TNF-α, IL-6, IFN-γ) and anti-inflammatory cytokines (IL-10) can affect directly the surface expression of the HLA-DR molecule on monocytes. Other mediators such as catecholamines, prostaglandins and immunosuppressive agents can also affect the surface expression of this molecule [9]. Clearly, the expression of this immunologically pivotal molecule is controlled by complex regulatory mediators in an autocrine and paracrine fashion.

As alluded to previously, the functional changes associated with low HLA-DR expression are those of reduced antigen presentation and an impaired ability to secrete pro-inflammatory cytokines (IL-1, IL-6, IL-8, TNF-α) whilst anti-inflammatory cytokine secretion (IL-10) is relatively preserved following single or repeated stimulation with LPS [8–11]. Thus, low monocyte HLA-DR expression is a mere reflection of a 're-orientation' to a predominately anti-inflammatory role. The shift to an anti-inflammatory monocyte phenotype must be taken in context with the time point at which it is detected in the evolution of the inflammatory response. An 'early' reduction in monocyte HLA-DR is likely to represent a physiological downregulation of monocyte function to prevent over stimulation, or an 'excessive' pro-inflammatory response. Persistence of the low HLA-DR monocyte phenotype is

more likely to be of major pathogenic significance as this is associated with an increased risk of recurrent infections and poor outcome [2, 3, 12–15].

Monocyte Function and the Endotoxin Tolerance Model

The functional monocyte deactivation observed in sepsis has striking similarities with the *in vitro* 'endotoxin tolerance' model (Fig. 1). In an elegant study, normal monocytes were repeatedly stimulated with LPS to reproduce the *in vivo* phenomenon encountered in sepsis [16]. These monocytes showed a marked reduction in HLA class II surface expression and a reduction in antigen presentation capacity and pro-inflammatory cytokine secretion [16]. The same group subsequently reported that *in vitro* administration of IL-10 to normal monocytes resulted in 'endotoxin tolerant' monocytes [11].

Influence of IL-10 on Monocyte Function

IL-10 is a well characterized anti-inflammatory cytokine. It inhibits pro-inflammatory cytokine expression and production (TNF-α, IFN-γ, IL-12) and reduces antigen presentation of monocytes [17]. It is released from the very start of the systemic inflammatory response concomitantly with TNF-α; the levels correlating with the magnitude of the inflammatory response evoked [17–19]. In septic shock, high circulating levels of IL-10 are associated with a reduced surface expression of HLA-DR [12, 14] and result in functional monocyte deactivation described in the previous section [19].

In summary, the systemic inflammatory response involves a complex interplay between pro-and anti-inflammatory components. A homeostatic imbalance is evident in septic shock where the anti-inflammatory response may prevail and give

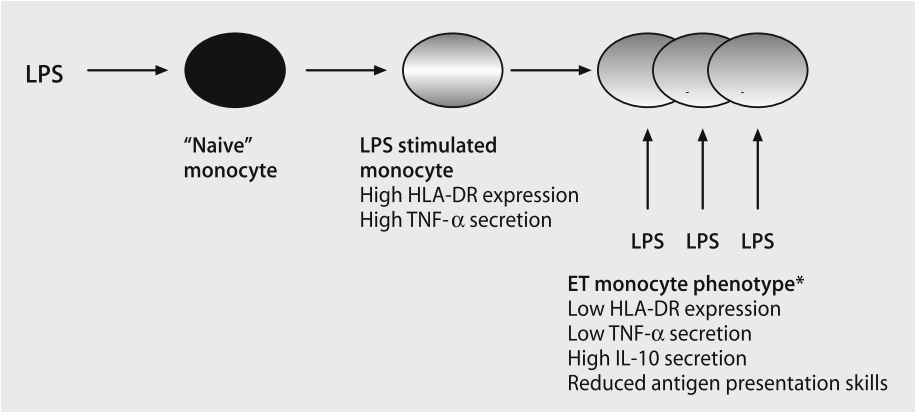

Fig. 1. Schematic representation of the *in vitro* model of functional monocyte deactivation – the endotoxin tolerant (ET) monocyte phenotype*, following repeated lipopolysaccharide (LPS) stimulation. This *in vitro* phenomenon closely resembles functional monocyte deactivation encountered *in vivo*. TNF: tumor necrosis factor; IL: interleukin

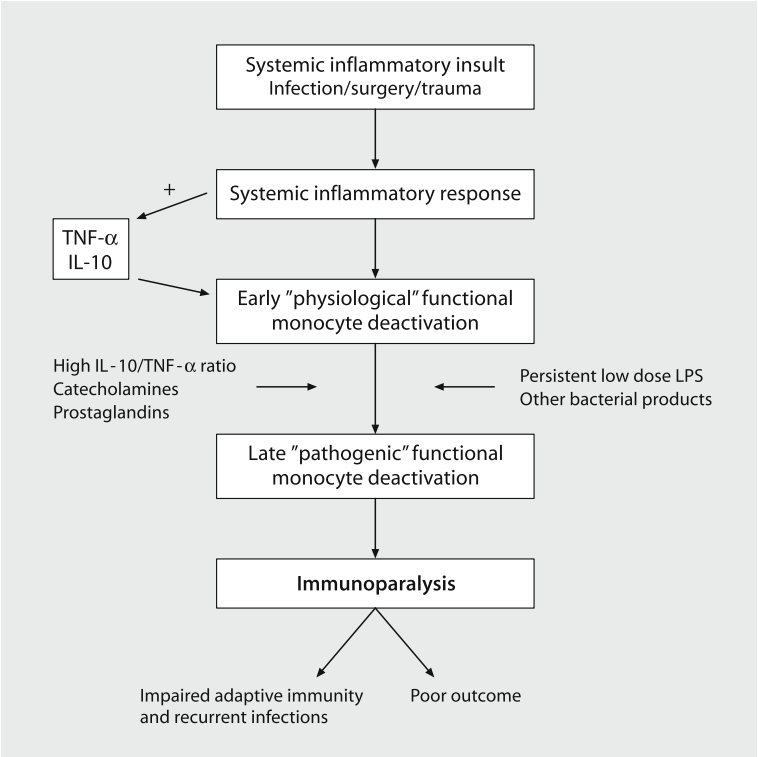

Fig. 2. Proposed temporal evolution of immunoparalysis in septic shock. TNF: tumor necrosis factor; IL: interleukin; LPS: lipopolysaccharide

rise to functional monocytic deactivation and immunoparalysis, the consequence of which being repeated infections and a poor outcome. A schematic representation of this is provided in Figure 2.

Immune Dysfunction in Acute Liver Failure

Infection is very common in patients with acute liver failure, with bacterial infection being identified in up to 80% of patients [20]. Furthermore, fungal infection is also a frequent occurrence in the later stages of this condition. This susceptibility to infection is a consequence of defects in immune function, more specifically relating to neutrophil and Kupffer cell function. Rolando et al. demonstrated that neutrophil function was impaired in acute liver failure and that administration of G-CSF could improve the function of these cells [21]. Kupffer cell function is also impaired, limiting the clearance of circulating endotoxin and thus allowing bacterial translocation from the gut to the systemic circulation [22], giving rise to the systemic endotoxemia encountered in this condition.

Macrophages, in particular Kupffer cells, and their inflammatory mediators are thought to play a role in propagating the initial toxin or viral induced hepatic in-

jury. In animal models of acute liver failure, such as carbon tetrachloride, endotoxin, galactosamine, acetaminophen-induced acute liver failure, there is a profound activation of the Kupffer cell population and release of inflammatory mediators such as TNF-α, IL-1, proteolytic enzymes and nitric oxide (NO) [23]. These inflammatory mediators are recognized endothelial cell activators and result in both 'local', namely hepatic, and systemic activation of the inflammatory response. Furthermore, there is some evidence to suggest that IL-10 is released concomitantly as part of a homeostatic response to hepatic injury in an attempt to counteract the damaging effects of the inflammatory cytokines [24].

The presence of the systemic inflammatory response syndrome (SIRS) in acute liver failure has been shown to confer a poor prognosis [25, 26]. Rolando et al. reported the presence of SIRS in 504 out of 887 (57%) admitted with acute liver failure [25]. The presence of SIRS on admission was associated with an increased severity of organ dysfunction, worsening of encephalopathy and death. Acute liver failure patients, in whom sepsis was responsible for SIRS, showed a rapid progression of their encephalopathy score and consequently a poor outcome. This finding was later reproduced by Vaquero et al. in a large multicenter US study [26]. In acute liver failure, SIRS is implicated in the progression of encephalopathy and a poorer prognosis.

Increased serum levels of pro-inflammatory cytokines (IL-1, IL-6, IL-8 and TNF-α) are thought to reflect the severity of the inflammatory response evoked in acute liver failure with some studies reporting higher IL-6 and TNF-α levels in non-surviving acute liver failure patients [27–33].

Monocyte HLA-DR Expression in Acute Liver Failure

Given the striking phenotypic similarities between septic shock and acute liver failure and the importance of the inflammatory response in determining outcome, we measured monocyte HLA-DR and circulating levels of both pro-and anti-inflammatory cytokines in acute liver failure patients admitted to our unit [34]. Monocyte HLA-DR was determined within 48 hours of admission to our unit in 50 patients with acetaminophen-induced acute liver failure and 20 with non-acetaminophen induced acute liver failure. Acetaminophen-induced acute liver failure patients were divided into those who died or received a liver graft ($n = 26$) and those who survived with conservative medical management ($n = 24$). Forty patients with chronic liver disease and 50 healthy volunteers served as controls. The serum levels (pg/ml) of IL-4, IL-6, IL-10, and TNF-α were concomitantly measured in 30 patients with acetaminophen-induced acute liver failure.

Compared to the healthy volunteers (75%) and patients with chronic liver disease (63%), HLA-DR % expression was lower in patients with acetaminophen-induced acute liver failure (15%, $p < 0.001$) and non-acetaminophen-induced acute liver failure (22%, $p < 0.001$). The levels of HLA-DR expressing monocytes were significantly lower in acetaminophen-induced acute liver failure patients who died or fulfilled transplant criteria compared to those who survived on conservative medical management (11 vs 36%, $p < 0.001$); indicating that this measurement is a marker of poor prognosis. Furthermore, we detected a strong correlation between the indices of acute hepatic injury (international normalized ratio [INR], arterial pH, arterial blood lactate, degree of encephalopathy and vasopressor requirement) and levels of monocyte HLA-DR expression.

We also observed higher circulating levels of both pro- (TNF-α, IL-6) and anti- (IL-10) inflammatory cytokines in patients with acetaminophen-induced acute liver failure with a poor prognosis compared to those who survived on conservative medical management. This finding indicating that the magnitude of the inflammatory response evoked reflects the severity of hepatic injury. Interestingly, monocyte HLA-DR levels had a striking negative correlation with circulating IL-10 levels suggesting that monocyte dysfunction may in part account for the fact that these patients suffer from recurrent infections and develop refractory MOF.

This work emphasizes the importance of the systemic inflammatory response in the pathogenesis and outcome in acute liver failure. The acute hepatic injury activates both resident (Kupffer cells) and circulating macrophages (monocytes) that release potent inflammatory mediators and gives rise to the systemic inflammatory response. Our knowledge of monocyte function during the evolution of this process is very primitive; however, it is increasingly apparent that monocyte dysfunction, or deactivation, is evident in acute liver failure and renders these patients immunodeficient, hence conferring poor outcome.

Immune Dysregulation in Cirrhosis and Acute-on-Chronic Liver Failure

Cirrhosis has long been associated with vulnerability to infection, especially in the hospitalized or decompensated patient. The high incidence, over 60%, of bacterial infection following variceal hemorrhage (prophylaxis against which has been shown to improve survival [35]) reflects the degree of immunosupression. A number of factors have been identified to explain this susceptibility, for instance reduced neutrophil opsonization and intracellular killing capacity [36, 37]. The role of the monocyte, and in particular HLA-DR expression and cytokine response in such situations is now being investigated.

Cirrhosis is associated with a degree of endotoxemia, which appears quantitatively related to the degree of liver disease [38, 39] and to the cardiovascular derangements (hypotension, reduced vascular tone) seen in this population [40]. Bacterial translocation and reduced hepatic clearance of LPS are the most likely reasons for this [41, 42]. Resting circulating peripheral blood mononuclear cells from cirrhotic patients have enhanced RNA expression of TNF-α [43], consistent with a state of immune stimulation. Further stimulation of monocytes by LPS results in higher than normal TNF-α production, and the concept of monocyte 'priming' has been developed [44], whereby in the cirrhotic patient immune cells are, through the effects of the milieu in which they circulate, ready to respond dramatically to intervening sepsis.

The number of HLA-DR expressing monocytes in stable cirrhosis is frequently increased [45], in keeping with the theory that these cells are in a stimulated state. So why should the patient be *more* prone to infection? It is in the monocytes' exaggerated response to intercurrent illness, and the over-extended release of cytokines, that we begin to see parallels with acute liver failure, and the sustained falls in HLA-DR described above.

Wasmuth et al. [46] compared HLA-DR expression in 27 patients with acute-on-chronic liver failure with a population of non-cirrhotic septic (n = 31) and uninfected, stable cirrhotic (n = 24) patients. Serum cytokine concentrations and *ex vivo* production of TNF-α by circulating monocytes were also assessed. Although infec-

tion was the cause of severe decompensation in 13 of the patients with acute-on-chronic liver failure, active infection was excluded at the time of testing. Median SAPS II scores and mortality were equivalent in the acute on chronic liver failure and sepsis groups. Although the patients with acute-on-chronic liver failure displayed significantly lower C-reactive protein (CRP), procalcitonin, IL-6 and IL-10 levels than the sepsis group, patients with acute-on-chronic liver failure had a more 'inflammatory' physiological profile than the stable population. Most interestingly, however, HLA-DR expression was equally suppressed in the patients with acute-on-chronic liver failure and in septic patients. *Ex vivo* TNF-α secretion showed the same pattern, affected as much by acute-on-chronic liver failure as severe sepsis. The authors concluded that acute-on-chronic liver failure represents a severe challenge to cellular immunity even in the absence of infection.

HLA-DR in the Complications of Cirrhosis

Inflammation is implicated in the complications of cirrhosis. Cytokinemia, NO production and their effect on an already hyperdynamic portal circulation may play a role in the onset of variceal bleeding [47]. Cytokines may also limit the myocardial response to the circulatory derangements seen in spontaneous bacterial peritonitis, and this has been associated with the onset of hepatorenal syndrome [48]. The presence of inflammation has been independently associated with worsening encephalopathy [49]. These observations, implicating inflammation in the specific complications of cirrhosis, have prompted further study into monocyte mediated immune dysfunction in chronic liver disease.

Hypothesizing that the common life threatening complications of cirrhosis, namely spontaneous bacterial peritonitis, variceal bleeding, renal failure and encephalopathy may either reflect or effect immune status, HLA-DR expression was studied in 100 consecutive cirrhotic patients admitted to hospital [50]. Overall clinical status (compensated cirrhosis, decompensated but stable cirrhosis [ascites], cirrhosis complicated by one of the conditions described above, or acute-on-chronic

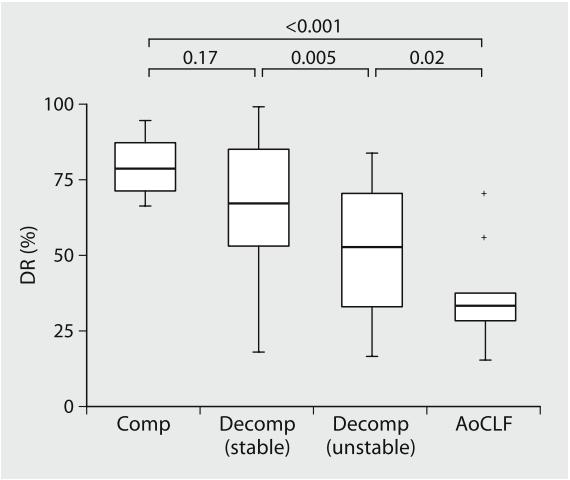

Fig. 3. HLA-DR% in relation to clinical state. AoCLF: acute on chronic liver failure; Comp: compensated; Decomp: decompensated

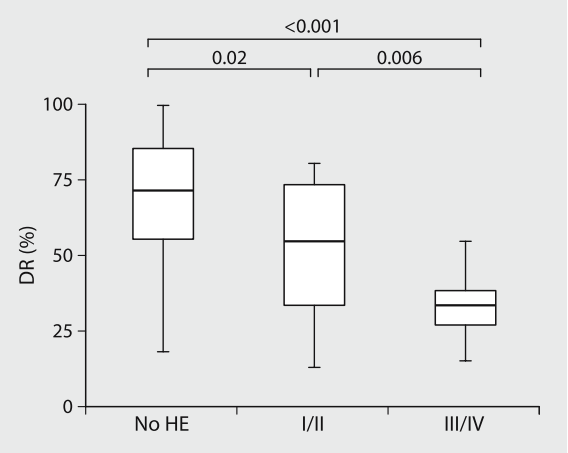

Fig. 4. HLA-DR% in relation to hepatic encephalopathy (HE)

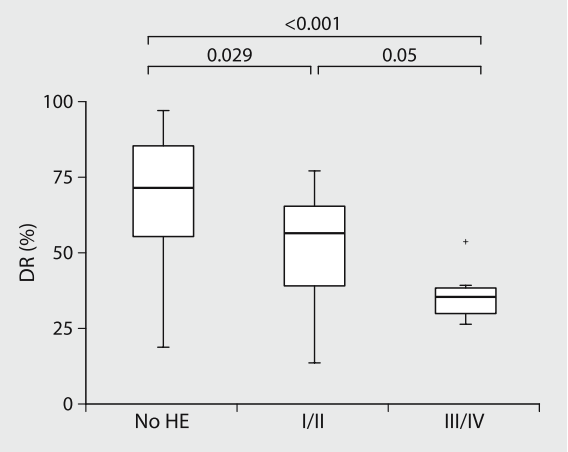

Fig. 5. Is this just the recognized effect of sepsis? HLA-DR% in relation to hepatic encephalopathy (HE) in 69 culture negative patients

liver failure) was noted, and in the case of encephalopathy the degree (I to IV) was documented. Significant relationships were found between HLA-DR expression and overall clinical status (Fig. 3). Moreover, a statistically significant trend existed between HLA-DR expression and degree of encephalopathy; the higher the degree of hepatic encephalopathy, the lower the expression of HLA-DR (Fig. 4). This relationship persisted when only those without positive bacterial cultures (69%) were analyzed (Fig. 5). Significant relationships were also demonstrated in terms of the pro- and anti-inflammatory cytokines IL-6 and IL-10.

These observations emphasize the interplay between cirrhosis, its complications, inflammation and cellular immunity. In the case of hepatic encephalopathy, we observe an inter-relationship between superficially quite disparate factors: inflammation, neurological deficit and immune dysregulation. Such variations in this aspect of immune function specific to cirrhosis and its complications will undoubtedly prompt further study.

Conclusion

Monocyte dysfunction in the systemic inflammatory response plays a major role in the pathogenesis of organ dysfunction in acute liver failure and acute on chronic liver failure. Our understanding of the processes that lead to immune 'dysregulation' in these conditions is limited at present; however, patterns revealed in the studies reviewed above have offered insights into this hugely complex field. Hopefully, further studies into monocyte function will allow clinicians to recognize at risk patients, and perhaps develop new therapeutic strategies.

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Hepatorenal Syndrome

P. Angeli

Introduction

Patients with liver cirrhosis and ascites often develop renal failure, even in its acute form. As of all forms of acute renal failure, prerenal failure (42%) and acute tubular necrosis (38%) are the most common, with hepatorenal syndrome (HRS) being somewhat less frequent (20%) [1]. The prevalence of HRS in patients affected by hepatic cirrhosis with ascites is in effect 18% after one year, rising to 39% at 5 years [2]. In almost half the cases of HRS, one or more precipitating factors may be identified, including: bacterial infections (57%), gastrointestinal hemorrhage (36%), and therapeutic paracentesis (7%) [2].

HRS is characterized by: a) marked renal vasoconstriction with a consequent reduction in renal blood flow and glomerular filtration rate (GFR); b) the absence of histological changes in the renal tissue; and c) preserved tubular renal function. HRS usually arises when chronic liver disease is associated with a circulatory dysfunction with very low values of arterial pressure [3]. The prognosis for cirrhotic patients who develop HRS is very poor with mortality rates of around 100%, and a median survival rate of two weeks from the moment of onset [2].

Physiopathology of HRS

At a pathophysiological level, the causes of the intense renal vasoconstriction underlying HRS are not completely known. In comparison to control subjects and the same cirrhotic patients with ascites but without HRS, patients with HRS consistently show lowered splanchnic vascular resistance. Renal vasoconstriction, therefore, develops where there is a marked reduction in effective circulating volume related to splanchnic arterial vasodilatation and to other factors (Table 1) [3, 4].

Considerable evidence exists both at an experimental and a clinical level to support this interpretation, leading to an elaboration of the theory of arterial vasodilatation where renal vasoconstriction is the result of an activated endogenous vasoconstrictor system (renin-angiotensin system, sympathetic nervous system and release by osmosis without vasopressin) as a result of a reduced effective circulating volume produced by splanchnic arterial vasodilatation [4, 5]. The involvement of endogenous vasoconstrictor systems in the development of HRS has been documented both at a clinical and/or experimental level. In particular, renal denervation in cirrhotic animals with ascites [6] or local anesthetic block of the lumbar sympathetic nervous system in cirrhotic patients with ascites or HRS [7] produces an improvement in renal perfusion.

Table 1. Determinants of effective circulating volume

systemic vascular resistance
blood volume
redistribution of blood volume
total vascular compliance
cardiac ouput
arterial compliance

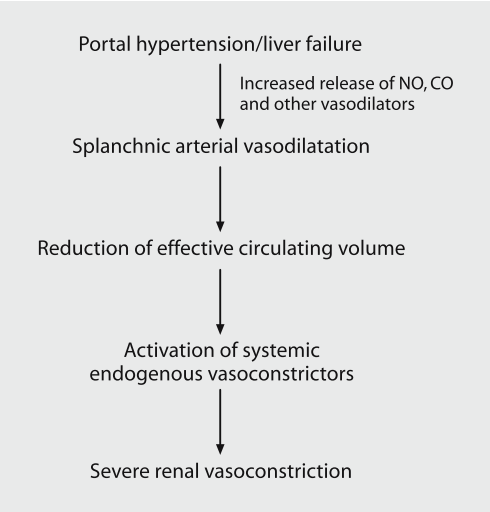

Fig. 1. Pathogenesis of HRS. NO: nitric oxide; CO: carbon monoxide

Despite recent findings [8], arterial splanchnic vasodilatation is thought to be mainly the consequence of an increased release of nitric oxide (NO) due to portal hypertension and/or hepatic failure [9] (Fig. 1).

A detailed analysis of all the possible mechanisms involved in HRS pathogenesis goes beyond the purposes of this chapter; however, one cannot fail to mention the fact that the arterial splanchnic vasodilatation theory leaves several issues still open, such as:

- to what extent are other factors involved in determining the impairment of the effective circulating volume?
- What role does intrarenal vasoconstriction play?
- What is the role of intrarenal vasodilators?

In relation to the first question, it must be emphasized that the effective circulating volume does not depend only on peripheral vascular resistance, but also on a series of other factors outlined in Table 1, which are subject to alteration in patients with cirrhosis [10]. To confirm the importance of some of these factors, it has been observed that in HRS caused by spontaneous bacterial peritonitis, a worsening of the degree of contraction of efficient circulating volume is not connected to any further reduction in peripheral vascular resistance but rather to a reduction in cardiac flow [11].

Regarding the second question it may be observed that the administration of an endothelin receptor antagonist improved renal perfusion in patients with HRS and no variation in arterial pressure systems, testifying to the fact that endothelin may act mainly as an intrarenal vasoconstrictor [12]. Further relative findings report an increased release at a strictly intrarenal level of other very potent vasoconstrictors among which are thromboxane, 20-hydroxyeicosatetraenoic acid (20-Hete), and leukotrienes [13–16].

Finally, concerning the third question, it may be observed that at an experimental level, acute renal failure may be provoked in cirrhotic animals with ascites via the administration of inhibitors of the release of substances that perform a vasodilatory action in the kidney and which include prostaglandin E2 and I2 (PGE2 and PGI2) [17], endogenous natriuretic peptides [18], and NO [17]. It may be added that in cirrhotic patients with ascites the administration of a non-steroidal anti-inflammatory drug may cause an acute form of renal failure that is quite similar to HRS as far as clinical and laboratory findings are concerned [19].

Criteria for HRS Diagnosis

The criteria for diagnosing HRS have been re-examined by members of the "International Ascites Club" and are distinguished by major and minor criteria. The presence of all major criteria has to be established before making a diagnosis of HRS, while the presence of minor criteria only provides additional diagnostic support [2] (Table 2).

By combining some major with more minor criteria we were able to differentiate HRS from other forms of acute renal failure and in particular from acute prerenal failure and acute tubular necrosis (Fig. 2).

In diagnostic terms, two types of HRS can be distinguished (Table 3). Type 1 HRS is characterized by a rapid progression of renal failure so that its main clinical

Table 2. Criteria for a diagnosis of HRS

Major Criteria

- A diagnosis of chronic liver disease with portal hypertension;
- reduced glomerular filtration rate as indicated by a serum creatinine > 1.5 mg/dl or a creatinine clearance < 40 ml/min;
- absence of shock, ongoing bacterial infection (presence of clinical biohumoral or cultural findings of infection in spite of adequate antibiotic treatment), recent use of nephrotoxic drugs, dehydration;
- no improvement in renal function after the withdrawal of diuretics and plasma volume expansion with 1.5 l of isotonic saline solution;
- proteinuria < 500 mg/dl and no ultrasound evidence of obstructive uropathy or parenchymal nephropathy.

Minor Criteria

- urinary volume < 500 ml/day;
- urinary sodium excretion <10 mmol/l;
- urine osmolality/plasma osmolality > 1;
- no significant findings in urinary sediment;
- natremia < 130 mmol/l.

Features	Prerenal failure	Hepatorenal syndrome	Acute tubular necrosis
Urine sodium concentration (mEq/l)	< 10	< 10	> 30
Fractional excretion of sodium (%)	< 1	< 1	> 1
Urine/plasma osmolality (mOsm/kg)	> 1	> 1	< 1
Urine sediment	normal	unremarkable	cast and cellular debris
Response to plasma volume expansion	Yes	No	No

Fig. 2. Differential diagnosis of acute renal failure in cirrhosis

Table 3. Clinical forms of HRS

	Type 1 HRS	Type 2 HRS
Characteristics	rapidly progressive reduction in renal function as defined by a doubling of the initial serum creatinine to a level > 2.5 mg/dl or 50% reduction of the initial 24-hr creatinine clearance to a level < 20 ml/min in less than two weeks	renal failure without a rapidly progressive course
Clinical pattern	acute renal failure	refractory ascites

Table 4. Precipitating events for HRS

- spontaneous bacterial peritonitis
- paracentesis without plasma expansion
- gastrointestinal hemorrhage
- severe acute alcoholic hepatitis
- unknown

feature is acute renal failure. By contrast, the degree of impairment of renal failure is more stable over time in patients with type 2 HRS. As a consequence the presence of refractory ascites is the main clinical problem in these patients. In addition, the two types of HRS differ substantially in prognostic terms in that the median survival for type 1 is about 2 weeks whilst for type 2 it is generally around 6 months [20]. Differences at a pathophysiological level between type 1 HRS and type 2 are unknown; however, at a clinical level it can be observed that type 1 HRS is often induced by the occurrence of a precipitating event (Table 4), in particular by the development of spontaneous bacterial peritonitis, in patients who are already suffering from type 2 HRS [21].

HRS Therapy

Orthotopic liver transplantation represents a unique therapeutic option in cirrhotic patients with HRS because it can remove the main causes of disease, which are portal hypertension and liver failure. However, the presence of HRS at the moment of the transplant compromises the transplant's success in terms of survival, costs, and quality of life [22]. Furthermore, the rapid evolution of type 1 HRS makes it unlikely that cirrhotic patients with ascites and type 1 HRS will ever undergo a liver transplant. This fact outlines the need for other efficient forms of treatment for HRS, and the necessity to at least locate a 'treatment bridge' towards liver transplant. Over the past five years, new treatment options have been proposed for this purpose, including transjugular intravenous porto-systemic shunts (TIPS), albumin and vasoconstrictors, and the molecular adsorbent recycling system (MARS).

A significant improvement in renal function was reported in 16 patients with HRS after placement of a TIPS [23] with a 3-month survival rate of 75%. However, it should be noted that a significant percentage of patients treated with TIPS had type 2 HRS. In addition, it should be remembered that there are many contraindications to treatment with TIPS (Table 5), including a Child-Pugh score >11 and a total serum bilirubin >5 mg/dl, which occur more frequently in patients with HRS, particularly in those with type 1 HRS. It therefore follows that TIPS is not often used in the treatment of type 1 HRS, but it is widely used as an alternative to therapeutic paracentesis in the treatment of refractory ascites associated with type 2 HRS. Five controlled studies have been published in which therapeutic paracentesis was compared to the use of TIPS in this situation [24–28]. Overall these studies have demonstrated that:

- TIPS is effective in the control of ascites,
- the risk of encephalopathy is greater in patients who are treated with TIPS, and
- with one single exception [28], a significant difference was not observed between the two treatments as regards survival.

The more interesting observations relative to the pharmacological treatment of type 1 HRS refer to a completely new approach based on more recently acquired knowledge regarding the pathophysiology of the renal functional abnormalities in cirrhosis and, in particular, to the relationship between splanchnic arterial vasodilatation and renal vasoconstriction. In particular, recent studies have demonstrated that the protracted use of a vasoconstrictor derived from vasopressin (ornipressin, terlipressin) [29–34] or of an α-agonist vasoconstrictor (norepinephrine, midodrine) [35–37] in association with the prolonged infusion of human albumin, can improve renal function in patients with type 1 HRS (Table 6).

Table 5. Contraindications to TIPS

Child-Pugh score >11
serum bilirubin >5 mg/dl
overt or chronic hepatic encephalopathy
age greater than 70
cardiac dysfunction
portal vein thrombosis

Table 6. New pharmacological therapies for HRS

▮ Albumin (1 g/kg during the first day and then 20–40 g/day intravenously)
▮ Terlipressin (0.5–2 mg/4 h intravenously or 2–10 mg/day by intravenous infusion)
▮ Albumin (20–40 g/day, intravenously)
▮ Midodrine (7.5–12.5 mg three times daily orally)
▮ Octreotide (100–200 μg three times daily, subcutaneously)
▮ Albumin (20–40 g/day, intravenously)
▮ Norepinephrine (0.5–3 mg/hr, intravenously)

Overall these studies have demonstrated that:

- recovery of renal function can be obtained in 60–80% of cases by using a vasoconstrictor plus albumin for a mean period of 10–15 days and for doses which are reported in Table 6 [29–37]
- the recovery of renal function can be maintained in over 70–80% of patients after the treatment is suspended [29–37]
- the use of albumin in association with the vasoconstrictor is essential for the success of the treatment [31]
- a marked impairment of hepatic function represents a negative predictive factor for response to the treatment [32–34].

These results, although still preliminary and therefore in need of further confirmation, do appear to be a quite encouraging development for the effective treatment of type 1 HRS. In particular, even in the absence of any controlled clinical study, the use of a vasoconstrictor in association with albumin appears to increase short-term survival in cirrhotic patients with ascites who develop type 1 HRS [38]. The mean survival in patients treated with a vasoconstrictor and albumin varies from a minimum of 21 days to a maximum of 11.9 weeks [31–35, 38] against 2.0 weeks in untreated patients [2] or in those treated with albumin or dopamine [35]. It should be noted that the prognosis in these patients is not only related to a recovery of renal function but, also, to the degree of liver failure. A marked impairment of liver function not only represents a negative predictor for the response to treatment with vasoconstrictor and albumin [33, 34] but is, also, a negative predictive factor for survival in patients with type 1 HRS undergoing the treatment. A retrospective study with terlipressin demonstrated that a Child-Pugh score greater than 11 was indicative of poor outcome regardless of any eventual recovery of renal function [33].

The recent introduction of innovative techniques for extracorporeal liver function support like MARS has made it possible to recover to some extent, even transiently, hepatic function. In addition, when MARS was applied to the treatment of type 1 HRS, the ability of the procedure to remove vasoactive substances carried by albumin, such as NO, tumor necrosis factor (TNF), and other pro-inflammatory cytokines, and to reduce serum urea and serum creatinine, was essential for its positive effects on systemic hemodynamics and renal function [40, 41]. Such improvements have also been shown to have a positive effect on 30-day survival in these patients (37.5 versus 0%) [39]. It is, therefore, easy to hypothesize that in the near future MARS will be used together with a vasoconstrictor and albumin in the treatment of type 1 HRS in patients with a marked impairment of liver function.

Meanwhile, however, it appears evident that in many patients the pharmacological treatment of type 1 HRS does not represent a treatment 'bridge' to liver transplant. This new therapeutic approach has likewise increased the percentage of patients that is subjected to transplant after developing HRS and has improved the 'outcome' of the transplant both in terms of average and long-term survival as well as in terms of costs [42].

The effects of vasoconstrictors and albumin in treatment of type 2 HRS has been less studied. The percentage response to treatment in terms of recovered renal function does not seem, however, to be different from that observed in patients with type 1 HRS [26], while survival appears decidedly longer (100% at 3 months). The data on survival should, however, be evaluated with the utmost care considering that survival in patients with untreated type 2 HRS is above that observed in patients with type 1 HRS [2], as described above. It also needs to be stressed that the clinical problem in these patients does not appear to be so much the recovery of renal function as the treatment of ascites, which often appears refractory to diuretic therapy.

