Novel hemodynamic therapies in septic shock; Effects on circulation, organ perfusion and immune system

Frank van Haren
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An academic essay in Medical Sciences

Doctoral thesis

to obtain the degree of doctor from Radboud University Nijmegen on the authority of the Rector Magnificus, prof. dr. S.C.J.J. Kortmann according to the decision of the Council of Deans to be defended in public on Thursday, 22th of April 2010 at precisely 15:30 hours

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The research presented in this thesis was performed at the Departments of Intensive Care Medicine of: Bosch Medical Centre, ‘s-Hertogenbosch; VieCurie Medical Centre, Venlo; Radboud University Nijmegen Medical Centre, Nijmegen; and Waikato Hospital, Hamilton, New Zealand. The laboratory research was performed at the Department of Pharmacology and Toxicology, Nijmegen Centre for Molecular Life Sciences; and at the Molecular Genetics Laboratory, University of Waikato, New Zealand.

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Novel hemodynamic therapies in septic shock; Effects on circulation, organ perfusion and immune system

Een wetenschappelijke proeve op het gebied van de Medische Wetenschappen

Proefschrift

ter verkrijging van de graad van doctor aan de Radboud Universiteit Nijmegen op gezag van de rector magnificus, prof. mr. S.C.J.J. Kortmann, volgens besluit van het College van Decanen in het openbaar te verdedigen op donderdag 22 April 2010 om 15.30 uur precies

door

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Part of this research project was financially supported by a grant from the Waikato Medical Research Foundation, grant number WMRF 127.
Nature and Nature’s laws lay hid in night;
God said ‘Let Newton be!’ And all was light.

Alexander Pope (1688-1744)
Epitaph, intended for Sir Isaac Newton

It did not last: the Devil howling ‘Ho!
Let Einstein be!’ Restored the status quo.

Sir John Collins Squire (1884-1958)

Courtesy of Colin L. Davey, MSc (Otago) PhD (Cantab.), biochemist
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INTRODUCTION

Sepsis: The context

Sepsis is the leading cause of death in critically ill patients. The yearly incidence is increasing, and more than 18 million cases of sepsis occur worldwide each year. Sepsis kills some 1,400 people worldwide every day. The mortality rates associated with sepsis are extremely high: 30% to 50% for severe sepsis and 50% to 60% for septic shock according to recent epidemiological studies. This imposes a huge burden on modern healthcare systems. Cost-of-illness studies focusing on direct costs per sepsis patient have yielded estimates between €20,000 and €30,000. Indirect costs arising from loss of productivity losses due to mortality are likely to be 3-4 times higher than the direct costs. In addition to the economical aspects, the loss of lives and long-term physical and psychological sequelae represent a significant burden in terms of human suffering.

Susceptibility to and outcome from infectious disease is heritable. For example, it has been shown that the genetic contribution to death from infection is five times greater than the genetic contribution to cancer. The promise that the age of genomic personalized medicine is within our reach may be true, but in the meantime optimization of currently available treatment approaches seems necessary.

Uniform definitions have been adopted for the spectrum of the sepsis syndrome, including the systemic inflammatory syndrome (SIRS), sepsis, severe sepsis, and septic shock. International treatment guidelines based on best available evidence have been developed and implemented worldwide. However, our understanding of the complex pathophysiology of sepsis remains incomplete, and therefore our approach towards diagnosis and treatment may often be ineffective or insufficient. Sepsis is characterized by complex abnormalities of the immune system and coagulation pathways. The hemodynamic changes found in septic shock, the most severe form of sepsis, include severe vasodilation, myocardial depression and impaired microcirculatory blood flow, resulting in redistribution of regional blood flow. If uncorrected, these perfusion abnormalities rapidly lead to multiple organ dysfunction and death.
New therapies and treatment strategies for severe sepsis are constantly being developed and investigated in an attempt to decrease the mortality as well as the short and long-term morbidity associated with severe sepsis.

**Gastrointestinal perfusion in sepsis**

Gastrointestinal mucosal hypoperfusion is thought to be important in septic shock patients, both as an indicator of inadequate resuscitation and potentially as a mechanism by which multiorgan failure may occur. Loss of gut barrier function may lead to translocation of bacteria, endotoxin, and other inflammatory mediators, thereby increasing systemic inflammation, and resulting in distant organ dysfunction. Most experimental sepsis models show intestinal microvascular vasoconstriction and hypoperfusion. The observed changes in microcirculatory blood flow in splanchnic organs are quite heterogeneous, both in early hypodynamic and in resuscitated hyperdynamic septic shock. Different control mechanisms have been implicated in this impairment of mucosal vascular perfusion. Abnormalities in the nitric oxide system induced by inflammation are considered to be one of the mechanisms responsible for the observed distributive defects. Cellular hypoxia resulting from these perfusion abnormalities, combined with the direct cytotoxic effects of inflammatory mediators, may result in increased gut permeability and impaired immunological gut barrier function. This in turn could lead to a vicious circle of augmentation of the inflammatory response and distant organ dysfunction: “The gut as motor of sepsis”. For example, hemorrhagic shock can induce the gut to become a cytokine-generating organ. In vitro studies have documented that mesenteric lymph, following hemorrhagic shock, activates neutrophils, is toxic to endothelial cells, and increases endothelial permeability. In other experiments, ligation of intestinal lymphatic flow prevented lung injury after hemorrhagic shock. In a recent porcine study, mesenteric lymph collected following hemorrhagic shock increased neutrophil activation and endothelial cell permeability as opposed to lymph from control animals. These results indicate that the lymphatic route could be the primary route by which gut injury causes distant organ injury.

Global hemodynamic measurements are routinely performed in septic shock patients and are used to guide fluid resuscitation and administration of vasoactive
agents. However, these measurements do not provide reliable data on gut perfusion, either because of regional redistribution of blood flow, or because gut blood flow may paradoxically be preserved when cardiac output is decreased. Conversely, therapy used to treat shock can apparently normalise clinical or global variables, but still allow the existence of occult defective tissue oxygenation. Measuring variables of splanchnic perfusion is thought to be a better predictor of the presence of uncompensated shock than markers of global perfusion. Abnormal gastrointestinal perfusion and failure to normalize these perfusion deficits is a strong predictor of poor outcome of sepsis. Sepsis treatment has variable and often unknown effects on the gastrointestinal perfusion. For example, a vasoconstrictive drug may increase the perfusion pressure of a vascular bed but at the same time decrease the actual flow. This could have potential detrimental effects on cellular oxygen delivery, organ perfusion and function. More importantly, currently there are no treatment strategies available that are able to improve gastrointestinal perfusion, and by doing so, improving clinical outcome parameters such as organ failure and mortality.

Different measurement techniques and tools have been developed and investigated to study the gastrointestinal perfusion. Tonometry is the most widely used and most accessible technique for this purpose. With this minimally invasive technique, intraluminal partial pressure of CO₂ is measured. Most studies have applied tonometry in the stomach, but other sites such as the jejunum, the rectum, and sigmoid colon have been examined as well. The difference between intraluminal and arterial partial pressures of CO₂ is called the CO₂ gap or the CO₂ gradient, which should be smaller than 10 mmHg. An increased gradient is indicative of gut hypoxia, especially when the gradient is greater than 20 mmHg. Ischemic hypoxia due to hypoperfusion results in a more pronounced increase in pCO₂ gradient than hypoxic hypoxia or anaemic hypoxia. The pCO₂ gap is therefore largely dependent on mucosal perfusion and is considered to be a valid measure for gastrointestinal metabolism perfusion ratio.

The prognostic value of tonometry has been well established. In an inception cohort study in 95 ventilated critically ill patients, the pCO₂ gap measured 24 hours after admission was an independent risk factor and marker for mortality.
Septic shock therapies

Standard septic shock therapy includes supportive treatment such as fluid resuscitation, administration of vasopressors (adrenergic and nonadrenergic drugs), and respiratory and renal support. These therapies may have beneficial or detrimental effects not only on systemic hemodynamics but also on splanchnic hemodynamics, at both the macrocirculatory and microcirculatory levels.

Vasopressors

Vasopressors are necessary to treat the vasodilation characteristic of hypotension in septic shock. The effect of conventional vasopressors (noradrenaline, dopamine, adrenaline) on mucosal microcirculatory blood flow is not straightforward. Vasoconstrictors can potentially have detrimental effects on regional perfusion, microcirculation and gastrointestinal mucosal perfusion. Normalization of global hemodynamic values therefore does not automatically guarantee an improvement in cellular oxygen delivery. The actual effect is dependent on the drug used, the clinical situation, and whether a patient has been adequately fluid resuscitated. Different drugs have different effects on the gastrointestinal mucosal perfusion.

The available clinically relevant data on the effects of "non-conventional" vasopressors such as vasopressin, methylene blue, and potassium channel blockers, on splanchnic circulation is very limited, and their safety has not been demonstrated. All these drugs can modify the perfusion and metabolism of splanchnic organs, changes which cannot be predicted from changes in systemic circulation or metabolism.

Vasopressin is a relatively new drug that has found its use in septic shock treatment as a potent vasoconstrictor. Vasopressin increases blood pressure, improves some measures of renal function and decreases catecholamine requirements. Despite its favourable effect on global hemodynamics in septic shock patients, few clinically relevant data are available on the effect of vasopressin on the splanchnic circulation in sepsis. Interestingly, it has been widely used to control the bleeding in patients with bleeding oesophageal varices because of its
vasoconstrictive effects on the splanchnic circulation in that specific clinical scenario.

Methylene blue (MB) has been shown to have vasocostrictive properties in sepsis. Several small studies in septic patients, including two prospective randomized controlled studies, have demonstrated an increase in blood pressure mediated by an increase in systemic vascular resistance (SVR). An important mechanism that is potentially responsible for the effects of MB infusion is selective inhibition of production of nitric oxide (NO) and cyclic guanosine monophosphate (cGMP). Abnormalities in the NO system can be regarded as one of the mechanisms responsible for the gastrointestinal mucosal perfusion defects observed. Animal studies have shown that NO acts as a final common pathway of mediators and neural pathways in the gastrointestinal tract and also is a major inhibitory component of gastrointestinal function.