Prevention of HRS

The prevention of HRS requires examination of several factors ranging from the correct management of diuretic therapy to adequate plasma volume expansion following therapeutic paracentesis in cirrhotic patients with ascites. Moreover, it should be remembered that the most common precipitating factor for HRS is bacterial infection and, in particular, spontaneous bacterial peritonitis. As a result of the development of better and quicker diagnostic strategies and more effective and safer antibiotic therapy over the last twenty years, the prognosis of spontaneous bacterial peritonitis has improved greatly. Hospital mortality rates relating to this complication have in fact decreased from 50 to 20%. Spontaneous bacterial peritonitis-induced renal failure is a common cause of death in these patients. The deterioration in renal function due to the bacterial infection is thought to be the expression of a further reduction in effective circulating volume and in 20–30% of cases it has the features of type 1 HRS. Recently, it has been observed that by treating spontaneous bacterial peritonitis-induced type 1 HRS it was possible to further reduce the in-hospital mortality due to the infection (13%) [43]. In this context, the latest progress in prevention demonstrates that an association between effective antibiotic therapy and plasma volume expansion with albumin is able to reduce the prevalence of spontaneous bacterial peritonitis-induced renal failure and related mortality [44]. Albumin appears to be able to prevent further reduction of the effective circulating volume induced by the bacterial infection both by increasing the cardiac preload and by improving cardiac contractility [45]. This latter effect of albumin is probably related to its ability to bind NO and pro-inflammatory cytokines that appear after the infection, and may develop a negative inotropic effect at a cardiac level [46].

Conclusion

HRS is a common and serious complication of advanced liver disease. It develops in the setting of major alterations of circulatory function, in particular, intense vasoconstriction in the renal arterial circulation due to a severe reduction of effective circulating volume. The reduction of the effective circulating volume is secondary to splanchnic arterial vasodilation due to an increased released of vasodilatatory substances, which include NO, prostacyclin, glucagon, and carbon monoxide. The diagnosis is based on the exclusion of other types of renal failure such as prerenal failure and tubular necrosis, and two distinct forms are characterized: a) type 1 HRS which is characterized by a rapid progression of renal failure and b) type 2 HRS with a stable degree of impairment of renal function over a period of weeks or months. Up to a few years ago the prognosis of HRS was very poor, particularly in type 1 HRS, and liver transplantation was the only therapeutic option in patients without contraindications to the procedure. However, very few patients with HRS and particularly with type 1 HRS survived long enough to undergo liver transplantation and the presence of renal failure at the time of liver transplantation had a negative impact on the outcome of the procedure both in terms of survival and costs. In the last few years, some new therapeutic options, tested in non-controlled clinical studies, have really changed the approach to the treatment of HRS. Vasoconstrictor drugs (terlipressin and α-agonists) with albumin and TIPS are able to improve renal function in patients with HRS, even in type 1 HRS. Although these new therapeutic options probably improve survival in patients with HRS, it still remains poor since it is largely dependent on the liver failure. The recovery of liver function is, therefore, an essential step in the treatment of HRS. Until results of controlled clinical trials on extracorporeal artificial and bioartificial support in this field are available, liver transplantation still remains the best option in suitable patients. In this context, it should be underlined, however, that treating HRS with vasoconstrictors and albumin improves the outcome of liver transplantation. Finally, it has been shown recently that HRS may be prevented in the course of spontaneous bacterial peritonitis by the administration of albumin together with antibiotics.

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Renal Failure

Sepsis and Acute Renal Failure

R. W. Schrier, E. Zolty, and W. Wang

Introduction

Severe sepsis is associated with acute renal failure in approximately 23% of patients, while septic shock is complicated by acute renal failure in 51% of patients [1]. Acute renal failure associated with sepsis has a mortality as high as 70–80% [2]. Much of the hemodynamic and inflammatory events which accompany sepsis are related to endotoxemia. The study of the renal effects of endotoxemia have, therefore, provided substantial insights into the mechanisms mediating acute renal injury during sepsis.

Early Events during Endotoxemia

Endotoxemia is associated with increased cytokine release such as tumor necrosis factor (TNF)-α [3]. Cytokine induction of inducible nitric oxide (NO) synthase (iNOS) increases circulating NO and contributes to the arterial vasodilation which is a hallmark of sepsis [4]. Arterial blood pressure is maintained during endotoxemia and sepsis by activation of the neurohumoral axis including the renin-angiotensin system and sympathetic nervous system. However, while the increase in renin-angiotensin system and sympathetic nervous system activity exerts a beneficial effect on blood pressure, at the same time renal vasoconstriction occurs. In an experimental model of endotoxemia, renal denervation was shown to afford protection against acute renal failure [5]. Unfortunately, selective renal vasodilators, which avoid worsening of the sepsis-related systemic arterial vasodilation, are not available.

In addition to the increases in circulating vasoconstrictors, there is also evidence that the intrinsic renal vasodilators, which attenuate the action of these vasoconstrictors on the kidney, are decreased during endotoxemia. There is experimental evidence that the increased plasma NO during endotoxemia is associated with a downregulation of endothelial NOS (eNOS) in the kidney [6]. This observation is supported by results which demonstrate that eNOS knockout mice are more susceptible to endotoxemia-related acute renal failure than wild type mice. The eNOS knockout mice have higher blood pressures but also have increased renal vascular resistance as compared to wild type mice [7]. At 24 hours after endotoxemia, a downregulation of renal cyclic GMP, the secondary messenger of NO mediated vasodilation, also occurs [8]. While the mechanism for this downregulation is not understood, it could further contribute to renal vasoconstriction during sepsis.

The renal vasoconstriction during endotoxemia is associated with intact tubular function during the early stages, as supported by enhanced tubular sodium reabsorption and decreased fractional excretion of sodium [9]. Prolonged renal ischemia during sepsis will, however, convert this prerenal azotemic stage to acute tubular necrosis (ATN) with impaired tubular function and a rise in fractional excretion of sodium. Early intervention must therefore be undertaken to prevent progression to ATN.

Early Intervention in Endotoxemia

Large volumes of isotonic saline have been routinely used for early resuscitation of septic patients with little effect on mortality. In fact, because of an increase in albumin distribution space and alterations in Starling forces during systemic arterial vasodilation, this aggressive volume expansion approach frequently leads to pulmonary edema, hypoxia and institution of mechanical ventilation [10] (Fig. 1.) We have termed these early events pseudo-acute respiratory syndrome (ARDS) since

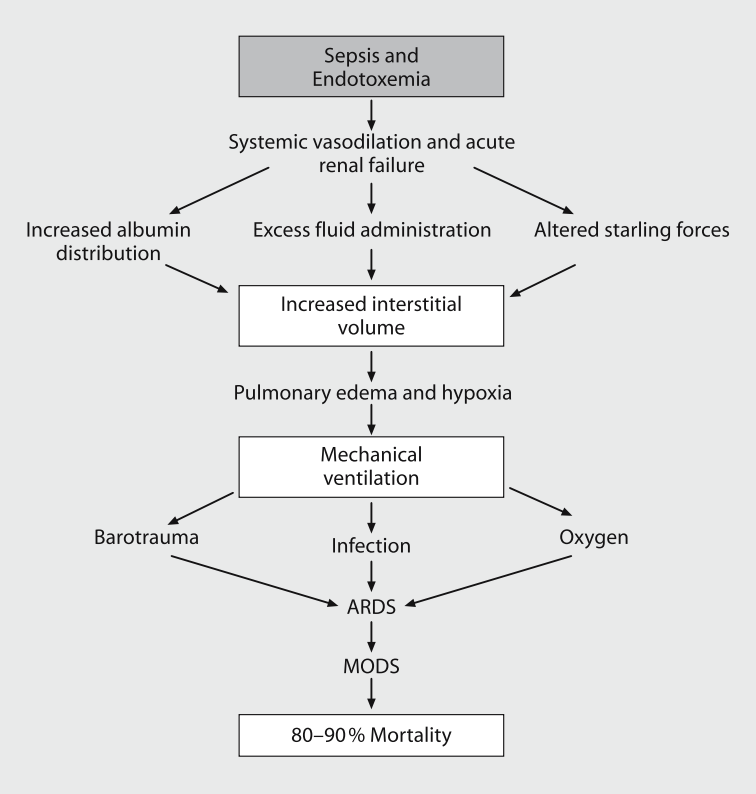

Fig. 1. Sequence of events whereby sepsis and endotoxemia can lead to non-cardiogenic pulmonary edema, mechanical ventilation, acute respiratory distress syndrome (ARDS), multiple organ dysfunction syndrome (MODS), and an extremely high mortality.

frequently the pulmonary compliance is normal [11]. However, prolonged mechanical ventilation frequently progresses to ARDS secondary to infection, barotrauma and oxygen toxicity. Studies have shown a much higher incidence of mechanical ventilation and mortality in sepsis-related acute renal failure as compared to acute renal failure in the absence of sepsis [12].

Since arginine vasopressin may be more effective than other pressor agents during sepsis [13], and may even afford protection to the kidney by constricting the glomerular efferent arteriole, clinical studies are needed to compare resuscitation of septic patients with arginine vasopressin and albumin versus volume loading with saline. Any therapeutic maneuver leading to a reduction in acute renal failure and the need for mechanical ventilation would no doubt be associated with improved survival in the septic patient.

Early goal-directed therapy within the first six hours of admission to the emergency department has been shown in a randomized study to decrease in-hospital mortality and multiorgan dysfunction score in septic patients with a mean admission serum creatinine of 2.6 mg/dl [14]. The goal was to maintain central venvous oxygen saturation at greater than 70% and mean arterial pressure (MAP) greater than 65 mmHg with pressor and isotonic saline boluses. If oxygen saturation was still less than 70%, red blood cell transfusion to increase hematocrit to 30% or more was used. Blood pressure, plasma lactate, pH, oxygen saturation, and fibrin split products were all improved in the early goal-directed therapy groups as compared to standard care [14].

Recently, pentoxifylline has been shown, in a normotensive mouse model of endotoxemia-related acute renal failure, to afford renal protection as assessed by glomerular filtration rate (GFR) [15]. The effect of pentoxifylline was also studied in eNOS knockout mice. The eNOS knockout mice have higher blood pressures and increased renal vascular resistance, indicating an important effect of eNOS in maintaining normal blood pressure and renal perfusion. Although pentoxifylline administration in the eNOS knockout mouse during endotoxemia was associated with a decrease in blood pressure, there was remarkable renal vasodilation associated with a protective effect on GFR and renal blood flow. Pentoxifylline is known to increase red blood cell deformability and thus diminish blood viscosity, an effect which could contribute to the agent's renal vasodilation effect during endotoxemia. Another possibility to account for the renal protective effect of pentoxifylline during endotoxemia is the agent's non-specific inhibition of phosphodiesterase. This effect on phosphodiesterase can be associated, depending on the dose, with increases in cyclic GMP and /or cyclic AMP, both secondary messengers mediating vascular vasodilation.

This protection by pentoxifylline was also associated with a decrease in TNF-α [15]. Earlier studies demonstrated that inhibition of TNF-α with soluble TNF receptor administration afforded renal protection during endotoxemia [4]. TNF-α receptor knockout mice also are protected against endotoxemia-related acute renal failure [16]. Of further interest was the observation that kidney transplants from these TNF-α receptor knockout mice to wild type mice induced renal protection against endotoxemia-related acute renal injury in the recipient mice. There is evidence that the renal injurious effect of TNF-α during endotoxemia may be related to several factors including leukocyte infiltration, apoptosis [16], and endothelin [17].

Oxidant Injury during Endotoxemia

Reactive oxidant species (ROS) are known to increase during endotoxemia [18]. The resultant renal oxidant injury can be assessed by measurement of 8-isoprostane or malondialdehyde. Endogenous scavengers of ROS are important in attenuating the oxidant injury during endotoxemia. In this regard, endotoxemia has been shown in the mouse to be associated with a decrease in extracellular superoxide dismutase (SOD), which is located primarily in the kidney and vasculature [19]. This reduction in an endogenous scavenger of ROS may predispose the kidney to oxidant injury by the increased superoxide anion (O_2^-) which is increased during endotoxemia. The increases in O_2^- and NO during endotoxemia combine to produce peroxynitrite which is a very injurious ROS (Fig. 2.)

Increased ROS during endotoxemia can be associated with dysfunction of the endothelium. Such an impairment of the endothelium, particularly when associated with a decreased eNOS, could lead to less attenuation of renal vasoconstriction and platelet-mediated intraglomerular thrombi during endotoxemia.

The observation of endothelial damage during endotoxemia has led to the development of transgenic mice with upregulated renal prostacyclin synthase and quanosine triphosphate cyclohydrolase I, the key enzyme for tetrahydrobiopterin synthesis (BH_4). BH_4 is a pivotal cofactor in the synthesis of eNOS [20] and prostacyclin synthase is key in prostacyclin synthesis [21], events which lead to mediators of renal vasodilation. Both BH_4 and prostacyclin are potential agents available to be examined in septic patients at risk of developing acute renal injury.

On this background, antioxidant agents have been investigated as potential protective agents against acute renal injury during endotoxemia [19]. Impressive renal protection has been shown to occur during endotoxemia with chemically dissimilar antioxidant agents. N-acetylcysteine, a readily available antioxidant for clinical use in septic patients, needs to be studied in endotoxemic mice.

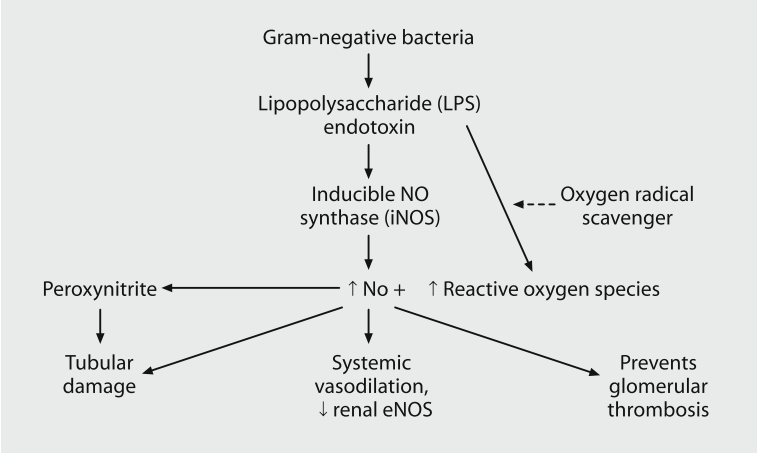

Fig 2. Good and bad effects of nitric oxide (NO) in sepsis. Induction of NO synthase (iNOS) and oxygen radical generation during sepsis causes peroxynitrite-related tubular injury, systemic vasodilation and down-regulation of renal eNOS. NO may however exert renal protection by inhibiting platelet aggregation as well as increasing renal NO and cyclic GMP.

Erythropoietin (EPO) is also known to have antioxidant and anti-inflammatory properties, thus preliminary studies were undertaken to examine the effect of human recombinant EPO during murine endotoxemia [22]. During endotoxemia the mice demonstrated an increase in hypoxia-inducible factor-1 (HIF-1) and a resultant increase in serum EPO. An increase in renal SOD was also observed in the endotoxemic mice. Although endogenous EPO was increased during endotoxemia, the possibility existed that pharmacological treatment with exogenous EPO might afford renal protection in the endotoxemic mouse. This indeed was the case. A dramatic increase in GFR in mice receiving endotoxin was observed with exogenous EPO administration.

Recent prospective randomized studies in critically ill patients with insulin resistance and stress hyperglycemia have been undertaken [23]. These patients are at a substantial risk of developing multiple organ failure and acute renal failure. Patients receiving conventional treatment (insulin therapy only when blood glucose exceeded 215 mg/dl) were compared with a group treated with an intensive insulin protocol to maintain blood glucose levels between 80 and 110 mg/dl. The intensive insulin treated patients had better ICU and in-hospital survival. In addition to the effect on blood glucose, the intensive insulin treated patients in the ICU longer than 5–7 days demonstrated an improvement in their lipid profile [24], decreased plasma NO and intercellular adhesion molecule (ICAM) [25], less inflammation as assessed by lower C-reactive protein levels [26], and decreased structural and functional mitochondrial damage in hepatocytes [27]. These metabolic effects of intensive insulin therapy were associated with:

- higher hematocrits despite fewer red blood cell transfusions,
- decreased serum creatinine and acute renal failure necessitating dialysis or continuous renal replacement,
- decreased nosocomial episodes of bacteremia, and
- decreased critical illness of polyneuropathy and fewer patients needing mechanical ventilation for longer than 14 days [24].

Since many of these critically ill patients are septic and at risk of acute renal injury, these results support an aggressive approach to insulin treatment of critically ill patients with stress hyperglycemia.

Conclusion

In summary, recent advances in understanding of the factors involved in experimental acute renal injury during endotoxemia suggest potential effective interventions. Prospective, randomized studies in septic patients at risk of acute renal failure are, therefore, needed to examine potential therapies to decrease the morbidity and mortality associated with this frequent and devastating clinical problem.

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Sixty Years of 'Extended Dialysis' in the ICU

J. T. Kielstein, C. Hafer, and D. Fliser

Introduction

One of the most challenging problems of nephrology and intensive care medicine is the treatment of acute renal failure. Due to its increased incidience and changing patterns, mortality rates remain high despite the advent of modern means of renal replacement therapy [1–4]. However, increasing the dose of renal replacement therapy may provide substantial benefits, particularly in patients with intermediate levels of illness severity [5, 6]. To achieve this high dialysis dose, there is renewed interest in prolonged or extended dialysis modalities for critically ill patients with acute renal failure in the intensive care unit (ICU).

From Extended Dialysis à la Kolff to CVVH (and Back)

The discussion of extended dialysis for the treatment of acute renal failure must begin with the seminal work of W. J. Kolff. Using his dialysis machine made of cellophane tubing (made for sausage casing) wrapped around a cylinder that rotated in a bath of fluid, Kolff started to treat patients in 1943. On 11 September 1945, Maria Schafstaat, was the first patient whose life was saved by dialysis [7]. He treated the 67-year-old woman for 690 minutes (i.e., 11.5 hours) with a blood flow rate of 116 ml/min. By simple, but well-structured, clinical observations Kolff had described a renal replacement therapy that has only recently become established as a treatment for severely ill patients with renal failure in the ICU – prolonged dialysis time with low flow rates.

As the technical complexity of dialysis machines increased and the need for dialysis rose, treatment time was shortened to more efficiently use equipment and the ever limited number of dialysis staff. The original way of Kolff to treat acute renal failure by lengthy dialysis sessions was abandoned and the short intermittent hemodialysis then employed led to hemodynamic instability, fluctuating fluid and electrolyte levels. This paved the way to the development of continuous renal replacement therapy (CRRT) by P. Kramer and colleagues from Göttingen. They employed continuous arteriovenous hemofiltration (CAVH) which had advantages summarized as follows: "This method, which needs no technical investment, is easy and simple to handle for the physician, bears only a very low risk for the patient, and ensures a negative fluid balance even at a mean blood pressure of only 60 mm Hg" [8]. Therefore, CRRT soon became a widely used method for treating acute renal failure in ICU patients. However, its limited capacity to remove nephrotoxins in

the presence of high catabolism and complications connected to the arterial access lead to the development of a venovenous pump-driven technique, continuous venovenous hemofiltration (CVVH), in order to become independent from the systemic circulation and arterial access. Further progress to improve solute clearance was made by combining the convective principle of hemofiltration with the diffusive transport of dialysis (continuous arteriovenous hemodialysis or hemodiafiltration). Currently this combination is thought to be the most effective renal replacement technique for treating acute renal failure in critically ill patients. To date, however, controlled studies have not detected a definitive advantage in terms of patient survival for CRRT as compared to intermitend hemodialysis [1, 2, 9, 10].

Many aspects of acute renal failure patient treatment have changed since the time of Dres Kolff and Kramer. Today, acute renal failure is generally one feature of a multiple-organ dysfunction syndrome in critically ill patients, which develops in response to major surgery, cardiogenic shock or a systemic inflammatory response syndrome. According to a recent multicenter, multinational study involving 30,000 patients, in almost 50% of the patients acute renal failure was associated with septic shock [4]. Mortality rates are high, despite modern intensive care medicine and extracorporeal renal replacement therapy [1–4]. Patients are usually hemodynamically unstable and hypercatabolic. They, therefore, require treatment with large doses of catecholamines and volume expanders, and parenteral nutrition, which inevitably causes hyperhydration, especially in the presence of oliguria/anuria. In light of these unfavorable clinical characteristics of critically ill patients, physicians have increasingly used CRRT in the ICU, particularly CVVH. CVVH seems to offer better cardiovascular stability in the critically ill patient than conventional intermittent hemodialysis. The efficacy of CVVH with respect to urea clearance is limited by the exchange volume and need for frequent interruptions for other interventions [9, 11]. CVVH requires continuous anticoagulation therapy in addition to making mobilization of patients difficult. The need for sterile hemofiltration fluid makes treatment costs considerable [12], particularly if higher rates of fluid substitution are employed. On the other hand, conventional intermittent hemodialysis is technically demanding, requiring trained (dialysis) personnel, a fresh water supply and hygienic removal of spent dialysate. Further, the relatively short treatment time increases the likelihood of cardiovascular instability in patients who need larger ultrafiltration volumes. The relative advantages and disadvantages of intermittent vs continuous renal replacement therapies are summarized in Table 1. More important than the choice of renal replacement modality in the ICU is delivery of a sufficient treatment dose to critically ill patients [2, 13, 14]. Large clinical trials have shown that survival of patients with acute renal failure can be improved by increasing the dose of renal replacement therapy [5, 6]. As such, current treatment strategies for patients with acute renal failure in the ICU focus on highly efficacious elimination of uremic toxins and concomitant gentle volume removal. This can be achieved with either daily intermittent hemodialysis or high volumes of substitution fluid during CVVH [2, 9]. Unfortunately, the costs (equipment, disposals, staff) associated with these labor-intensive techniques are increasingly important obstacles to their implementation. Alternative strategies have, therefore, been developed, with the aim of providing an easy to perform and less expensive treatment with reduced solute clearances that can be maintained for prolonged periods.

Extended Dialysis – Reinvented

As Kolff had no way to forsee the benefits of his initial strategy for treating acute renal failure in the decades to come, the theoretical basis and clinical implications of the reinvented modality of extracorporeal renal replacement therapy called 'slow continuous hemodialysis' [15, 16] dates back to 1988 [17]. This technique utilizes equipment originally developed for treatment of patients with chronic renal failure and does not require industrially produced substitution fluid [15, 18]. The term 'sustained low efficiency dialysis' (SLED) is the most widely used, but alternatives used in the literature include 'extended dialysis' and 'slow continuous dialysis'. This means of renal replacement combines several advantages of both intermittent and continuous renal replacement therapies [19–21], most notably excellent detoxification and cardiovascular tolerability akin to that associated with CVVH.

Clinical Studies of Extended Dialysis

Several controlled studies [19, 21–23] and accounts of long-term experience [24] have been published by groups that use SLED to treat ICU patients with renal failure (Table 1). Marshall et al. [21, 23] used a standard intermittent hemodialysis

Table 1. Comparison of intermittent and continuous renal replacement therapy.

Intermittent hemodialysis

Advantages
- Short duration makes more time available for (out-of-unit) diagnostic and therapeutic procedures
- Lower risk of (systemic) bleeding due to less heparin use
- More suitable for severe hyperkalemia
- Optional online bicarbonate dialysate production
- Less labor-intensive and therefore less expensive

Disadvantages
- Technically sophisticated requiring specific infrastructure (e.g. water supply)
- Qualified (dialysis) staff required to supervise the labor-intensive procedure
- Periodic solute control with subsequent disequilibrium
- Dialysis dose and nutritional support may be inadequate with low treatment frequency
- Frequent hypotensive episodes with aggressive ultrafiltration
- Dialysis department necessary

Continuous renal replacement therapy

Advantages
- Machines are generally easy to operate and do not require specific infrastructure
- ICU staff can operate and perform monitoring (but increases workload)
- Prolonged, gradual solute and volume removal achieves superior solute and fluid control
- Ultrafiltration over a longer period provides better hemodynamic stability
- Adequate nutritional support possible
- No dialysis department necessary

Disadvantages
- Higher requirement for heparin and higher risk of (systemic) bleeding
- Impairs mobilization of patients
- Treatment frequently interrupted due to filter problems, and diagnostic and therapeutic procedures
- Expensive sterile substitution solutions substantially increase treatment costs

machine (2008H®, Fresenius Medical Care North America, Lexington, USA) at a reduced dialysate flow rate of 100 ml/min. They have used this approach to treat critically ill patients in whom intermittent hemodialysis had repeatedly failed due to intradialytic hypotension, patients in whom hemodynamic intolerance was likely to occur, and patients in whom the prescribed solute control goals were not achieved despite daily intermittent hemodialysis. In these settings, the authors achieved ultrafiltration goals and adequate solute removal in most of their 37 patients with 145 extended dialysis procedures. Dialysis quantification in nine oliguanuric patients revealed a mean delivered double-pool Kt/V of 1.36 ± 0.38 per treatment. Hospital mortality was 62%, not significantly different from expected mortality determined from the APACHE II illness severity score. Moreover, ICU staff accepted extended dialysis well [21].

Kumar and colleagues [19] compared extended dialysis with standard CVVH in a prospective study. They also used the 2008H® machine to treat 25 critically ill patients with SLED (total treatment days, 367). An additional 17 patients were treated with CVVH for a total of 113 days. Median daily treatment time was 7.5 h for extended dialysis and 19.5 h for CVVH. No differences in mean arterial blood pressure (MAP) or use of catecholamines were observed between the treatment groups, despite similar median net daily ultrafiltration rates (3,000 vs 3,028 ml/day). By contrast, requirement for anticoagulation was significantly less in patients treated with extended dialysis (median heparin dose 4,000 U/day vs 21,100 U/day with CVVH). Kumar et al. [24] have also published an account of their 2-year experience with extended dialysis. They concluded that this technique is well tolerated and offers many of the benefits of continuous techniques, but is technically much simpler to perform and therefore well accepted by the ICU team.

The devices used in these [19, 21, 23, 24] and other [15, 25, 26] studies of extended dialysis are sophisticated dialysis machines, usually used for intermittent hemodialysis (the 2008H® [15, 19, 21, 23, 24] and the AK200® Ultra [Gambro, Lund, Sweden]) [25]. The next important step in developing a straightforward extended dialysis treatment for the ICU setting is simplification of apparatus. We have used the Genius® single-pass dialysis system (Fresenius Medical Care Germany, Bad Homburg, Germany) to treat patients with acute renal failure in the ICU [20, 22]. Currently this dialysis system is only available in Europe and South America. The technical principle underlying Genius® is based on the very first dialysis systems, the 'tank' or 'batch' devices. Briefly (the technical features are described in detail elsewhere [22, 27, 28]), the dialysis machine does not require the usual infrastructure such as multilocal water supply or waste removal. Ultrapure dialysis fluid is added and removed from the individual machine at one central 'filling station', where the sterile dialysis fluid is prepared, filled into the machines and the used dialysate is drained after the treatment. Further advantages are reduced thrombogenicity (tubing is completely fluid-filled [i.e. air-free]), simple and reliable control of volumetric ultrafiltration, 100% bicarbonate ultrapure dialysis fluid (with attendant beneficial effects on patient survival [29]) and the option of individualized treatment duration without software or hardware changes. Due to different combinations of dry and liquid concentrates as well as supplemental magnesium and glucose, up to 480 possible dialysate compositions can readily be mixed to tailor the individual treatment to the patient's need. This extremely flexible yet highly efficient treatment modality fulfils all ICU requirements: it offers immediate, highly effective dialysis therapy for acute hyperkalemia, whereas for less urgent indications treatment durations can be extended up to 18 h [20]. Thus, dialysis can be performed overnight, facilitating mobilization of the patient during the day. At

our institution, the dialysis machine is completely supervised by ICU nursing staff provided that blood flow does not exceed 200 ml/min, but there is no scientific evidence governing this practice.

In a prospective randomized controlled study, we treated ventilated critically ill patients suffering from oliguric acute renal failure with either CVVH ($n=19$) or extended dialysis ($n=20$). The urea reduction rate achieved with 11.7 ± 0.1 h of extended dialysis was comparable to that achieved after 23.3 ± 0.2 h of CVVH, even though a substitution fluid exchange rate of at least 3 l per hour was used [22]. These data support kinetic models which indicate that both CVVH and extended dialysis provide very effective control of azotemia in hypercatabolic acute renal failure patients [30]. In this study we also performed hemodynamic monitoring by using a pulse-induced contour cardiac output (PiCCO) device [22]. This device is comparatively less invasive than the traditionally used pulmonary artery catheter. MAP, heart rate, cardiac output and systemic vascular resistance (SVR) were similar in patients treated with extended dialysis and in patients treated with CVVH. With respect to these hemodynamic variables there were no significant differences between extended dialysis and CVVH neither before nor immediately after the start of the treatment/nor during the observation period. In addition, total ultrafiltration volume was comparable with extended dialysis and CVVH (2.97 ± 0.55 vs 3.28 ± 0.39 l/24 h; n.s.). The use of catecholamines in the extended dialysis and CVVH treatment groups was not significantly different either. The norepinephrine dose was 0.47 ± 0.11 (extended dialysis) vs 0.47 ± 0.14 (CVVH) μg/kg/min at the start of treatment and 0.45 ± 0.12 (extended dialysis) vs. 0.42 ± 0.13 (CVVH) μg/kg/min at 12 h of treatment. It was 0.39 ± 0.13 μg/kg/min after 24 h of CVVH therapy.

These results confirm that extended dialysis, at absolutely equivalent hemodynamic stability, is at least as efficacious as classical CVVH. The significantly reduced need for heparin associated with extended dialysis can be a decisive advantage, especially in patients at high risk of bleeding [19, 22]. Preliminary data indicate that the survival outcome of patients treated with SLED does not differ from that of those treated with state-of-the-art CVVH; more definitive information will become available through impending multicenter prospective randomized trials (The Acute Renal Failure Network Trial [lead investigator, P Palevsky]; and CRRT vs EDD-Substudy of the Stuivenberg Hospital Acute Renal Failure Trial [lead investigator, R Lins]).

Night-time Extended Dialysis

Nocturnal extended dialysis allows unrestricted physician access to patients for daytime procedures, thereby minimizing disruption of ICU activities by renal replacement therapy. Several centers have reported their experience with overnight extended dialysis (Table 1). As described above, Marshall and co-workers [21, 23] used standard intermittent hemodialysis equipment, and reduced blood (200 ml/min) and dialysate (100 ml/min) flow rates, so that treatment could be supervised by ICU staff (following appropriate training from dialysis personnel). Importantly, their primary intention was to perform 12 h treatments overnight [21]. Although the nephrology team assumed medical responsibility, the overnight extended dialysis treatments were more or less in the hands of the ICU staff.

We have used the Genius® system for night-time extended dialysis [22]. In general, there are no practical differences with respect to performing day-time and night-time extended dialysis. Machines are supervised solely by ICU personnel during the overnight shift; a dialysis nurse is available on-call for advice and trouble-

shooting. Currently, about 90% of all extracorporeal renal replacement treatments in the Hannover Medical School ICUs are extended dialysis sessions (approximately 3,000 per year). Almost half are performed overnight.

Other Extended Dialysis Variants and Indications

Some important modifications of the extended dialysis technique have been recently developed. Sustained low-efficiency daily diafiltration (SLEDD-f) combines diffusive and convective solute transport [31]. SLEDD-f is primarily used to improve clearance of putative middle-molecule inflammatory mediators, which are thought to have a role in the pathophysiology of systemic inflammatory response syndrome in critically ill patients. Considerable efficient removal of larger molecules has been reported even for 'standard' extended dialysis with high-flux dialyzers [22]. As mentioned previously, controlled studies have failed to detect a patient survival advantage for CRRT over intermittent hemodialysis, despite the convective transport that more efficiently eliminates middle molecules.

Another extended dialysis variant is regional citrate anticoagulation [32–34] Morgera et al. [34] have used the Genius® system together with a low calcium dialysate concentration (1 mmol/l) to test the safety and feasibility of a regional citrate anticoagulation protocol with respect to acid–base and electrolyte changes in 27 critically ill patients with acute renal failure. These investigators infused a 4% sodium citrate solution into the arterial line of the extracorporeal circuit, and adjusted the citrate dose according to the post-filter ionized calcium concentration (target values, 0.5–0.7 mmol/l) without routine calcium substitution. They observed no significant untoward effects on blood levels of calcium and sodium, and acid–base values remained equilibrated during citrate anticoagulation. Excellent filter patency and cardiovascular stability of patients were maintained [34]. This was confirmed in a smaller study by Finkel and Foringer who reported that in 14 patients, no clotting occurred during 1500 hours of extended dialysis with citrate infusion [32].

Encouraged by its efficient elimination of uremic toxins, physicians increasingly use extended dialysis to counteract intoxications [35–38]. For this indication, the advantages of extended dialysis include fewer complications (especially in comparison to charcoal perfusion) and use of regular dialysis machines (minimizing staff load). Many reports of short standard dialysis followed by extended dialysis to prevent rebound of the offending toxin have been published [36, 38, 39]. Experience is, however, limited to case reports, hence the optimum role for SLED in management of intoxications merits further study.

Extended Dialysis and Pharmacokinetics

High efficiency renal replacement therapy has profound effects on both the pharmacokinetic and pharmacodynamic properties of all drugs administered to critically ill patients. Despite this, few studies have evaluated this problem, even for high-volume CRRT [40]. The degree to which extended dialysis eliminates drugs differs greatly from standard thrice-weekly intermittent hemodialysis or CRRT. Moreover, dosing and pharmacokinetic data obtained from patients receiving either intermittent hemodialysis or CRRT might not be applicable to patients treated with extended hemodialysis because duration of treatment, the filters used, and rates of blood flow are quite different. It is somewhat surprising that there have been only four pharmacokinetic studies in patients undergoing extended dialysis [25, 41–43].

These studies confirmed that there are significant differences in rates of drug removal by extended dialysis compared with intermittent hemodialysis and CRRT. Aiming for high-dose renal replacement therapy while adhering to outdated drug dosing recommendations could, therefore, lead to underdosing of important drugs such as antibiotics, in turn having a detrimental effect on critically ill patients suffering from life-threatening infections. Therapeutic drug monitoring should be performed whenever possible. Dosing recommendations for patients with renal failure in the ICU treated with SLED must be developed if excessive mortality due to underdosing of life-saving medications is to be avoided.

Prolonged extended dialysis using the Genius® single-pass system causes slight but significant cooling of the patient. The entire volume of dialysis fluid is loaded at once, such that the 90 l glass container is completely filled and, therefore, air-free. Excessive cooling of the pre-warmed dialysis fluid is prevented by the thermal insulation of the transparent container, but the lack of a separate heater leads to some cooling. Lonnemann et al. [20], using the 75 l tank of a previous model over 18 h, measured a temperature drop in the venous blood line of the extracorporeal circuit from $35.3 \pm 0.7\,^{\circ}C$ to $30.2 \pm 0.8\,^{\circ}C$, which is equal to an average temperature loss of $0.28\,^{\circ}C/h$. When we performed SLED over a period of 12 h in mainly septic patients, at a blood flow rate of 200 ml/min and a dialysate flow rate of 100 ml/min [22, 21], the core temperature (measured by a thermistor in the femoral artery) decreased slightly but significantly from $37.4 \pm 0.3\,^{\circ}C$ to $36.7 \pm 0.2\,^{\circ}C$. The decrease in dialysate temperature and consequently the patient's core temperature could actually be advantageous as it increases peripheral resistance and improves cardiovascular stability (as observed in patients on chronic hemodialysis) [44].

Microbiological Advantages of Extended Dialysis with the GENIUS System

Bicarbonate-based buffers are susceptible to bacterial contamination, especially when the system used for CRRT is frequently opened to connect a bag of replacement fluid and discard the used filtrate [45]. This can occur as often as forty times per day at a substitution rate of 37.5 ml/kg/h in a 100 kg patient. Extended dialysis of any kind does not involve this risk. Moreover, the Genius® system need not be opened at all once filled. Its smooth, crack-free, straight glass surfaces, UV-radiator and requirement for ultrapure water make colonization by microorganisms difficult, and facilitate effective cleansing and sterilization. In one analysis, no bacterial growth was found in spent extended dialysis dialyzate, even after 18 h [22, 20]. So, the spent extended dialysis dialyzate almost meets the threshold of sterility ($<10^{-6}$ cfu/ml) [20]. As backfiltration of pyrogens from contaminated dialyzate into the blood can induce a drop in blood pressure during high-flux hemodialysis, the high bacteriological quality of the Genius® dialyzate might contribute to cardiovascular stability during extended dialysis. The Genius® apparatus also permits easy access to the entire complement of substances removed during a dialysis session. This feature generates exciting opportunities for clinical research in the fields of uremic toxins [22, 46], pharmacokinetics [43, 47, 48] and intoxications [35–37].

Economic Considerations

Substantial cost reduction can be achieved if the equipment used for extended dialysis is also used for chronic renal replacement therapy in the same hospital. In fact, all centers offering extended dialysis use various standard intermittent hemodialysis

machines such as the 2008H® or the Genius® single-pass dialysis system without adding or altering software or hardware. In some hospitals, flexible treatment modalities allow the same machine to be used for two intermittent hemodialysis sessions and one overnight extended dialysis treatment in a 24 h period. This dual usage has been recognized by major manufacturers of dialysis equipment. Newer machines like the Fresenius 4008 series (4008K in the US, 4008S ArRT-Plus [Fresenius Medical Care-Asia Pacific Pty, NSW, Australia] and the 4008S elsewhere) have a built-in option for extended dialysis, which is selected from the startup screen without any delay or requirement for further adjustment. Several economic evaluations have shown extended dialysis to be less expensive than CRRT, both within the setting of the USA healthcare reimbursement scheme and within a more widely applicable nationalized healthcare system [49, 50]. The main sources of cost savings are reduced staff load and need for industrially produced sterile substitution fluid.

Conclusions

Indications for extended dialysis have the potential to expand to include prolonged high-volume treatment of severely ill patients; for example, of highly catabolic patients with systemic inflammatory response syndrome. Extended dialysis permits normalization of indicators of uremic intoxication (e.g., blood urea concentration) in a short time, and hemodynamic stabilization [13, 28]. High-volume CVVH is currently too expensive to be widely used in critically ill patients. Moreover, extended dialysis offers an alternative to conventional intermittent hemodialysis in treatment of acute intoxications with a variety of drugs including carbamazepine and salicylate [35–38]. We reported that extended dialysis can be a safer and less costly alternative to hemoperfusion of substances that are believed to be poorly responsive to dialysis (e.g., life-threatening intoxication with valproic acid in a thombopenic patient) [36].

In summary, extended dialysis is an increasingly utilized renal replacement therapy that facilitates efficient detoxification and has a favorable cardiovascular tolerability profile, even in critically ill patients with acute renal failure on the ICU. The technically simple, single-pass batch dialysis systems are easy for ICU staff to operate and offer a high degree of flexibility with regard to the timing of treatment. The evolution and use of extended dialysis as an alternative to classical intermittent or continuous renal replacement therapies is likely to continue and grow over the next decade. The true potential of renal replacement therapy for critically ill patients is currently being explored in comparative studies of treatment outcomes with extended dialysis, intermittent hemodialysis and CRRT.

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Anticoagulation in CRRT: Systemic or Regional?

H. M. Oudemans-van Straaten

Introduction

During continuous renal replacement therapy (CRRT), anticoagulation of the extracorporeal circuit is generally required in order to prevent clotting of the circuit, to preserve filter performance, optimize circuit survival and prevent loss of blood due to circuit clotting.

For anticoagulation of the extracorporeal circuit, heparins are most commonly used. Although widely used, heparins have drawbacks. Most importantly, anticoagulation of the circuit is associated with systemic anticoagulation, which may be indicated in some, but is undesirable in the majority of intensive care patients. Systemic anticoagulation may cause bleeding [1], to which critically ill patients are at increased risk, due to recent surgery, trauma or mucosal lesions causing endothelial disruption and coagulopathy. Simultaneously, acute renal failure may be associated with a procoagulant state [2–4], related to the expression of tissue factor (TF) on activated mononuclear and endothelial cells [5, 6], downregulation of natural anticoagulants [7–9], inhibition of fibrinolysis and activation of platelets. Therefore, CRRT without anticoagulation is generally associated with early filter clotting, increasing costs, greater workload for the nurse and most importantly with the loss of blood in the extracorporeal circuit and increased transfusion. Transfusion of foreign blood should be prevented rigorously, because it carries the risk of transmission of pathogens and decreases immunocompetence. Transfusion is associated with increased mortality in the critically ill [10].

In addition, the use of both unfractionated heparin, low molecular weight heparins (LMWH) and danaparoid can induce thrombocytopenia. Heparin-induced thrombocytopenia (HIT) can present as a non-immunogenic, early, limited and self-recovering fall in platelet count (HIT I), whereas HIT II is a severe clinicopathologic syndrome associated with a major drop in platelets, risk of bleeding, paradoxical hypercoagulability with thrombosis, and heparin/PF4 antibodies [11, 12]. In a recent meta-analysis, the risk of HIT with LMWH was 0.2% and with unfractionated heparin 2.6% [13]. Cross-reactivity of danaparoid with heparin/PF4 antibodies is reported in 5–10% of the patients [14].

Therefore, anticoagulation for CRRT steers between the *Scylla and Charybdis* of clotting and bleeding.

Minimal Systemic Anticoagulation

Although there are several attractive strategies for circuit anticoagulation yielding minimal systemic anticoagulation [15–21], they have drawbacks as well. While prostaglandins may cause hypotension and are expensive, nafamostat is not available outside Japan and may cause anaphylaxis. Non-pharmacological measures to optimize circuit survival interfere with stasis of extracorporeal flow [22] or hemofiltration-related hemoconcentration. Among these, improvement of vascular access and training of nurses are of utmost importance [23]. Predilution may reduce anticoagulant needs or prolong circuit life, but requires more fluids and reduces the efficacy of substance removal [24, 27]. The ideal option would be a circuit anticoagulation that does not influence systemic coagulation. The application of CRRT should not dictate obligatory systemic anticoagulation and CRRT should be safely applicable in a patient after major surgery or who is actually bleeding or at risk for bleeding. Regional anticoagulation seems, therefore, the most attractive option in the critically ill, if and when the method is safe and not associated with major side effects.

Regional Anticoagulation using Heparin-protamine

Regional anticoagulation can be realized by the post-filter infusion of protamine in a dose equal to the pre-filter infused heparin. The method combines, however, the negative effects of both unfractionated heparin and protamine (platelet activation, activation of inflammatory mediators, systemic hypotension and pulmonary hypertension) [28]. Its use should, therefore, be discouraged.

Regional Anticoagulation using Citrate

The most attractive alternative is regional anticoagulation with citrate. Intensivists hesitate to implement the method because they have no clear view of the metabolic consequences (Table 1). In the following, we aim to describe the metabolic consequences of citrate and summarize the pros and cons of the use of citrate in CRRT.

The prefilter infusion of citrate inhibits coagulation by chelating calcium. As a result ionized calcium decreases (iCa) (see Fig. 1). An iCa concentration below 0.35 mmol/l is required to inhibit coagulation. Calcium- and magnesium- citrate complexes are partially cleared by convection or diffusion and the remaining citrate enters the systemic circulation. In the patient, iCa rises again due to dilution of extracorporeal blood, the replacement of calcium and the liberation of chelated calcium when citrate is metabolized. Within minutes, citrate is converted to citric acid, which is readily metabolized in the citric acid circle by liver, kidney and muscle cells leaving sodium and bicarbonate. As a result, systemic effects on coagulation do not occur. Each molecule of citrate potentially yields three molecules of bicarbonate. The net-reaction is as follows:

$$Na_3\,\text{citrate} + 3H_2CO_3 \leftrightarrow \text{Citric acid}\,(C_6H_8O_7) + 3NaHCO_3$$

Anticoagulation with citrate has complex metabolic consequences. These are summarized in the table. Metabolic complications include metabolic alkalosis, metabolic acidosis and hypocalcemia. They are preventable if the local protocol is strictly followed, metabolic monitoring is applied and the composition of the CRRT fluids is adjusted to the type of citrate solution and citrate flow. It should be noted that if metabolic function of the liver fails, anticoagulation with citrate is not applicable [29, 30].

Table 1. Metabolic consequences of anticoagulation with citrate

Metabolic effect	Metabolic consequence	Consequences for implementation
▮ Citrate acts by chelating Ca^{2+} in the filter	At [iCa^{2+}] < 0.35 mmol/l coagulation is inhibited	For stable and safe anticoagulation: couple citrate flow to blood flow
▮ Ca/Mg-citrate complexes are freely filtered	Greater loss of Ca^{2+} and Mg^{2+}	Monitor iCa^{2+} Replace additional Ca and Mg systemically
▮ Citrate is a buffer	1 mol citrate → 3 mol bicarbonate	Use replacement fluid with no or less buffer
▮ The amount of citrate lost by ultrafiltration varies with ultrafiltrate flow	Varying amounts of buffer enter the systemic circulation *if* filtrate flow is not fixed: if filtrate flow drops, more buffer enters the patient	Monitor acid-base Use fixed filtrate flow, or adjust the amount of buffer in the replacement fluid. Change filter if ultrafiltrate flow drops below a set limit
▮ If the liver fails, no buffer is generated and citrate accumulates	Accumulation of citrate → metabolic acidosis, anion-gap ↑, iCa^{2+} ↓, totCa/iCa^{2+} ↑	Monitor acid-base, iCa^{2+} and anion-gap Stop citrate, use bicarbonate buffered replacement
▮ If too much citrate infusion is infused accidentally	Metabolic alkalosis occurs if liver function is normal	Reduce citrate dose, use buffer-free replacement, administer calcium
▮ The trisodium citrate solution contains a substantial amount of sodium		Replacement fluid should contain less sodium

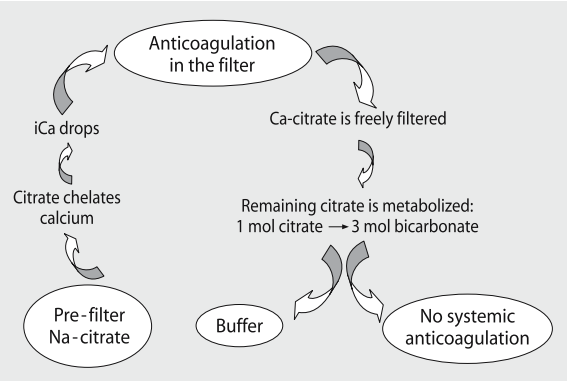

Fig. 1. The prefilter administration of citrate provides regional anticoagulation and buffer

Numerous studies have appeared with several home made options for the implementation of anticoagulation with citrate in clinical practice, using hemo-dialysis or -filtration, pre- or postdilution, different citrate solutions and different approaches for monitoring anticoagulation or adjusting acid base balance [31–44]. Citrate is either administered as a separate sodium citrate solution or added to the predilution replacement fluid (see Fig. 2). For stable anticoagulation, citrate flow should be adjusted to blood flow. Strict coupling is not possible if citrate is in the replace-

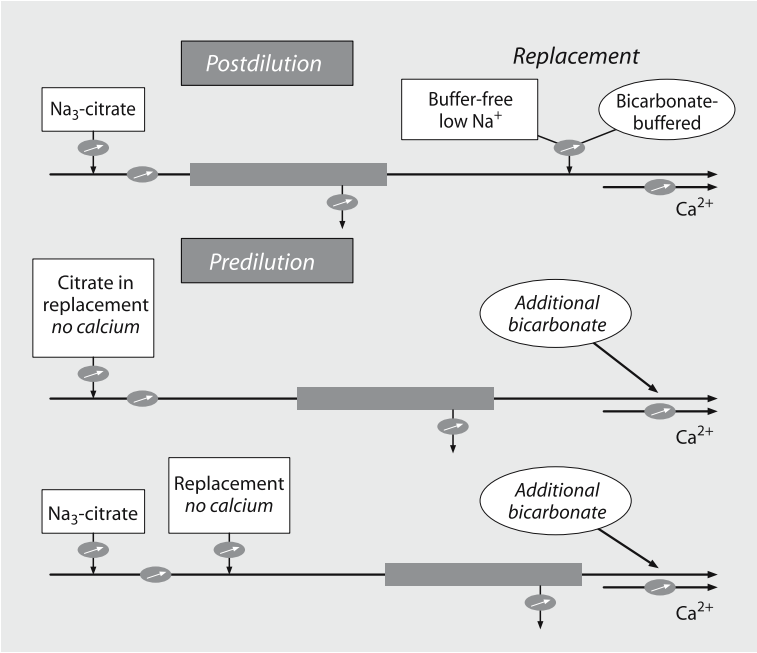

Fig. 2. Scheme of different options for regional anticoagulation with citrate for continuous venovenous hemofiltration in pre- or postdilution modes. Bicarbonate-buffered replacement or additional bicarbonate is indicated if higher volumes are exchanged or if the patient is highly catabolic

ment fluid. Calcium/magnesium and sodium are either administered by separate infusion pumps (adjusted to citrate and filtrate flow) or included in the dialysis or postdilution replacement fluid. Predilution replacement cannot contain calcium. With severe acidosis or higher ultrafiltrate flow the replacement fluid needs additional buffer.

Most studies have an observational design describing the feasibility of the method, some compare circuit life and bleeding with historical or contemporary nonrandomized controls. Up to now, only two small randomized studies have appeared as full publications, including 20 and 30 patients. They compared unfractionated heparin to citrate. Both showed a longer circuit survival and less bleeding or transfusion during citrate [42, 43].

A Large Randomized Trial Comparing Systemic to Regional Anticoagulation

We are now performing a large randomized controlled trial to determine the safety and efficacy of regional anticoagulation with citrate in critically ill patients with acute renal failure *without* an increased risk of bleeding. Until now, 144 patients have been included (mean age 70 years, mean APACHE II score 28, mean SAPS II 60) and we will continue enrolment until 200 patients have been included. Regional anticoagulation with citrate is compared to systemic anticoagulation with nadropar-

in. CRRT is provided as continuous venovenous hemofiltration in postdilution [44], initiated at 4 l/h for at least 24 h. In stable patients, the dose can be reduced if concentrations of urea and creatinine are low, to minimum of 2 l/h. At interim analysis, we found that the study medication had to be stopped prematurely on predefined criteria in 5% of citrate patients (citrate accumulation) and in 18% of nadroparin patients (bleeding or suspicion of HIT). In case of bleeding (pre-defined criteria), nadroparin was discontinued (per protocol) and regional anticoagulation with citrate was initiated to prevent excessive bleeding. At the end of the CVVH period, the hemoglobin concentration was significantly lower in the nadroparin group than in the citrate group ($p = 0.002$) while transfusion requirements were not significantly different (median total volume of transfused red blood cells 270 ml [range 0–3510 ml] in the citrate patients versus 540 ml [0–5400 ml] in the nadroparin patients, $p = 0.65$). ICU mortality was lower in the citrate group (25% versus 30%, $p < 0.01$) and hospital mortality tended to be lower (40% versus 48%, $p = 0.065$).

Conclusion

The major advantage of regional anticoagulation with citrate is that adequate anticoagulation of the circuit can be obtained without systemic anticoagulation and thus without increasing the patient's risk of bleeding. Compared to systemic anticoagulation with heparin, circuit survival is favorable, there is less bleeding and transfusion requirements are lower. Furthermore, in our ongoing large randomized controlled trial comparing citrate to nadroparin, there is a trend toward a higher survival rate in the citrate group. Whether this benefit is due to a lower rate of bleeding and transfusion or additionally to improved biocompatibility due to less activation of coagulation and leukocytes [45, 46] is not known. It should be noted that anticoagulation with citrate is feasible and safe, if and when liver function is adequate, the method is guided by a strict protocol, and electrolytes, and ionized calcium and and acid base are monitored. It should also be stressed that for safety reasons, future CRRT devices should be designed with six pumps, which are coupled to prevent ongoing infusion of citrate when the blood pump stops.

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Plasma Filtration Adsorption Dialysis: A New Experimental Approach to Treatment of Sepsis and MOF

F. Nalesso and C. Ronco

Introduction

Severe sepsis and multiple organ failure (MOF) represent significant challenges in critical care. Despite all the developments achieved in infectious diseases, organ substitution and critical care, the mortality rates from these conditions remain unacceptably high. The pathophysiology of severe sepsis and MOF is only partially understood. Circulating pro-inflammatory and anti-inflammatory mediators appear to participate in the complex cascade of events which leads to deranged microcirculatory function with consequent MOF as detailed in the peak concentration hypothesis [1]. The cytokines and other pro-inflammatory mediators in the systemic circulation, where major biological effects take place, determine vasopermeability, hypotension and shock. At the same time, the monocytes of the septic patient seem to be hyporesponsive to inflammatory stimuli to a certain extent. Due to the short half-life of cytokines and other mediators spilled over into the circulation, it is extremely difficult to approach the problem at the right moment with the right pharmacological agent. For these reasons, the peak concentration hypothesis suggests that continuous renal replacement therapies due to their continuity and non-specific capacity of removal might be beneficial in cutting the concentration peaks of inflammatory molecules [2].

Several *ex vivo* studies have shown that it is technically possible to adsorb inflammatory mediators using specific sorbents [3] or to remove them by high volume hemofiltration [4]. In severe sepsis, several blood purification techniques, such as continuous veno-venous hemofiltration (CVVH) [5], high volume hemofiltration (HVHF), pulse high volume hemofiltration (pHVHF) and coupled plasma filtration adsorption (CPFA) [6] have been proposed but such techniques appear to have both theoretical as well as practical limitations. Current data demonstrate that these techniques appear to have limitations related to the characteristics of molecules implicated in the pathophysiology of sepsis and MOF. Cytokines and other inflammatory molecules have high molecular weights and their removal by ultrafiltration and dialysis is less efficient than by adsorption on specific resins. Individual techniques, such as CVVH, HVHF, pHVHF, appear to have theoretical as well as practical limitations because of their single principle of purification (convection). CPFA is more specific and efficient since it combines convection on whole blood with plasma adsorption.

To improve the effectiveness of extracorporeal purification in sepsis and MOF, we focused our attention on the vehicle of transport of pathologic molecules. In whole blood, molecules are transported by plasma water if soluble or by carriers in the plasma (albumin or other specific carriers) if insoluble. Thus, the real vehicle

of toxic molecules is identified as plasma and the best extracorporeal purification technique could act directly on the plasma. Focusing attention on the characteristics of plasma, we can also identify the possibility of using plasma itself as a medium of purification thanks to its carriers and solute composition [7]. In this new concept, cytokines and other inflammatory molecules could be removed directly from the plasma by a new process, which employs purified plasma as a medium of further purification. The single techniques described above (CVVH, HVHF, etc.) can act on plasma water (convection, diffusion or their combination) or on protein carriers (adsorption on specific resin beds) presenting technical limitations. Plasma filtration adsorption dialysis (PFAD) [8] is a new extracorporeal treatment which combines different principles of blood purification in a single device using and acting on a patient's plasma. The core of this technique is a new dialyzer composed of three compartments that each provides specific functions.

The PFAD technology allows molecules (cytokines and other inflammatory molecules) to be removed from plasma water and albumin/protein carriers (bound molecules) thanks to the sequential processes in the plasma circuit. In addition, all these processes result in a complete removal of uremic toxins and recovery of hydro-electrolyte and acid-base equilibrium.

In conclusion, PFAD technology combines and improves convection, diffusion, adsorption on bed resin and adsorption on dialyzer membrane as a synthesis of past and present blood purification techniques applied in sepsis and MOF. The specificity and effectiveness of purification is improved by using plasma as both the substrate and the medium of purification in the same processes.

Introduction of Plasma as the Medium and Substrate of Purification

In whole blood, all molecules are transported either in the plasma water or bound to albumin (or other protein carriers). Albumin and other carriers are present in solution in plasma water. Thus, transport of molecules depends on their hydrophobic characteristics and their molecular weights. Molecules with elevated solubility are transported in solution in plasma water whereas hydrophobic molecules are transported bound to specific or non-specific carriers in plasma water.

Hence, the plasma is the only carrier of all molecules. A physico-chemical equilibrium can be achieved among tissues and plasma through the capillary endothelium. Thanks to this equilibrium every molecule can establish a dynamic equilibrium in plasma. An extracorporeal epurative treatment acting directly on plasma is able to remove toxic molecules from tissues working on this dynamic equilibrium.

By analyzing this concept, we can understand the potential of a patient's plasma to be used as dialyzate after purification into its components (electrolyte composition, acid-base status, water, protein carriers). The plasma does not contain cells so they can be used in extracorporeal purification systems using non-biocompatible materials such as some sorbents for specific molecules (cytokines, bilirubin, lipopolysaccharide [LPS]…).

With this new concept, the patient's own plasma achieves a very important role in blood purification, removing all molecules with a high molecular weight or hydrophobic characteristics. First, the plasma is the medium through which all molecules can be transported to the site of purification. Second, due to its intrinsic capacity to bind and transport toxins we can use regenerated patient plasma at the

site of purification to purify the 'dirty' plasma coming from the patient. In this case, the plasma is the medium which we can use for the purification process.

PFAD System: Description

The PFAD technology is based on new principles of purification and utilizes a tricompartment dialyzer, to purify the patient's plasma by a combination of 3 sequential techniques: convection/diffusion and adsorption followed by a process of 'whole blood dialysis' provided by the regenerated plasma of that patient (Fig. 1).

The tricompartmental dialyzer is the core of this new technology (Fig. 2). It is composed by hollow fibers like a regular dialyzer for hemodialysis. The compart-

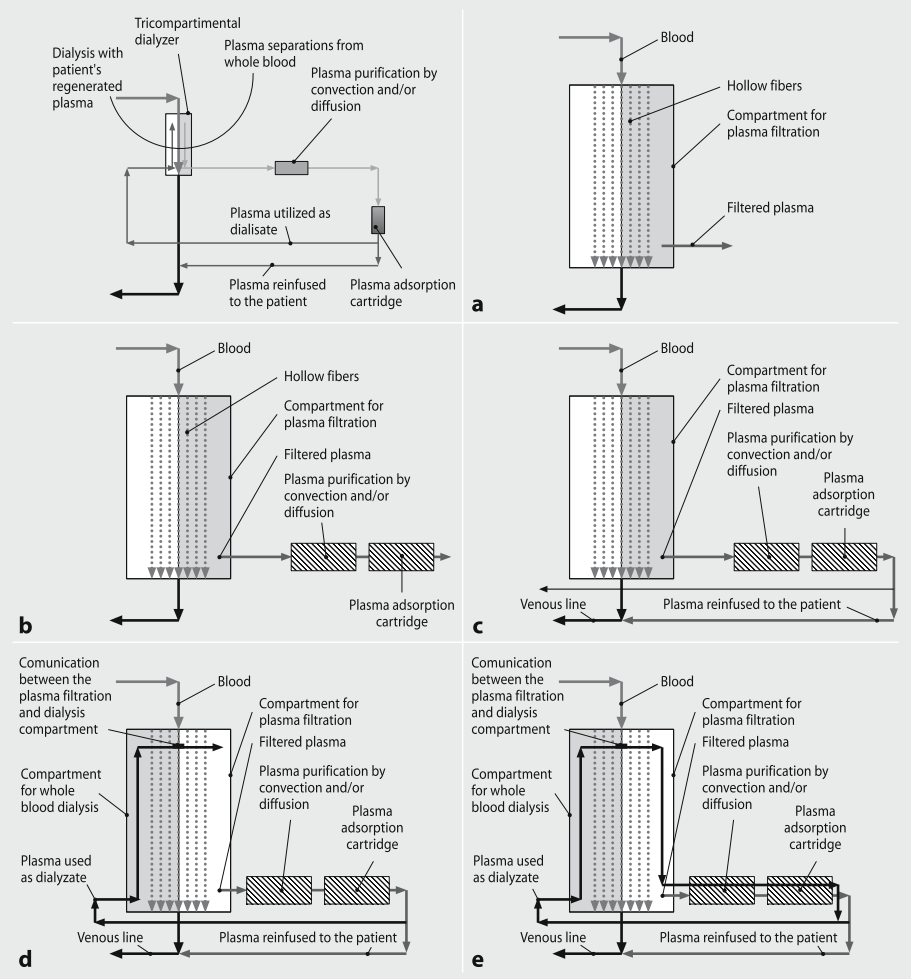
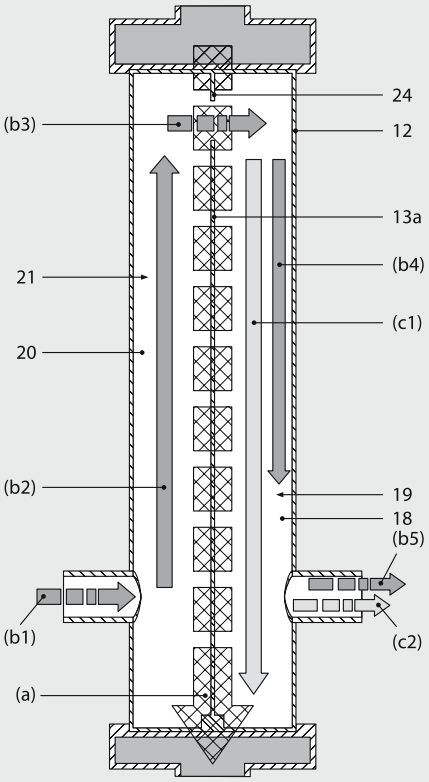


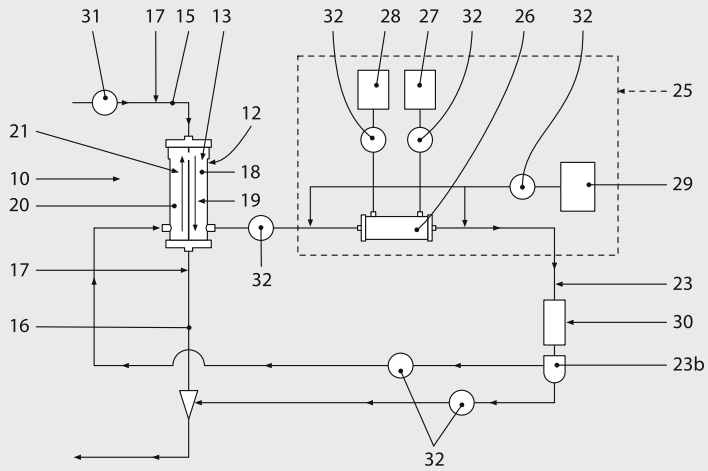
Fig. 1. Schematic representation of the plasma filtration adsorption dialysis (PFAD) system (see text for details)



a: the arrow designates the blood that flows in the permeable capillaries
b1: the arrow designates the purified plasma that arrives from the plasma circuit
b2: the arrow designates the plasma that, in countercurrent, purifies the whole blood of the capillaries (acting as a fresh dialyzate)
b3: the arrow designates the plasma (exausted dialyzate) which goes to the plasma filtration compartment through a communication
b4: the arrow designates the plasma (exausted dialyzate) mixed with the filtered plasma
b5: the arrow designates plasma (exausted dialyzate) in output from the filter 12 in order to enter the plasma purification circuit
c1: the arrow designates the plasma filtered by the capillaries
c2: the arrow (c2) designates the filtered plasma, which together with the used plasma as dialyzate (b5) enters the plasma purification circuit
13a: wall separating the dialyzer into two compartments
18: compartment for plasma filtration
19: space for the plasma filtration from whole blood flowing in the hollow fibers
20: compartment for whole blood dialysis
21: space for the dialysis of whole blood flowing in the hollow fibers
24: communication between the plasma filtration and dialysis compartment

Fig. 2. The tricompartment dialyzer of the PFAD

ments are located in different areas, and each has its particular function. The hollow fibers form three compartments along the extension of the dialyzer: the first compartment is formed by the inner space of a hollow fiber in which the blood passes through the whole fiber length thanks to a roller pump (blood pump). The internal compartment of the dialyzer is divided, along the extension of the hollow fibers, into two compartments separated by a wall; respectively a (second) compartment, which forms a stage for filtering plasma, and a (third) compartment, which forms a stage for dialysis. The second compartment is the delineated space where the patient's plasma can filtrate from the whole blood across the hollow fiber membrane (Fig. 1a). The third compartment is the space where the patient's regenerated plasma performs a process of purification based on a 'diffusive method'; in this way the regenerated patient's plasma is used as a dialyzate in countercurrent to purify the blood flowing in the first compartment (Fig. 1 d). The second and third compartments have two different cut-offs of hollow fiber membrane according to their function with adequate area to perform the processes (filtration and dialysis). The second and the third compartments communicate through a hole in the arterial extremity of the dialyzer (Fig. 2).



10: tricompartment dialyzer
12: second compartment of three compartment dialyzer
13 : filtered plasma from the whole blood and exhausted dialyzate (plasma) from the dialysis compartment (third compartment of the three compartment dialyzer)
15: artery line
16: venous line
17: whole blood line
18: compartment for plasma filtration
19: space for the plasma filtration from whole blood flowing in the hollow fibers
20: compartment for whole blood dialysis
21: space for the dialysis of whole blood flowing in the hollow fibers
23: plasma line
23b: venous chamber
25 : circuit for plasma purification
26: filter to purify the plasma by convection/diffusion
27: Bag for fresh dialyzate (according to the selected treatment)
28 : Bag for ultrafiltrate and exhausted dialyzate (according to the selected treatment)
29 : Bag for the infusate fluid in pre- or post-dilution (according to the selected treatment).
30: cartridge for adsorption
31: blood pump
32: pumps

Fig. 3. The plasma filtration adsorption dialysis (PFAD) circuit

The first step of the process aims to separate the plasma from whole blood (Fig. 1 a). This process determines the plasma filtration from the inner space of hollow fibers (membrane with very high cut-off) to the space of the second compartment. The patient's plasma then goes from the second compartment to the plasma purification circuit where it is purified by two different and separated methods: convection or diffusion or convection plus diffusion followed by adsorption (Fig. 1 b). The plasma flow is obtained by a roller pump that assures a stable and continuous flow to the plasma purification circuit according to the blood flow that determines the maximal filtration fraction of plasma (Fig. 3).

The plasma purification circuit (Fig. 3) is composed of two separated devices in order to remove first water-soluble and dialyzable toxic molecules by convection

and/or diffusion then hydrophobic and non-dialyzable molecules by adsorption on a specific cartridge (Fig. 1 b). The convection or diffusion or convection plus diffusion are obtained performing high volume hemofiltration or hemodialysis or high flux dialysis on plasma, respectively. It is known that high volume ultrafiltration using a super high flux filter has achieved better cytokine clearances compared to those currently achieved for urea during standard continuous renal replacement therapy [9]. Since these processes are applied to the plasma, not to the whole blood as in standard techniques, the expression 'high plasma filtration', 'plasma dialysis', or 'high flux plasma dialysis' would be more correct. The convective and dialytic processes are performed on plasma with a high cut off dialyzer and standard solution for hemodiafiltration. The device used to perform these plasma treatments, uses the same technologies to perform them on whole blood. The convective/diffusive process is able to re-establish hydro-electrolyte, acid-base equilibrium and fluid balance acting directly on plasmatic water.

After the convective/diffusive purification, the plasma is adsorbed on one or more specific cartridges able to remove hydrophobic or non-dialyzable molecules (Fig. 1 b). The cartridge is specific for the molecules implicated in the patient's disease (sepsis, hepatorenal syndrome, acute and chronic liver failure, etc.). The cartridge resin for adsorption presents high homogeneity, good pressure-flow performance and excellent mechanical and chemical stability in order to perform the best adsorption on plasma.

After these two processes of purification, the purified plasma can take two separate routes (Fig. 1). In the first, the purified plasma returns to the patient through the venous line (Fig. 1 c); in the second, the patient's regenerated plasma is used as dialyzate in the suitable compartment of the tricompartment dialyzer in order to perform the dialysis process based on 'diffusion' acting on the whole blood (Fig. 1 d). In this way, the patient's filtered plasma and exhausted dialyzate, (regenerated plasma previously used as dialyzate), can be purified again in the plasma circuit (Fig. 1 e). In fact there is a connection between the second and third compartments at the arterial extremity of the tricompartment dialyzer, thus, the exhausted dialyzate can go to the plasma circuit through the second compartment (Fig. 2).