**Fluid resuscitation**

Adequate fluid resuscitation is considered to be an essential part of sepsis treatment. For example, early goal-directed resuscitation of severe sepsis and septic shock has been shown to reduce in-hospital mortality from 47% to 31%. The choice of the type of resuscitation fluid is not straightforward and subject of much debate. Resuscitation fluids are not innocent bystanders, but impact on organ dysfunction and outcome. Recent examples include the potential increase in mortality associated with the use of albumin, and an increase in the incidence of renal failure needing dialysis in septic patients with the use of a certain type of colloid (10% pentastarch).

Hypertonic fluid resuscitation has been studied extensively in experimental and human traumatic hemorrhagic shock. The macrocirculatory effects of hypertonic infusion in hemorrhagic shock are characterized by rapid plasma volume expansion. Experimental evidence also suggests a possible improvement of myocardial contractility. Hypertonic fluids have been shown to cause hemodilution and to reduce shock-induced endothelial and red blood cells oedema, increasing blood flow at the capillary level by reducing viscosity and hydraulic resistance. In addition to immediate blood volume expansion and
restoration of cardiac output in animal models of hemorrhagic shock, hypertonic fluid administration has been shown to correct microcirculatory alterations as assessed by intravital microscopy. Data concerning the effects of hypertonic fluids in septic patients are scarce. Although several previous studies in patients with sepsis have shown transient improvements of global perfusion, its effect on gastrointestinal perfusion, microcirculatory abnormalities, and the immune system in septic shock patients is currently unknown.

**Thesis**

The main aim of this thesis was to investigate the effects of different novel hemodynamic sepsis treatments on the gastrointestinal perfusion. However, because an intervention to improve the hemodynamic status in sepsis often has several other effects e.g. on other regional vascular beds, on the microcirculation, and on the immune system, we expanded our methods to include relevant other measurements, as to further improve our understanding of the effects and potential of these treatments.

In part 1, an overview is presented on the role the gastrointestinal perfusion plays in sepsis. Its place in the complex pathophysiology of septic shock and multi-organ failure is discussed, as well as its potential target for treatment and intervention. Currently available measurement tools and techniques to evaluate gastrointestinal perfusion are being reviewed.

In part 2, we examine the effects of the novel vasopressor agents vasopressin and methylene blue in septic shock patients. In chapter 2.1 we describe a study that aims to quantify the effects of vasopressin on the gastrointestinal perfusion by using gastric tonometry. In chapter 2.2, the effects of methylene blue on gastric tonometry and on intestinal mucosal cellular damage are investigated. In addition, the effect of MB on the production of nitric oxide and on kidney damage is described in chapter 2.3.

In part 3, we compare hypertonic fluid with isotonic fluid administration in septic shock patients. The global cardiovascular effects and differences are described in chapter 3.1. In chapter 3.2, we examine whether hypertonic fluid exerts intrinsic
effects on the gastrointestinal perfusion as assessed by gastric tonometry, and on the sublingual microcirculation as assessed by Sidestream Dark Field (SDF) imaging. To further understand the in vivo effects of hypertonic fluid administration in sepsis, we describe the immunomodulating effects by measuring inflammatory mediator gene expression using real-time reverse transcriptase polymerase chain reaction (RT rtPCR) in chapter 3.3.

The thesis ends with a summary.
References


Gastrointestinal perfusion in septic shock


PART I

GASTROINTESTINAL PERFUSION
IN SEPTIC SHOCK

Effects on circulation, organ perfusion and immune system
NOVEL HEMODYNAMIC THERAPIES IN SEPTIC SHOCK;
Gastrointestinal Perfusion in Septic Shock

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Summary

Septic shock is characterized by vasodilation, myocardial depression, and impaired microcirculatory blood flow, resulting in redistribution of regional blood flow. Animal and human studies have shown that gastrointestinal mucosal blood flow is impaired in septic shock. This is consistent with abnormalities found in many other microcirculatory vascular beds. Gastrointestinal mucosal microcirculatory perfusion deficits have been associated with gut injury and a decrease in gut barrier function, possibly causing augmentation of systemic inflammation and distant organ dysfunction.

A range of techniques have been developed and used to quantify these gastrointestinal perfusion abnormalities. The following techniques have been used to study gastrointestinal perfusion in humans: tonometry, laser Doppler flowmetry, reflectance spectrophotometry, near-infrared spectroscopy, orthogonal polarization spectral imaging, indocyanine green clearance, hepatic vein catheterization, and measurements of plasma D-lactate. Although these methods share the ability to predict outcome in septic shock patients, it is important to emphasize that the measurement results are not interchangeable. Different techniques measure different elements of gastrointestinal perfusion. Gastric tonometry is currently the most widely used technique because of its non-invasiveness and ease of use.

Despite all the recent advances, the usefulness of gastrointestinal perfusion parameters in clinical decision-making is still limited. Treatment strategies specifically aimed at improving gastrointestinal perfusion have failed to actually correct mucosal perfusion abnormalities and hence not shown to improve important clinical endpoints.

Current and future treatment strategies for septic shock should be tested for their effects on gastrointestinal perfusion; to further clarify its exact role in patient management, and to prevent therapies detrimental to gastrointestinal perfusion being implemented.
**Introduction**

Intestinal mucosal hypoperfusion is thought to be important in septic shock patients, both as an indicator of inadequate resuscitation and potentially as a mechanism by which multiorgan failure may occur. Loss of gut barrier function may lead to translocation of bacteria, endotoxin, and other inflammatory mediators, thereby increasing systemic inflammation, and resulting in distant organ dysfunction.

*Global* hemodynamic measurements are routinely performed in septic shock patients and are used to guide fluid resuscitation and administration of vasoactive agents. However, these measurements do not provide reliable data on gut perfusion, either because of regional redistribution of blood flow, or because gut blood flow may paradoxically be preserved when cardiac output is decreased. Conversely, therapy used to treat shock can apparently normalise clinical or global variables, but still allow the existence of occult defective tissue oxygenation. Measuring variables of splanchnic perfusion is thought to be a better predictor of the presence of uncompensated shock than markers of global perfusion.

In this review we will discuss the splanchnic circulation and the different techniques that are available to measure adequacy of gastrointestinal perfusion. We specifically focus on the gastrointestinal perfusion deficits found in septic shock, including relevance for the pathogenesis of multiple organ dysfunction, and for prognosticating and treating septic patients.

References were obtained from Pubmed and Medline databases from the earliest records to November 2006. We used the following keywords: intestinal, gastrointestinal, splanchnic, perfusion, blood flow, circulation, sepsis, septic shock, vasodilatory shock, shock, and microcirculation. We also reviewed reference lists of all relevant articles. Laboratory, animal, as well as human studies were included to describe the underlying pathophysiological mechanisms implicated in gastrointestinal perfusion abnormalities in septic shock. We then focused on studies describing techniques that have been used to measure gastrointestinal perfusion in human septic shock situations. Retrospective as well as prospective studies that reported mortality were included to describe the prognostic value of measurement of gastrointestinal perfusion in septic patients. Only prospective controlled studies were included to determine the effects of treatment guided by gastrointestinal perfusion on outcome of septic patients. Finally, animal and human studies reporting the effects of various interventions
on gastrointestinal perfusion are discussed, as to give an overview of potential
treatment strategies that need further studies.

Regulation of gastrointestinal perfusion

Splanchnic blood flow, at both macrovascular and microvascular levels of
perfusion, is regulated to perform two basic gastrointestinal functions: to digest
and absorb ingested nutrients, and to sustain barrier function to prevent
transepithelial migration of bacteria and antigens.

Three direct branches of the aorta supply the human gastrointestinal system:
the celiac artery, the superior mesenteric artery, and the inferior mesenteric artery.
Total blood flow in this system consumes approximately 25% of cardiac output.
Following ingestion of food, blood flow increases by as much as 200% for 2-3
hours. The mucosal layer receives 70-80% of the total blood flow, and is capable
of rapidly recruiting closed capillaries. Blood flow to the mucosa is autoregulated
by metabolic factors such as decreases in pO2, pH, or osmolarity, increases in
pCO2, or adenosine. Other potential vasoactive mediators of the enteric circulation
are neural mediators (e.g. sympathetic and parasympathetic tone), circulating
humoral mediators (e.g. vasopressin, adrenomedullin, catecholamines), and
paracrine and autocrine mediators (e.g. nitric oxide, endothelin-t)

Gastrointestinal perfusion in septic shock

Septic shock is characterised by cardiac depression, vasodilation, and
microcirculatory defects resulting in redistribution of regional blood flow. In a
broader perspective, severe dysfunction of the microcirculation in sepsis has been
well described and classified.

The metabolic demand for oxygen in the splanchnic region during sepsis is
increased, partly by an increased hepatic metabolism. In hyperdynamic shock
states, mucosal vascular perfusion is compromised, despite decreased total
peripheral resistance and increased total splanchnic blood flow. This appears to
be comparable to the situation in post-cardiac surgery patients, where there is no
consistent association between local intestinal mucosal perfusion and global
splanchnic blood flow. Most experimental sepsis models show intestinal
microvascular vasoconstriction and hypoperfusion. The observed changes in microcirculatory blood flow in splanchnic organs are quite heterogeneous, both in early hypodynamic and in resuscitated hyperdynamic septic shock. In early septic shock, autoregulation of microcirculatory mucosal blood flow is largely intact, and blood seems to be diverted from the muscularis towards the mucosa. Acute bacteraemia causes both endothelial alterations and vascular smooth muscle cell changes. Vasomotion (a normally occurring rhythmic process of dilation and contraction) is impaired in magnitude and frequency in both inflow and premucosal arterioles following E. coli infusion.

Different control mechanisms have been implicated in this impairment of mucosal vascular perfusion. Abnormalities in the nitric oxide system induced by inflammation can be regarded as one of the mechanisms responsible for the distributive defects observed. Local intestinal oxygen-derived free radicals also play a role in the intestinal microvascular sequelae; as the administration of lazaroids, which are antioxidants that scavenge radicals and block lipid radical chain reactions, prevents vasoconstriction in both inflow and premucosal arterioles in an animal sepsis model. Gene expression of endothelin 1, a potent vasoconstrictor, is upregulated in sepsis models and implicated in the shift towards a more tonically constricted state. Other mediators that have been associated with the observed mucosal perfusion abnormalities in sepsis include platelet-activating factor and adrenomedullin.