The plasma circuit is an open circuit at the venous line, therefore, the dialysis system does not work in recycling modality, each time using new freshly regenerated plasma from the plasma circuit that is fed by new plasma from the second compartment of the tricompartment dialyzer (Fig. 1 c, d). The thermal balance is maintained or modified by changing the temperature of the fluids used in the plasma purification circuit.

PFAD System: Rationale

The PFAD system works on plasma instead of whole blood. The physical and chemical principles of purification act directly on the patient's plasma. This allows the use of non-biocompatible adsorbents. The association of convection/diffusion, adsorption and the plasma used as dialyzate synergistically improve the capacity of purification of hydrophobic and high weight molecules (i.e., cytokines). The ability to choose the best association among diffusion, convection, convection-diffusion and specific adsorption allows removal of the pathologic molecules implicated in the pathophysiology of disease (sepsis, hepatorenal syndrome, . . .).

According to the concept that a patient's plasma can have a double role in the purification system, we can use the plasma as a medium through which we can transport pathologic molecules from whole blood and tissues to the purification site, then we can use the intrinsic capacity of purified plasma to shift and bind molecules in order to obtain the best removal of these toxins from the patient's body.

In the specific case of sepsis, the convective treatment and the specific cytokine adsorption on plasma produces regenerated plasma that can be reinfused into the patients and utilized as dialyzate. Thanks to the specific capacity of binding of albumin and protein carriers, the dialyzate can shift and bind toxics and cytokines from the whole blood. The same mechanism is obtained in the patient's body where the reinfused regenerated plasma can shift and bind molecules from tissues through the capillary endothelium.

In other diseases such as hepatorenal syndrome and chronic liver failure (before and after liver transplant) the specific adsorption of bilirubin and salt acids in association with a diffusive-convective treatment for the other hepatic toxins can achieve the best purification improving the patient's clinical condition.

Cytokine removal and the consequent protective role in systemic inflammation response syndrome may suggest a future role of PFAD in brain dead donors.

PFAD System: Studies in Progress and Results

The preliminary results from an experimental model of endotoxic shock in pigs demonstrate a protective effect of PFAD on all organs. In this model, we administered a very high dose of LPS intravenously in nine pigs. We divided the animals into three groups: group A (n = 3, only standard medical therapy of endotoxic shock); group B (n = 3, standard medical therapy and CPFA treatment); and group C (n = 3, standard medical therapy and PFAD treatment). Severe hypotension, tachycardia, tissue hypoperfusion and organ dysfunction/failure (oligo-anuria, pulmonary edema, cardiac failure) were characteristic signs of severe septic shock. In our animal model, all these signs were present after the induction of endotoxic shock. All groups presented a severe hypotension and tachycardia after the LPS bolus.

Group A (control) presented cardiovascular instability not responsive to fluid administration and high doses of dobutamine and norepinephrine. The progressive decrease in blood pressure caused organ dysfunction and consequently the death of all animals. Survival time was less than 2 h.

Group B (CPFA) also demonstrated cardiovascular instability even though the CPFA treatment achieved fewer oscillations and a less rapid decrease in blood pressure. This effect is probably responsible for the increased survival of the animals compared to those of group A. Survival time was about 2 h and 30 min.

Group C (PFAD) presented the same cardiovascular instability as the other groups in the first hour and half of induced shock only. After about 90 minutes of PFAD treatment, all animals presented a spontaneous, and progressive increase in blood pressure that allowed suspension of norepinephrine and dobutamine infusions. After the second hour of shock, animals in the PFAD treatment group demonstrated an improvement of all vital signs in all animals. In this group the stable and good general conditions of animals allowed the treatment to be stopped after 4 hours and the pigs were extubated. All animals survived the endotoxic shock presenting a stable cardiovascular compliance and blood pressure comparable to their

status prior to the LPS injection. The re-established permeability and integrity of the endothelium due to the PFAD allowed animals to recover from the shock and survive. All animals of Group C survived and were euthanized at 36 h after the experiment in order to execute the necroscopy.

These clinical findings are supported by the macroscopic and microscopic (standard microscopy and immunohystochemistry) findings at necroscopy. One of the main lesions related to LPS administration was neutrophil margination. The histopathological signs of neutrophil margination observed in animals of groups A and B, were not present in subjects of group C. In group C, none of the animals showed histological signs of neutrophil margination, as the endotoxic shock had been overcome.

In group A, without the support of a dialysis machine, and in group B after about 2 hours of CPFA extracorporeal epuration, the gross and histopathological lesions observed were similar and were characterized by a diffuse, severe lymphadenopathy and systemic congestion. On the other hand, all pigs of group C, sacrificed 36 hours after LPS administration and about 30 hours after the epurative process, were clinically in good general body condition, ate the same amount of food as before the trial, interacted normally with the keepers and did not have depressed sensory function. In conclusion, clinical, anatomical and histopathological lesions observed could be summarized as severe (control group), moderate-to-severe (CPFA control group), and mild (PFAD group). Thus, therapy using the latter treatment overcame the critical phase of endotoxic shock. The minimal lesions observed in the animals treated with PFAD reflects the active removal of those molecules involved in the inflammatory processes and endothelial injury, which, in untreated pigs, were the cause of irreversible multiorgan failure and death.

Conclusion

The PFAD system has proved to be an efficient treatment in endotoxic shock in an animal model. This new method of plasma purification utilizes the capacity of the plasma as a means of transport for toxins and can hence be used to bring them to the filters, clearing the organism. Also based on our preliminary results, it appears that extracorporeal treatment using the PFAD system is one of the most promising therapies in treating the dysfunctions related to circulating mediators, such as cytokines, bilirubin, biliary acids and endotoxemia, all of which are the basis of other more complex pathologies (sepsis, systemic inflammatory response syndrome [SIRS], hepatorenal syndrome, chronic and acute liver failure, brain death donors).

Considering the positive results obtained so far, it would be enlightening to study how subjects with other similar medical conditions respond to this system, thus verifying the effectiveness of the PFAD system in other critical diseases.

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Sleep and Delirium

Sleep in the ICU

B. Cabello, L. Brochard, and J. Mancebo

Introduction

The concept of sleep has evolved from that of the first philosophers such as Democritus, who defined it as "a small group of atoms leaving the body" to its present definition as "a readily reversible suspension of sensorimotor interaction with the environment, usually associated with recumbency and immobility". The invention of the human electroencephalogram (EEG) by Hangs Berger in 1929 [1] and the consequent development of polysomnography by Rechtschaffen and Kales in 1968 [2] played a key role in the progress of sleep science. Nowadays, polysomnography is an essential tool for sleep studies. This field of research has grown rapidly in ambulatory patients. Nevertheless, probably due to the complexity of sleep exploration and the wide use of sedation in the intensive care unit (ICU), the incidence of sleep disorders in ICU patients has historically been underestimated. Sedation can carry the patient from a state of superficial artificial sleep to pharmacological coma, while physiological sleep is a state of rapidly reversible unconsciousness. Differentiation between sleep and sedation is, therefore, the first essential point.

Polysomnography studies in ICU patients have objectively revealed an increase in sleep fragmentation. Noise and patient care activities are considered responsible for approximately 20% of this fragmentation. Indeed, the majority of the causes of sleep fragmentation still remain unknown. The severity of the underlying disease, the sedatives and narcotics administered to patients, and mechanical ventilation seem to play a role in the pathophysiology of sleep derangement. Strategies aiming to decrease sleep disruption in ICU patients have had limited success, and we do not yet know how they can affect outcome.

Physiological Sleep

Sleep is a non-homogeneous state and the standard tool for its study is polysomnography. Polysomnography generally includes monitoring of the following: patient's airflow through the nose and mouth, arterial blood pressure, electrocardiogram (EKG), pulse oximetry, EEG, eye movements, and movements of the respiratory muscles and limbs. Polysomnography findings classify sleep in different stages. From a physiology point of view, sleep is classified into rapid eye movement (REM) sleep and non-REM sleep (Fig. 1). Non-REM sleep is divided into four stages. The EEG reading is normally made by dividing the recordings into 30 second periods called epochs.

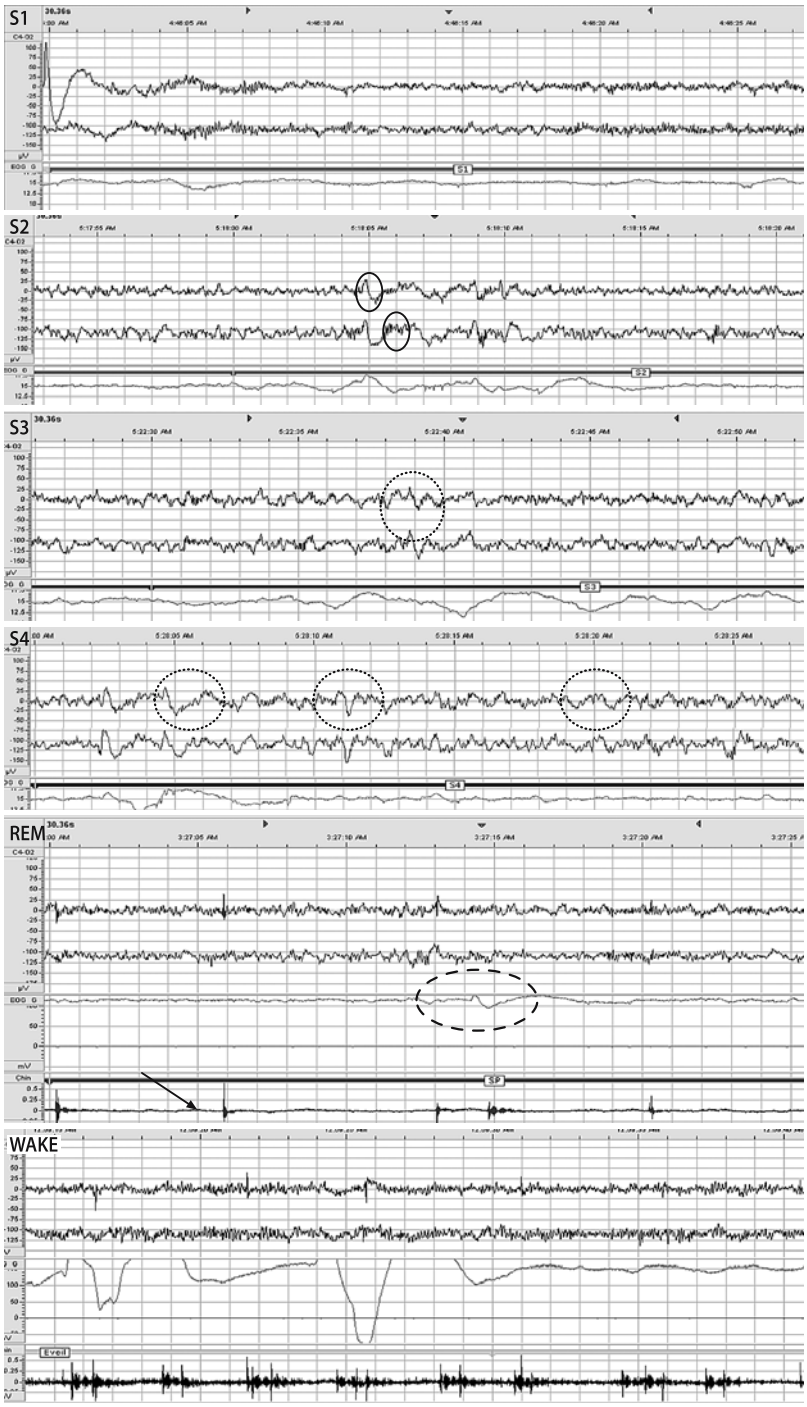
S1
S2
S3
S4
REM
WAKE

Fig. 2. Hypnogram of an ICU mechanically ventilated patient. The polysomnography was performed during 24 hours. The patient was not under sedatives. From top to bottom: Wakefulness (Eveil), REM sleep (SP), Stage 1 (S1), 2 (S2), 3 (S3) and 4 (S4). A high fragmentation of sleep is observed, because the patient switches often between stages. The small percentage of REM sleep (gray color) is also remarkable

Stage 1 is a transition phase which represents a small percentage of total sleep (2–5%). It is characterized on the EEG by low amplitude and mixed frequency with less than 50% of alpha activity. Stage 2 represents a high percentage of total sleep (50%) and is characterized by at least one sleep spindle or K complex, typical EEG findings, and less than 20% of slow wave activity. Stages 1 and 2 represent light sleep. Deep sleep includes stages 3 and 4, i.e., 20% of total sleep, and it is characterized by slow wave activity in the EEG. In stage 3, there is less slow wave activity (between 20 and 50%) than during stage 4, where it represents more than 50%. REM sleep together with stages 3 and 4 represents the restorative phase of normal sleep. REM sleep usually makes up 25% of total sleep time. It is characterized by a low-voltage, mixed frequency EEG, occurring in an episodic way, every 90 minutes, and with a relatively low amplitude in the electromyogram (EMG). This amplitude is lower than or equal to the lowest amplitude in non-REM sleep. During REM we find characteristic rapid eye movements, irregularities in respiration and heart rate, high variability in arterial tension, absence of thermoregulation, paralysis of the respiratory muscles except the diaphragm, a low threshold for wakefulness and emergence of dreams.

Sleep can be fragmented to some degree due to episodes of arousal, defined as abrupt variations in EEG frequency with a minimal duration of 3 seconds, and awakenings, which are episodes compatible with an EEG of wakefulness (i.e., low amplitude, high frequency waves).

From the EEG readings, the quantity, distribution and fragmentation of sleep can be represented in a figure known as a hypnogram, as described by Kales and Kales [3] (Fig. 2).

Propensity for sleep and wakefulness, as well as hormonal secretion and other physiological variables, cycle regularly each day (circadian rhythm). This circadian rhythm is based on a biological clock.

Fig. 1. Six sleep recordings in an ICU patient. Stage 1 (S1), 2 (S2), 3 (S3), 4 (S4), REM and wakefulness (wake). Each figure shows from top to bottom electroencephalogram and electrooculogram. In Stage 2 Complex K (gray circle) and spindle (black circle) is observed. In Stage 3 and 4 slow waves (broken circle) are observed, with a higher number in Stage 4. In REM we observed the characteristics eye movement (dashed circle) and no activity of the electromyogram (black arrow)

Sleep Characteristics in ICU Patients

In ambulatory patients, sleep alterations are classically classified in terms of quantity, architecture, level of fragmentation and distribution through the day. Studies have shown that sleep in ICU patients is severely altered [4–6]. This alteration has been subjectively evaluated in a survey among patients recovering after ICU admission. Simini [7] showed that more than two thirds of ICU patients complained of a poor quality of sleep. In another large study involving 203 ICU patients, sleep quality was subjectively evaluated as lower than that at home [8].

Sleep has been objectively analyzed by polysomnography in ICU patients. Results regarding sleep quantity are contradictory. Some studies show a decrease in total sleep time [4, 9], while others show an almost normal total sleep time [5–7]. Besides quantity, quality alterations involving architecture, distribution and fragmentation, are always present [4–6]. Regarding architecture and fragmentation, a decrease in stages 3 and 4 with REM, and an increase in the number of arousals and awakenings, are common findings in ICU patients [10]. The high level of fragmentation is not unlike that observed in sleep apnea patients [11]. In some ICU studies [6, 12, 13], the fragmentation index has been calculated as the number of arousals and awakenings per hour of sleep. Depending on the study, this index ranges from 22 to 79 events per hour [10, 12]. The fragmentation index in normal subjects varies from 5 to 15 events per hour.

Concerning the distribution of sleep alterations, the circadian rhythm is commonly altered and almost half of total time sleep occurs during the daytime [5, 6, 13]. Cooper et al. [6] studied sleep characteristics in ICU patients treated with mechanical ventilation. Polysomnography recordings were made over 24 hours. Based on EEG findings, patients were classified in three groups. Only one of these groups showed EEG characteristics compatible with physiological sleep. The other two groups showed coma phases or 'atypical' sleep phases. In these last two groups, without physiological sleep, the amount of sedation and the APACHE II acute physiology scole component were higher as compared to the 'physiological sleep' group. The authors proposed selection criteria to evaluate sleep based on gravity, Glasgow Coma Score (GCS) and the level of sedation. It should be taken into account that the patients were under sedation in this study. In a subsequent study where the majority of patients were not under sedation, Freedman et al. [5] showed how sepsis altered the polysomnography readings. They found low voltage mixed frequency waves with variable amounts of theta and delta waveforms, making it difficult to differentiate wake and sleep states. Such studies by Cooper et al. [6] and Freedman et al. [5] in ICU patients illustrate that the difficulties in the reading and interpretation of polysomnography recordings are magnified by the use of sedation.

Pathogenesis of ICU Sleep Alterations

The pathogenesis of sleep alterations in ICU patients is not well known and several factors have been proposed as responsible for sleep disruption. Environmental factors, such as patient-care activities, light and noise, have been implicated as the main origin, probably due to the particular characteristics in this scenario. In an initial study on noise, Freedman and colleagues [5, 8] showed how patients per-

ceived the assessment of vital signs and phlebotomy as more disruptive than noise [8]. No EEG recordings were performed and the information was obtained by means of a questionnaire completed by recently discharged ICU patients. In a subsequent study, using polysomnography and time-synchronized recordings, these authors surprisingly found that episodes of noise were only related to 15% of the total number of arousals and awakenings. Similar results from Gabor et al. [13] showed that both noise and patient-care activities were responsible for 30% of episodes of the total number of arousals and awakenings, while the vast majority (70%) remained unknown. These two studies used very similar criteria to define arousals and awakenings related to noise. Noise was defined as a sound elevation of 10 decibels (db). Arousals or awakenings occurring during or within 3 seconds of the termination of the sound were attributed to noise. Because of the high number of peak sounds in the ICU environment, 36.5 per hour of sleep in the Gabor study [13], as well as the high number of arousals and awakenings in ICU patients, from 22 to 79 per hour of sleep [6, 12, 13], a definitive cause-effect relationship between these two events could not be established (Fig. 3).

Besides environmental factors, there are other possible causes of sleep fragmentation, such as pharmacological treatment [14], gravity of the illness [6] and mechanical ventilation [12]. In the ICU, the number and dose of drugs given to patients is non-negligible. Some of them are almost always used, like sedatives and narcotics. Regarding sedatives, benzodiazepines can improve behavioral aspects of sleep by decreasing the time necessary to fall asleep, by decreasing awakenings, and by increasing sleep efficiency. Benzodiazepines can also increase stage 2, suppress restorative sleep, and decrease EEG amplitude and frequency. This last effect occurs at high doses [10]. Insomnia, hallucinations and nightmares have been described when using benzodiazepines. Narcotics suppress REM and slow wave sleep, i.e., restorative sleep [15–17]. The combination of sedatives with narcotics is a com-

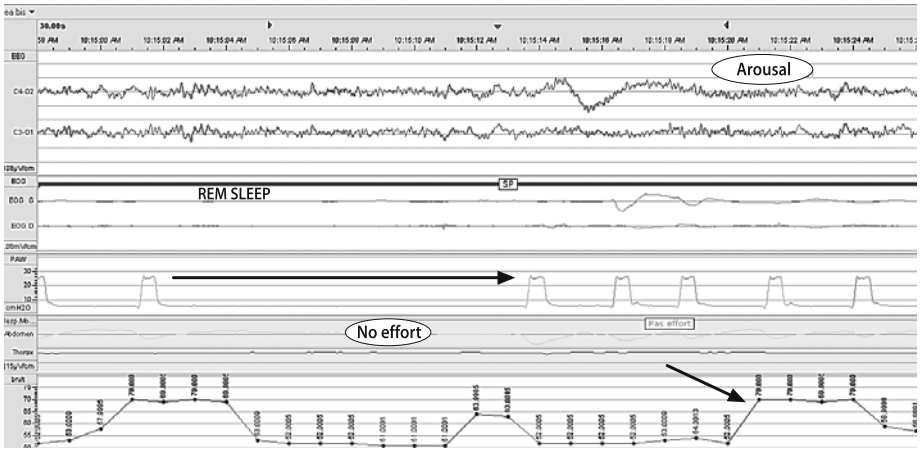

Fig. 3. From top to bottom: Electroencephalogram (EEG), electrooculogram (EOG), airway pressure (PAW), thoraco-abdominal plethysmography and noise scale. This is an ICU patient, during REM sleep, ventilated with pressure support. Patient presents a central apnea (solid black arrow) and an increase in noise of more than 10 dB followed (broken black arrow). Both of these were followed by an arousal (black circle). Knowing which triggered the arousal is difficult to ascertain

mon treatment in ICU mechanically ventilated patients, and leads patients to a sleep-like stage, which is far away from the physiological sleep architecture. The clinical relevance of this abnormal drug-induced sleep architecture is not known.

The gravity of the illness is another hypothesized cause of sleep derangement in ICU patients. It has been observed that sleep architecture normalized almost simultaneously with illness recovery [18]. When investigating this relationship, Parthasarathy and Tobin [19] found that acuity of illness, measured from the APACHE severity of illness score, was correlated with the level of fragmentation. However, in another study, the same authors [12] did not find this correlation.

Sleep and Mechanical Ventilation

The influence of mechanical ventilation on sleep quality is unknown. As previously mentioned, one confounding factor in this scenario is the administration of sedation, widely used in mechanically ventilated patients. Besides pharmacological treatment, the ventilator *per se*, the ventilatory mode and settings, and the patient-ventilator interaction could influence sleep quality in ICU patients. Two thirds of patients under mechanical ventilation complained of sleep alterations due to ventilator adaptation difficulties, but also because of anxiety related to ventilator use [20].

The cortical stimuli to breathe is lost during sleep, and in this state breathing depends mainly on chemoreceptors. Therefore, a decrease in PCO_2 during sleep is a powerful inhibitory factor of ventilation. If the PCO_2 is reduced below the hypocapnia-apnea threshold, central apneas may occur. Indeed, there are clinical conditions that can trigger central apneas due to hypocapnia such as hyperventilation, stroke or congestive heart failure. The pathophysiology of central apneas in congestive heart failure patients is explained by a decrease in cardiac output and an increase in left ventricular filling pressure. These abnormalities cause, via pulmonary afferent stimulation, hyperventilation, hypocapnia, and subsequent development of central apneas [21].

Concerning patient ventilator interactions and sleep, a recent study showed that the ventilatory modality could play a role in sleep quality [12]. Parthasarathy and Tobin compared the effect of two different ventilatory modalities in sleep fragmentation. The same patients were consecutively ventilated with pressure support ventilation (PSV) and assist control ventilation (ACV). The PSV level was set to achieve the same tidal volume as with ACV. Tidal volume in ACV was 8 ml/kg and the mean PSV level was around 17 cmH_2O. These authors found an increase in the number of arousals and awakenings when the patient was ventilated with PSV as compared to ACV. The increase in sleep fragmentation was proportional to the increase in apneas during PSV. In the group of patients with apneas, 80% had been diagnosed with congestive heart failure, a condition which is highly susceptible to the development of central apneas. The most important determinant factor of the apneas was the difference between the level of end-tidal CO_2 and the apnea threshold point. This threshold point was individually measured as the average of six determinations of end-tidal CO_2 before the onset of an apnea. After adding dead space, the number of apneas decreased and sleep quality improved. This could probably have been achieved by selecting lower levels of pressure support. High respiratory drive could be a protective factor for the development of central apneas. Indeed, apneas were not present in patients with a high ventilatory demand [22].

Meza et al. [23] showed that sleep fragmentation in healthy subjects could be induced by an augmentation of pressure support level. It should be kept in mind however, that there is a non-negligible variation among individual patients. The main mechanism of apnea in this group of subjects was the decrease of PCO_2 to below a threshold level. Because the ventilatory demand is lower during sleep, the PCO_2 diminishes and goes below the threshold if alveolar ventilation remains constant. The pressure support level during wakefulness can be excessive and inadequate during sleep.

In their recent study, Fanfulla et al. [24] compared two different settings of non-invasive mechanical ventilation (NIV). Nine stable patients with neuromuscular disease requiring long term ventilation were evaluated. Two settings for NIV were adjusted, one based on clinical parameters and the other according to the patient's respiratory effort. Improvements in sleep quantity and quality, the latter expressed as an increase in the percentage of REM stage, were observed when patients were ventilated with pressure support and when positive end-expiratory pressure (PEEP) was adjusted according to the physiological parameters of the patient's respiratory effort. The increase in REM stage was strongly correlated to the reduction in the number of ineffective efforts during NREM sleep. In three patients ventilated with NIV adjusted by clinical parameters, the authors found a higher index of central apneas, associated with episodes of oxygen desaturation. No significant difference was observed for the level of pressure support between the two groups. However, in the group of patients with central apneas, when the pressure support level was decreased, apneas almost disappeared. Additionally, a statistically significant difference was found for the average PEEP level, this being higher in the group of patients in whom pressure support was clinically adjusted. The authors suggested that clinical adjustment of pressure support and PEEP during wakefulness may cause patient-ventilator asynchronies and central apneas during sleep, thus leading to a deranged sleep quality as represented by a decrease in REM stage. A decrease in ineffective efforts when the pressure support level was adjusted from physiological parameters could be explained by the decrease in intrinsic PEEP which could have contributed to the improvement in trigger sensitivity. The authors also hypothesized that adjusting the ideal pressure support and PEEP level with polysomnography could be useful in ambulatory patients needing NIV.

The applicability of the latest study findings to the ICU scenario remains uncertain due to the high instability in respiratory mechanics and the complexity of sleep studies in ICU patients. However, this study is extremely interesting because it introduces a new idea and a new tool for setting an 'ideal' pressure support and PEEP levels, at least during sleep.

Consequences of Sleep Alterations in ICU Patients

The consequences of sleep alterations in ICU patients are unknown. Early studies (1946) in rats showed that long sleep deprivation killed the rat. In the days prior to the animal's death, a notable weight loss, a decrease in central temperature and an increase in norepinephrine and thyroxine plasma levels was observed [25]. Recent studies in healthy human subjects have shown an increase in pro-inflammatory cytokines, interleukin (IL)-6 and tumor necrosis factor (TNF)-alpha [26], C-reactive protein [27], and natural killer lymphocyte activity [28] lasting approximately

24 hours after acute sleep deprivation. Consequences on hormonal function have also been observed. Aldosterone, for example, decreases at night-time in these circumstances and alters the water and salt balance [29]. A decrease in insulin sensitivity has also been described [30]. Other clinical effects due to sleep deprivation, have been observed in healthy subjects, such as a diminished strength and endurance of respiratory muscles [31] and a decrease in host defenses [32].

Concerning ICU patients, it has been suggested that sleep deprivation could lead the patient to agitation and/or delirium [33]. An impaired sleep architecture, mostly due to a decrease in the percentage of REM sleep, has been associated with a higher incidence of delirium in ICU patients [34]. The development of delirium during the ICU stay has emerged as an independent predictive factor associated with an increase in mortality [35]. In a group of posttraumatic patients with coma, Valente et al. [36] showed that prognosis, in this case evaluated as survival and functional recovery, was better in the group of patients who had a near normal sleep architecture. This index was a better outcome predictor than conventional prognostic indexes such as age, GCS and neuroradiological findings.

Treatment of Sleep Alterations in ICU Patients

Therapeutic interventions to improve sleep quality have not been well defined. This appears to be the consequence of a lack of knowledge about the pathogenesis of sleep alterations in ICU patients. Minimizing noise in the ICU and giving patients earplugs could be useful [37]. Setting optimal ventilatory parameters, not only to avoid non-triggered breaths but also to avoid apneas and episodes of desaturation, seems helpful to diminish sleep fragmentation [12]. It is conceivable that changing ventilator settings during sleep so as to avoid hyperventilation could help to increase sleep quality. Limitations of this approach include alterations in circadian rhythm in ICU patients, and the high percentage of sleep occurring during daytime. Consequently, knowing when the patient is sleeping is a relevant challenge. A system providing continuous adaptation of the level of assist, avoiding hypoventilation, could be helpful for this purpose.

Regarding pharmacological treatment, the ideal hypnotic remains to be found. The drugs most commonly used these days to induce sleep are sedatives, but such agents lead the patient to a non-physiological sleep stage. Drugs such as atypical antipsychotics and melatonin have been hypothesized as potentially good hypnotics in ICU populations [14, 38], but further investigation in this field should be carried out before they can be recommended for sleep purposes.

Conclusion

Sleep in the ICU is an avenue of scientific research which is only now starting to evolve. There are at least three factors that make the ICU scenario unique. The first of these is the particular environment, characterized by a background of noises, changes in lighting and constant patient-care activities. Surprisingly, the environment has been shown to play but a small role in sleep disruption. The second factor is the acuity of illness; some studies suggest a relationship between normalization of sleep architecture and recovery from an illness. And the third factor is the

widespread use of sedation associated with mechanical ventilation. The ventilatory modality and its settings have an impact on sleep quality, suggesting there is a relative hyperventilation during sleep, adding to sleep fragmentation. Regarding sedatives, we know that these drugs disrupt physiological sleep. All these factors, either alone or interacting together, have some impact on ICU patients' sleep architecture. A deeper understanding of sleep physiopathology and the use of a more physiological approach to setting the ventilator could help to improve sleep quality.

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Sleep and Delirium in the Critically Ill: Cause or Effect?

A. C. Trompeo, Y. Vidi, and V. M. Ranieri

Introduction

The intensive care unit (ICU) is perceived by patients and families as a hostile environment: with analgesic requests not yielding the expected pain relief, sleep deprivation and disruption, anxiety, isolation, pain and lack of information often being reported [1–4]. Using patient questionnaires and sleep monitoring, numerous studies have demonstrated that exposure to the ICU environment consistently results in reduced sleep efficiency, reduced restorative sleep, and increased sleep fragmentation. Common aspects of the ICU, such as sleep disturbances and deprivation, induce additional stress in critical care patients and may impair immunity and cause increased catabolism [5–7].

Acute brain dysfunction, a general term including delirium, stupor, and coma, is exceedingly common in ICU patients. One of these diagnoses, delirium, is of great clinical concern since:

- it is associated with mortality and morbidity in other populations;
- it is the most frequent complication of hospitalized elderly patients, affecting more than 2.3 million older patients each year [8–12].

Although internists and psychiatrists have specifically considered delirium as an important area of focus regarding patient outcome for many years, only recently have data regarding delirium been reported in the critical care literature. Delirium is commonly unrecognized or overlooked by physicians during their practice, especially in patients in a critical condition or receiving mechanical ventilation [9, 13, 14]. A delay in recognition or ignorance of the development of delirium in those patients may lead to poor outcome and an increase in medical costs [9, 14–16].

In this chapter we will explore the potential links between delirium and sleep disruption in the critically ill.

Delirium in the Critically Ill

Delirium is defined as an acute change or fluctuation in the course of a patient's mental status, plus inattention and either disorganized thinking or an altered level of consciousness and it is not a disease itself but a marker of acute cerebral insufficiency affecting the brain in many ways, and is recognized as a sign of brain failure where the risk of death increases with the number of organs failing [9, 13, 15]. Three subtypes of delirium ('hyperactive', 'hypoactive', and 'mixed') have been described based on psychomotor activity and arousal levels. Although the hyperactive

Table 1. Risk factors for delirium in critically ill patients

▮ Sleep disturbances	▮ Tube feeding or total parenteral nutrition
▮ Administration of benzodiazepines or narcotics	▮ Renal failure (creatinine > 2 mg/dl)
▮ Rectal or bladder (Foley) catheters	▮ BUN/Creatinine ratio
▮ Visual or hearing impairment	▮ Liver disease (bilirubin > 2 mg/dl)
▮ Central venous catheters	▮ History of congestive heart failure
▮ Hypo or hyperglycemia (< 80 or > 120 mg/dl)	▮ History of stroke, epilepsy
▮ Hypo or hypernatremia (< 135 or > 145 mg/dl)	▮ Use of physical restraints or posey vest
▮ Hypothermia or fever (< 36° or > 38 °C)	▮ Prior history of depression
▮ Cardiogenic or septic shock	▮ Hypo- or hyperthyroidism
▮ Age over 70 years	▮ Human immunodeficiency virus infection

type is most easily recognized, a substantial proportion of patients present with hypoactive delirium. Patients with hyperactive symptoms are much more likely to be diagnosed with delirium than patients with hypoactive symptoms [15–17]. Patients in the ICU are at very high risk for the development of delirium because of factors such as multiple-system illnesses and co-morbidities, use of psychoactive medications, age, and mechanical ventilation (Table 1). This reversible organic mental disorder is often thought to be a reflection of these aspects and not a cause-and-effect of being in the ICU [9, 10, 16, 17]. The incidence of acute respiratory failure requiring mechanical ventilation rises tenfold from the age of 55–85 years [10–12], resulting in greater numbers of elderly patients treated in our ICUs. Without appropriate preventive and management strategies, the aging of the population will likely result in an increased burden of delirium among mechanically ventilated patients across the country, a factor which could strongly effect discharge rates to nursing homes following hospital discharge [9, 14, 15].

▮ Delirium and Sleep

Sleep is most accurately and objectively assessed by means of polysomnography (PSG), the simultaneous recording of several electroencephalographic (EEG) and physiologic parameters. Sleep periods are generally classified as non–rapid eye movement (NREM) and rapid eye movement (REM) sleep. NREM sleep is further subdivided into four stages, with stages 3 and 4 (also referred to as delta sleep or slow-wave sleep) being a deeper and more restful sleep and occupying approximately 15 to 20% of the total sleep time in a healthy middle-aged individual. REM sleep is also considered to be restful, although the brain is much more active and, as the name suggests, distinctive rapid eye movements occur intermittently. Sleep onset occurs with stage 1, which is usually short-lived, and progresses to stage 2, which occupies about half of the total sleep time, and eventually to stages 3 and 4. Each sleep cycle is approximately 90 to 110 minutes in duration, with REM sleep periods in each increasing in length as the night progresses, usually alternating with stage 2 and accounting for 20 to 25% of the total sleep time in a healthy middle-aged individual [2].