Cellular hypoxia from these perfusion abnormalities and from possible mitochondrial dysfunction, combined with the direct cytotoxic effects of inflammatory mediators, is thought to result in increased gut permeability and impaired immunological gut barrier function. Under ischemic conditions, enhanced paracellular permeability and epithelial destruction increases the mucosal permeability for endotoxin in human endothelial cell lines, an effect that has also been described in vivo in rats.

An impaired gut barrier is thought to play a role in subsequent amplification of systemic inflammation and distant organ dysfunction. Numerous animal experiments have shown the importance of bacterial translocation and systemic spill of mediators in the pathogenesis of multiple organ dysfunction syndrome. Haemorrhagic shock can induce the gut to become a cytokine-generating organ. In vitro studies have documented that mesenteric lymph, following haemorrhagic shock, activates neutrophils, is toxic to endothelial cells, and increases endothelial
permeability. In other experiments, ligation of intestinal lymphatic flow prevented lung injury after haemorrhagic shock. In a recent porcine study, mesenteric lymph collected following haemorrhagic shock increased neutrophil activation and endothelial cell permeability as opposed to lymph from control animals. These results indicate that the lymphatic route could be the primary route by which gut injury causes distant organ injury, and could explain the reported absence of detectable bacteraemia in human studies of increased gut permeability. However, in one case series several cases of Saccharomyces boulardii fungemia have been described in critically ill patients who received this agent enterally as a probiotic, possibly as a result of translocation.

Increased gut permeability has been found in sepsis, pancreatitis, trauma, burns, and following cardiopulmonary bypass surgery. Some of these studies used differential absorption of various polysaccharides of differing molecular weights to estimate gut permeability. Specific pitfalls to the use of these tests in ICU patients have been described elsewhere. The association between splanchnic mucosal perfusion and systemic inflammation has been studied in different patient groups. In septic shock patients, the gastric to arterial pCO₂ gap correlated well with systemic levels of tumour necrosis factor-alpha and interleukin-6, indicating that gut injury and the inflammatory response are associated. In patients following cardiopulmonary bypass surgery (characterized by a systemic inflammatory response) gastrointestinal permeability increased despite normal global splanchnic blood flow and oxygen delivery.

Intestinal mucosal injury is associated with a poor outcome in critically ill patients. In one small study, levels of serum and urine intestinal fatty acid binding protein were measured, which are sensitive and specific markers for intestinal mucosal injury. The presence of detectable intestinal fatty acid binding protein was associated with a poor prognosis.

Techniques to measure gastrointestinal perfusion

Gastrointestinal perfusion can be measured in many different ways. Some techniques are able to directly measure intestinal mucosal perfusion (e.g. laser Doppler studies of blood flow); other techniques measure endpoints of oxygen delivery (e.g. lactate measurement with intraluminal microdialysis). Some techniques have only been used in animal studies and are therefore not discussed.
Table 1
Summary of techniques to measure gastrointestinal perfusion in patients

<table>
<thead>
<tr>
<th>Method</th>
<th>Variable</th>
<th>Advantages</th>
<th>Limitations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tonometry</td>
<td>Gastric-arterial pCO₂ gap, mucosal-end tidal pCO₂ gap, pH</td>
<td>Minimally invasive; measure of mucosal perfusion; validated; reproducible; commercially available</td>
<td>Limited by site of measurement; gastric feeding interferes with measurement; requires equilibration (no real time monitoring)</td>
</tr>
<tr>
<td>Laser Doppler flowmetry</td>
<td>Mucosal hematocrit, mucosal red blood cells velocity, blood flow in arbitrary perfusion units</td>
<td>Useful to evaluate endothelium-dependent vascular responses; real-time velocity measurement</td>
<td>Small sampling volume for blood flow measurement; does not reflect heterogeneity of blood flow; no measurement of absolute blood flow</td>
</tr>
<tr>
<td>Reflectance spectrophotometry</td>
<td>Tissue microvascular haemoglobin oxygen saturation, relative haemoglobin concentration</td>
<td>Estimates adequacy of oxygen delivery; commercially available; real time monitoring possible</td>
<td>Not a measure of blood flow; endoscopic introduction; blind probes need validation; luminal contents interfere with measurement</td>
</tr>
<tr>
<td>Near-infrared spectroscopy</td>
<td>Quantitative tissue oxygenation index (ratio oxygenated haemoglobin to total haemoglobin)</td>
<td>Minimally invasive; measures tissue oxygenation</td>
<td>Penetrates into muscularis layer; nasogastric probes not tested in human studies; transcutaneous liver oxygenation measurement not feasible in adults</td>
</tr>
<tr>
<td>Orthogonal polarization spectral imaging</td>
<td>Functional capillary density, percentage of perfused small vessels, semi-quantitative blood flow</td>
<td>Easy; non-invasive; reproducible; commercially available</td>
<td>Semi-quantitative; sublingual measurements may not represent splanchnic perfusion; operator-dependent</td>
</tr>
<tr>
<td>Indocyanine green clearance</td>
<td>Plasma disappearance rate ICG</td>
<td>Commercially available; measures splanchnic perfusion as well as global liver function</td>
<td>Multiple variables determine results (hepatic blood flow, hepatocellular uptake, excretion into bile)</td>
</tr>
<tr>
<td>Hepatic vein catheterization</td>
<td>Lactate, venous saturation, indocyanine green extraction</td>
<td>Allows measurement oxygen and indocyanine-green extraction</td>
<td>Invasive; requires radiological guidance; dual hepatic blood flow limits use</td>
</tr>
<tr>
<td>Plasma D-lactate</td>
<td>Plasma D-lactate levels</td>
<td>Easy; correlates with gastric tonometry results</td>
<td>Assay not commonly available; not a direct measure of gastrointestinal perfusion; unable to detect rapid changes in perfusion</td>
</tr>
</tbody>
</table>
in detail. Table 1 summarizes currently available techniques and the advantages and limitations for their use in septic patients.

**Tonometry**
There is now more than 20 years experience with tonometry in humans. With this minimally invasive technique, intraluminal partial pressure of CO\(_2\) is measured.

Most studies have applied tonometry in the stomach, but other sites such as the jejunum, the rectum, and sigmoid colon have been examined as well. The difference between intraluminal and arterial partial pressures of CO\(_2\) is called the CO\(_2\) gap or the CO\(_2\) gradient, which should be smaller than 10 mmHg. An increased gradient is indicative of gut hypoxia, especially when the gradient is greater than 20 mmHg. Ischemic hypoxia due to hypoperfusion results in a more pronounced increase in pCO\(_2\) gradient than hypoxic hypoxia or anaemic hypoxia \(^{31,32}\). The pCO\(_2\) gap is therefore largely dependent on mucosal perfusion and is considered to be a valid measure for gastrointestinal perfusion \(^{33}\). The use of the pCO\(_2\) gap has replaced the use of mucosal pH (pHi, calculated from luminal pCO\(_2\) and blood bicarbonate content) because of increased sensitivity and specificity for regional ischemia \(^{34,35}\). Saline tonometry has been replaced with automated air tonometry because of technical problems, including long equilibration time, variability and lack of quality in the determination of saline pCO\(_2\), which is discussed elsewhere in more detail \(^{36-38}\).

The variability of gastric pCO\(_2\) in intensive care patients has been compared with systemic hemodynamic parameters. In patients with acute respiratory or circulatory failure, the coefficient of variation for gastric-arterial pCO\(_2\) gradient was 15%, compared to 10% for thermodilution cardiac output measurements \(^6\). One of the limitations of tonometry is that it only provides information of the site of measurement (e.g. gastric perfusion), which is important in view of the known heterogeneity in microvascular perfusion within the gastrointestinal tract.

**Laser Doppler flowmetry**
Laser Doppler flowmetry provides continuous measurement of microcirculatory blood flow. The principle of this method is the Doppler shift, the frequency change that light undergoes when reflected by moving objects e.g. red blood cells \(^{39}\). The output voltage varies linearly with the product of mean red blood cell velocity and red blood cell concentration. This is referred to as red cell flux, which is
proportional to blood flow at all but very high haematocrits. The tissue penetration of the laser is approximately 1-3 mm, allowing the study of mucosal blood flow without interference from the greater muscularis blood flow. Due to variable optical properties of different tissues, absolute blood flow cannot be measured. Laser Doppler devices indicate microcirculatory blood flow in arbitrary perfusion units. The results are usually expressed as changes relative to baseline. It has been used to measure microcirculatory blood flow in many tissues including muscle and intestine. As a non-invasive instrument, it can also provide information on endothelium-dependent vascular responsiveness in the skin microcirculation.

To assess intestinal mucosal perfusion in humans, custom-made laser Doppler catheters have been developed for placement in the lumen of the proximal jejunum under fluoroscopic guidance. Jejunal mucosal perfusion is calculated as the product of jejunal mucosal hematocrit and red blood cells velocity, both of which are measured variables. For example, this technique has been used to study the effects of different vasoconstrictors on jejunal mucosal perfusion after cardiac surgery. In a prospective randomized crossover study in 10 patients, noradrenaline and phenylephrine were randomly and sequentially infused to increase mean arterial blood pressure by 30%. Neither of the vasoconstrictors asserted a significant effect on jejunal mucosal perfusion, or on gastric to arterial pCO₂ gradient.

Positioning of the probes is essential to ensure continuous and steady contact with the surface of the measurement site. The technique gives an average of blood velocities and does not take into account the heterogeneity of blood flow in the window studied, making it less suitable for monitoring of gut perfusion in septic patients.