Despite significant progress in many aspects of delirium and sleep, the pathophysiology continues to be the least understood; however, they are thought to be related both to anatomic deficits and imbalances in the neurotransmitters which modulate the control of cognitive function, behavior and mood. Sleep is not merely a passive state but requires a complex and fine regulation performed mainly by the brainstem and diencephalic structures [18]. Clinically, both the cortical and subcortical areas of the brain are involved in delirium but there is no distinct anatomical structural defect common to all causes of delirium [18–21]. The physiology of aging partially explains why elderly patients are more likely to develop delirium compared with younger patients when presenting with an acute medical illness. In normal aging, cerebral blood flow declines by 28%, neuronal loss occurs, and there are lower concentrations of important brain neurotransmitters, such as acetylcholine, dopamine, [gamma]-aminobutyric acid (GABA), and norepinephrine [22]. It has been suggested that these changes result in less physiologic reserve to combat the additional neurologic stress that occurs with metabolic disturbances or infection [21, 22].

Hypothetically, this kind of neurotransmission may have a role in the regulation of the sleep/wake cycle since it can work in a slow and long-term modulation over diverse and widespread systems producing a sustained function that may collaborate in the maintenance of the wake and sleep phases. Cytokines such as tumor necrosis factor (TNF)-α, interleukin (IL)-1, and IL-2 are thought to play some role in the pathogenesis of delirium. Usually, cytokines are found extracellularly at low levels in the central nervous system (CNS), but after stress, inflammation, infection, and trauma, cytokine release increases, which leads to activation of the hypothalamic-pituitary-adrenal (HPA) axis, increased permeability of the blood-brain barrier, and interference with neurotransmitter synthesis and transmission. It has been postulated that in healthy brains, cytokine production may have little deleterious effect but can enhance neurodegeneration when neuronal damage is already present. Activation of the CNS's immune response is accompanied by the peripheral production of TNF-α, IL-1, and interferon-δ. It is plausible that the delirium experienced among these patients not only is a marker of end-organ damage but also acts directly as a promoter for other organ system failures. Much as the lung can become an engine of inflammation in the setting of acute lung injury (ALI), it is conceivable that the brain could become a promoter of the inflammatory cascade in the setting of ICU delirium [24–26].

Clinical Aspects

The accurate diagnosis of delirium in the ICU setting is limited by the difficulty of communicating with mechanically ventilated patients and in the past by the lack of a validated clinical instrument for the identification of delirium [13]. The "Diagnostic and Statistical Manual of Mental Disorders", Fourth Edition (DSM-IV), is the most popular tool used in medical research and therapy to evaluate the development of delirium. It requires verbal communication between the professional psychiatrist and patient. However, this is not applicable in mechanically ventilated patients who cannot communicate verbally due to endotracheal intubation. The Confusion Assessment Method for the Intensive Care Unit (CAM-ICU), based on the Confusion Assessment Method, is a new method for the detection of delirium in the ICU. It consists of four categories of nonverbal assessment items and has

been validated in the evaluation of delirium in critically ill and mechanically ventilated patients [27, 28]. This newly developed tool was also documented to be easily accessible by medical staff without formal psychiatric training and can reliably detect delirium in mechanically ventilated patients with a high degree of sensitivity and specificity.

Routine monitoring for delirium is neither difficult nor time consuming. Importantly, the CAM-ICU takes an average of 1 min to perform at the bedside and can be accurately and reliably performed with minimal training. In a recent study of >22,000 patient days of monitoring, the bedside nurses demonstrated excellent accuracy and reliability in using the CAM-ICU. They reported that this tool did not represent additional work but rather allowed for a more organized and clinically meaningful neurologic examination in an area of critical care medicine that has traditionally been nebulous [13, 16, 28].

It is very important to establish a correct differential diagnosis. Some symptoms in delirium are common to other conditions, which may prove especially challenging when these conditions coexist. Cognitive disturbance occurs in both delirium and dementia, but even patients with moderate dementia remain alert and are still able to focus and maintain attention. Caregivers and families can detail the duration and course of the patient's cognitive impairment. Acute cognitive decline may occur in vascular dementia. Hypoactive delirium may be mistaken for depression but the timing of the onset of symptoms and cognitive impairment will help clarify the presence of delirium or depression [17, 29, 30].

Delirium in mechanically ventilated patients is associated with a prolonged ventilation, nosocomial pneumonia, longer length of stay in the ICU after adjusting for age, gender, race, and severity of illness, longer hospital stay, and may be related to negative long-term neuropsychological outcomes. In addition, development of delirium in patients receiving mechanical ventilation at some point during the ICU stay is an independent predictor of higher 6-month mortality [14–16, 31, 32].

Considering that the possibility of being left cognitively impaired following a serious illness is the primary determinant of a patient's treatment choice, it will be imperative for intensivists to consider the relationship between delirium and long-term cognitive outcomes. Previous studies have reported that preventive intervention strategies targeting risk factors could reduce the incidence and duration of delirium in older hospitalized patients [29, 30, 34].

Risk factors for delirium include acute systemic illnesses, age, medical comorbidities, preexisting cognitive impairment, sleep deprivation and sleep disturbances and medications. It has been postulated that aspects of the ICU, such as sleep disturbances and deprivation common in critically ill patients, contribute to the occurrence of delirium [13–29]. We recently tested the hypothesis that abnormalities of sleep in the ICU cause delirium in critically ill patients. We studied adult, post-surgical patients who needed mechanical ventilation for more than 48 hr admitted to the general ICU at the University of Turin, Italy. The major finding of our observational study was that the occurrence of delirium was associated with a selective deprivation of REM phase sleep, and although patients had a similar clinical status at the time of the sleep study, those with REM deprivation had a longer stay in the ICU and required mechanical ventilation for a longer time [33].

There is no consensus regarding the best methods for preventing or treating delirium; however, a number of specific objectives should be carefully considered including the identification and treatment of the cause of delirium, environmental modification, and the control of symptoms [8, 17, 23, 29]. Identification and treat-

ment of the cause of delirium include the correction of metabolic disturbances and hypoxia, protocol-directed ventilator weaning using spontaneous breathing trials to expedite liberation, early mobilization and physical therapy, frequent reorientation of the patient by the nurse and/or family, restoration of normal sleep patterns, goal-directed delivery of sedation and analgesia (i.e., using daily awakening trials to avoid oversedation and expedite ventilator liberation), avoidance or rationing of specific psychoactive medications, and thoughtful use of specific antipsychotic medications when appropriate [30].

Management of delirium involves intervention by nursing and medical staff. Older people are susceptible to environmental change, and an unfavorable environment may exacerbate delirium. Use of calendars, clocks, adequate rest/sleep times, maintaining normal night and day patterns by opening the blinds by day and turning off lights at night, and reorientation by staff are recommended. Educating the family is important to decrease stress when seeing a loved one with delirium. Involving family and friends can make the patient feel more secure and may be very helpful in reorienting and reassuring the patient [17, 23, 29]. When discussing the management of delirium, it is important to remember that patients who develop delirium are a very heterogeneous group, and one approach does not fit all.

Pharmacological treatment is limited to symptom treatment while identified causes are being simultaneously treated. There are no placebo-controlled trials or large prospective trials. Most of the study designs are case series or case reports. Many drugs have been used, including haloperidol, risperidone, rivastigmine, olanzepine, ondansetron, quetiapine, trazodone, and lorazepam. Haloperidol is the most studied drug in the treatment of delirium and works as a dopamine receptor blocker. Reserpine and tetrabenazine block the release of dopamine, but these drugs are non-specific and also block the release of norepinephrine and serotonin, leading to multiple unwanted effects. Haloperidol is considered the standard treatment for delirium and the drug of choice. Although there are side effects (extrapyramidal side effects and prolonged QT interval), the benefits are rapid onset, relatively wide therapeutic window, reasonable safety profile over many years of use, and various routes of administration (can be administered orally, intramuscularly, subcutaneously, and intravenously). It has no active metabolites, does not cause significant hypotension, and is the most studied neuroleptic agent. The onset of sedation is rapid, but the antipsychotic effects can last 3 to 5 days because its elimination half-life is estimated at between 10 and 18 hours. It may be used in diverse patients with severe illness, including congestive heart failure, renal failure, and chronic obstructive airway disease. Intravenous haloperidol rarely causes extrapyramidal side effects. It can be associated with prolonged QT interval, and cardiac monitoring is recommended especially when using high doses, but high doses of the drug have been given safely to patients with severe cardiac disease. When a patient has been given haloperidol, it is better to taper the dose over the next 3 to 5 days because abrupt withdrawal can lead to rebound symptoms. Olanzapine (2.5–10 mg) and risperidone (1.0–4 mg) have been used successfully in uncontrolled case series. These drugs have fewer extrapyramidal side effects, cause less sedation, are more costly, and may only be given orally. Benzodiazepines are the first-line treatment when the cause of delirium is alcohol and or substance withdrawal. In delirium, benzodiazepines are sometimes used in conjunction with neuroleptics because they are synergistic, and lower doses of neuroleptics can be administered with good effect with fewer extrapyramidal side effects. Lorazepam is often the drug of choice because it is short acting, has no active metabolites, and works rapidly [8–17, 23, 29, 30].

Conclusion

A review of the literature raises the question of how important it is to monitor the quality of sleep in the ICU to avoid clinical consequences such as delirium that seem to be independently associated with worse outcomes, such as prolonged ventilator dependence, ICU length of stay, and hospital length of stay, and is an independent predictor of higher 6-month mortality. Every day spent by ICU patients in a state of delirium is associated with a 10% higher risk of death and worse long-term cognitive function, and delirium is associated with an increase in costs. Taking into consideration the increasing number of elderly patients treated in our ICUs, this burden will rise. Future research should focus on the pathophysiology, cause, and treatment of sleep deprivation and of the occurrence of delirium.

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Delirium, Recall and the Post-ICU Challenge

R. D. Griffiths and C. Jones

Introduction

In recent years, evidence has started to emerge of the impact on patients of their experiences while they were critically ill on an intensive care unit (ICU). Numerous studies in the past had shown that patients' recall for their time on the ICU was often fragmentary and that a significant number of patients reported remembering delusional memories, such as hallucinations and nightmares [1–4]. However, the effect of such distorted memories on the patients' psychological health during their recovery had, until recently, not been examined. It is only with the advent of critical care follow-up that the struggle that some patients have coming to terms with their memories for ICU has become clear [5].

Delirium or 'ICU Psychosis'?

A wide variety of psychological and behavioral disturbances displayed by patients within the ICU have been unhelpfully called 'ICU psychosis or syndrome'. Recent research has shown that most patients have a period of delirium at some stage during their period of critical illness, which may or may not be accompanied by agitation but increasingly is recognized to be associated with the patient experiencing delusions and hallucinations. It is not surprising given the serious medical conditions, metabolic derangements, and numerous drugs received by ICU patients that the incidence of delirium varies considerably dependent on the patient population, between 19% [6] to over 80% [7] within intensive care (see Table 1 for possible causes of delirium). The presence and duration of delirium is a strong predictor of length of hospital stay apparently even after correcting for severity of illness, age, and days of sedative use [7]. Predisposing factors for delirium in the ICU continue to be closely associated with markers of the underlying illness such as anemia, metabolic acidosis, uremia, jaundice, and use of common ICU drugs including dopamine, and duration of ventilation [7–10]. The most frequent risk factor within the ICU was the use of benzodiazepines or narcotics while a history of smoking, alcohol, and hypertension were prior risk factors [6]. Of course, intensive care patients are not a simple cross section of society, rather a select population where the very diseases that lead to ICU admission are themselves often related to smoking and excess alcohol consumption. Further clues to the etiology of delirium in critically ill patients come from the recognition that neuropathological changes to the brain, especially during severe sepsis, may be common [11, 12]. Delirium develops over a short period of time, usually hours to days,

Table 1. Causes of delirium [14]

- Metabolic encephalopathies
  - Acid-base or electrolyte imbalance
  - Hypoglycemia
  - Hypoxia, hypercapnia
  - Hepatic or renal failure
  - Wernick's encephalopathy and other B-vitamin deficiencies
  - Endocrine disorders, e.g.,Cushings or Addisons disease
  - Porphyria
- Intoxication by drugs and poisons
  - A wide range of drugs, including anticholinergics, hypnotic-sedatives, anti-parkinsonian agents, anticonvulsants, digoxin etc.
  - Alcohol, illicit drugs and inhalants
  - Industrial poisons
- Withdrawal syndromes, especially alcohol and hypnotic-sedatives
- Infections, both intracranial (meningitis, encephalitis) and systemic
- Multifocal and diffuse brain disease
  - Anoxia, fat embolism
  - Vasculitis
  - Cerebrovascular disease
  - Raised intracranial pressure, hydrocephalus
- Head trauma
- Epilepsy, including post-ictal and non-convulsive states

and tends to fluctuate during the course of the day. The features are disturbances of consciousness (i.e., reduced clarity of awareness of the environment), reduced ability to focus, sustain, or shift attention, and a change in cognition or the development of a perceptual disturbance [13]. This results in a dense amnesia for the period of the confusion [14]. Islands of memory can remain and coincide with the patient's consciousness level as it fluctuates. As part of the delirium there can be increased (hyperalert) or decreased psychomotor activity (hypoalert) and vivid hallucinations or paranoid delusions are more frequently associated with the former state, particularly following withdrawal from alcohol and/or sedative-hypnotics [11]. In the hypoalert state of delirium, patients appear quiet and still and when not stimulated they fall asleep, although patients may still experience hallucinations and delusions in this state.

A validated, practical and reliable assessment tool is available to diagnose delirium in the ICU, the confusion assessment method for the ICU (CAM-ICU) [15]. Routine use has been recommended by the practice guidelines for sedation of the American Society of Critical Care Medicine [16]. In addition to delirium other problems such as patients suffering from anxiety or depression have been reported [17–19]. It is, however, very difficult to reliably establish these diagnoses within the ICU where patients cannot freely talk due to oral or tracheal intubation. A patient appearing anxious may in fact be experiencing hallucinations. An example of this can be given from our own practice. A patient was weaning on continuous positive airway pressure (CPAP) through a tracheostomy. She appeared very anxious to the staff and they tried to reassure her. When she was extubated one of the authors spoke to her and she said her recall of that period was that everyone, including her

family, looked right but had really been replaced by aliens (Capgras delusion) and if she relaxed and fell asleep that she would be replaced as well [20]. She was, therefore, quite reasonably anxious but the reassurance she had received was counter-productive as she interpreted this as the aliens trying to get her to go to sleep and be replaced! The criteria used to assess depression are equally unclear. A withdrawn patient may be labelled as depressed when they are actually in the hypoalert phase of delirium. In this case, the administration of an antidepressant may further cloud cognition in this acute setting.

Alternatively, mood may be affected by a delusional memory, for example, one gentleman had convinced himself his wife had died because he could not remember her visiting him due to intermittent confusion, usually in the evening when she was visiting. His mood improved considerably when she was asked to visit earlier in the day when he was not delirious.

Experience through intensive care follow up coupled with the appreciation that delirium is a frequent occurrence in the ICU has identified a number of key points to help in patient management:

- Patients have different kinds of hallucinations and delusions. While some may be pleasant and transitory others are unpleasant delusional experiences that may be firmly held beliefs. The most strongly held beliefs tend to be persecutory and even life-threatening [21].
- The behavior of patients may be strongly influenced by the delusion they are experiencing but this may not be apparent to their carers. The Capgras delusion mentioned above is an example of a failure of face recognition leading to a delusional belief.
- Patients have difficulty in remembering real events and can misinterpret events if sufficiently aware. However, when patients remember real events this enables them to orientate and explain any hallucination or delusional experience. However unpleasant many aspects of an ICU stay may be, the underlying experience for patients when they are aware is that someone is looking after them, i.e., providing security and safety.
- The nature of the delusional experience can be influenced by surrounding events or experiences immediately before the illness while its impact on the patient is determined by its meaning to the patient. For instance, the incidence of delusions containing military themes was observed to appear during a period of intense media focus on a recent war and occurred uniquely in patients who would have been young adults during World War II [22].
- Patients that are amnesic of any real ICU event may have all experience replaced by a strongly held delusional belief. If this is persecutory and in particular life-threatening, this memory is maintained and predisposes to developing post traumatic stress disorder (PTSD) after ICU as discussed later. Key to this is the combination of a vivid life-threatening experience and the loss of personal safety due to amnesia of the ICU care.

Within intensive care, excluding delirium is an important first step and involves a reasoned medical approach with some formal testing for instance using the CAM-ICU if practical. However, even with delirium excluded, the inability for our patients to vocalize makes establishing alternative diagnoses difficult and the indiscriminate use of anxiolytics and antidepressants is to be deplored without careful longitudinal assessment. Rather, programmed management of drug or substance withdrawal is more profitable and may be combined with cautious use of appropriate medical sedation.

Post ICU Discharge

Following serious illness, anxiety, panic attacks, and depression are common in many patient groups [23]. ICU patients are no exception to this and anxiety and depression are common in both patients and their families after discharge. One large study involving 3655 patients reported problems with emotional behavior, sleep and alertness, particularly with younger patients aged between 30–50 years [24]. The delusional memories that patients recall from their stay in ICU can be vivid and be a powerful precipitant of PTSD-related symptoms. The patient's memory of their illness may be disturbed with some suffering amnesia for the ICU stay [25]. Where delusional memories are the sole recollections of the ICU, with no factual memories being recalled at all, the risk of developing high levels of PTSD-related symptoms has been shown to be even higher (Fig. 1) [26]. In addition, there seems to be a strong correlation between symptom levels in family members and patients [27]. This may be because the patient and relatives are unable to support each other. PTSD can be extremely debilitating affecting the patient's own ability to socialize, work, or engage with health services in the future. PTSD is characterized by a range of symptoms such as re-experiencing the traumatic event (flashbacks), avoidance of situations that remind patients of the event, a numbed reaction and symptoms of increased arousal, for example, sleeplessness [28]. PTSD can only be diagnosed when the symptoms have been present for more than one month. There are many tools for diagnosing and screening for PTSD. A few tools have been used with ICU patients. A short, easy to use tool indicating levels of symptoms requiring intervention (e.g., counselling or clinical psychology) is useful and an early short screening tool for use in the clinic or over the telephone allows quick assessment. The PTSS-14 may be a suitable tool as it performed well in a pilot study [29]. The

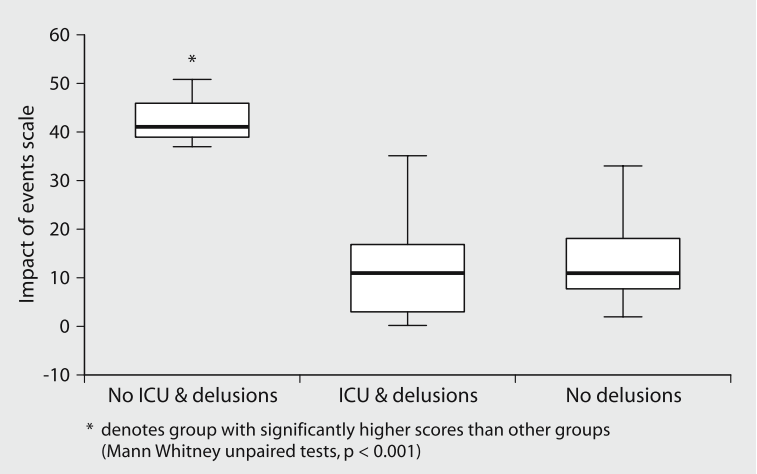

Fig. 1. Box and whisker plots of PTSD-related symptoms at 8 weeks post ICU for the 3 patient groups categorized according to factual and delusional memories of ICU recalled at 2 weeks. No ICU & delusions: no factual memories of ICU plus delusions; ICU & delusions: factual memories of ICU plus delusions. From [26] with permission

PTSS-14 is a short questionnaire with 14 items covering 14 of the 17 symptoms of PTSD. It takes 3–5 minutes to complete, making it suitable for outpatient clinic or telephone use. In the pilot study, the PTSS-14 scores at 2 months predicted PTSD at 3 months using a full interview diagnostic tool. A review of the issues involved in the diagnosis of acute stress reactions in ICU patients is available [30].

Although physical recovery can be accelerated by the use of a self-directed rehabilitation package, the ICU Recovery Manual, early in the convalescent phase, psychological recovery may not be as simple [31]. While depression may be improved, the self-directed package had less impact on anxiety and PTSD-related symptoms at 6 months post ICU discharge. This is particularly so when patients reported recalling delusional memories from their ICU stay, so emphasizing the importance of recognizing such patients and ensuring appropriate further treatment.

In addition to psychological distress, critical illness can also have a prolonged impact on cognitive function, particularly on strategic thinking and memory, possibly reflecting frontal lobe problems. At the present time, it is unclear if the cognitive deficits noted so far are permanent due to neurological damage or may recover with time [32–34]. One recent study suggests that although some patients improve with time a significant number of patients remain cognitively impaired and may have problems with the performance of mental tasks such as financial management and driving [35].

Clinical Implications

It has been the tradition in the UK and many Northern European countries to sedate patients to ensure their compliance with the ventilator. This practice has been defended in terms of patient comfort and to reduce anxiety while in the ICU. Clearly such drugs have significant impact on the patient's cognitive function and may affect the ability to understand and remember information given to them. Research is now starting to explore the ideal depth of sedation to ensure that patients are not traumatized by their experience of ICU. Sedation may have a role to play in the formation of delusional memories by clouding cognition. In a recent retrospective study, the impact of waking the patient daily from their continuous sedation while on the ICU was evaluated in terms of their later psychological distress [36]. There was a trend towards those patients who were woken daily being less distressed and less likely to develop PTSD than those who were kept continuously sedated and only woken up to get them to breathe on their own without a ventilator. This may have major clinical implications for the way patients are cared for on the ICU.

Once ICU patients are discharged home even where follow-up services exist there may be little provision for counselling or referral to clinical psychology. A few hospitals around the UK have dedicated counsellors and/or clinical psychologists with sessional time for the ICU. For the majority of patients the first person they may tell about their problems will be their General Practitioner. While some General Practitioners now have counsellors attached to the surgery the waiting list can be lengthy. Little research has been undertaken on particular therapeutic approaches to address psychological distress in this patient group.

Possible Therapeutic Interventions

A simple intervention, which originated from Sweden and is well received by patients, is to keep a diary with photographs while the patient is on the ICU [37]. This can be written by staff and family and is given to the patient afterwards. The language used is everyday and any medical words are explained. Periods of confusion or agitation are also written about with a possible reason for this. A small pilot study in Sweden showed that patients felt that the diary helped them come to terms with their illness and recall what had happened [38]. For those who could not remember their ICU stay the diary seemed to fill in the lost time. This intervention needs formal evaluation to ensure that some patients are not made more distressed by reading a diary and seeing photographs of themselves in the ICU.

Conclusion

Further research is needed to understand the processes behind the formation of delusional memories on the ICU and to examine the impact of therapeutic interventions undertaken within the ICU such as altering sedation practice on later psychological health.

The growth of counselling services for patients diagnosed with cancer is in recognition of the psychological impact of severe illness. At present only about a third of ICUs in the UK follow up their patients in a dedicated clinic. The provision of counselling or psychology services specifically for this patient group is sparse. Psychological services for post-ICU patients are required so that each patient is appropriately assessed and given the necessary support. Physical recovery after critical illness is on the whole good, although it is a lengthy process. If psychological services are not available for some patients, their optimal quality of life may never be achieved.

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Contemporary Issues

Morbid Obesity as a Determinant of Outcome in the Critically Ill

I. Kim and S. A. Nasraway Jr.

Introduction

Obesity is a growing epidemic and holds significant health and economic consequences [1–3]. It has been shown to dramatically increase the risk of other diseases including type II diabetes, serious cardiovascular and pulmonary conditions such as coronary artery disease, and stroke. In addition, obesity directly correlates with mortality and can decrease life expectancy by nearly two decades [4]. The economic ramifications of obesity are also profound [5, 6]. One study reported that $93 billion of health care expenditure in the USA was allocated to obesity-related illnesses. Between the periods of 1987 and 2001, there was a 27% rise in inflation-adjusted per capita spending for obesity-correlated disease in the USA [5]. While first originating in the USA as a medical epidemic, obesity has also risen sharply in other parts of the world, including Europe, Russia and Latin America. In the last fifteen years, the prevalence of obesity has tripled in England and Wales and has risen by 20% in Eastern Europe [7, 8]. The International Obesity Task Force considers obesity to be a global epidemic, and estimates 300 million people around the world are obese [9]. The significance of obesity in health care cannot be overemphasized – obesity has dictated the way in which health care providers manage and strategize their treatment to this unique patient population.

Obesity is defined as having a high amount of body fat in relation to lean body mass as measured by body mass index (BMI) $\geq$30 kg/m^2. Overweight classification is defined as having a BMI $\geq$25 kg/m^2, whereas extreme obesity (also termed morbid obesity) exceeds a BMI $\geq$40 kg/m^2. In 1999–2002, 65.1% of the USA population was overweight, 30.4% of adults were obese, and 4.9% were extremely obese [1, 2].

Because of the alarming rise in the obese and morbidly obese populations, recent research has centered on outcomes of obesity in all health care sectors, including critical care medicine. BMI is a relatively simple anthropometric measurement to calculate. Classically, the relationship of BMI to mortality is reflected in a U-shaped curve with increased risk of death sustained in those individuals at the lowest and highest percentiles of BMI, even when controlling for confounding variables such as age and smoking. Given its facility of calculation, BMI and classifications of obesity could potentially prove a potent predictor of mortality in seriously ill patients as in general populations [10].

Special Needs of Obese Patients in the Intensive Care Unit

Morbidly obese patients often have different needs than non-obese patients, resulting in a shift in the prevailing standard of care. Relatively simple tasks, such as obtaining a suitable bed or providing routine perineal care for the morbidly obese patient, can impose unexpected burdens in many institutions. Nasraway et al. examined the multidisciplinary care of obese patients in a surgical intensive care unit (ICU), revealing multiple challenges that were met when looking after the needs of the chronically ill obese patient [11]. Obtaining accurate blood pressure measurements with standard-sized sphygmomanometer cuffs, determining appropriate drug dosing in patients with an enormous volume of distribution, or securing central venous access in individuals with difficult or unrecognizable anatomic landmarks can be formidable tasks.

Imaging studies, which are widely and routinely available for most patients are often entirely unavailable in the critically ill, morbidly obese patient due to weight or circumferential limitations (computed tomography [CT] scans) or due to adipose impedance for ultrasound signals (diagnostic ultrasound). Radiographs of morbidly obese patients in the ICU are often of very poor quality from insufficient x-ray penetration, making it difficult to diagnose pathology. This leads to obvious delays, both in diagnosis and in starting timely treatment for life-threatening disorders. Surgical interventions, including infectious source control, can also be delayed due to inability to accommodate large body habitus on operating room tables, or more commonly because of limitations rendered by body habitus in blunting the reliability of physical findings, such as with the abdominal exam. In our experience, morbidly obese patients with peritonitis routinely do *not* demonstrate classic peritoneal signs, which are masked by a ponderous and redundant abdominal wall. Appropriate surgical exploration consequently can be delayed in the morbidly obese patient who demonstrates ambivalent physical findings, and whose pathology cannot be further elucidated with imaging studies.

Morbidly obese patients often suffer from obesity hypoventilation syndrome or from obstructive sleep apnea [12]. The former, typically found in the super obese population (BMI $\geq$50 kg/m^2), is a condition that results in hypercapneic respiratory failure caused by a diminished response to CO_2 and hypoxia. The excess of adipose tissue can lead to narrowing of the upper airway, leading to obstructive sleep apnea. When this constriction occurs, there is an increased work of breathing, which can militate against successful extubation in these high-risk patients. For these pulmonary conditions, there is a greater risk of respiratory failure and long-term dependence on mechanical ventilation.

Pharmacokinetic dosing in obese patients can also be inaccurate, leading to inappropriate medical treatment. For example, dosing lipophilic medications like benzodiazepines and propofol, commonly used sedatives in the ICU, requires administering appropriate amounts of drug, while avoiding overdosage that can arise because these compounds will accrue in fat stores [13]. Appropriate sedation in morbidly obese patients can be difficult to achieve.

Sepsis is a frequent cause of death in critically ill patients, underscoring the importance of proper antibiotic dosing. Antibiotics are commonly dosed according to creatinine clearance, which is thought to be an especially unreliable calculation in the morbidly obese [14]. Antibiotics are frequently underdosed in obese patients, in part because of a significant increase in the volume of distribution associated with this casetype [15]. These roadblocks when compounded can impede the quali-

ty of drug delivery, which can inadvertently lead to undertreatment of serious infections in obese patients.

Care of the critically ill obese patient can be particularly rife with challenges while addressing the special needs of this patient population. Protocolizing care, early assessment of potential difficulties in treatment, and acquiring the proper equipment to care for these patients can help patient management and, hopefully, lead to better outcomes.

Obesity as a Risk Factor for Death in Adult Intensive Care

Until recently, the influence of morbid obesity on mortality rates in the critically ill population was not known. Early investigations failed to demonstrate an effect of morbid obesity on outcome. The first investigation to study morbid obesity as an influence on ICU patients compared extremely obese (BMI >40 kg/m^2) patients with non-obese (BMI <30 kg/m^2) patients in a medical ICU [16]. Investigators retrospectively selected 113 extremely obese medical ICU admissions and matched them with a cohort of 132 non-obese patients. Unfortunately, the extremely obese group had an increased severity of illness; suboptimal cohort matching by itself may have accounted for the increase in mortality and length of stay (LOS) observed in the extremely obese cohort [16], mitigating against drawing any clear conclusions from this analysis. Tremblay and Bandi studied the relationship between BMI and outcome in 41,011 critically ill patients using the Project Impact ICU database that was developed by the Society of Critical Care Medicine [17]. High BMI was not associated with an increased risk of death; however, the median ICU LOS in this study was very short, ≤ 2.0 days. The relatively short stay for this large cohort of patients may not have been sufficient to observe the emergence of complications from the natural difficulties in caring for the morbidly obese that would arise during a more prolonged ICU stay.

A larger study of medical ICU admissions ($n=2148$) analyzed outcomes across the complete spectrum of BMI, with categories ranging from underweight to morbid obesity [18]. There were no differences observed in severity of illness, mortality, length of mechanical ventilation, ICU LOS or ICU related complications among groups stratified by BMI. The investigators concluded that BMI has little effect on overall ICU outcome. However, it is noteworthy that the mean ICU LOS in these patients was less than 5 days, including that of the morbidly obese. Again, there is the possibility that these patients did not experience a sufficient duration of hospitalization during which the burdens of caring for patients with extreme obesity might become more evident.

More recent data have better characterized the relationship between morbid obesity and outcome in the ICU. Our group examined the effects of obesity in one surgical ICU over a four-year period [19]. Data were collected examining 1471 critically ill patients admitted to various surgical services, including general surgery, vascular surgery, transplantation, and cardiothoracic surgery. Height and weight measurements were recorded and assigned a simplified acute physiology score-II (SAPS-II) and a mortality prediction model (MPM-24) score to evaluate the severity of illness in patients. The patients were further stratified into four cohorts in which 6.8% were extremely obese, 19.8% obese, and 29.7% were overweight; the remaining patients were of normal size. A multivariate analysis was carried out to control for differences in age, gender, and severity of illness among those patients

in the subset of prolonged ICU stay. Importantly, morbid obesity independently increased the risk of death 7-fold when compared with non-morbidly obese, critically ill surgical patients. Increased mortality was directly related to increasing BMI after controlling for other confounding variables.

The only other study to conclude that obesity is an independent risk factor for death came from a matched cohort study from an adult medical-surgical ICU in France [20]. The patients in this study were sicker than the population examined in the Boston data and were matched according to eight criteria including cause of illness, cardiac status, probability of death, gender and acquisition of severe events appearing within 24 hours before admission. Bercault et al. [20] presented data on 170 patients requiring mechanical ventilation for >48 hours and compared the obese with non-obese patients. ICU mortality was higher in obese patients than in non-obese patients (32% v. 17%, $P<0.01$). They concluded that obesity was strongly associated with mortality in critically ill patients with respiratory failure, doubling the odds of death (OR, 2.1; 95% confidence interval, 1.6–3.6). Obesity-related mortality was found in the youngest patients and in patients with a high probability of ICU death. The researchers attributed increased mortality to higher obesity-related complications such as nosocomial infections.

More research is needed to reinforce the link between obesity and mortality in the critically ill. Preliminary data show that obesity may be an independent risk factor for death, due to an increased risk for infection and due to complications that arise from the burdens of care imposed by obesity itself.

Obesity Leads to a Higher Rate of ICU Complications

In addition to increasing the risk of death in hospitalized patients, morbid obesity has also been implicated in contributing to a myriad of comorbid conditions and ICU complications. Extreme obesity has been associated with left ventricular hypertrophy and resultant systolic and diastolic dysfunction [21–23]. These cardiopulmonary derangements lead to sudden death, which is increased 40-fold [23]. Morbid obesity increases the risk of type II diabetes and serious cardiovascular diseases, including coronary artery disease. In fact, studies have shown the reversal of diabetes, hyperlipidemia, cholelithiasis, and other diseases following weight loss after gastric bypass surgery [24].

Hyperglycemia in the critically ill has been found to nearly double ICU mortality. The mechanism of this observed mortality is attributed to increased susceptibility to sepsis and ultimately, multiple organ failure. Obesity leads to greater insulin resistance and hyperglycemia in the critically ill [25]. Obesity also has been shown to lead to respiratory disorders and complications such as increased risk of pulmonary embolism and severe sleep apnea. Nearly one third of extreme obese patients have severe sleep apnea, leading to other cardiopulmonary disorders including cardiac rhythm disturbances. Aspiration pneumonia, more common in obese patients, is attributed to larger gastric content and acid secretion compounded with a higher incidence of gastroesophageal reflux disease [26].