Reflectance spectrophotometry
The technique of reflectance spectrophotometry for assessment of gastric mucosal blood flow was first described by Sato et al. in 1979. This optical technology uses white light (wavelengths between 390 nm and 780 nm) to illuminate tissues. The light that returns (reflects) to the detector is analyzed quantitatively. Using visible light, the recovered signal is predominantly due to haemoglobin absorption in the superficial 0.25 mm of tissue. To measure mucosal microvascular haemoglobin oxygen saturation and relative haemoglobin concentration, light from a xenon high-pressure arc lamp is transmitted to the mucosal surface using a single flexible microlightguide. This microlightguide is introduced through the
operation canal of a gastroscope, and attached to the mucosal surface under visual control. With newer commercially available instruments, it is also possible to obtain a stable signal without endoscopy by using a probe that is embedded in a nasogastric or rectal tube. Validation studies applying these probes in patients are awaited.

In typical gastrointestinal mucosa, the average haemoglobin saturation is approximately 70%. The microvascular haemoglobin oxygenation reflects the balance between regional oxygen delivery and oxygen uptake. Decreased values may be caused by decreased delivery, enhanced uptake, or a combination of both. This technique has been applied to measure intestinal mucosal perfusion in ulcer disease, portal hypertension, cardiopulmonary bypass, septic shock, and in response to infusion of vasoactive agents. For example, septic shock patients showed a decrease in average gastric mucosal haemoglobin oxygen saturation to 51%, compared to 70% in healthy controls. Infusion of dopexamine increased mucosal saturation in the septic patients by an average of 10%.

Because reflectance spectrophotometry measurements are easily obtained several times per second, real-time monitoring of perfusion is possible. However, and in contrast to laser Doppler flowmetry, reflectance spectrophotometry does not measure blood flow, but provides an estimate of the adequacy of oxygen delivery to the mucosa. Several factors can impact the quality of measurements, such as the presence of optically active materials in the lumen (e.g. bile, stool, blood), the effect of pressure when the probe touches the mucosa, and the endoscope light. Newer systems correct for an uneven baseline and obtain measurements without touching the mucosa.

**Near-infrared spectroscopy**

Near-infrared spectroscopy is another non-invasive optical technique to measure tissue oxygenation. Near-infrared light penetrates more deeply into tissues than visible light, therefore enabling measurements in the muscularis propria or deeper structures. Tissue penetration is directly related to the spacing between illumination and detection fibres. At 25 mm spacing approximately 95% of the reflected optical signal is from a depth of 0-23 mm. Absorption and hence reflection of near-infrared light depends on the oxygenation state of haemoglobin. Each tissue type has a so-called path length through which the near-infrared light travels. The absolute concentrations of oxygenated and deoxygenated haemoglobin cannot be measured without knowledge of this path.
length. This problem has been overcome by the development of newer generations of near-infrared spectrometers, often referred to as spatially resolved spectroscopy, allowing additional measurement of a quantitative tissue oxygenation index, which represent the ratio of oxygenated haemoglobin to total haemoglobin.

This technique has been applied in superficial muscles as a non-invasive measure of peripheral perfusion in haemorrhagic and septic shock. It has also been used to measure liver tissue oxygenation transcutaneously in critically ill children, and a good correlation ($r=0.72$, $p<0.0001$) with invasively measured central venous oxygen saturation was shown. However, there are several limitations to the latter observations that prevent this tool from being generally applicable in the measurement of hepatosplanchnic perfusion in septic patients. Measurements of single point liver tissue oxygenation show a large inter-individual variation. Also, in adults, the liver is usually not as accessible as in small children, and differences in subcutaneous fat and oedema may result in significant inter-individual differences in measured liver tissue oxygenation. Furthermore, liver oxygenation may not be a good marker for splanchnic perfusion because of the dual hepatic blood supply and lack of autoregulation.

In animal studies, side-illuminating near-infrared spectroscopy nasogastric probes have been shown to rapidly reflect changes in splanchnic perfusion. In an experimental haemorrhagic shock model, bowel pH obtained with intraluminal near-infrared spectroscopy correlated well with pH measured with microelectrodes. Human studies of intraluminal near-infrared spectroscopy in septic shock are currently awaited.

**Orthogonal polarization spectral (OPS) imaging**
This recently developed and non-invasive technique uses reflected light to produce real-time images of the microcirculation. It allows microscopic visualization of the microcirculation as well as of the flow of red blood cells in the microvessels. In order to improve spatial resolution and allow for better visualization of the smallest capillaries, an improved imaging modality called sidestream dark-field imaging has been developed. With this technique, the light guide is surrounded by 530 nm light-emitting diodes. Because this wavelength is absorbed by the haemoglobin of red blood cells, these cells can be seen as dark cells flowing in the microcirculation.

Tissue perfusion is assessed and quantified by using the functional capillary
density, which is the length of perfused capillaries per observation area in cm/cm², and by semi-quantitative measurement of blood flow velocities in capillaries. Although the technique can be used to visualize microcirculation in many organs (e.g. during surgery), it is most commonly applied sublingually. Hand-held devices are commercially available. Limitations include artefacts secondary to movement and secretions, and observer-related bias including the amount of pressure that is used to obtain the images. Nevertheless, in a recent validation study, agreement and kappa coefficients were >85% and >0.75, respectively, for interrater and intrarater variability in quantification of flow abnormalities in vascular beds of the sublingual and stoma region during sepsis. Interpretation of the obtained images is time-consuming, and software has been developed to analyze and quantify the images.

Although the oropharynx can be considered as part of the gastrointestinal system, perfusion of the tongue is regulated in a complex way, which is different from the way the splanchnic perfusion is controlled. Sublingual OPS imaging is therefore not a direct measure of gastrointestinal perfusion. However, OPS-derived measurements have been shown to correlate well with measurements of sublingual capnometry and gastric tonometry in septic patients, but more validating studies are needed.

**Indocyanine green clearance**

Global liver function and splanchnic perfusion can be quantified with measurements of indocyanine green dye clearance. After venous injection, indocyanine green is eliminated unchanged by the liver into the bile without enterohepatic recirculation. Elimination of indocyanine green is determined by hepatic blood flow, hepato-cellular uptake, and excretion into the bile. After administration of a bolus of indocyanine green, the blood clearance can be calculated by taking repeated venous samples or by using intra-arterial fibreoptic spectroscopic measurements. Alternatively, the plasma disappearance rate can be assessed transcutaneously, with comparable results to venous sampling. The major advantage of the latter is that it is a non-invasive technique. Normal values in healthy control subjects for indocyanine green clearance and for indocyanine green plasma disappearance rate are > 700 ml/min/m² and 18%/min, respectively.

As a method to measure splanchnic perfusion, this technique has several limitations. Results are not only dependent on perfusion, but also on hepatic uptake and excretion, which makes it difficult, to determine what exactly is being
measured. Estimated hepatic blood flow calculated from systemic indocyanine
green clearance correlates poorly with values obtained using hepatic vein
catheterization and the Fick principle, because of the variability in dye extraction 6.

**Hepatic vein catheterization**
Monitoring of hepatic vein oxygen saturation is invasive, and involves the
insertion of a hepatic catheter. Fluoroscopic or ultrasound guidance is commonly
necessary for adequate insertion. A new technique for blind insertion has been
described, using galactose infusion combined with a specific biosensor 53. The
use of these catheters appears to be safe. Several studies have documented that
the gradient between mixed-venous and hepatic vein saturation is commonly
increased in septic shock 54. However, as this measurement reflects portal and
hepatic arterial flow, maintained hepatic venous oxygen saturation does not
exclude gut hypoperfusion. Hepatic vein lactate measurements can be used to
detect splanchnic hypoxia, with similar limitations.

Hepatic blood flow can also be determined by hepatic venous sampling during
constant infusion of indocyanine-green, therefore enabling the calculation of the
indocyanine-green extraction, as discussed earlier. The coefficient of variation for
splanchnic blood flow in septic shock patients using hepatic vein catheterization
derived indocyanine green clearance measurements was 9% 55. In another study
using the same technique, the standard error for repeated measurements was
31% 56.

**Plasma D-lactate**
Measurement of plasma D-lactate has been suggested to be a marker of
splanchnic hypoperfusion. D-lactate is produced by bacterial fermentation in the
colonic lumen and subsequently absorbed into the blood. Bacterial overgrowth
and increased gut permeability are both features of gastrointestinal dysfunction
during septic shock. During hypoperfusion in the gut, both L-lactate and D-lactate
are produced in increased amounts. Because humans lack the enzyme D-lactate
dehydrogenase, liver metabolism of D-lactate is slower than that of L-lactate. D-
lactate may therefore reflect the intestinal perfusion more closely than L-lactate.

Increased D-lactate levels were found in acute intestinal ischemia, acute
pancreatitis, and severe burns 57-60. In a study in 20 septic shock patients, D-
lactate levels but not L-lactate levels correlated significantly with gastric
tonometry results, indicating that D-lactate is a better marker for splanchnic
hypoperfusion and increased splanchnic luminal CO₂ production than L-lactate in septic patients. These results need to be reproduced by subsequent studies. Further clinical validation of the usefulness of this assay is needed. This technique may not be able to rapidly reflect changes in gastrointestinal perfusion (e.g. to study specific interventions) because of the relatively long half-life of D-lactate. Current clinical use is also limited by the fact that the D-lactate assay is not available in most institutions.

**Future techniques**

Other techniques are being developed to measure gastrointestinal (mucosal) perfusion or oxygenation. Currently, these methods have limited use in humans, and as such are not within the scope of this review. Examples include contrast-enhanced ultrasonography and intestinal luminal microdialysis.

**Conclusion**

Different techniques have been developed to measure gastrointestinal perfusion. Realistically, most methods are actually indirect estimates of gastrointestinal perfusion. The clinical usefulness and relevance of the different techniques is dependent on the problem that is being investigated, and results of different methods can sometimes appear seemingly conflicting (e.g. normal global splanchnic blood flow does not equal adequate oxygen delivery to the cells). Also, there is no technique that is considered the ‘gold standard’.

In sepsis, heterogeneity in microcirculatory flow seems to be a key characteristic. Interruption or decrease of red blood cell velocity, as well as hyperdynamic microcirculatory flow patterns (shunting) has been observed. Techniques that evaluate the adequacy of mucosal oxygenation (e.g. reflectance spectrophotometry and tonometry) are likely to better reflect the microcirculatory disturbances in sepsis than techniques that simply measure flow (e.g. laser Doppler flowmetry). Orthogonal polarization spectral imaging is a useful technique to quantify microcirculatory abnormalities in sepsis, but studies are awaited to further correlate sublingual measurements with gastrointestinal mucosal perfusion.
Table 2
Clinical studies reporting the effects of splanchnic perfusion abnormalities on mortality in critically ill patients

<table>
<thead>
<tr>
<th>Reference (year)</th>
<th>n</th>
<th>Patients</th>
<th>Splanchnic perfusion</th>
<th>Prediction of mortality</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>[65] (2003)</td>
<td>95</td>
<td>Ventilated critically ill</td>
<td>PCO₂ gap</td>
<td>OR 1.57 (95% CI 1.10-2.24)</td>
<td>After 24 hours Automated air tonometry</td>
</tr>
<tr>
<td>[87] (2000)</td>
<td>210</td>
<td>Critically ill</td>
<td>pHi</td>
<td>pHi lower in non-survivors</td>
<td>Intervention trial, pHi guided resuscitation</td>
</tr>
<tr>
<td>[66] (1997)</td>
<td>62</td>
<td>Critically ill</td>
<td>pHi PCO₂ gap</td>
<td>pHi but not PCO₂ gap predicted mortality</td>
<td></td>
</tr>
<tr>
<td>[67] (1998)</td>
<td>19</td>
<td>Critically ill trauma</td>
<td>pHi</td>
<td>RR 4.5; Mortality 50% vs. 11%</td>
<td>After 24 hours</td>
</tr>
<tr>
<td>[68] (1997)</td>
<td>19</td>
<td>Pediatric septic</td>
<td>pHi PCO₂ gap</td>
<td>PCO₂ gap but not pHi predicted mortality</td>
<td>After 24 hours</td>
</tr>
<tr>
<td>[69] (1996)</td>
<td>57</td>
<td>Trauma with organ failure</td>
<td>pHi</td>
<td>Mortality 54 % vs. 7%</td>
<td>Failure to normalize pHi predicts mortality</td>
</tr>
<tr>
<td>[70] (1995)</td>
<td>8</td>
<td>Pediatric septic shock</td>
<td>pHi</td>
<td>Mean pHi lower in non-survivors</td>
<td></td>
</tr>
<tr>
<td>[71] (1995)</td>
<td>35</td>
<td>Severe sepsis</td>
<td>pHi</td>
<td>pHi lower in non-survivors</td>
<td>At 0, 4, and 24 hr</td>
</tr>
<tr>
<td>[72] (1994)</td>
<td>20</td>
<td>Critically ill trauma</td>
<td>pHi</td>
<td>Mortality 50 vs. 0 %</td>
<td>Low pHi on admission that did not correct after 24 hrs</td>
</tr>
<tr>
<td>[73] (1993)</td>
<td>30</td>
<td>Ventilated critically ill</td>
<td>pHi</td>
<td>pHi predicts mortality</td>
<td></td>
</tr>
<tr>
<td>[74] (1993)</td>
<td>83</td>
<td>Acute circulatory failure</td>
<td>pHi</td>
<td>Likelihood ratio 2.32</td>
<td>After 24 hours</td>
</tr>
<tr>
<td>[75] (1991)</td>
<td>80</td>
<td>Intensive care</td>
<td>pHi</td>
<td>Mortality 65% vs. 44%</td>
<td>On admission</td>
</tr>
<tr>
<td>[76] (1999)</td>
<td>114</td>
<td>Trauma</td>
<td>pHi PCO₂ gap</td>
<td>OR 4.6 OR 2.9</td>
<td></td>
</tr>
<tr>
<td>[77] (1999)</td>
<td>68</td>
<td>Cardiac surgery</td>
<td>pHi PCO₂ gap</td>
<td>No difference between survivors and non-survivors</td>
<td>On admission and after 12 hours</td>
</tr>
<tr>
<td>[78] (2005)</td>
<td>28</td>
<td>Sepsis (severe sepsis excluded)</td>
<td>pHi PCO₂ gap</td>
<td>OR 4.8 (95% CI 1.5-14.6) OR 3.0 (95% CI 1.4-8.3) OR 3.9 (95% CI 1.1-13.8)</td>
<td>Regional but not global variables predict mortality after stabilization</td>
</tr>
<tr>
<td>[79] (2001)</td>
<td>21</td>
<td>Septic shock</td>
<td>ICGC</td>
<td>Low value and failure to increase in non-survivors</td>
<td></td>
</tr>
<tr>
<td>[80] (2002)</td>
<td>336</td>
<td>Critically ill</td>
<td>ICG-PDR</td>
<td>Lowest value lower in non-survivors than in survivors</td>
<td>ROC AUC 0.8</td>
</tr>
<tr>
<td>[82] (2004)</td>
<td>6</td>
<td>Ventilated with septic SIRS</td>
<td>ISO₂, IHB</td>
<td>Observed mortality 83% vs. predicted mortality 25%</td>
<td>Unexpectedly high observed mortality</td>
</tr>
<tr>
<td>[83] (2006)</td>
<td>37</td>
<td>Septic shock</td>
<td>D-lactate</td>
<td>Decrease between day 1 and 2 in survivors</td>
<td></td>
</tr>
</tbody>
</table>

pHi, intramucosal pH; PCO₂ gap, gastric mucosal-arterial gradient of PCO₂; ICGC, indocyanine green clearance; ICG-PDR, indocyanine green plasma disappearance rate; ISO₂, endoscopic reflectance spectrophotometry recorded index of gastroduodenal mucosal oxygen saturation; IHB, endoscopic reflectance spectrophotometry recorded index of gastroduodenal mucosal hemoglobin concentration; OR, Odds Ratio; RR, Risk Ratio; ROC AUC, Receiver operating characteristic Area under curve.
Prognostic value of impaired gastrointestinal perfusion

Gastrointestinal mucosal hypoperfusion in critically ill patients is associated with a poor outcome, as illustrated in table 2.

Several studies in different patient populations, have investigated the association between gastric intramucosal pH (pHi), gastric mucosal pCO2, or the gastric to arterial pCO2 gradient, and outcome. In the majority of these studies, a low pHi at admission, or failure to normalize pHi after a set time period (most often 24 hours), discriminates non-survivors from survivors. However, pHi is closely related to the systemic acid-base status, which may partly explain why pHi has been shown to be a good prognostic marker. Only one study investigated the prognostic role of the gastric to arterial pCO2 gap using automated air tonometry, convincingly demonstrating that the pCO2 gap is a marker of mortality in ventilated ICU patients.

In a recent study, global hemodynamic variables were compared with different regional variables in 28 septic patients. After initial resuscitation aimed at improving global pressure-related hemodynamics, hepatosplanchnic variables but not global hemodynamic variables were independent predictors of outcome. Gastric mucosal pH, mucosal-end tidal CO2 gap, and indocyanine green blood clearance were the most important predictors of outcome. Indocyanine green elimination rate has been shown to correlate with survival in critically ill and septic shock patients in other studies as well. Sakka et al. retrospectively studied 336 critically ill patients who were monitored with transpulmonary double indicator dilution technique. The lowest value of indocyanine green plasma disappearance rate (ICG-PDR) was significantly lower in non-survivors (n=168) than in survivors (n=168) [median, 6.4%/min vs. 16.5%/min]. The area under the ROC curve as a measure of accuracy was 0.8 when using the lowest ICG-PDR in each patient and found to be comparable with values obtained with APACHE II and SAPS II scores. The predictive value of ICG-PDR was found to be independent from the underlying disease, as subgroup analysis showed that in sepsis, ARDS, and all other patients, the ICG-PDR was always significantly higher in survivors.

In one small study looking at endoscopic reflectance spectrophotometry, values for gastroduodenal blood flow in mechanically ventilated septic patients were approximately 40-50% of values found in healthy control patients. This impairment of gastroduodenal perfusion was associated with a higher in-hospital mortality than predicted by APACHE II (83% vs. 25%).
Persistent abnormalities in the sublingual microcirculation of septic patients (as visualized with orthogonal polarization spectral imaging) are associated with organ failure and death. Sakr et al. found that at the onset of shock, survivors and non-survivors had similar vascular density and percentage of perfused small vessels. However, small vessel perfusion significantly improved over time in survivors but not in non-survivors. Despite similar haemodynamic and oxygenation profiles and use of vasopressors at the end of shock, patients dying after the resolution of shock in multiple organ failure had a significantly lower percentage of perfused small vessels than survivors (57.4% vs. 79.3%) \(^8\).

Plasma D-lactate has also been studied as a prognostic marker. A rapid decrease in plasma D-lactate levels, but not L-lactate levels, between day 1 and 2 in septic shock patients, discriminated between survivors and non-survivors.\(^8\)

In summary, these results show that measurements of gut perfusion can be used to predict outcome in septic patients.

**Treatment of gastrointestinal perfusion defects**

Gut injury has been shown to be associated with infectious complications, systemic inflammation, and organ failure in septic patients. In view of the above-described correlations between splanchnic perfusion and prognosis, interventions targeted at preventing or ameliorating gut injury are warranted. However, many of the studies that we report on below show disappointingly negative results; presumably in part due to small sample size and ineffective therapies that do not reach their treatment goals. They do clearly demonstrate that hyper-aggressive use of some “traditional” method of macrovascular resuscitation (usually applied in the treatment arm of the study) often fails to improve gastrointestinal microcirculatory function. It is likely that radical new approaches to microvascular resuscitation are needed before we can expect to achieve positive survival benefits. This is further elaborated on in the discussion section.

**Global and regional resuscitation**

Resuscitation strategies aimed at global hemodynamic parameters often fail to improve gastrointestinal perfusion and microcirculation. Prospective randomized controlled studies that have investigated the effect of treatment strategies
Two small controlled studies in different patient populations have examined whether treatment aimed at increasing gastric intramucosal pH (pHi) improves outcome. No significant differences were found in mortality or morbidity between the treatment groups and the control groups. However, both studies were not adequately powered to detect differences in outcome. Moreover, failure of the intervention (fluids and more vasoactive agents) to improve pHi strongly predicted poor outcome in both studies.