El-Solh et al. [16] retrospectively reviewed 117 morbidly obese patients (BMI >40 kg/m^2) admitted to the medical ICU in two hospitals. Hospital LOS was significantly longer for morbidly obese patients compared to the non-obese group (17.7 vs. 11.3 days, $P=0.007$). Morbidly obese patients had a longer ICU LOS (9.3 vs. 5.8 days, $p=0.007$) and a longer duration of mechanical ventilation (7.7 vs. 4.6 days,

$p = 0.0004$). Furthermore, weaning the morbidly obese patient from mechanical ventilation required more time when compared to the non-obese cohort. After adjusting for APACHE II scores, gender, and comorbidities, the researchers found that BMI was a significant determinant in accounting for LOS and the need for prolonged ventilator assistance.

In another study looking at overall outcomes between obese (BMI = 30–40 kg/m^2) and morbidly obese patients (BMI > 40 kg/m^2) in an ICU, one group also studied the effects on mechanical ventilation, pneumonia, line infections, need for tracheostomy, and acute renal failure [27]. They retrospectively analyzed 63 patients diagnosed with obesity and subdivided the patients between obese and morbidly obese arms. The morbidly obese patients had a higher mortality rate (23.3 vs. 6.1%, $p = 0.016$), higher ICU complications including sepsis (26.6% vs. 6.1%, $p = 0.028$), nosocomial pneumonia (33.3% vs. 3%, $p = 0.002$), acute respiratory distress syndrome (ARDS) (33.3% vs. 3%, $p = 0.002$), and line infection (23.3 vs. 3.0%, $p = 0.019$). In addition, need for tracheostomy, and acute renal failure were also observed more frequently in the morbidly obese cohort.

Morbid Obesity in Cardiac Surgery

Obesity is seen as a risk factor for developing coronary artery disease, but it is not widely known whether obesity leads to adverse outcomes following cardiac surgery. Extremes in body weight are relative contraindications in cardiac transplant surgery. Obese patients suffer from a larger percentage of wound infections, including sternal dehiscence after cardiac surgery, but little is known regarding mortality [28]. Several studies have examined obesity as a predictor for mortality, morbidity and a cause for readmission after coronary artery bypass grafting (CABG) or valve replacement. Rockx et al. looked at 1310 patients undergoing cardiac surgery and prospectively examined the relationship between high BMI and mortality [28]. Only 10 postoperative complications, including cardiac arrhythmias and pulmonary complications, were attributed to obesity; they concluded that an increased BMI did not lead to a higher incidence of postoperative death. Another study looked only at BMI related to outcomes from CABG surgery [29]. In this paper, the researchers enrolled 4372 patients of when 3.0% were underweight, 49.7% were overweight, 17.1% were obese, and 3.6% extremely obese. Compared with the normal weight bracket, the obese and extremely obese patients had a higher percentage of being female, with hypertension and diabetes, but fewer of these patients had serious ischemic heart disease and poor ventricular function. Of the different groups, the underweight group fared the worst having a higher incidence of renal failure and greater length of stay. Overweight, obese and severely obese patients sustained no difference in adverse outcome compared to non-obese patients and were found to have less need for transfusions. They concluded that obesity was not a predictor of mortality following cardiac surgery.

Survival outcome has also been studied in obese patients undergoing implantation of left ventricular assist devices (LVAD). One study showed that higher BMI did not adversely affect outcome after LVAD implantation [30]. The researchers concluded that obesity should not be a contraindication to LVAD placement.

Table 1. Summary: Obesity as a predictor of death and complications in adult ICU

	Design	Results	Conclusions
El-Solh AA (2001) [16]	Retrospective; n = 117 Medical ICU	Overall mortality for morbidly obese patients was 30% and for non-obese patients was 17% ($p = 0.019$). Groups differed in severity of illness, casting doubt on findings	Critically ill morbidly obese patients are at increased risk of morbidity and mortality compared to the nonobese patients
Tremblay A (2003) [17]	Retrospective; n = 41,011 Mixed medical – surgical from national ICU database	Increased mortality in underweight patients. ICU and hospital LOS increased in both severely obese and underweight groups (OR 0.81, 0.83, respectively)	Low BMI, not high BMI, is associated with increased mortality and worsened hospital discharge functional status
Bercault N (2004) [20]	Prospective; n = 340 Primarily medical ICU (89%)	Obesity associated with ICU mortality (OR, 2.1; C.I. 1.2–3.6). Obesity-related excess mortality in youngest patients (OR 2.5, CI 1.6–6.1)	Obesity is an independent risk factor for ICU death and should be regarded as a severe comorbidity in such units
Ray DE (2005) [18]	Prospective; n = 2148 Medical ICU	Patients stratified based on BMI; no difference in APACHE II score, mortality, ICU LOS, hospital LOS among groups	BMI has minimal effects on ICU outcome
Yaegashi M (2005) [27]	Retrospective; n = 63 Medical ICU	Morbidly obese patients sustained increased risk of death (>7x); increased rates of pneumonia, acute renal failure, catheter related infection. Prolonged ICU LOS and duration of mechanical ventilation	Morbid obesity associated with increased mortality and morbidity in Medical ICU population
Nasraway SA (2006) [19]	Retrospective; n = 406 Surgical ICU, prolonged stay	ICU and hospital mortality were significantly increased in extreme obese patients compared to all other patients (33.3% vs. 12.3%, $p = 0.009$); odds of death increased 7.4×	Morbid obesity is an independent risk factor for death in prolonged stay surgical patients

LOS: length of stay; BMI: body mass index

Morbid Obesity in Trauma and Transplant Surgeries

Obese patients who sustain trauma have a risk of death that may be as large as 8-fold greater than that of non-obese patients [31]. One study at an academic Level I trauma center observed outcomes between obese and non-obese patients who were critically injured from blunt trauma. The two cohorts had similar demographics and injury patterns, but the obese patients fared worse than nonobese patients. Of the 242 patients studied, the morbidly obese patients had a higher mortality (32% vs. 16%, $p = 0.008$) than the non-obese group, showing that obesity was an independent predictor of death after blunt trauma [32].

Increased BMI is apparently also a risk factor in transplant patients. Obese patients undergoing orthotopic liver transplantation in one study sustained a greater incidence of wound breakdown; obesity did not affect other common transplant complications, such as graft failure or acute rejection [33]. Sawyer et al. found that morbidly obese patients undergoing liver transplantation are more likely to need transfusion, develop wound infections and sustain early death from multiple organ failure when compared to non-obese patients [34]. Overall, it is accepted that obesity is not a contraindication for organ transplantation, but may lead to unusual sequelae and additional complications in comparison with transplantation in non-obese patients.

Conclusion

Obesity is a growing worldwide epidemic and is observed with increasing prevalence in the critically ill. Diagnosing obesity and calculating BMI is a relatively simple task when first assessing patients who are admitted to the ICU. Recent studies have shown that increased BMI is an independent determinant of death in the extended critical care setting (Table 1). Morbid obesity increases complications, most especially infection, and prolongs length of stay in selected subgroups of critically ill patients (Table 1). Standardizing care and protocols, and developing an appropriate equipment and resource infrastructure specifically geared for these special patients should improve patient outcome and ease the burdens imposed on care providers.

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Patient Safety Management System in Pediatric ICUs

C. van der Starre, Y. van der Tuijn, and D. Tibboel

Background: Epidemiology of 'Errors' in Health Care

Patient safety has become an increasingly important topic of discussion in health care organizations all over the world. The publication of the report *To err is human* by the Institute of Medicine in the United States [1] triggered awareness of the importance of safer practices in health care, among both health care professionals and the public. In 1990, Rubins and Moskowitz [2] reported that 14% of studied patients experienced complications during their ICU stay; the hospital mortality rate in these patients increased to 67% (versus 27% in patients without complications, $p < 0.0001$). Giraud et al. [3] observed iatrogenic complications in 31% of adults admitted to the intensive care unit (ICU), and the patients with a major complication had a twofold increase in mortality risk. Stambouly et al. [4] reported on a prospective study on complications in a pediatric ICU (PICU); complications occurred in 8% of the admissions. The authors observed that any complication increased PICU stay from 2 to 12 days ($p < 0.0001$). They also found an increased mortality risk for patients experiencing any complication (0.010 to 0.022). Another study on the impact of adverse events in the PICU [5] reported that infectious complications gave rise to approximately a $50,000 increase in total costs and an increased length of stay of 15.6 days. In another context, Zhan and Miller [6] reported on the effect of medical injuries during hospitalization using the Agency for Healthcare Research and Quality (AHRQ) Patient Safety Indicators (PSIs). The impact of these injuries was highly variable; postoperative sepsis increased length of stay to 11 days, total costs by $57,727 and mortality to 22% ($p < 0.001$).

In 2001, the Institute of Medicine released the statement "the biggest challenge to moving toward a safer health system is changing the culture from one of blaming individuals for errors to one in which errors are treated not as personal failures, but as opportunities to improve the system and prevent harm" [7]. To provide safer patient care, we have to realize that health care is a high-risk 'industry' that is inherently hazardous. Therefore, health care providers have to step away from the 'blame and shame' culture that prevents acknowledgement of errors and (near-) incidents and thus prevents learning from those errors and (near-) incidents. A profound culture change is necessary to create a culture of safety among health care workers. Cultural changes are also necessary to successfully implement innovations that are designed to improve patient safety. A patient safety management system will only be successful when accompanied by these changes in culture and attitude [8]. For example, adverse event reporting systems can only work in a blame free environment; in a culture where errors are not acknowledged or are punished, underreporting will remain a serious problem. In 2004, Shell Netherlands presented

a report entitled "Here you work safely or you don't work here at all" to the Dutch government and Ministry of Health [9]. This survey of patient safety practices in Dutch healthcare showed that in Dutch hospitals 1500 to 6000 people are estimated to die every year because of preventable incidents. The authors claim that a reduction of 75% is possible within 15 years. The Dutch Pediatric Association in collaboration with the Dutch Order of Medical Specialists therefore decided to start and/or support projects and initiatives on patient safety. Apart from local initiatives this has so far resulted in two nation-wide projects, a complication registry for pediatric practices and the so-called Neosafe® project for blame free incident reporting in neonatal ICUs. Besides the professional interest in safer health care, raised public awareness made the Ministry of Health order each hospital to have a validated patient safety management system up and running by January 2008.

Our Achievements: Safety First Project

The setting for one of the local initiatives is the surgical PICU of the Erasmus MC Sophia Children's Hospital in Rotterdam, the Netherlands. A level 3 multidisciplinary PICU, it is one of eight academic PICUs in the Netherlands and has 550 admissions per year. Children with the following diagnoses are admitted to the surgical PICU: major congenital malformations, traumatic brain injury/major trauma patients, brain tumor surgery, scoliosis surgery, and renal transplants. In addition, the unit has a supraregional function for craniofacial surgery and extracorporal membrane oxygenation (ECMO).

In 2003, coinciding with the decentralization of incident reporting in the University Hospital Erasmus MC, we started a project called Safety First. Incident reporting was now going to be dealt with on the unit itself. The Safety First project encompasses four elements: Complication Registry, Safety First reports (blame-free reporting of potential hazards/ (near-) incidents), Critical Nursing Situation Index (CNSI), and Crew Resource Management (CRM). The Complication Registry started as a pilot study on the frequency of complications occurring in patients admitted to general pediatric wards, wards in referral hospitals in academic centers, and neonatal/pediatric ICUs. We aim at clarifying not only the numbers but also the nature of these complications, which will enable further analysis of possibly preventable causes and development of preventive measures. The blame free reporting of all accidents, (near-) misses, errors and faults, whether the incident affects the patient or not, will generate more data on preventable causes of adverse events than looking at the actual adverse events alone. For that reason we introduced so-called Safety First reports in May 2004. All unit staff members are invited to report anything that did not proceed as it should have or that went wrong. For instance, medication errors, deviant ventilator settings, but also lack of communication, etc., can be reported. The CNSI is a validated method for detecting flaws and errors of (nursing) care in ICUs [10]. The aim of implementing the CNSI is twofold: scrutinizing and updating nursing protocols and analyzing whether (non-)adherence to nursing protocols has any impact on incidents/adverse events. CRM is a training method, adapted from the world of aviation, aimed at improving communication and cooperation between teamworkers. The parallels between aviation and health care (especially emergency and intensive care) warrant implementation of CRM training for healthcare personnel. Integrating these four elements (complication registry, safety

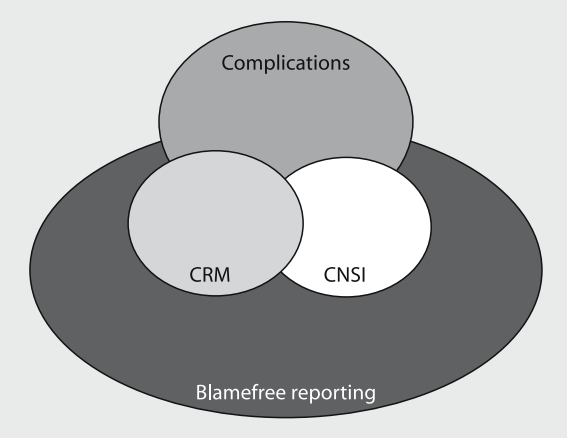

Fig. 1. A model for a patient safety management system. CRM: crew resource management; CNSI: critical nursing situation index

first reports, CNSI, and CRM) has created a model for a patient safety management system which will reveal flaws and provide for the development of preventive strategies. Its final aim of course is improving quality of care (Fig. 1).

Data Analysis

In our hospital, a longstanding interest in quality of care has not only resulted in studies on preventable mortality and iatrogenic complications [11], but also on cost effectiveness [12, 13]. Another factor contributing to the demand for safer health care practices is the fact that a small proportion of admissions on the ICU accounts for a considerable part of the admission days. To further clarify this observation, we evaluated length of stay over a three-year period, with a special focus on episodes of prolonged length of stay, which we arbitrarily defined as three times the mean length of stay. The total number of admissions from January 2000 till January 2003 was 1475, amounting to 12,431 admission days. Mean length of stay, therefore, was 8.4 days. Sixty-seven patients, i.e., 4.5% of all admitted patients, appeared to have been admitted for more than 24 days. These 67 patients had been admitted for 5299 days, 42% of the total admission days! Causes for prolonged stay can be either non-preventable, i.e., a consequence of the underlying disease, or preventable causes. Preventable causes occur due to errors in management, operative/non-operative incidents, procedural errors, and nursing-related adverse events.

Again, 4.5% of the patients took up 42% of the total admission days, and in half of these patients the prolonged length of stay was due to preventable causes. These numbers correspond well to those reported by Marcin et al. [14] who found that long stay patients represented 4.7% of the population in the PICU, representing 36% of the days of care.

A child's prolonged stay leads not only to considerable morbidity and stress on the child and parents, but also to higher costs; 2.3% of the patients account for 24.3% of the costs, that is 4,085,100 in a 3-year period. There are few reports on the costs of adverse events in PICUs. Dominguez et al. [5] reported an increase in

costs due to infectious complications of $50,000 per patient, and Miller et al. [15] reported 2- to 20-fold higher median total charges in case of pediatric hospitalizations during which patient safety events occurred.

Complication Registry

A complication is defined as an unintended and unwanted event or situation during or following medical specialists acts, which are so disadvantageous for the health of the patient that adjustment of medical therapy is necessary or the patient suffers irreparable damage. We undertook this study to improve quality of care by means of using complications as subjects for discussions and education, and by developing and improving protocols. During a 3-month period, the medical and nursing staff reported on all possible complications, which then in turn were evaluated by a study group of the Dutch Pediatric Association. Not only numbers and nature of complications were registered, also the best way to register (on paper, using a pocket portable computer, using a patient data management system) was evaluated. The minimum dataset was also determined, i.e., what patient data, treatment data, context, etc. is needed for an adequate complication registry. Over 3 months we recorded 122 complications (8.1 complications per 100 admission days). This is a higher rate of complications than that reported by Stambouly et al., 2.7 complications per 100 admission days [4]. This difference may be attributed to the fact that our unit predominantly admits surgical patients, whereas Stambouly reported on a general PICU, with only 20% surgical admissions. Case selection might be a significant confounder in this respect.

Safety First Reports

A new reporting system was created to gain more insight into the prevalence of (near-) incidents and errors. As reported, for example, by Taylor et al. [16], underreporting of errors is a serious problem in healthcare. Nursing staff members are considerably more likely to report medication errors compared to the medical staff, but still nurses do not report everything that should be reported. A blame free environment for health care workers is a necessity for a successful voluntary incident reporting system. In addition, a shift needs to be made from the person approach to the system approach to errors [17]. The focus of attention needs to be the fact that the mistake was made and not the person who made the mistake.

The 'classical' reporting system in many hospitals in the Netherlands was a voluntary reporting system of faults or near-accidents (FONA). Within this system, only 50–60 reports per year were released in our department. We created a new form, based on the forms used in The Hospital for Sick Children, Toronto and by different Dutch neonatal ICUs incorporated in the so-called Neosafe® project. We instituted a committee for the assessment of all the reports, the Safety First Committee. The new system was then introduced to all unit staff and came into use in May 2004. Because underreporting had been partly caused by lack of a definition of what should be reported [16], staff were asked to report "everything that did not go as it should have gone". The result was a staggering amount of reports. The first year yielded 1784 reports, and each month 100–150 forms are still written, a 30-fold increase compared to the FONA reports. More than one third of the reports

Table 1. Safety First reports from May 2004 to May 2005

Type of incident	N	%
Medication errors	608	35
Respiratory equipment	309	17
PDMS	242	13
Catheters/tubes	181	10
Environmental	168	10
Enteral nutrition	105	6
Laboratory	61	3
Equipment	23	1
Skin integrity	24	1
X-ray	1	1
Other	48	3
Total	1784	100

* PDMS = patient data management system

concern medication errors, for instance dosage errors, wrong infusion speed and omission errors (medication either not prescribed or not given). Ventilator-related incidents are reported in approximately 20% of cases, varying from accidental extubation to wrong ventilator settings to power failure of the ventilator, etc. (see Table 1). Apart from the nature and number of incidents we also studied the consequences for the patients. To this end we used an industry-based risk assessment matrix. The person who reports an incident is asked to rate the anticipated consequences as either none, minor, major, serious, or unknown. A minor consequence is defined as minimal discomfort, without lesion, longer stay, or intervention needed. A major consequence is discomfort or a temporary lesion, with minimally longer stay or minimal intervention. Serious consequences are serious discomfort, lasting damage, severe prolonged stay, major intervention (i.e., resuscitation, surgery) or death. The Safety First Committee assesses the actual consequences within one week after the report. We found that very few incidents actually resulted in major or serious consequences (Table 2).

A possible reason for underreporting in our unit under the FONA-system, but also in other hospitals [5], was the lack of feedback to the 'reporters'. The sheer number of reports makes it impossible to give personal feedback in our project. So we decided to publish a monthly *Safety First Journal* including the top 5 most frequent incidents and the results of the analysis of major incidents. In addition, new preventive strategies are announced or results of the implementation of these strategies are made public. We believe this has helped in keeping the staff motivated for reporting.

More knowledge on how and why incidents occur can be obtained by means of a detailed analysis of the reports. Without such an analysis it is impossible to develop preventive strategies for recurrent (near-) incidents and errors [18]. In fact, the ultimate aim is not the observation of errors but to change the attitude of all involved in patient care towards pro-active registration and prevention. A major concern with the different techniques for analyzing incidents is the lack of evaluation and evidence. In the nationwide project Neosafe®, which promotes blame-free

Table 2. Comparison of anticipated and actual consequences of Safety First reports

		Actual consequences					
		None	Minor	Major	Serious	Unknowm*	Total
Anticipated consequences	None	168	2	0	0	1	171
	Minor	461	51	3	1	17	533
	Major	375	61	41	3	28	508
	Serious	249	48	45	20	13	375
	Unknown*	148	14	8	1	19	190
	Total	1401	176	97	25	78	1777

* unknown = missing data or not yet known at time of assessment of report

incident reporting on neonatal ICUs, the PRISMA analysis is now being validated. This Prevention and Recovery Information System for Monitoring and Analysis was developed by Van der Schaaf, associate professor at the Safety Management Group of the Technical University, Eindhoven in the Netherlands [19]. This system is adapted from incident analysis systems used in aviation and the petrochemical industry. The end result of an analysis using the PRISMA methodology is a number of root causes, categorized into human, technology, organization, and patient related factors. As a rule each incident has root causes in at least two different categories. The PRISMA analysis system is now being applied to the incident reports concerning three major patient safety indicators: medication, mechanical ventilation, and arterial/venous catheters. Insight into the root causes of these incidents will enable us to develop preventive strategies, to reduce the frequency of adverse events and finally to perfect quality of care.

Critical Nursing Situation Index

A Critical Nursing Situation is any observable situation which deviates from good clinical practice and which may potentially lead to an adverse event. The CNSI is a list of items to be checked at the bedside of patients. The aim of the CNSI is to detect flaws in safety and quality of care in the ICU. The CNSI was originally developed and validated for an adult ICU by Binnekade et al. [10]. For each CNSI item, a protocol was selected to be evaluated. Protocols were obtained from the Protocol net (the hospital wide computerized network), or were unit-specific or derived from other disciplines (e.g., the hospital pharmacy). Each protocol was then checked for being up-to-date, evidence based, and/or according to 'good clinical practice'. When found inadequate, a protocol was revised by two experienced PICU nurses and approved by one of the pediatric intensivists. The result was an adapted list of 192 items to be scored for the CNSI. Ten enthusiastic PICU nurses volunteered to test the new $CNSI_{picu}$ for interrater-reliability and usefulness on the unit. Paired scoring of 30 CNSIs showed a good interrater-reliability: Cohen's kappa 0.76. This pilot study made it clear that many nurses were still afraid to be "caught mak-

ing mistakes" when patients they were taking care of were scored. Pilot scoring led to much debate among the scoring nurses about the "right way to do things" and "what is good clinical practice". In other words, they had to revise some of the protocols again to make sure the protocols are clear and unquestionable. Now a second pilot study on CNSI is underway. Every day, one randomly assigned patient is scored by one of the CNSI trained nurses. A monthly report on the most frequently occurring CNSI items is published and appropriate action undertaken, for instance education, protocol revision, etc. The $CNSI_{picu}$ consists of two parts: a generic part and an ICU-specific part. The items on the ICU-specific part are determined by the patient mix admitted to the particular ICU and each ICU needs to create its own list of specific items for the CNSI.

Crew Resource Management

Crew Resource Management is a training method that has evolved in aviation over recent decades. Aviation has a longstanding history of collecting and analyzing safety data. Over a 7-year period, Billings and Reynard analyzed 35,000 reports and found that nearly half resulted from flight crew errors [20]. An additional 35% was attributed to errors made by air traffic controllers. Root cause analysis of these incidents revealed that insufficient communication and cooperation between teamworkers contributed largely to the errors. CRM training programs are now widely used to improve the safety and operation of flight crews. Aside from attitudinal surveys and peer performance rating questionnaires, no other tools are available for determining the effectiveness of CRM [21]. A number of studies found that overall crew performance increased after implementation of CRM [22, 23]. We believe there are considerable similarities between aviation safety and health care safety. The similarities are: a significant knowledge base, rapidly changing information patterns, complex systems, high risk environment, and team dependent activities. The big difference in case of 'errors' is the high profile media coverage in aviation versus the secret healthcare society based on patient confidentiality and error non-disclosure.

In the same context, Sexton and colleagues compared 1033 operating room personnel and more than 30,000 flight crew members on several items, mainly dealing with attitude towards teamwork, attitude about error and safety, and perceptions of stress and fatigue [24]. Medical respondents were more likely to agree to the item "even when fatigued, I perform effectively during critical times ", compared to flight crews (60 versus 26%). There was also a remarkable difference in preference for flat hierarchies. While 55% of the consultant surgeons advocated flat hierarchies, as many as 94% of cockpit crews indicated this to be their preferred model. This study and other analyses suggest that safety-related training as used in aviation may also be useful in health care. In a number of health care settings, CRM applications have been or are being incorporated, e.g., the operating room, the emergency department, and labor and delivery units. The medical application of the CRM methods required adjustment of the training approaches to better cover the areas in which human factors contribute to errors in health care. Our project aims to assess the applicability of CRM for (pediatric) ICUs. Together with the Center for Man in Aviation of the Dutch Royal Airforce, a tailored CRM training course was developed for health care workers. All medical and nursing staff are to participate in the training course, which includes:

- assessment of information
- human errors
- effects of stress on acting and perceiving
- communication between members of the team
- group dynamics, effects of working in a team
- leadership and team structure, responsibilities and authorities
- decision making
- risk management

Note that the effects and effectiveness of CRM training are hard to assess. Proving that CRM training helps to reduce medical errors or adverse events is very challenging. Grogan et al. found that the CRM training of 489 health care professionals appeared to have a positive impact on attitudes toward leadership, coordination, and communication in teams in particular [25]. So far there are no published data about the effect of CRM training on medical errors or patient safety in general. Although CRM has been adopted in the aviation industry without objective data as to its effectiveness, we feel it has a high potential in health care.

Evaluation

For a working safety management system, medical and nursing staff, but also, e.g., hospital administrators and managers, need to work together to create a safe environment in which reporting and discussing any incident is a routine procedure. Creating this safe and blame free working environment is the first step towards safer patient care. The next step constitutes eliciting the potential hazards, the adverse events and the outcomes related to the adverse events. Then, analysis of adverse events and (near-) incidents must lead to the development of interventions to prevent errors/incidents and to mitigate the effect of errors/incidents that do occur.

The patient safety management system that has been developed in our unit is a growing and evolving system. There are still controversies about the effects and effectiveness of any strategy to improve patient safety. In the relatively new field of science of safety in health care a number of basic issues are still controversial [26]. What is patient safety? Does safety comprise any adverse event of medical treatment, or is safety dealing with adverse events caused by errors that are preventable with specific safety strategies? The Institute of Medicine defines medical error as "the failure of planned action to be completed as intended or the use of a wrong plan to achieve an aim" [1]. But what about errors of omission (not doing something that should be done)? Another problem is the widespread use of different definitions and terms for errors and near misses, etc. Collecting evidence to support some of the safety practices was very difficult. Should we only use evidence-based practices or should we also use as yet unproven strategies, such as CRM training?

We believe that collecting and studying reports on "everything that did not go as it should have gone" will reveal all potential hazards and provide baseline data for individual units. The result will be the development of preventive strategies and in due time we will be able to prove a reduction in adverse events and complications by goal directed and predetermined interventions. So far our experience is consistent with nursing and medical staff being very motivated to contribute to improving quality of care, and the Safety First project has been well received by all

co-workers on the unit. Furthermore, the involvement of other disciplines has proved to be very useful. For instance, good cooperation with the hospital pharmacy and medical technology and radiology departments helped reduce the occurrence of incidents. It has also become clear that creating a blame-free environment is difficult and takes a long time.

Conclusion

The multidisciplinary approach of the project is improving patient safety by continuous quality assessment and modification guided by monthly, real data. Cost effectiveness of patient safety management systems is an important issue, especially in relation to the increasing costs of health care, which should be considered as an integral part of such a system.

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Information Exchange in Intensive Care: How can we Improve?

B. Venkatesh, A. Miller, and A. Karnik

Introduction

Medical error is thought to contribute to a significant proportion of adverse events in hospitalized patients. An adverse event is an injury caused by medical management rather than the underlying condition of the patient [1]. Three sentinel studies which have examined adverse events in hospitalized patients include those of Leape et al., Bates et al. and Thomas et al. [2–4]. The data on adverse events rates and preventable adverse events are detailed in Table 1.

The consequences of adverse events range from prolonged hospitalization, death, loss of productivity and cost to the health care provider. Thomas et al. undertook an additional analysis of the data from the Quality in Australian Health Care Study (QAHCS) in order to understand more fully the causes of the adverse events (adverse events) identified and to assist in developing prevention strategies [4]. The top three causes included complication of, or failure in, the technical performance of a procedure, failure to synthesize and act on available information and failure to request or arrange an investigation. It was also felt that in 3.6% of cases, better transfer of information could have led to a significant reduction in the error rate.

Not surprisingly, reduction of errors remains an important focus of quality and safety improvement programs in medicine today. Whilst drug administration errors have been a focus of attention from 1960 [5] to the present day [6] only recently has attention turned to the adequacy of information exchange between health professions [7–9]. The purpose of this chapter is to examine the etiology of failure of information exchange in the ICU, and review strategies for improving the flow of information in day-to-day practice in the critical care setting between clinicians.

Table 1. Adverse event rates in hospitals

	Adverse event rate	Preventable adverse events
Leape et al. [2]	3.7%	69%
Bates et al. [3]	6.5%	28%
Thomas et al. [4]	10.6%	51%

Etiology of Information Transfer Errors in the Intensive Care Unit (ICU)

There is a paucity of good data on the incidence and prevalence of medical errors in an ICU environment. Cook et al. [10] maintain that gaps and discontinuities can result in adverse events as a consequence of information loss. Donchin et al. [11] published a sentinel study investigating the nature and causes of human error in the ICU, adopting a human factors approach. The study was conducted over a 4-month period and identified 1.7 errors per patient per day over a 4 month period. Only half of these were discovered and self reported. About a third of them were graded as potentially devastating if not discovered in time. These errors included prescription and medication errors, equipment failure and communication problems. The latter was thought to be the largest contributor to medical error rate in ICU but the nature or causes of communication errors were not clearly articulated. Although medical order charts were strongly implicated the etiology of information transfer errors required further description.

ICU patients have complex life-threatening medical conditions that resolve over days to weeks. Monitoring these patients may involve tracking up to 200 variables per patient per day. Available information must be processed and assimmilated even if much of it is not immediately relevant to treatment decisions. Organizing, prioritizing and separating important from less important information can be cognitively demanding especially for junior staff members and when staff members are fatigued.

ICU patients are monitored on a 24-hour basis by medical and nursing subteams. The intensive nature of monitoring necessitates shiftwork and with it the need to pass patient care responsibilities from one staff member to another during shift handovers. Information gaps and discontinuities may occur from physician to physician, from nurse to nurse, from physician to nurse and nurse to physician during the same set of handovers, e.g., the morning or evening shift handovers. In addition, for long stay patients with evolving pathologies information including relevant care plans made in the earlier stages may not be incorporated into ongoing care plans thereby facilitating further losses of information.

Other factors in the ICU environment which contribute to poor information flow include ambient noise and failure of basic principles of human-computer interaction design [12–14]. The major factors which preclude effective information transfer are summarized in Table 2.

Whilst the factors discussed above come into play throughout the patient's ICU stay, these become more critical during shift handovers or transfer under the care

Table 2. Factors precluding effective information transfer in the critical care setting

Prolonged admission
Complex life threatening admission
Information load
Rapidly changing clinical problems
The presence of a shift system (usually 2–3/day) which in turn necessitates medical and nursing handover 2–3 times a day
Ambient noise
Poor human-computer interaction design

of another physician during a weekend on call or transfer to another facility for specialized investigation or treatment.

Ineffective Information Transfer: Magnitude of the Problem

Petersen et al. [15] studied the relationship between house staff coverage schedules and the occurrence of preventable adverse events. They reviewed the adverse event rate in 3146 patients over a 4-month period; 127 adverse events were noted. On both univariate and multivariate analyses, cross coverage by a physician from another team was strongly associated with a higher incidence of adverse events. This study identified the role of cross coverage and, therefore, the potential for inadequate transfer of information as an important factor in the genesis of adverse events. Data from the study by Norgaard et al. [16] also seem to confirm those of Petersen et al. These authors used a checklist to assess trainee performance during handover of information and concluded that the more junior the trainee, the less effective was the information transfer. Lee et al. [17] introduced a computerized sign out card for doctors to hand over information and was able to demonstrate a reduction in the adverse events rate from 14.9% to 5.8%. Similar pattern of results emerge from nursing data [18].

Methods Used for Handover of Information

Published data from both the UK and Australia reveal that handover of patient care continues to be a problem, the process is not formalized and there is lack of advice and guidance on the process [19, 20]. Current methods of handover can be classified in a number of ways. It can be either by the bedside or within the confines of an office. Only one published study has compared the effectiveness of these methods of handover [21]. This study explored the effectiveness of bedside and office handover techniques amongst nurses in five acute care settings. While no one type of handover was shown to be more effective than the other, it identified the potential strengths and weaknesses of both types. Some of these are summarized in Table 3.

Handover can also be classified based on the method employed to facilitate handover of information. These include use of handwritten lists, computerized sign

Table 3. Comparison of bedside and office handovers

	Bedside	Office
Bedside visual cues	Utilized and may improve quality of handover	Not available
Interaction with bedside nursing staff	Possible	Possible
Process	Less structured, may be more formal	More structured, but potential for informality to creep in
Patient confidentiality	May be compromised	Preserved
Opportunity for staff debriefing	Minimal	Significant
Assessment of patients at handover and ability to check charts and medications	More likely	Less likely

out cards and personal digital assistants (PDAs). Verbal handovers are frequently employed by senior staff. Although published data are not available to testify to the greater effectiveness of one over the others, there appears to be a consensus that verbal handover is probably the least effective process [21, 22] with the potential for incomplete and inaccurate transfer of information.

Cultural and Technological Factors

Cultural factors may present barriers to the effective transfer of information within and between handovers. Medical and nursing staff each has their own training curricula, language and career pathways. Attitudes, values and behaviors shaped by these different influences may affect communication styles and relationships between team members. Leonard et al. [23], for example, found that medical and nursing staff use different communication styles during handovers. Nurses tend towards a more narrative style while doctors tend towards a more truncated, fact only style. Sexton et al. [24] also found that perceptions of hierarchical status influenced junior doctors' willingness to speak up during handovers, which might impede information transfer. The study found varying degrees of rejection of steep hierarchical status amongst different medical practitioners (94% amongst intensive care staff vs. 55% amongst surgeons). The study also surveyed airline pilots who traditionally retained a strong sense of hierarchy. Interestingly, following the introduction of techniques such as Crew Resource Management (CRM) used in the aviation industry, 97% of airline pilots who were surveyed also rejected steep hierarchical behaviors. Cultural factors may also influence clinicians' denial of the negative effects of fatigue on performance [24, 25].