Three larger controlled studies that investigated the influence of splanchnic perfusion directed therapy in critically ill patients were performed in 260, 210 and 151 patients. In all 3 studies, patients in the intervention groups with a low pHi received additional fluid resuscitation and vasoactive agents in an attempt to correct the abnormal pHi and to improve outcome. In one study, patients in a second intervention group additionally received therapies specifically aimed at...
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optimising splanchnic perfusion and minimising reperfusion damage, including mannitol, hydrocortisone, antioxidants, glutamine, and vasodilator therapy. Only the Gutierrez trial showed a beneficial effect of pH guided treatment. In this study, patients admitted with a low pH had similar survival rates in both the intervention and the control group (36% vs. 36%). However, in patients with a normal admission pH, survival was significantly better in the intervention than in the control group (58% vs. 42%, p < .01). These patients received additional resuscitation whenever pH fell below a certain level. This drop in pH indicated potential inadequate resuscitation despite normal global hemodynamics. The authors suggested that timing of the intervention could be crucial, and that their therapeutic approach could be ineffective after prolonged (irreversible?) mucosal acidosis as seen in the patients admitted with a low pH. However, treatment in the control arm of this multicenter study was not standardized. This, combined with the high mortality in the control group, suggests that the difference in mortality may have been unrelated to the treatment of low pH. Interestingly, in general, attempts at correction of pH in the 3 trials usually failed to alter this parameter (compared to baseline or to the control group) in a sustained and significant manner. This suggests that the additional treatment provided, which consisted of significantly more administration of fluid and vasoactive agents, in fact was ineffective in resuscitating or recruiting the gastrointestinal microcirculation. In the two trials that measured pH in the control patients as well, admission pH was significantly lower in non-survivors compared to survivors, confirming the prognostic value of tonometry. An important limitation of these studies is the use of saline tonometry derived pH as marker for gastrointestinal hypoperfusion (as discussed earlier, this has been replaced by the more accurate automated gas tonometry). Also, it is important to realize that the patient population studied was heterogeneous and not limited to a diagnosis of septic shock. Moreover, different resuscitation protocols were used, including infusion of crystalloids, colloids, and blood products. The effect of fluid resuscitation on gastrointestinal perfusion is variable and dependent on the type of fluid used. Simply optimizing cardiac output by fluid loading in septic patients, with or without subsequent reduction of vasopressor dose, is not necessarily associated with an increase in splanchnic blood flow. Different fluids have different properties (e.g. composition and tonicity), which may result in different biological effects in septic shock. Specific to gastrointestinal perfusion, hydroxyethyl starch (HES) has shown to have a more pronounced beneficial effect...
on splanchnic perfusion than Ringer’s solution or modified fluid gelatin. In a recent uncontrolled study in septic patients, the effect of HES fluid challenge on gastric mucosal pCO₂ was variable between patients. Improvement was more pronounced in septic patients with an abnormal baseline pCO₂ gap, indicating the actual presence of gastrointestinal mucosal hypoperfusion. Theoretically and supported by animal studies, hypertonic fluid resuscitation (e.g. 7.5% hypertonic saline solution) has the potential to recruit gastrointestinal microcirculation. In a recent animal study, hypertonic saline decreased thermal injury induced bacterial translocation in the gut, and enhanced host response to bacterial challenge by augmenting Toll-like receptor expression of inflammatory cells. The effect of hypertonic fluid resuscitation on gastrointestinal perfusion, microcirculation, and immunomodulation is the subject of an ongoing randomised controlled study in septic shock patients. Finally, transfusion of red blood cells to enhance systemic oxygen delivery in septic patients failed to improve gastric pH. In conclusion, resuscitation of septic shock patients on the basis of tonometry has not convincingly been shown to improve outcome. Patient heterogeneity, lack of statistical power, the use of saline tonometry, and failure of the treatment protocols to actually improve pH may explain this lack of efficacy. Persistently low pH despite treatment in these studies identified patients at risk for poor outcome, and illustrates the fact that the therapies studied seem generally not effective in actually resuscitating mucosal microcirculation.

Vasoactive therapy
The influence of classic vasoactive therapies on gastrointestinal perfusion in septic shock has been well described in a recent review article, and will not be further elaborated on. In summary, increasing perfusion pressure with vasopressor therapy in septic shock patients does not significantly improve or impair splanchnic perfusion. However, from a microcirculatory perspective, vasopressors should be applied with caution. We have previously shown that vasopressin infusion in septic shock patients treated with high dose noradrenaline results in a significant increase in gastric-arterial pCO₂ gap. Preliminary results from the Vasopressin and Septic Shock Trial (VASST) show that vasopressin infusion in patients with septic shock may be beneficial in patients that require low dose noradrenaline, but demonstrate no improved outcome in patients with ‘high’ dose (>15 μg/min) noradrenaline dependency. Although no significant
increase in overt mesenteric ischemia was reported, this potentially may have been caused by inadvertent effects on gastrointestinal perfusion.

While pressure-guided resuscitation in septic patients has consistently been found to be effective in restoring systemic blood pressure, it does not, by definition, have an equivalent correcting effect on microcirculatory perfusion.

Vasodilator therapy increases the driving pressure at the entrance of the microcirculation and is potentially able to recruit microcirculatory perfusion. One of the problems interpreting the results of administration of vasodilator drugs is that these drugs often have other pharmacological effects as well (e.g. reduction in leukocyte adhesion and platelet aggregation). Nitric oxide donors such as nitroglycerin improved gut microcirculation in a number of animal experiments and also improved sublingual microcirculatory blood flow in an observational uncontrolled human study, as measured by orthogonal polarization spectral imaging. However, as part of a complex therapeutic intervention protocol to resuscitate trauma patients and prevent ischemia-reperfusion damage, vasodilators failed to correct a low pH.

Prostacyclin and its analogue iloprost have vasodilator and cytoprotective properties. Administration of these drugs in septic patients either intravenously or aerosolized increased gastric intramucosal pH in two studies. In a prospective uncontrolled study in 20 septic shock patients, iloprost was infused and plasma disappearance rate of indocyanine green (ICG-PDR) increased significantly 24 h after start of iloprost infusion (baseline: 13.9±1.7% vs. 18.6±2.2%/min) and significantly decreased 1 h after end of infusion (13.7±1.7%/min). Iloprost also improved cardiac index and global hepato-splanchnic perfusion in another small uncontrolled study in septic shock patients.

The complex interactions between the exogenous administered vasodilators and the endogenous sepsis-induced vasodilatory mechanisms (e.g. through iNOS activation) are not well understood. This makes it difficult to resolve the clinical conundrum of attempting to administer vasodilator drugs to a critically ill septic patient, who is concurrently requiring vasoconstrictors to maintain adequate macrocirculatory parameters.

**Enteral nutrition**

Under normal conditions, blood flow to the gastrointestinal system increases during the digestion and absorption of nutrients. Regulation of this postprandial...
hyperaemia is complex. In several clinical situations, enteral feeding has been shown to prevent or ameliorate the increase in gut permeability and systemic inflammation induced by the disease state. For example, in one study, multiple injured patients who had recovered from shock within 6 hours, where randomized in an early enteral nutrition group and a late enteral nutrition group.

Intestinal permeability was measured using a lactose/mannitol (L/M) clearance assay. On post injury day 4 L/M ratio was significantly higher in the second group; suggesting that immediate enteral nutrition protects against an increase in intestinal permeability induced by multiple injury. In surgical critically ill patients, early administration of enteral nutrition decreases the number of infectious complications and the length of hospital stay. The mechanisms by which enteral nutrition is thought to decrease the pathological gut permeability, include an increase in hepato-splanchnic blood flow, prevention of gut-associated lymphoid tissue atrophy, and modulation of immunological phenomena.

**Experimental therapies**

Endothelium-derived substances such as endothelin and nitric oxide are recognized as important mediators of systemic inflammation. Selective inhibition of inducible nitric oxide synthase blunted the progressive increase in ileal to arterial pCO₂ gap in an animal model of long-term endotoxemia. The use of endothelin receptor antagonists has been studied in several animal models of septic shock. In one porcine study, bosentan completely restored the gut oxygen delivery with a reversal of intestinal mucosal acidosis as measured by ileal tonometry. These findings were reproduced in another study with the use of laser Doppler flowmetry, confirming the hypothesis that endothelin plays an important role in the regulation of splanchnic microcirculatory flow in septic shock. In patients with severe sepsis, endothelin plasma levels are markedly increased. To our knowledge, there are no studies published to date that look at the effects of endothelin receptor antagonists in septic patients. Timing of these potential interventions would seem to be crucial. The selective inhibition of inducible nitric oxide synthase during hyperdynamic, earlier phase of sepsis combined with the blockade of endothelin receptors at a later stage may represent a novel promising strategy for the therapy of septic shock.

Blockade of the angiotensin II type 1 receptor ameliorates splanchnic hypoperfusion in acute experimental circulatory failure. In animal models of...
Gastrointestinal perfusion in septic shock

Septic shock however, administration of the angiotensin II type I receptor antagonist candesartan had no effect on mucosal acidosis. Although survival improved when used as pre-treatment, survival decreased compared to controls when candesartan was administered during endotoxemia.

Discussion

Septic shock is associated with splanchnic perfusion abnormalities, which can be measured with several techniques as discussed above. These perfusion abnormalities are associated with a poor outcome. The main question now is: If impairment of splanchnic mucosal perfusion in sepsis adversely affects outcome, why have trials aimed at improving gut perfusion failed to show survival benefit? Several explanations may be considered. First of all, the majority of studies have looked at intermediate physiologic effects (e.g. gastric intramucosal pH) and not at mortality as primary outcome measure. Clearly, if an intervention which is targeted to improve mucosal microcirculatory perfusion fails to do so it is also unlikely to find a significant difference in outcome. The effect of those treatment strategies that are actually potentially capable of effectively restoring systemic and gastrointestinal microcirculatory defects (e.g. hypertonic fluid resuscitation) on outcome in septic patients has not yet been studied.