Individual and team situations can be compromised when information is not represented in a way that it can be perceived. As noted, the tendency of some clinical information systems to bury information in multiple screens [12–14] is one way that information can be effectively lost. In this context, the effect of blended memories may become relevant. Separate information sources when activated closely together in time can lead to memory blends resulting in errors. This was illustrated in a study by Azuma et al. [26] when examining the effect of homophones. Homophones are words which sound similar but have different spellings – for example, PAWS and PAUSE. The study found that homophones could result in blended memories with the potential for confusion. When volunteers were presented with PAWS and CAUSE, PAWS was subsequently misspelled as PAUSE. An analogy in medical practice would be Gram-positive cocci and Gram-negative bacilli being frequently mis-associated as Gram-positive bacilli from an effect of blended memory.

Towards a Solution

Etiologically, the adverse effects of information loss during handovers arise from a loss of situation awareness of the patient and of what medical and nursing teams and subteams are doing. Situation awareness is a person's ability to perceive important information, to comprehend its meaning, and to project the status of information into the future [27]. Team or shared or common situation awareness is the awareness a team has about what the situation they are dealing with is, what should be done about it, when and by whom.

Handover processes that focus on ensuring that team members do in fact have a common understanding of the patient and the team situation may be more important than the simple transfer of information. Removing cultural barriers that may prevent staff from calibrating their awareness of the situation against the awareness of others may also be important. Other processes which might improve information transfer include the use of structured handovers, avoiding informal verbal handovers and allowing longer duration of overlap between the 'handing-over' and 'taking-over' teams.

Evidence that behavioral and cultural modifications improve outcome has been demonstrated in the aviation industry. An investigation into air crashes determined that almost 50% of air crashes result from human error. This led to the development of a program termed CRM, which typically includes education of crews about the limitations of human performance, role of cognitive errors, and how stressors (such as fatigue, emergencies, and work overload) contribute to the occurrence of errors. Although the effectiveness of CRM has not been demonstrated in terms of a reduction in accidents or near misses, improved attitudes, better staff coordination and improved teamwork have been shown in simulated mishaps [28, 29]. Courses integrating CRM principles into medical training in a variety of disciplines (anesthesia, emergency medicine and labor room) have been conducted and data from these published [30, 31]. There is uniform agreement amongst course participants that CRM principles relevant to medicine are inadequately taught during the training program.

All of the above potential solutions make intuitive sense and appear to have face validity. These have now become an integral part of training of staff the aviation industry. However in medicine, the awareness of such programs is just being raised. The implementation of these programs has a significant cost implication and given the current climate of increasing financial constraints in the health care budget, ultimately the acceptance of these recommendations will depend on the clear demonstration of their usefulness in appropriately conducted clinical trials.

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Catastrophic Anachronisms: The Past, Present and Future of Disaster Medicine

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Introduction

Disasters, particularly earthquakes, volcanoes, floods, war-related complications, famine and infectious epidemics, have been a part of recorded human experience. From Pompeii to the Johnston Flood and World War II and the Black Plague to the Spanish Influenza, there have been catastrophic occurrences that will not long be forgotten by either legend or history books. Nevertheless, those occurrences were relatively few and far-between before the mid-twentieth century. Indeed, the nature of disasters has changed since then. From terrorists taking advantage of 'new technology' to weather-related events that cause trillions of dollars worth of damages and economic loss, the world has evolved.

In the last 50 to 60 years alone, the risk and frequency of events with multiple injuries and deaths has increased dramatically [1–4] and it is bound to increase logarithmically over the next half century. Not only is the earth more heavily-populated with human beings settling across many more regions of the planet, but there are also larger concentrations of human inhabitants dependent on other resources for sustenance. Dependency on others for complicated food supply chains, refrigeration, fuel and power sources and public health hygiene (sewage handling), makes human populations more vulnerable than ever before and less self-sufficient. In addition, these large, vulnerable population centers are often concentrated in high-risk locales such as metropolitan cities where very frequent and multiple person-to-person contacts occur. Often they are along disaster prone areas, be they in coastline or sub-sea level reclaimed lands.

In addition, the world now faces a broadened spectrum of disasters, ranging from terrorist bombings, unconventional wars, nuclear releases, transportational mishaps, worldwide infectious disease epidemics and chemical discharges (technology-generated) to floods, famine, earthquakes, tornadoes, cyclones, fires, and other 'natural disasters' [2, 4]. It may also be that human technology has increased the likelihood of more severe and more frequent natural disasters. Whether it is global warming (from industry and omnipresent internal combustion machines) leading to stronger and more frequent hurricanes or the jumbo jet that rapidly translocates deadly influenza viruses around the planet, the threat continues to evolve far beyond the world of our grandparents.

Therefore, although famine, war, plagues, earthquakes and such threats have darkened the human experience, the on-going spiral of human populations, the rapid growth of technology, relatively easy world-wide travel for millions and the exponential expansion of at-risk industries and domiciles have now conspired to dramatically increase human exposure to catastrophic events. In turn, the potential for

casualties resulting from each incident is more likely to be much larger [2] and the magnitude of the economic costs beyond our current comprehension.

The recent undersea earthquake in the Indian Ocean that sent towering, 700 kilometer per hour tsunamis across Southern Asia resulted in a tremendous number of deaths and morbidity because of the sheer volume of exposures in vulnerable sites. The 1950s seaside fishing villages of 5,000 local inhabitants had become cosmopolitan, internationally-populated, destination resorts hosting hundreds of thousands supported by multi-national industries and technology. Such expanding population bases living and working in vulnerable situations along the at-risk seashores such as Phuket, Thailand, or in major metropolitan settlements built below sea level such as New Orleans, USA, provided unprecedented fodder for disaster, regardless of continent. Likewise, the recent earthquake in Pakistan that killed tens of thousands in a few minutes might not have had such an effect 50 years ago when such concentrated populations centers did not exist.

If that were not enough, beyond a higher risk for casualties and frequency of events, the events have now become global with far-reaching psychological and sociological impact. While still true in some respects, the old adage that "all disasters are local" may have become somewhat anachronistic since the mid-20th Century. Although the Christmas 2004 tsunami disaster occurred in Southern Asia, it affected many other countries worldwide because those nation's citizens and many international businesses were involved. Mass air travel, economic strategic initiatives and evolving technology have all changed the face of 'local' disasters. With the rapid growth of air travel and relatively affluent worldwide economies, travel and sightseeing have morphed from an occasional pastime of the very privileged and the eclectic adventurer into a prevailing norm of worldwide mass tourism. Likewise, industries have also become global, often seeking heavily-populated sites where labor may be more economical and local workforces readily available to work for lower wages. A large number of the victims of the Indian Ocean tsunamis were foreign nationals operating businesses and many, many tourists, including celebrities, were caught up in this Asian calamity as well. Thus, in many ways, disasters throughout the world have now become multinational in nature, having global impact, an impact amplified by mass media coverage and internet streaming of the events.

Even so, a disaster in New Orleans involving mainly geography of only a few tens of kilometers, had ramifications in major cities hundreds of miles of way. Both before, and especially after, the strike of Hurricane Katrina in late August of 2005, many tens of thousands of evacuees had to settle in Dallas and Houston affecting resources in those cities and taxing the local medical system. For example, one Dallas shelter (750 kilometers from New Orleans) treated twice the number of medical problems and emergencies (among the evacuees) than it would on a day-to-day basis in the city's main trauma center. This sudden surge in medical problems required the establishment of a 'field' medical facility for more than two weeks after the storm had dissolved. Not only did those patients have their day-to-day medical, dental and psychological needs, but they were now at greater risk in terms of compromised immune systems (nutrition/sleep deprived and psychologically-stressed), but also because of crowded conditions.

From another perspective, despite the relatively fewer number of deaths than the 2004 tsunami or the 2005 Pakistani quake, the September 11, 2001 attack on the World Trade Center in New York could be considered a multi-national event because of the hundreds of persons from dozens of countries working there. It also

pointed out the concepts of modern vulnerability. Not only were the victims highly concentrated in one building making it easier to target and cause many casualties, but modern technology facilitated the incident. A large modern transportation device laden with explosive fuel made such a dramatic disaster possible, one that would not have occurred to such an extent before 1950.

While such technology makes a local disaster global in nature, the same technology can bring a global disaster to each locality. The threat of worldwide spread of contagious disease, both naturally-occurring and malicious (bioterrorist) promulgation [5, 6] is accelerated not only by exponential population growths, but the air ship as well. Again, with more people on the planet who have the potential to become an infected vector and with more global concentrations of highly-mobile populations moving around in jet aircraft (and thus more opportunities for exposure), the risk for pandemics has clearly become greater than ever before. Although the overt threats of cold war nuclear holocaust have seemingly dissipated, the underlying devices of mass destruction still exist, particularly in a world where terrorists are further spurred on by another form of technology, the mass media and internet. Such threats are unimaginable, even for those who must anticipate how to deal with the aftermath [2, 4].

As inferred previously, we are no longer a self-sufficient agrarian culture. Major populations are now fully dependent on day-to-day energy and water supplies, sewage, garbage removal, and food products from others. Therefore, in addition, to the initial impact of injury and illness are the subsequent public health sequelae such as insufficient food supplies, contaminated water, lack of shelter and the subsequent threat of associated diseases. Likewise, the psychological impact of disasters on populations is only just now beginning to be better appreciated, not just for those directly affected, but for the population as a whole [2]. For example, the economic downturns in the United States, Europe and elsewhere after the events of fall 2001 are often considered one of the casualties of the terrorist attacks. It emphasizes the under-recognized widespread affects that disasters can have on the international public psyche.

Even when loss of life and infrastructure are relatively minimal in the grand scheme of the particular nation involved, disasters can have significant and far-reaching psychological impact (e.g., the 2001 USA anthrax postal system attacks). The mass media, internet and other modern technology not only race information to all of us worldwide, but often do so in a graphic and emotionally-stimulating manner. Even though the death and long-term morbidity toll was not as high as the staggering consequences of the Indian Ocean tsunamis, the public health risk management issues associated with the 2001 USA anthrax attacks and other identified *potential* threats were still enormous [2, 5, 6]. Facilitated by obsessive mass media mania, the potential threat of other public health crises (involving large populations) as a result of either terrorism or natural disease (e.g., small pox and avian flu pandemic scenarios) became more of a reality in terms of public perception. As a result, tremendous political pressure has developed for clinicians and public health officers alike to become better prepared to protect the public from all disasters, let alone doomsday scenarios.

Such psychological affects on the public play out in political venues as evidenced in the USA with the development of the Department of Homeland Security and improved worldwide intelligence-gathering cooperation both domestically and internationally. Nevertheless, while some improvements in surveillance and pharmacological resource allocations have evolved, the medical aspects of homeland security and

public health emergency preparedness remain worrisome and clearly fall short of public expectations, appropriate expectations or not.

Sociologically, it is logical that wealthier countries might stand a better chance of mitigating mortality and morbidity with their robust health care systems, solid public infrastructures, substantial community resources and early detection-warning systems. This would occur not only through forewarned prevention of injury and illness, but also through rapid access to sustenance, medical and rescue assets. It could be emphasized that much of the loss of life and morbidity from the 2004 Indian Ocean tsunamis and their subsequent sequelae actually resulted from relative limitations in terms of such characteristics. Nevertheless, the events of the fall of 2001 in the USA, the subsequent Toronto experience with severe acute respiratory syndrome (SARS) and the recent aftermath of Hurricane Katrina in the Gulf Coast, also exposed the vulnerability of even relatively healthy and well-resourced nations.

In fact, these recent events emphasized our vulnerability in surge capacity. Regardless of disaster events, on a daily basis, the great majority of healthcare facilities and emergency medical services (EMS) systems, even in prosperous Western nations, are overwhelmed in terms of emergency care capacity, be they government-based or private entities. Many of the world's key trauma centers and EMS crews are deluged on a day-to-day basis, with in-patient facilities brimming with fully-occupied beds, sub-optimal nurse and ancillary personnel staffing, despite increasing demands for service and a higher acuity of illness and injury.

One could argue that these existing health care services, including ambulances, emergency centers, operating rooms, and intensive care units (ICUs) are facing a disaster each day as available medical resources outstrip the daily demands for urgent and critical care. This tenuous situation causes the looming threat of additional surges from disasters, be they technological or natural, to become truly deep concerns for the future of disaster medicine.

Table 1. Some key reasons for increases in the frequency, magnitude and impact of disasters (natural, unintentional and intentional) since the mid-20th century

▮ Exponential growth of human populations, concentrated in high-risk venues (e.g., shorelines, earthquake zones and prior uninhabited regions), particularly those externally dependent upon on others for sustenance (food supply chains), power resources and public health hygiene, making human populations increasingly vulnerable when infrastructures disrupted.
▮ Increasing exposures to zoonoses; more mutations of microbes (more human vectors); potential effects of global warming on storm formation; more sites (e.g., large buildings, subways) that are larger destructive traps.
▮ Technology that facilitate disasters (e.g., jet aircraft allowing more rapid spread of disease globally or terrorist use as a massive explosive device).
▮ Growth of hazardous industries and transportational systems (e.g., chemicals in plants, trucks, boats); genetic engineering/technology that facilitates bio-terrorism and weapons of mass effect across populations.
▮ Mass media (television, internet) that provide instantaneous imagery and disaster threat concerns, affecting populations psychologically worldwide, and, in turn, affecting markets/economies (also a stimulus for terrorists).
▮ The global, multinational, multi-jurisdictional nature of disasters because of international-global industries, businesses, and tourist activities, and the associated global vulnerability through computer/internet disruption

In essence, there is a spiraling risk for catastrophic events involving multiple casualties and population-based medical morbidity, including proximal injury and illness and subsequent psychological and public health concerns (Table 1). Such events will likely be multinational in nature with global ramifications, even when localized to a particular venue. Therefore, this will require international cooperation in terms of prevention, mitigation and relief. However, the medical care infrastructure, even in wealthy countries already seems to operate at capacity, making a major multiple injury event or even an influenza pandemic a true challenge.

Key Problems in Disaster Medicine Today

Just as the threats of disasters have increased and become multi-faceted, so have the medical sequelae and complications [2, 4, 6]. Explosions carry the triple threat of thermal, penetrating and blunt trauma [2, 4]. Associated building collapses not only cause crush injury syndromes, but a higher risk of being trapped, and modern fires induce chemical inhalation injuries as well as carbon monoxide poisoning and traditional respiratory tract impairment [2, 4]. Earthquakes cause all of the above, but killer tsunamis as well because of concentrated population centers. Hurricanes, floods and tidal waves result in drowning, snake bites, and contaminated water supplies and subsequent mosquito-borne/zoonotic illnesses [2]. Chemical releases can result in pulmonary injury, burns, nerve system dysfunction, liver damage and cellular dissolution [2, 4]. Severe radiation exposure causes burns, immunological suppression, and diffuse epithelial damage, internally and externally. Biological agents result in a myriad of physiological insults. From pneumonia, coagulopathies, and central nervous system compromise to cardiac suppression and liver failure, the viral and bacterial agents provide often-insidious challenges to clinicians and public health officers alike, regardless whether the root source is a natural epidemic or a malicious dissemination.

While using existing medical personnel would be best, developing coordinated plans to incorporate them into a disaster plan (and provide them with timely respite and staggered shifts) is the challenge. While additional personnel theoretically could be imported from nearby (unaffected) medical facilities or from other regions or countries, the local personnel work best in their own environments and still would need to provide coordinating leadership roles. This even plays out politically as was seen in the aftermath of Hurricane Katrina where local medical personnel still wanted control despite the loss of resources (hospitals, supplies), both out of sense of loyalty to 'their' patients, and their own sense of duty.

In some respects, using the local personnel is in keeping with the number one rule of multiple casualty incident management, namely to follow day-to-day routines as closely as possible or modify day-to-day routines as much as possible to meet the unique demands of a disaster [7–9]. The logic here is that unfamiliar activities or settings result in logistical and procedural learning curves for clinicians and that such medical care obstacles can be amplified in a strange venue with overwhelming patient care demands. Learning how the laboratory or pharmacy works or how to operate less familiar equipment or communications systems, can delay and impair the true focus of patient care.

In the Hurricane Katrina scenario or the Pakistani earthquake circumstance, however, much of that infrastructure was wiped out, diluting such arguments. If, then, outside teams are to be brought in, one solution is to bring in a team that al-

ready works together well. In the aforementioned Dallas field hospital built to handle the surge from Katrina evacuees, the medical team was used to working together. Likewise, the arrival into New Orleans of the USS Comfort, a floating naval hospital replete with extensive operative capacity, neurosurgeons, multiple ICUs and airlift capacity, worked well because the team had been in place and worked well together. The question remains, however, how long could such 'borrowed teams' last in the disaster zone. What is an appropriate tour of service?

Therefore, if they are still standing and operational, the utilization of highly-experienced trauma centers and ICUs would optimize the medical care skills needed and the efficiency of the delivery of care assuming that they could be off-loaded from some of their day-to-day activities. For example, a busy emergency department could off-load sore throats, urinary tract infections, and broken arms to clinics and field infirmaries (places where surge and delays in care would have less concerning consequences). Through prospective, government-moderated agreements, ICUs at major receiving centers for disaster victims could transfer certain critical care patients to other community hospitals if necessary. This kind of arrangement has been worked out to significant degree in venues like Miami, USA, a frequent target for major hurricanes in which certain hospitals themselves are at risk of damage. In any case, there will be a need for external surge capacities outside the day-to-day facilities.

Contrary to popular perception, *most* disastrous events do not create an immediate influx of critical patients [7–9]. Victims are either killed outright or they have generally survivable injuries. In most traditional situations, experience has shown that less than 10% of patients (those not killed outright) will require critical care or critical care monitoring [7–10]. The main threat is actually displaced persons who need their day-to-day medical care, prescription, optometrist, dentist, and, in some cases, their dialysis or chemotherapy. In the aftermath of Hurricane Katrina, hundreds of thousands needed to be evacuated for extended periods of time. Even if most were healthy, some of these healthy evacuees were bound to have their unanticipated appendicitis or their typical sore throats. More to the point, tens of thousands, especially the elderly nursing home patients, needed so much more. And how does one address the situation of a woman who says she is due for her chemotherapy when she has no medical records or physician contact? This becomes the real challenge, not necessarily the threat from the actual disaster event. Most conventional disasters can be handled by existing resources when those resources are not involved or currently overwhelmed [8].

If the major problem becomes the destruction of the local facilities and these concerns become further confounded by legal issues such as licensure and credentialing of medical professionals coming to volunteer at other alternative hospitals or the disaster zone itself. A physician coming to provide aid at another locale or hospital may not be authorized to do so if they are not licensed to practice medicine in that jurisdiction (i.e., country, state, province). Moreover, even if licensed, hospital accreditation, in most venues, requires prospective scrutiny of physicians with relevant background checks and certain administrative requirements. All of these procedures take time and are, therefore, essentially impossible to provide at the time of a catastrophic event. While some communities have set up mechanisms to cross-credential physicians for a disaster, only a few have done so and this does not account for the issues of familiarity and skills utilization.

Furthermore, the key practitioners that might be needed to provide assistance at alternative locations in a disaster are skilled nurses, respiratory therapists, dialysis

technicians, pharmacists and the like. While all of these practitioners could also be 'cross-credentialed', it still does not account for motivators to have any of those persons participate. Motivators are not just the dedication of a zealous volunteer or the lure of a financial incentive, but they also include care for those practitioners' families in the midst of a disaster during which time the families may be vulnerable as well (no food or water, loss of electricity, trapped by flooding, possible exposure to contagious disease, etc.). Many of the persons trapped in the flooded New Orleans hospitals were actually family members of the health care workers. Finally, there could be concerns about liability coverage and protection from malpractice lawsuits when providing services outside of one's routine location [5].

All of these concerns strengthen the argument that existing facilities, particularly major trauma centers and critical care hospitals, should all be fortified and better prepared for surge capacity. Not only are there issues of skills, experience, familiarity, learning curves, licensure, credentialing, motivation and liability protection with which to be concerned, but, again, this paradigm follows the basic disaster tenet to follow one's day-to-day routines as closely as possible [7–9]. It relies on the premise that one should prepare for such events by modifying day-to-day activities to accommodate requirements for a multi-casualty event. One of the major reasons why disaster scenarios, be they drills or actual events, often go awry is that they are encumbered by plans or procedures that fall outside normal routines. Therefore, working in environments that facilitate familiar clinical and procedural behaviors in a disaster is the most advisable strategy.

Future Concerns in Disaster Medicine

While local, reinforced facilities might be best suited for the more traditional 21st Century disasters, a major nuclear or biological event can pose a more regional-national threat, if not a global risk [4–6]. For example, even if only a fifth of a population becomes infected with a highly contagious disease over a several week period and only 5% of such patients require critical care, this can mean 10,000 critical care patients in a city of a million residents. Assuming a week's stay for each patient whether they live or die, it still translates into the need for thousands of ICU beds at any given time. Also, under such circumstances, patients more than likely would not be transferred out to other communities because those other venues also may be experiencing similar, or even worse, surges in patient demands in such scenarios. Similarly, certain catastrophes may involve those 'routine facilities' and transport services themselves such as that seen in the Hurricane Katrina aftermath. They may be destroyed or inaccessible due to flood, earthquake, terrorist bombing or contamination. This scenario also entreats a plan for working outside one's normal routine or enormous surge at other facilities, either local or at a distance, established or makeshift.

Nevertheless, if current, day-to-day facilities are to be fortified (as they should), then these modifications will have significant fiscal impact. Already strapped with budgetary challenges, such infrastructure changes would be unreasonable without full public support (government assistance) and commitment that all other facilities would share in similar burdens. In fact, far beyond the costs of constructing better ventilation mechanisms, improved water supply systems, well-placed generators and circumferential barricades (e.g., mitigating the effectiveness of car-bombs) as well as better control of hospital entrances, are the sociological conflicts. Such security

measures defeat the purpose and current philosophy of hospitals, clinics and medical facilities which should strive to provide even easier, patient-friendly access to medical care, particularly for the elderly, sick and chronically ill who need comfortable entrance.

Even with infrastructure changes, they will be of little avail without standardized training of the healthcare providers. During the last quarter of the 20th Century, standardized, multi-disciplinary courses had been developed for the management of other major threats to life, namely cardiac arrest and trauma (e.g., the American Heart Association's Advanced Cardiac Life Support and the American College of Surgeons' Advanced Trauma Life Support). Such standardized training has even been demonstrated to be effective in increasing life-saving. However, unlike cardiac arrest and trauma cases that can present in certain facilities on a daily basis, disaster events are uncommon and infrequent events, even worldwide, making additional training and practice even more critical.

Even if the infrastructure is addressed and personnel trained, the complexity of nuclear explosion or 'simply' a pandemic may be overwhelming. It is feared that with the typical current processes for producing vaccines, an entirely new influenza virus could sweep through even healthy worldwide populations in a matter of months, long before a vaccine could be developed, processed, and distributed, not to mention the time it takes for inoculated persons, particularly children, to develop adequate protective antibodies. A similar genetic jump from animal populations (e.g., 'avian flu') could proliferate with the same scenarios. In both cases, with more people to infect, more ways of rapidly transmitting the virus around the globe, and more persons with immunological suppression alive today, the risk for pandemic will become even more of a threat, not only for the population as a whole, but also for the ambulance crews, emergency department staff and ICU practitioners. If they become ill as well, there will be even fewer healthcare providers available to care for the throngs of ill persons, making the disaster scenario even worse. Such as seen in the SARS situation, the medical facilities and healthcare workers may become a main vector themselves.

More worrisome is the unknown. The concept of SARS was unknown three years ago. With tremendous advances in genetic engineering, it is feared that both malicious and unintentional contagion threats will become new realities. And while threats for disaster are increasing, medical care resources are being spread thinner and thinner, from sociological factors (nursing shortages) to financial constraints (decreasing reimbursements for medical care).

Ironically, with increased demands for protection from terrorism and other public health threats, financial resources have been diverted from healthcare to national defense and homeland security efforts. Unfortunately, most security efforts are overhead costs and not at all revenue-generating. Even within hospitals, dealing with disaster management is generally administrative in nature (training, equipment, procedures, personnel) and consumes and diverts medical care professionals' time and efforts from their day-to-day patient care activities. These concerns are likely to become worse and resources further drained. Moreover, the actual costs of the disasters are accelerating and public expectation for assistance stronger than ever before.

Some Potential Solutions and Improved Preparations

Many lessons have been learned in our era of expanding and more frequent disasters. In a cyclone, tornado or hurricane belt, reinforced hospitals should have their ICUs and operating rooms secured within the center of the edifice, well above the level of potential storm surges and flood levels and far below the roof-top levels where spin-off tornadoes can rip off the top floors and blow out exterior windows. Secondary and back-up generators should be protected in a similar fashion. All hospitals, particularly those in recognized earthquake zones, should have 'earthquake-proof' designs with applicable structural integrity. When a hospital is a potential terrorist target such as any main receiving facility, trauma center, burn center, or children's hospital, security measures will dictate the need for barricades to bombs and specialized ventilation systems that detect and can control the spread of aerosolized poisons or biologicals. These hospitals should have a disproportionate number of negative pressure rooms and well-established decontamination zones around the potential entrance and receiving personnel (e.g., trained triage nurses and security personnel will be staged further out into the periphery and prepared with universal precautions and easy access to decon (decontamination) suits, including high level personal protective equipment (PPE). Within the next ten years, spurred on by additional major terrorist events and further recognition of the vulnerability of the medical safety net, governmental resources will likely begin to make these changes and architects for new facilities will incorporate them into future design. But, economically intensive, this aspect of preparation will lag behind other efforts.

This first consideration to enhance the structural aspects of disaster management would be of most value to handle the more likely conventional disasters. However, one might then return to entertaining the concept of a specialized facility or facilities to manage pandemics and disseminated bioterrorism incidents such as a mutated smallpox organism. In this case, to overcome the concepts of lack of familiarity with equipment and resources, credentialing, liability and medical skills utilization, a mitigating solution would be to dedicate a reserve team of medical personnel in the way a government entity deploys a fire service or army as a dedicated standing force. Currently, a hybrid for this type of concept is accomplished through the mobile medical teams and tent hospitals designed by the National Disaster Medical System (NDMS), an element of the Federal Emergency Management Agency (FEMA) in the United States Department of Homeland Security. Although this does not employ a round-the-clock standing team, it does utilize a 'stand-by' team of medical and allied health volunteers who are trained and routinely exercised to operate mobile hospitals. Currently, these teams would likely be inadequate for a mass casualty scenario with tens of thousands of victims, but still they are somewhat helpful in areas where the standing facilities have been destroyed, impaired or are inaccessible. The Hurricane Katrina incident demonstrated the clear value of expert, co-trained military medicine assets.

Mobile hospitals have reasonable value in some circumstances and should always be part of the planning including safe and secured storage of required equipment and assets, but community-wide plans to enhance existing facilities for surge capacity should still supercede all other plans. Inter-hospital agreements to facilitate transfers of patients to balance out surges throughout the system should be in play in the best prepared communities. Such agreements will also include specialized storage facilities for antidotes and antibiotics, perhaps in a separate, undisclosed

and protected storage facility, but close enough for easy access. The regional hospitals should band together and purchase these items in bulk to leverage economies of scale (cheaper prices), but they should also coordinate receipt so that expiration dates on the drugs will be staggered and not all expiring at once. Such coordination would also include the use of healthcare and ancillary personnel in case of overload at a given hospital or incapacity of another.

Not only will prospective cross-credentialing be accomplished ahead of time using familiar mechanisms, but the process may even be facilitated by course completion in Advanced Disaster Life Support and Hospital Disaster Life Support types of courses [1, 2, 11]. In all likelihood, arrangements should first focus intra-murally. For example, they may prioritize the use of nurses from the same hospital system (i.e., many hospital systems operate more than one hospital) because of the similarities in policies, procedures, liability coverage and payroll considerations. Such coordination of efforts would be a massive under-taking, but well-prepared communities will want to cooperate in such approaches. Also, since many disasters are multinational and often occur in under-resourced countries with relatively poor infrastructures, the creation of dozens of international disaster teams by the United Nations or other organizations worldwide could be considered. Equipped with fast transport aircraft and mobile emergency units, these teams would best be available on any site within less than 6 hours and will work closely with local organizations. Very likely, each disaster team will be staffed and operated by a multinational force, and most likely a standing military team. As in any other military organization, teaching and training in disaster medicine will be part of military education in all countries and the teams will continuously drill and work together in coordination on a routine basis. If anything has been learned in recent disasters, it has been the clear value of the military.

Another lesson learned was the value of standardized training, not only for the military, but for the civilian population as well. After 2001, the American Medical Association (AMA), working with several major academic centers, began to help development of a family of standardized, interoperable, multi-disciplinary, all-hazards courses to deal with the medical aspects of disaster medicine and counter-terrorism [1–3]. Working in close conjunction with university-based trauma center teams as well as multiple federal and military agencies and, more recently, other professional societies such as the American College of Emergency Physicians (ACEP) and the Society of Critical Care Medicine (SCCM), the AMA courses are beginning to lay the groundwork for standardized training and improved personnel preparations for disasters [1–3, 11]. During Hurricane Katrina, the Louisiana State Department of Health attributed many of the medical evacuations that were accomplished quite successfully, to the use (and previous training in) ADLS as well the use of a table top disaster scenario called “Hurricane Pam”. Like Advanced Cardiac Life Support and Advanced Trauma Life Support, the Advanced Disaster Life Support course provides hands-on, multi-disciplinary scenarios in which participants learn to provide antidotes and other resuscitative skills in simulated austere, hazardous conditions requiring the donning and use of high-level PPE in insecure environments [1]. In addition to the Advanced Disaster Life Support course, a prerequisite Basic Disaster Life Support course [2] provides intensive exposure to the didactic elements of disaster preparation, be it chemical, biological, radiological, or traumatic in nature [2, 4]. Within the next ten years, it is predicted that the Basic Disaster Life Support course will be required for every medical student, paramedic student, nursing and other applicable allied health personnel in the USA [2]. The

Advanced Disaster Life Support course will also be provided to applicable trainees and nursing staff in critical care areas including advanced life support ambulances, emergency departments, and critical care areas. It is also predicted that specialized in-hospital spin-off courses (e.g., Hospital Disaster Life Support) will address elements of decontamination tactics by custodians, engineers and others. The SCCM has already laid much of the groundwork for such training with some of their course developments [11]. Such endeavors will hopefully become similar to the efforts conducted by the International Liaison Committee on Resuscitation (ILCOR) for cardiac resuscitation medicine using evidence-based approaches and worldwide consensus.

In addition to being involved in such standardized training, paramedics and emergency medical technicians should also be trained to deliver prophylaxis in a public emergency preparedness situation such as a smallpox or yersinia outbreak or even an influenza pandemic. Prospective rules about who can be denied or provided vaccination or antibiotics and how one receives protection from liability should be addressed ahead of time in the most prepared systems. In addition to healthcare workers, certain societal infrastructure personnel (e.g., elected officials, water, power, communications, media personnel) may be prioritized as well. Also, anticipating a major event, facilities should develop a cadre of antidotes, antivirals and antibiotics for biological threats. More importantly, they will need to ratchet up ICU equipment, ventilators and respiratory care equipment as well as PPE and decontamination equipment. Ambulances and emergency departments and hospitals at large should be fortified by new computerized technology that enhances detection and discrimination of abnormal gases, chemicals, aerosolized biologicals and other threats in ventilation systems and ambient air, just as a carbon monoxide detector or Geiger counter would provide sentinel detection of carbon monoxide or radioactivity in one's home. Medical records will be electronically stored and also duplicated and stored at distant sites in case of medical facility destruction and population displacements. New ventilators will need to be impervious to chemical and biological agents and provide protected ventilation in such environments. In addition, artificial hemoglobin-based oxygen carriers that can be stored in ambulances or in far-forward military conditions should be researched and eventually placed in massive storage places as well and ready to use for mass casualties [12]. Some products now in early test stages can be stored without refrigeration for several years and are likely to be standard equipment in all critical care settings, be it prehospital, emergency center or ICU settings [12].

Again, all of this will require a new level of international cooperation particularly because many natural disasters can be superimposed upon on-going complex emergencies, including on-going famines, civil wars or rebel insurgencies [13]. Such circumstances can further complicate rescue and restoration of normalcy [13].

Conclusion

There is a worldwide spiraling risk for more frequent catastrophic events involving multiple casualties, not only in terms of acute injury and illness, but also subsequent psychological and public health concerns. Today, such events will likely be multinational in nature, even when localized to a particular venue and this require international cooperation in terms of prevention, mitigation and relief. The best approach to preparing for disasters is to expand, modify and enhance current local

infrastructures and capabilities for managing the multiple types of disaster scenarios and create a number of inter-facility cooperative agreements in advance. Aside from safer internal locations for ICUs and surgical theaters, certain structural changes will need to be installed such as modified ventilation systems, protected water supplies, decontamination mechanisms and security renovations. A key strategy will be to proliferate interoperable, multi-disciplinary, all-hazards training initiatives such as the AMA National Disaster Life Support courses. Purchases of cadres of antidotes, antibiotics and hemoglobin-based oxygen carriers should be coordinated regionally, stored in secure locations and made readily-available for the applicable disaster scenario.

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Health Services Research and Critical Care

K. Hillman and J. Chen

Introduction

Much of our clinical practice is historically based on the individual doctor-patient relationship. While this provides the foundation for the practice of medicine, we view work in a complex environment where care of the seriously ill has to be provided at the same level for 24 hours a day. Moreover, the care involves the seamless interaction between other areas of the hospital, such as emergency departments, general wards and operating rooms as well as between many different specialists. In order to evaluate these interactions and systems we will need to familiarize ourselves with the principles of Health Services Research (HSR).

Background History of Acute Hospitals

The specialty of critical care in terms of the history of medicine is short; developing since the Second World War and complementing the way acute hospitals have developed over the same period.

About the time of the crusades, the hospital was given up as non-core business by monks, as monasteries devolved caring for the sick to separate and dedicated institutions. Public hospitals remained almost exclusively for care of the poor, up until around the Second World War. At about that time, popular movies moved from black and white to become color and the cry that we heard from actors on discovering the seriously ill or injured, changed from – "call a doctor" to "call an ambulance"! All patients, rich or poor, began going to hospitals when they became ill. In other words, the contents of the doctor's black bag no longer reflected the limited treatment options that hospitals could provide. Hospitals were about to rapidly expand their role and differentiate themselves from individual medical practitioners.