Measurement of treatment effects on physiologic variables such as splanchnic perfusion has potential intrinsic problems. Measurement errors can produce false positive as well as false negative results. The magnitude of treatment effect has to be greater than the baseline physiologic variability of the variable studied. Knowledge of this variability is essential in interpreting the results of these measurements. In healthy subjects, splanchnic blood flow has been demonstrated to exhibit a circadian variation. Animal experiments suggest that splanchnic haemodynamic parameters could exhibit a more pronounced variability than systemic haemodynamics. Some of the physiologic variability in splanchnic blood flow is explained by abdominal pressure variation during the respiratory cycle. However, in patients with acute lung injury, lung recruitment manoeuvres and prone positioning interestingly had no significant effect on gastric mucosal perfusion.

There is also the possibility that splanchnic hypoperfusion is a marker of disease or an epiphenomenon rather than a factor in its pathogenesis. However,
as outlined above, current experimental evidence supports the hypothesis that mucosal microcirculatory abnormalities play a role in the pathogenesis of systemic inflammation and multiple organ failure. From this pathophysiological understanding, it would seem likely that the main therapeutic benefit of effective mucosal resuscitation treatments would be to prevent an ongoing inflammatory insult to the patient. As a consequence, we would expect a benefit in reducing late sepsis-associated deaths (usually from multiple organ failure). However, the subset of septic patients who die from the primary infective insult (such as fulminant meningococcemia) would not be expected to benefit from treatments that act to reduce secondary inflammatory processes.

Clinical research in this specific area is also subject to a more common dilemma present in sepsis studies. The pathogenesis and pathophysiology of sepsis is complex. Sepsis can be looked at as a self-regulating complex system, with multiple cascading non-linear interactions and feedbacks, acting in series and in parallel, to form a "scale-free" network. As such, interfering with one variable is unlikely to change the course of the disease process\(^2\). Many 'magic bullets' have been considered, and subsequently shown to yield disappointing results in clinical trials. An approach targeted at different elements of that complex system therefore seems more rational. Examples are resuscitation strategies that at the same time modulate the immune response, such as hypertonic fluid resuscitation and the use of selective nitric oxide inhibitors as vasopressor.

**Conclusion**

Normal regulation of gastrointestinal blood flow is impaired in septic shock, resulting in mucosal hypoperfusion. Gastrointestinal mucosal hypoperfusion is an important marker, and also probably a cause, of poor prognosis in septic patients. Impaired gut barrier function could play a role in amplification of systemic inflammation, causing distant multiple organ dysfunction.

The aim of monitoring splanchnic perfusion is to detect, prevent, and reverse tissue hypoperfusion. Several techniques have been developed to measure gastrointestinal perfusion. These methods share the ability to predict outcome in septic shock patients. Gastric tonometry is the most widely used technique because of its non-invasiveness and ease of use. However, as treatment strategies
Gastrointestinal perfusion in septic shock

aimed at improving gastrointestinal perfusion have not been able to correct mucosal perfusion abnormalities and hence have not shown to improve outcome, the use of these tools in clinical decision-making is currently limited.

New treatment strategies for septic shock should be tested for their effects on gastrointestinal perfusion; to further clarify its exact role in patient management, and to prevent therapies detrimental to gastrointestinal perfusion being implemented.
References


Gastrointestinal perfusion in septic shock


PART 2

NOVEL VASOPRESSORS
Chapter 2.1

THE EFFECT OF VASOPRESSIN ON GASTRIC PERFUSION IN CATECHOLAMINE-DEPENDENT SEPTIC SHOCK PATIENTS

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Abstract

Objective: To study the effect of continuous infusion of vasopressin on the splanchnic circulation in severe septic shock patients.

Design: Prospective clinical study.

Setting: Intensive care unit in a teaching hospital.

Patients: Eleven consecutive patients with documented septic shock who remained hypotensive despite norepinephrine-infusion at a rate of ≥ 0.2 μg/kg/min.

Interventions: Insertion of a gastric tonometry catheter; continuous infusion of vasopressin 0.04 U/min during 4 hours.

Measurements and Main results: P(g-a)CO2 gap, blood pressure, and cardiac index were recorded at baseline and after 15, 30, 60, 120, and 240 minutes. The median P(g-a)CO2 gap increased from 5 mmHg at baseline to 19 mmHg after 4 hours (p=0.022). Blood pressure increased from 61 ± 13 mmHg at baseline to 68 ± 9 mmHg after 4 hours (p=0.055). No significant changes in cardiac index were noted.

Conclusions: In norepinephrine-dependent septic shock patients, continuous infusion of low dose vasopressin results in a significant increase of the P(g-a)CO2 gap compatible with gastrointestinal hypoperfusion.
Novel vasopressors

Introduction

Septic shock is often characterized by profound vasodilation that requires treatment with catecholamines. Norepinephrine-resistant hypotension associated with septic shock has a high mortality rate.

Patients with septic shock, compared to other forms of shock, have low levels of circulating endogenous vasopressin. This may be related to depletion of vasopressin stores in the neurohypophysis. In experimental septic shock, release of vasopressin is inhibited by central nitric oxide production arising from the inducible nitric oxide synthase pathway. Other experimental data indicate that sepsis also causes cytokine-mediated downregulation of vasopressin V(1A) receptors.

Vasopressin is a potent vasoconstrictor in patients with septic shock. Vasopressin increases blood pressure, improves some measures of renal function and decreases catecholamine requirements. Terlipressin, a long-acting vasopressin analogue, also restores blood pressure in patients with catecholamine-resistant septic shock.

Despite its favorable effect on global hemodynamics in septic shock patients, few clinically relevant data are available on the effect of vasopressin on the splanchnic circulation. In patients with bleeding esophageal varices, vasopressin leads to vasoconstriction of the splanchnic circulation and may stop the bleeding.

The aim of our study was to investigate the effect of vasopressin infusion on the splanchnic circulation in septic shock patients. We hypothesized that vasopressin, being a potent vasoconstrictive agent, decreases gastrointestinal blood flow and therefore may be potentially harmful.

Patients and Methods

Patients
The study was approved by the medical ethical committee of the Jeroen Bosch Hospital. Informed consent was obtained from the nearest relative. Thirteen consecutive patients with documented septic shock were screened for inclusion in the study. Septic shock was defined according to the ACCP/SCCM consensus conference. All patients were resuscitated with fluid until no further
improvement of cardiac output was obtained and received dobutamine based on
the judgment of the treating physician. All patients received a continuous
infusion of low dose hydrocortisone. Norepinephrine was added in case of
persistent hypotension. Patients were included when they met the criteria for
septic shock and had mean arterial pressures ≤ 70 mmHg despite
norepinephrine infusion at a rate of ≥ 0.2 μg/kg/min. Exclusion criteria were
age below 18 years, pregnancy, and myocardial ischemia or infarction less than
6 months prior to the study.

Interventions. A gastric tonometry catheter (TonometricsTM-catheter, TONO-
16F, Datex-Ohmeda Division, Helsinki, Finland) was inserted in the stomach.
Calibration was performed according to the manufacturers guidelines. Enteral
feeding was discontinued and all patients received omeprazole 40 mg
intravenously 1 hour before the first measurements. After data collection at
baseline, patients received vasopressin 0.04 U/min by continuous central venous
infusion for 4 hours. Catecholamine doses were not changed during the study
period unless mean arterial pressure dropped below 50 mmHg despite fluid
resuscitation. Vasopressin was continued after 4 hours in case of persistent
hypotension, based on the judgment of the treating physician.

Measurements
We recorded demographic data as well as the severity of illness using APACHE
II (first 24 hours of admission to the ICU) and SOFA-scores (24 hours prior to
inclusion in the study). Carbon dioxide partial pressure was measured in the
stomach (PgCO₂ in mmHg) by automated air tonometry using an equilibration
time of 10 minutes. Arterial carbon dioxide partial pressure (PaCO₂ in mmHg)
was measured simultaneously (blood gas analyzer, Bayer, Meijdrecht, The
Netherlands) and P(g-a)CO₂ gap was calculated. Blood pressure and cardiac index
were also recorded. Plasma levels of vasopressin were measured
(radioimmunoassay kit, Nichols; Wizard gamma counter, Wallac, Finland). All
measurements were performed at baseline and after 15, 30, 60, 120, and 240
minutes. Data on hospital mortality were collected after completion of the study.
Statistical and data analysis. Data are presented as mean ± 1 SD or as median (25-
75th) percentile depending on their distribution. Changes over time were analyzed
by ANOVA. All statistics were done using SPSS 10.0 (SPSS, Chicago, IL).
Table 1: Demographic and clinical characteristics

<table>
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<tr>
<th>Patient No.</th>
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<th>SOFA</th>
<th>Outcome</th>
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<td>74</td>
<td>34</td>
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<td>Died</td>
</tr>
</tbody>
</table>

Mean ± SD | 67 ± 14 | 26.6 ± 8.7 | 10.8 ± 1.8 |

Results

Patient characteristics
Thirteen consecutive patients were eligible for inclusion in the study. In two patients it was not possible to measure PgCO₂ due to technical problems. The demographic and clinical characteristics of the patients are shown in table 1. Septic shock was caused by pneumonia in 5 patients, by abdominal sepsis in 5 patients and by urosepsis and aspiration pneumonia in 1 patient. All patients were mechanically ventilated. Mean APACHE II score was 26.6 (± 8.7), mean SOFA score was 10.8 (± 1.8). Hospital mortality was 82% (9/11 patients died).