Up until just after the Second World War, doctors carried with them a range of diagnostic tools (e.g., stethoscope, tongue depressor and torches to inspect ears, eyes and throats), as well as almost the entire range of drugs available at that time (e.g., pain relief, sedation, antihistamines, and the first antibiotics). Hospitals at the time had a similar, limited range of diagnostic and treatment tools. This particularly applied to physicians. But surgery was also rapidly expanding its repertoire; anesthesia was beginning to be practiced by specialists; and post-operative care was being taken seriously in recovery rooms and specialized wards.

Before the Second World War, most people who could afford it, were treated by a visiting general physician. Most accepted that it was normal to die at home. It

was implicit that medicine could only offer limited options. Doctors were healers, not treaters of disease. Even King George VI had his thoracotomy in 1951, at home – albeit, in Buckingham Palace.

Background History of Critical Care

Critical Care followed the explosion of treatment options which became available in acute hospitals during the early 1950s. The poliomyelitis epidemic inspired anesthetists with artificial ventilation skills to support patients with potentially reversible respiratory failure [1]. The same skills were then applied to support patients with complex surgery in the post-operative period. Soon, patients with severe infections were also being supported by more sophisticated means, while they, hopefully, made a recovery. Artificial ventilation, invasive vascular access, cardiorespiratory monitoring, sedation, inotropes and vasopressor, intravenous fluid administration; and artificial feeding became the mainstays of treatment in critical care.

The development of critical care occurred against the background of a rapidly changing acute hospital. While there were many revolutionary new drugs and operations, the way in which services were delivered within hospitals remained much the same as it had over the previous centuries. Specialist surgeons and physicians remained in charge of patients; nurses recorded vital signs and cared for patients in the same basic ways; and medical specialists sporadically visited the hospital to see their patients who were managed by trainee doctors and nurses for the remainder of the time. Emergency Departments (ED) and intensive care units (ICUs) were just beginning to evolve but remained largely as appendages in the hospital. Even now, some countries do not have specialist physicians specifically trained in emergency and intensive care medicine.

Changing Nature of Acute Hospital Patient Population

The geographical, professional and functional boundaries within acute hospitals remain much as they always have. General wards accommodate most patients. The ED assesses stabilizes and triages patients to appropriate destinations. Operating rooms perform their role, often independently of the rest of the hospital, and ICUs and their staff are usually confined within their own four walls.

This may function perfectly well for patients being cared for in the ICU and probably facilitates the traditional individual patient – doctor relationship. However, it may not provide effective and safe care in a systematic way for the seriously ill across the whole hospital.

Increasingly acute hospitals are caring for the seriously ill. At least 50% of surgery is now conducted on a day-only basis. Even for complex cardiac and neurosurgery, patients are admitted on the morning of their operation. Ambulant patients are supported in their homes or managed in institutions or environments which are appropriate for their condition and importantly, operate at lower costs than the traditional acute hospital. The hospital of the future will increasingly be for the management of serious illness with a largely acute and reversible component [2, 3].

As more patients, who would have been managed in an acute hospital, are treated in an ambulant setting, the remainder will become more seriously ill. Thus, the latest additions to the acute hospital, such as EDs, ICUs and operating rooms

will become major features of the new acute hospital. There will be increasing emphasis on step down ICUs – or high dependency units (HDUs), probably to such an extent that the current concept of low acuity general wards will be a thing of the past. Most patients will, at least, have minimal monitoring such as non-invasive blood pressure and oxygen saturation. Patients will increasingly be in the hospital because the nature of their illness has a mainly acute reversible component and requires complex management. The rapid decline in acute beds in most developed countries support this trend [3].

Scientific Evaluation of Systems

These changes are occurring with little in the way of evaluation and research and are often being driven by cost or political imperatives. Sometimes effective solutions can be achieved which simultaneously achieve cost savings and greater efficiencies as well as better patient outcomes and improved patient/carer satisfaction. An example is the admission of most elective surgical patients on the same day as their surgery [4] – patients prefer it, clinical outcomes are improved and substantial cost savings are achieved. However, in other areas, such as early hospital discharge, there are obvious cost savings in the short term but little knowledge about patient outcome in the longer term; nor the appropriateness of the non-hospital component of the health system in supporting the patient and what burden is transferred to the patient carers.

The radical ways in which acute hospitals are being reconfigured and the effect of those changes on patients/carer outcomes are the subject of HSR. The definition of HSR is various [5], but it is essentially a multi-disciplinary field of inquiry, both basic and applied, that examines the use, costs, quality, accessibility, delivery, organization, findings and outcomes of health care services to increase knowledge and understanding of the structure, processes, and effects of health services for individuals, groups and populations; or research related to a conceptual framework other than that of contemporary applied biomedical science; or all strategic and applied research concerned with the health needs of the community as a whole, including the provision of services to meet those needs.

HSR has the potential to inspire and evaluate new system interventions which may have an equally or perhaps more important impact on patient outcomes than a new drug, procedure or monitoring device. It can also be instrumental in improving our understanding and practice in instituting changes for the health care sector.

An example of this sort of impact is a system to identify seriously ill patients across a whole hospital and provide immediate resuscitation in order to optimize patient outcome, possibly preventing serious complications such as death, cardiorespiratory arrest and unanticipated admission to the ICU. It involves changing the concept of a cardiac arrest team to a medical emergency team (MET) [6–8]. The MET responds to specific abnormalities in patient vital signs such as tachypnea, tachycardia and hypotension. The system works across the usual professional and geographical barriers and boundaries present in an acute hospital.

Developing such a system presents challenges not usually encountered in research and resistance to change is a complex, multidimensional response with emotional, cognitive and intentional components [9]. For example, all the individual hospital specialists must agree to early intervention in their patients – this may be

interpreted by colleagues as losing control or even income. Other challenges involve educating and orientating all hospital staff; changing the culture of a hospital to a patient-focused pre-emptive one; gaining the support of other staff such as nursing; and receiving support from administration in terms of overseeing organizational components and agreeing to any extra costs incurred.

The introduction of a new system is equivalent to the preclinical phases of drug research. For example, in this case, pre-MET calling criteria had to be validated, organizational theory had to be utilized, and assistance from medical anthropologists and educationalists was used in defining the cultural changes. The system had to be fine-tuned and many lessons had to be learnt before it was ready for evaluation in the pilot institution [6–8]. Different research methodologies were then necessary to test its effectiveness in other institutions [10, 11]. A multicenter national trial has been completed with the support of a national professional body, the Australian and New Zealand Intensive Care Society (ANZICS). The trial is a cluster randomized one, which lends itself to the evaluation of a new system as opposed to a drug or procedure, as it is based on randomization of institutions rather than individual patients [12].

Health Services Research

There are many new challenges to conducting HSR. The obvious one is that health system reconfiguration threatens existing stakeholders who may either be suspicious of change or may suspect that they have something to lose. HSR involves researchers thinking outside their existing 'box' (in this case, the ICU) and considering seriously ill or at-risk patients in the wider context of them being future customers with a more serious and intractable illness, or even worse, dying on general wards, because they received inappropriate management.

Funding HSR is not easy – private industry, a major driver of 'magic bullet' research, is unlikely to fund systems where profits are difficult to achieve. Governments, acting as patient advocates, are the most likely supporters but their budgets are always under strain, not the least by the existence of ICUs themselves. Cases for funding need to be made in a convincing way and governments need to be convinced that it may be in everyone's interest to reconfigure health services and to evaluate those new services.

What is the future of HSR in critical care medicine? We believe intensivists will have a potentially exciting and significant impact on the way we manage the seriously ill in hospitals and how we evaluate that impact. It will require shedding the historical determinants of how an acute hospital was shaped and, instead, concentrate on organizational and health care delivery issues within the ICU, and across the whole hospital as well as longer term outcomes after the patient has been discharged from hospital.

Our current research agenda has been largely confined to evaluation of supportive measures and new drugs and the underlying pathophysiological and pharmacological basis for the practice of critical care medicine.

There are many other interesting questions to be answered by HSR (Table 1): defining roles and relationship between doctors, nurses and other staff in an ICU; what are optimum staff:patient relationships and skills levels; what new roles and relationships could there be between, for example, single organ specialists, intensivists and emergency physicians or other hospital based specialists within a hospi-

Table 1. Examples of research questions addressed by a health services research approach

What is the optimum size of an ICU?
Do we need high dependency units?
What is the best staffing mix and numbers needed to staff an ICU?
What is the best system for caring for the seriously ill outside the ICU?
What is the most efficient way of dealing with seriously ill patients on arrival to hospital?
How should critical care services be best configured to support a population?

tal; what would be the effect of standardizing triage and care of the seriously ill across a hospital, in much the same way as has occurred for trauma; how should the ICU play a role in facilitating patient-flow through a hospital and what is the effect on long term outcome for the patient, carer and society on interventions in the ICU.

Conclusion

As we move from single therapeutic interventions such as new drugs or procedures to complex system interventions aimed at improving the care of the seriously ill we need to familiarize ourselves with the tools made available by HSR and increasingly work with other researchers such as social scientists, health economists and medical anthropologists.

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Healthcare Disparities in Critically Ill Patients

G.S. Martin

Introduction

Despite the tremendous technological advancements in the recent history of medicine, disparities in healthcare continue to exist. The preponderance of peer-reviewed literature on healthcare disparities focuses on conditions in the USA, yet disparities in one form or another exist universally throughout the world. Much attention has been focused on the USA because of a seminal publication from the National Academy of Sciences Institute of Medicine, entitled "Unequal Treatment: Confronting Racial and Ethnic Disparities in Healthcare" [1]. In this book, it is very clearly stated that minorities tend to receive a lower quality of healthcare than non-minorities, and that the sources of these disparities are complex and multifactorial. Directly relevant to the presence of disparities is that variations in healthcare and poorly managed-care results in expensive and avoidable complications. Disparities in healthcare are not simply related to patient insurance, income or access to healthcare. Nor are they wholly related to the delivery of medical care in our current healthcare systems. In this chapter, we will discuss the types of healthcare disparities identified in critically ill patients and the gaps in existing information, and explore how these disparities may differ from disparities in other conditions.

Magnitude of Healthcare Disparities

Disparities have been discussed at length in many medical venues. The presence and magnitude of disparities depends on the operative definition of disparity. The word 'disparity' can be simply defined as 'the condition or fact of being unequal.' For the purposes of varying agendas, the definition of disparity may change. Most generally, healthcare disparities are defined as differences in the development of disease, the management of a medical condition, or relevant clinical outcomes based upon a factor specific to the patient (e.g., age, race, or gender). However, in some circumstances disparities may be defined more specifically, such as differences outside of healthcare access-related factors, (i.e., exclusive of insurance status or income), or only when unrelated to fundamental patient differences (i.e., genetics). While these definitions are more specific and may be important for examining discrete contributors towards disparities, they restrict the overall understanding of disparity occurrence and the scope of contributing factors. In addition, while the validity of race has been questioned as a contributing factor for disparities in the USA (recognizing that race in that country represents a social construct more than

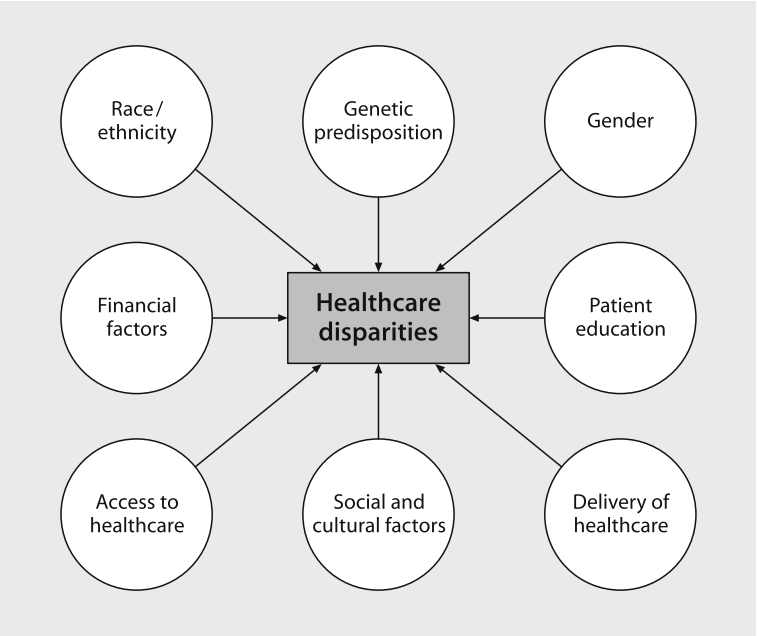

Fig. 1. Potential contributors to healthcare disparities in critically ill patients

a biological construct) [2], we will consider the factors of race and ethnicity as real and potential contributors towards disparities in critically ill patients. Broadly defined, the potential contributors to healthcare disparities are shown in Figure 1.

Disparities are often considered in terms of racial and ethnic background, but also exist based on age, gender, financial means, and geography. These differences, such as geographic differences, may relate directly to the availability of healthcare resources. In many areas of the world, healthcare resources are not universally scarce but are concentrated within populated areas, creating large healthcare disparities within the rural populations [3, 4]. Healthcare disparities based upon patient age are often considered in terms of healthcare delivery, with differences in aggressiveness of care because of either patient preference or physician preconception [5]. Gender differences, whether in the general population or in critically ill patients, have been postulated to result from hormonal differences between sexes [6]. Some of the most interesting areas that are evolving now include the consideration of predetermined biological differences (such as genetics) that may contribute to disparities in disease [7–9]. Addressing these healthcare disparities may occur on a variety of levels, including access to and delivery of healthcare, patient and physician education, and disease management.

Disparities in healthcare have been documented for nearly every major disease [10]. Both lower socioeconomic class and minority race are associated with poorer health and shortened survival [11], hypothesized to result from differences in the process and delivery of healthcare. Black Americans have an average life expectancy six years shorter than that of white Americans, with an approximate 20 year disparity in survival between white persons in the healthiest USA counties compared

to black persons in the least healthy counties [12]. Blacks undergo fewer cardiovascular procedures [13], fewer lung resections for cancer [14], fewer organ transplantations [15], fewer orthopedic procedures [16], and less blood transfusion [17]. Healthcare disparities of similar magnitude exist outside the USA, although they are frequently based on factors other than race [3, 4, 18, 19].

Disparities in Critically Ill Patients

In the USA, it appears that non-white patients receive less intensive hospital care compared to white patients [20, 21]. Whether this translates into a difference in care specifically for critically ill patients remains uncertain. In 1987, it was observed that after controlling for hospital and insurance plans, non-white patients were admitted to intensive care units (ICUs) significantly less often than white patients with the same diagnosis [22, 23]. Of studies examining the effect of race on outcome after ICU admission, there is no conclusive evidence that critically ill non-white patients have significantly different mortality rates after controlling for primary diagnosis and physiologic status [24]. Small cohort studies have documented an increased rate of death for black patients admitted to the ICU, but differences in mortality were not apparent after accounting for imbalances in disease severity and admitting diagnoses [25]. The largest study to date examined 17,440 consecutive critical care admissions from 42 ICUs in 40 hospitals across the USA [26]. In this study, black ICU patients were younger and had a higher severity of illness at admission, as well as a greater proportion of non-operative and emergency admissions. Despite having fewer measured chronic co-morbid medical conditions, black patients were more likely to have severe compromise in their activities of daily living, in addition to a higher prevalence of diabetes, chronic renal failure and intravenous drug abuse. As with the prior studies, there was no difference in risk-adjusted mortality. However, black ICU patients had less resource consumption in the first seven days and a shorter adjusted ICU length of stay. Despite these differences, it appears that once a black patient is admitted to the ICU, there is little or no significant difference in outcome. Whether the finding of black patients being less likely to receive ICU care still holds true in the 21st century remains to be determined.

Disparities in Sepsis

Sepsis is an important and life-threatening condition that is relatively common and increasing in frequency [27]. It occurs in approximately 2% of all hospitalized patients and is the leading cause of death in non-coronary ICUs, with a fatality rate of 20 to 60% depending on the severity of disease [28]. Incidence rates for sepsis fall in the range of 200–300 cases per 100,000 people, and for severe sepsis are between 50 and 100 cases per 100,000 [27–30]. Despite reductions in the sepsis-associated fatality rate in the past quarter-century, sepsis ranks as the 10th leading cause of death overall in the US [27]. Hospitalizations with sepsis are prolonged and expensive, with an average length of stay of more than two weeks and consuming more than $ 17 billion in healthcare resources in the USA alone [28].

The typical distribution of sepsis patients is more than 50% male. This finding of male predominance is true in epidemiological cohort studies [30–34] and in

controlled clinical trials [35, 36]. In a multivariable analysis adjusting for other predictors of the development of sepsis in France, male gender was associated with a 20% increased likelihood of sepsis (odds ratio 1.2, 95% confidence interval 1.0–1.4) [31]. When the frequency of sepsis in the USA is normalized to the population distribution of that country, males are approximately 30% more likely to develop sepsis than females (relative risk 1.28, 95% confidence interval 1.24–1.32) [27]. This gender disparity in sepsis has been hypothesized to result from hormonal differences [7]. However, hormones are unlikely to reflect the entire measure of disparity given that differences in gender-specific incidence are also evident in the neonatal and pediatric sepsis populations [37–39].

Disparities based upon race are the most frequently considered form of healthcare disparity. It has been shown that racial disparities in infectious diseases results in a tremendous number of life-years lost, second only to cardiovascular disease [40]. In sepsis, non-Caucasian races are at nearly twice the risk for developing sepsis compared to Caucasians (relative risk 1.90, 95% confidence interval 1.81–2.00). The existence of this racial disparity has also been supported by the pediatric sepsis literature [37].

The reasons for both racial and gender disparities in the incidence of sepsis remain uncertain. As with some of the above-described data in general ICU populations, there does not appear to be a disparity in fatality rates with sepsis, implying that males and minority races are more likely to develop sepsis-predisposing infections or to experience differential healthcare access and delivery. There are clear differences in the distribution of types of infections based upon gender, and both in the types of infection and in the distribution of chronic co-morbid medical conditions based upon race [41, 42]. As with nearly all medical conditions, socioeconomic status also plays a role in healthcare disparities for critically ill patients. Genetics as well may contribute, although these analyses are confounded by the racial mixtures present in so many parts of the world [2]. Initial genetic epidemiology studies have identified polymorphisms relevant to infectious diseases based upon both race and gender [7–9]. Overall, disparities in sepsis incidence are almost certainly multifactorial in origin.

Disparities in Acute Lung Injury

Acute lung injury (ALI) and acute respiratory distress syndrome (ARDS) are severe forms of acute hypoxemic respiratory failure. While much focus has been placed on the epidemiology of ALI/ARDS in the past two decades, there is very little information regarding disparities in these conditions. The most rigorous data comes from death certificates in the USA, where it was observed that males and blacks were more likely to die with ALI/ARDS than their respective counterparts (i.e., higher population-adjusted mortality rates) [43]. The magnitude of the racial disparity is at least as large as has been documented for sepsis, and the gender-based disparity may be even larger than identified in sepsis. The reasons for the different mortality rates in ALI/ARDS may reflect an increased number of cases in males and blacks, rather than a differential effect on fatality. This appears to be the case based on the limited data available from epidemiological studies [44] and by the male predominance of ALI/ARDS patients enrolled into large randomized clinical trials [45]. Further research is required to determine the amount to which disparities in the incidence of ALI/ARDS may be explained by disparities in sepsis, representing the most common cause of ALI/ARDS.

Disparities in Venous Thromboembolism

Venous thromboembolism is a common and under-diagnosed condition. Population-based studies have shown that age and morbid obesity are strong predictors of the risk for venous thromboembolism [46]. Aside from these risk factors, men (hazard ratio 1.4, 95% confidence interval 1.1–1.9) and blacks (hazard ratio 1.6, 95% confidence interval 1.2–2.2) are at higher risk for developing venous thromboembolism than their counterparts. These differences do not appear to relate to a diagnostic bias or a difference in evaluative strategy based upon race or gender [47, 48]. Other studies suggest that the racial origin of the patient may be relevant, as white persons of European descent have intermediate venous thromboembolism rates compared to higher rates observed in persons of African descent and lower rates in persons of Asian descent [49]. Based on USA death certificate data, age-adjusted mortality rates for blacks are approximately 50% higher than for whites, which are approximately 50% higher than for people of other races [50]. Controlling for race, mortality rates are 20 to 30% higher among men than women [50]. These differences in mortality rates are at least in part accounted for by differences in the incidence of venous thromboembolism, as discussed previously.

Healthcare Disparities – A Global Problem?

While much of the information on healthcare disparities has come from the USA, it is likely that disparities based on various factors may similarly exist throughout the world. For instance, while the racial heritage of the USA may lend itself to disparities based on race, it is likely that racial or ethnic disparities exist sporadically throughout the world, as in some parts of Africa. Alternatively, gender-based healthcare disparities are likely to exist in countries where education, finances and access to medical treatment differ according to sex. The healthcare delivery systems in technologically and socially advanced countries throughout the world may limit the existence of healthcare disparities. However, non-critical care healthcare disparities have been documented in most areas of the world, based on finances, geography, age, race and gender [3, 4, 18, 19]. Given that documented healthcare disparities between the USA and other countries are more similar than dissimilar, ICU disparities are also likely to exist to a similar degree. While future research must be focused geographically, being mindful of physical and political barriers to the creation or perpetuation of disparities, additional global information on healthcare disparities in critically ill patients is sorely needed.

Conclusion

Numerous international governments and federal funding agencies have placed the elimination of healthcare disparities on a spate of agendas targeted for completion in the next 10 to 20 years. Disparities in ICU patients are often less recognized, as efforts to identify and eliminate disparities frequently focus on common clinical conditions. Greater efforts are required to characterize the magnitude of healthcare disparities in critically ill patients and to seek the root causes of these disparities.

The elimination of healthcare disparities will require different interventions depending on the type of disparities, the underlying cause(s) and the type of healthcare system in which they occur.

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International Comparisons of Intensive Care: Understanding the Differences

H. Wunsch and D.C. Angus

Introduction

Since the first steps towards modern critical care during the Copenhagen polio epidemic in 1952, the use of intensive care to treat acutely ill patients has blossomed into an integral part of health care across the globe. Like other aspects of medical care, each country and culture has developed its own approach to building and using intensive care. Yet, little emphasis has been placed on fully understanding the multitude of differences that exist between countries with regard to resources, patients, and outcomes.

Large observational studies are frequently conducted at the international level. Whether focusing on variation across countries for end-of-life treatment, or pooling data to generate severity of illness prediction models, researchers continually cross borders with patients and outcomes from intensive care units (ICUs), both combining data to come to general conclusions and contrasting data to highlight differences [1–4]. For instance, data on the rates of infection in ICUs come from studies conducted across multiple European countries [5, 6], recent data on incidence and outcomes from acute renal failure come from data pooled from 23 different countries, and an understanding of the magnitude of intensive care concerns such as acute lung injury (ALI) are generated from pan-European data [7, 8].

In addition, more and more, randomized controlled trials in intensive care also include patients enrolled in ICUs in many countries [9, 10]. This trend towards international trials will only continue, as the types of questions being asked necessitate larger and larger enrolment to power studies looking for small differences in outcomes. In 2002, Cook et al. reported on collaborative groups conducting multicenter research in critical care, highlighting the growth of international cooperation for intensive care research [11]. Finally, as interest in genetics and genetic predisposition to illness also increases, there may be movement towards conducting a whole new type of study with large international cohorts, as important genetic variants may be missed with smaller sample sizes. Thus, in order to interpret the very research we are producing, we need to have an understanding of some of the fundamental differences in intensive care in countries that contribute to these studies.

Individual Country Data

Many papers are published in the literature that attempt to describe and quantify the resources, admissions and outcomes in the USA alone [12, 13], and some baseline data exist for countries such as the UK where there is a large, national cohort

of intensive care patients [14]. Hundreds of papers have also been published on specific topics within intensive care based on data from individual countries; most of these papers focus on the within-borders question of implications for policy and change in that country alone, or are faced with the problem of comparing and contrasting their findings with data from other countries that have potentially been collected and analyzed in a different manner.

International Comparisons of Healthcare

The difficulty of conducting research that directly compares two or more very different systems may have acted as a deterrent. Some studies have attempted to examine the similarities and differences between healthcare systems as a whole in different countries with mixed success. A recent attempt at a comparison in healthcare contrasted California's Kaiser Permanente health maintenance organization with the UK's National Health Service (NHS) in terms of performance and economics [15]. The conclusion that Kaiser achieved better performance for the same price as the NHS [16] was met with a slew of letters in response pointing out the very different nature of the two systems and a disbelief that these differences were fully accounted for in the analysis. A series of articles published in the *New England Journal of Medicine* has explored differences in healthcare between the USA and Canada, with an emphasis on health care costs, examining such aspects as rates of coronary angiography [17]. All of these studies seem to confirm that, overall, the US has a greater use of technology and higher expenditure for healthcare [18, 19].

Economic Differences

These large differences in resource distribution and funding are, of course, also evident in intensive care. There are now over 70,000 intensive care beds in the USA alone and approximately 1 in every 5 Americans who die do so after a stay in intensive care [20–22]. But, we know that the number of ICU beds available per capita varies wildly even between European and North American countries (anywhere from 8.6 ICU beds/100,000 population in the UK to 38.4/100,000 in France in the early to mid 1990s), and spending on intensive care is similarly variable [20, 23].

The true impact of economic differences on intensive care may be impossible to isolate and quantify. However, a knowledge of baseline differences in resources (such as ICU bed numbers) may allow us to have a better handle on how the economics confound our examination of other, narrower aspects of intensive care, such as patient selection, organization of ICUs and ultimately outcomes.

Patient Selection in Intensive Care

Studies in critical care typically only analyze patients admitted to the ICU. Thus, there is often very little information regarding the outcome of patients who may have benefited from, but did not receive, ICU management. As with any limited resource, decisions are constantly being made regarding who should or should not be admitted to intensive care [24]. Even within a single health care system, large

differences may exist with regard to healthcare access in general and intensive care access in particular. A survey by the Commonwealth Fund examining views of and experiences in healthcare in five nations (Australia, Canada, New Zealand, UK and the USA) found that the highest proportion of adults who had problems accessing healthcare because of costs was in the US, constituting 22–35% of those surveyed [25]. Within the US, Angus et al. explored the likelihood of gaining admission to an ICU for a similar level of illness severity between rural and urban populations and found differences of up to 50% [26]. Given the large differences in intensive care resources at the international level, different policies must exist regarding admission to ICU [20]. Therefore, the first question that we recommend addressing when comparing countries is who is or is not admitted to the ICU? Complicating this issue of admission policies is the question of differences in prevalence of disease and overall population age and health that also exist between countries.

A study from New Zealand and the USA compared the patient selection for intensive care in the two countries (2 New Zealand hospitals and 13 USA) and found very large differences in even basic factors such as average age (42 years in New Zealand versus 55 years in the USA) and case mix (trauma, drug overdose and asthma accounted for half the admissions in New Zealand but only 11% of US admissions) [27]. Rapoport et al. compared Alberta (Canada) and Western Massachusetts (USA) with regard to ICU use and found that the USA area devoted a greater proportion of its hospital facilities to ICU beds [28]. Tracking all patients admitted to hospital, a much greater percentage ended up in the ICU in the USA compared with Canada.

A further problem is one of definitions in intensive care. Rubenfeld and Christie, discussing the challenges for the epidemiologist in the ICU, point out the chronic difficulty of identifying patients with syndromes such as ALI and sepsis when the diagnostic criteria have poor reliability [29]. Care, therefore, needs to be taken to ensure that definitions are interpreted in comparable ways when attempting to make comparisons between countries.

Differences in ICU Organization

As well as differences in admission policies and, therefore, case mix, the organization of ICUs varies greatly even within countries and can confound any attempt to examine outcomes for patients [30]. We know, for instance, that most European ICUs function on a closed system, with intensivists leading care, while estimates in 1997 from the USA were that only one third of all ICU patients were treated by intensivists [13, 22, 31]. Furthermore, only 26% of ICUs in the USA ensure that all patients are managed by intensivists [22]. Given recent data suggesting that high-intensity ICU physician staffing is associated with reduced hospital and ICU mortality and length of stay [31], these substantial differences between countries are likely to have a significant impact on outcomes.

Certainly other differences in ICU organization between countries may also contribute to differences in patient care. For instance, given the small number of ICU beds in the UK, between-hospital transport of ICU patients to an open bed is a relatively common occurrence there [32], yet relatively rare in the USA. Differences in nursing to patient ratios, overall size of hospitals housing ICUs, the use of ancillary healthcare workers, such as respiratory therapists, as well as the mix of specialty versus general ICUs in any given country may or may not shape the experience of intensive care delivery.

Comparing ICU Outcomes

Some attempts have been made to characterize overall similarities and differences in intensive care between countries in order to address the question of outcomes. As far back as 1982, differences between French and USA ICUs were examined by Knaus et al. [33]. All of these ICUs were tertiary-care institutions (five University hospital in the USA and seven large public hospitals in France). They noted different observed to expected death rates amongst patients with gastrointestinal diseases, but not in other major categories of disease [33].

With the advent of severity of illness scoring systems (only the Acute Physiology Score was available in 1982) comparisons of patients and outcomes across countries should become easier, yet few recent studies have attempted direct comparisons. One study that has highlighted some of the differences between countries compared critical care in Japan and the USA [34]. Only 1% of hospital beds in Japan were ICU beds and many fewer older patients received intensive care (1.2% in Japan versus 4.6% in the USA over the age of 85). The authors used acute physiology chronic health evaluation (APACHE) III scores to stratify patient risk, but modified the hospital mortality model so that it fit the Japanese data. They concluded that overall mortality was similar in the two countries, although on average Japanese patients had a much longer hospital stay.

The problem of calibration of models in different countries is well recognized; either the models were developed in one country (APACHE II [35]) and do not appear to perform as well when applied to other countries, or the models were developed internationally but with the participation of only a few ICUs in each country (simplified acute physiology score [SAPS] II [3], mortality probability model [MPM] II [4]), resulting in similar calibration problems [3, 35–38]. Either way, applying a model to a new set of patients with a potentially different case mix has proved problematic and will continue to be a challenge in studies involving international data [36, 39].

Another attempt to examine international differences (although not specifically intensive care patients) by Bennett-Guerrero et al. compared surgical outcomes in the USA and the UK [40]. While the differences were striking, the study only included a single center from each country. We caution that a non-random, small sample of hospitals or ICUs may bias any estimates of national performance despite the best attempts at appropriate risk adjustment and inclusion of potential confounders.

Cultural Differences in Intensive Care

Intertwined with the differences in resource allocation are potentially large cultural differences between countries that may influence the delivery of intensive care. Many of the differences apparent in studies of health care as a whole and intensive care in particular may at least partially represent the tailoring of services to what people want based on cultural norms. The role of family in end-of-life decision-making is an example of a situation where cultural preferences may partially dictate how patients are cared for in the ICU. We know, for example, that even across Western Europe, large differences appear to exist with regard to use of treatment withholding and withdrawing [2]. While some of these differences may be attribut-

able purely to necessity born of more limited resources in one country than another, cultural differences in the expectation of how long to continue intensive care and whether families participate in this type of decision-making can vary widely [41]. Even laws across different countries vary with regard to who is legally responsible for the decision to withhold or withdraw care; many European countries give physicians much of the say, while in the USA it is almost impossible to withdraw care without family consent.

Cultural differences may also manifest at a smaller level, namely in the culture of the physicians and ICUs themselves. There have been recent moves towards implementing protocols for some components of intensive care; demonstration of improved mortality with tighter glucose control [42], and shorter time to extubation with appropriate weaning protocols [43], have led some ICUs to institute more uniform treatment of patients with regard to these and other aspects of care. The Surviving Sepsis Campaign has sought to influence mortality from sepsis by as much as 25% worldwide with its recommendations regarding a "sepsis change bundle" [44]. However, adoption of 'cook-book-based' medicine (as its detractors call it) can be slow. Differences in willingness and speed of adopting these ideas is known to differ between countries [45, 46].

Conclusion and Perspective

Studies involving international comparisons force us to grapple with differences in funding, laws, cultural values, and disease prevalence – modified by the genetic make-up of the population (Fig. 1). These differences will continue to make international research in intensive care challenging. Why continue to pursue these comparisons? There is currently a large disconnect in the rapid increase in large multinational studies and our understanding of these differences. For the quality of research in intensive care to continue to improve, many of these differences need to be further elucidated. As well as providing important information for future international study design, these same country differences are potentially informative and exciting in themselves, as we may begin to leverage these differences between countries for natural ecological experiments. For instance, if we can begin to isolate

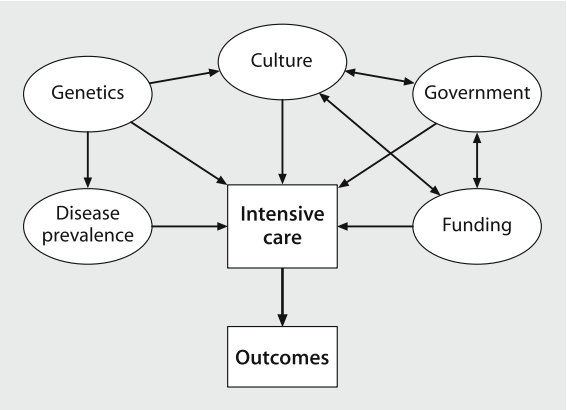

Fig. 1. Factors influencing delivery of critical care in individual countries

the differences in genetics of patients, or organization of ICUs between countries, we may gain a better understanding of the true impact of these factors using much larger cohorts than previously available. As differences in the implementation of protocols and availability of drugs occur between countries, we may also begin to assess the true impact of these new treatments that will ultimately allow for a superior understanding of their benefits. With improved and automated data collection, recognition of the importance of large sample sizes, and more sophisticated and robust methods for risk adjustment, research involving international comparisons will only become easier and more rewarding.

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Subject Index

C

I

J

K

L

O

P

U

V

W

Z

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