Measurements
The P(g-a)CO₂ gap at baseline and following vasopressin infusion is shown in figure 1A. The median P(g-a)CO₂ gap increased from 5 mmHg at baseline to 19 mmHg after 4 hours; this increase was statistically significant (p=0.022). Vasopressin infusion resulted in an increase in mean arterial pressure from 61 ± 13 mmHg at baseline to 68 ± 9 mmHg after 4 hours (p=0.055; figure 1B) without a significant decrease in cardiac index (p=0.978). Plasma vasopressin levels increased from 17 pg/ml at baseline to 230 pg/ml after 4 hours of infusion (p<0.001; figure 1C). There was a strong correlation between median plasma levels of vasopressin and the median P(g-a)CO₂ gap (r²=0.98) as is shown in figure 2.
Figure 1A
Median $P(g-a)CO_2$ gap in mmHg during vasopressin infusion with below 25th and 75th percentiles

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<th>Time (min)</th>
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<tr>
<td>240</td>
<td>13.3</td>
<td>26.8</td>
</tr>
</tbody>
</table>

Figure 1B
Mean arterial blood pressure ± SD in mmHg during vasopressin infusion
Figure 1C
Median plasma levels of vasopressin in pg/ml during infusion with below 25th and 75th percentiles

<table>
<thead>
<tr>
<th>Time (min)</th>
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<th>30</th>
<th>60</th>
<th>120</th>
<th>240</th>
</tr>
</thead>
<tbody>
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<td>25th percentile</td>
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<td>31</td>
<td>47</td>
<td>166</td>
<td>166</td>
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<tr>
<td>75th percentile</td>
<td>33</td>
<td>105</td>
<td>152</td>
<td>235</td>
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<td>274</td>
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</table>

Figure 2
Correlation between median P(g-a)CO₂ gap in mmHg and median plasma levels of vasopressin in pg/ml during vasopressin infusion
Discussion

In this study, we investigated the effect of low dose vasopressin infusion on gastric perfusion in septic shock patients who remained hypotensive despite high dose norepinephrine infusion. Infusion of vasopressin increased plasma levels of vasopressin to values that were more than ten times higher than those reported during comparable degrees of hypotension from other causes such as cardiogenic shock. This resulted in an increase in blood pressure, which was not accompanied by a reduction in cardiac output, compatible with an impairment of the baroreflex in septic shock patients. These findings are consistent with those previously reported by others.

We found that vasopressin infusion leads to an immediate and important increase in P(g-a)CO₂ gap in a dose-dependent fashion. This P(g-a)CO₂ gap is a reliable measure of gastrointestinal hypoperfusion. In animal experiments, increased levels of circulating vasopressin in different states of shock have been shown to contribute to redistribution of blood from the peripheral to the cerebral circulation. Although low concentrations of vasopressin have been shown to have vasodilatory effects in selected organs, the results of the only placebo-controlled trial that has been conducted with vasopressin in septic shock patients show that vasopressin treatment results in peripheral vasoconstriction. Our study shows that vasopressin probably also leads to vasoconstriction of the splanchnic vasculature. This finding is consistent with studies in human gastroepiploic arteries that demonstrate a concentration-dependent vasoconstrictive effect starting at levels that are lower than those obtained in our study. An increase in gastric pCO₂ indicating splanchnic vasoconstriction has also been shown in patients who received ornipressin (a vasopressin agonist specific for the V₁ receptor) to reverse the hypotension associated with combined general/epidural anesthesia. In animal experiments, endogenous release of vasopressin during endotoxin administration has been associated with gastric, duodenal, and jejunal microcirculatory and mucosal injury. Several studies have demonstrated that gastrointestinal hypoperfusion, reflected by a low gastric intramucosal pH, is a good predictor of poor outcome. Gut intramucosal hypoxia is thought to be important both as an indicator of inadequate resuscitation and as a mechanism by which multiorgan failure may occur. However, resuscitation based on the results of gastric tonometry has failed to show improvement in outcome.

Other vasoconstrictive agents used in septic shock don’t share the apparent
Novel vasopressors

detrimental effect of vasopressin on the gastrointestinal perfusion. In one study for example, both epinephrine and the combination of norepinephrine and dobutamine in septic shock patients increased gastric mucosal perfusion. Gastrointestinal hypoperfusion can be reversed by infusion of prostacyclin. In our study, all patients developed gastric hypoperfusion despite standard use of low dose vasodilating agents.

There are several limitations to this study. The number of patients studied is small, but an increase in the P\((g-a)\)CO\(_2\) gap was seen in 10 out of 11 patients. Furthermore, the patients included in this study were severely ill which makes generalization to all septic shock patients difficult. Although hospital mortality was unusually high, the study design permits no further speculations to be made on this observation.

Because all patients received high dose norepinephrine infusion as well as vasopressin, interaction between these two vasoconstrictive agents cannot be ruled out. Several studies indicate that subconstricting doses of vasopressin are able to potentiate the constricting effects of catecholamines. In a recent study, low dose terlipressin without administration of other catecholamines increased ileal microcirculation in fluid-challenged endotoxic rats. It is possible therefore, that the effect of vasopressin observed in our patients reflects the potentiating effect of vasopressin to infused and endogenous catecholamines.

Finally, the vasopressin dose administered to the patients may have been too high. We showed that higher levels of vasopressin led to more profound gastric hypoperfusion in a dose-dependent fashion. Landry et al. showed in six septic shock patients that administration of vasopressin at a lower infusion rate of 0.01 U/min resulted in plasma concentrations expected for the level of hypotension (around 30 pg/ml) with an increase of systolic arterial pressure from 83 to 115 mmHg.

In conclusion, in this prospective study infusion of low dose vasopressin in severe septic shock patients resulted in a rapid increase in P\((g-a)\)CO\(_2\) gap compatible with gastrointestinal hypoperfusion. In our view, vasopressin treatment of septic shock patients should be limited to controlled clinical trials until its effect on clinical outcome such as organ failure and mortality has been clarified.

Acknowledgments
We thank Mr. M.P.W.J.M. Bouw for his contribution to this study.
References


Novel vasopressors

Effects on circulation, organ perfusion and immune system
PART 2

NOVEL HEMODYNAMIC THERAPIES IN SEPTIC SHOCK;
Chapter 2.2

The effects of methylene blue infusion on gastric tonometry and intestinal fatty acid binding protein levels in septic shock patients

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2 Intensive Care Department, Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands
3 Intensive Care Department, VieCuri Medical Centre, Venlo, The Netherlands
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Journal of Critical Care, in press.
Abstract

Objective: We prospectively studied the effect of methylene blue (MB) infusion on gastric mucosal metabolism perfusion ratio, assessed by gastric tonometry, and on mucosal cell damage, assessed by urinary levels of intestinal fatty acid binding protein (iFABP), in septic shock patients.

Methods: MB infusion (img/kg/hr) during 4 hours in 10 consecutive patients with a proven or suspected bacterial infection and with severe vasodilatory shock, defined as a mean arterial pressure ≤ 70 mmHg for at least 1 hour despite adequate volume resuscitation and norepinephrine infusion at a rate ≥ 0.2 μg/kg/min.

Results: MB infusion did not significantly change the P(g-a)CO₂ gradient (p=.16). Post-hoc analysis of the subgroup of patients with an elevated baseline P(g-a)CO₂ gradient, defined as ≥ 20 mmHg, showed that the median P(g-a)CO₂ gradient (IQR) decreased from 45 (41-56) mmHg before infusion to 41 (28-52) at the end of the 4 h infusion and decreased further to 32 (26-36) mmHg 2 h after cessation of MB infusion (p=.012). The median urinary iFABP concentration at baseline was elevated (210 [79-437] pg/μmol creatinine) and did not change significantly after 24 h (116 [53-601] pg/μmol creatinine, p=.15). The median MAP [IQR] increased from 70 [69-71] mmHg at baseline to 77 [67-83] mmHg after 1 h (p=.04), the norepinephrine dose did not change significantly. The median [IQR] cardiac index decreased from 4.4 [3.2-5.5] l/min/m² at baseline to 3.6 [3.3-4.7] l/min/m² after 2 h, returning back to baseline values after cessation of MB infusion (p=.02).

Conclusion: Although MB infusion in patients with septic shock and advanced multi-organ failure increases MAP and decreases CI, it does not compromise the gastric mucosal perfusion metabolism ratio as indicated by tonometry, and by the release of a mucosal cellular injury marker.

Trial registration at Australian New Zealand Clinical Trials Registry (ANZCTR) www.anzctr.org.au The effects of methylene blue infusion on gastrointestinal perfusion and gut mucosal damage in septic shock patients Registration number ACTRN1260800090314
Introduction

Septic shock, resulting in refractory hypotension and progression to multiple organ failure, is the major cause of prolonged stay and death in noncoronary intensive care units with an estimated mortality between 50% and 60% \(^1\) \(^2\). Part of the pathogenesis of vasodilation and organ dysfunction in septic shock involves the excessive production of nitric oxide (NO) by inducible NO synthase (iNOS) that comes to expression during inflammation \(^3\). NO stimulates the soluble intracellular enzyme guanylate cyclase (sGC), which increases the generation of cyclic guanosine monophosphate (cGMP). Ultimately, this pathway leads to vasodilation, myocardial depression, increased vascular permeability, loss of gut barrier function and organ dysfunction.

Non-selective inhibition of NOS by L-NMMA (NG-monomethyl-L-arginine) resulted in an overall increase in mortality in a phase III trial in septic patients \(^4\). Methylene blue (MB) has been shown to inhibit inducible NOS, scavenge NO, and to inhibit sGC, therefore acting in a more selective fashion in comparison with L-NMMA, potentially preserving other physiologically beneficial NO pathways \(^5\) \(^6\). In septic patients, small trials, including two prospective randomized controlled studies, have demonstrated an increase in blood pressure mediated by an increase in SVR \(^7\) \(^9\). MB has been shown to be safe in humans and has been used for treatment of cyanide poisoning, methemoglobinemia and malaria for many years \(^10\).

Gastrointestinal mucosal hypoperfusion is thought to be important in septic shock patients, both as an indicator of inadequate resuscitation and as a mechanism by which multiple organ failure may occur \(^11\). Loss of gut barrier function may lead to translocation of bacteria, endotoxin, and other inflammatory mediators, thereby augmenting and sustaining systemic inflammation, and possibly resulting in distant organ dysfunction. Abnormalities in the NO system can be regarded as one of the mechanisms responsible for the gastrointestinal mucosal perfusion defects observed \(^12\).

Gastric automated air tonometry is an easy and reproducible technique to estimate adequacy of splanchnic perfusion, and an increased gastric to arterial pCO\(_2\) gradient (P(g-a)CO\(_2\) gap) is a marker of mortality in ventilated intensive care patients \(^13\).

Intestinal fatty acid binding protein (iFABP) is an intracellular epithelial protein in the intestinal mucosa. It has been shown to be a useful biochemical
marker of enterocyte injury and gut ischemia in experimental models and in humans \(^{14-16}\) and iFABP levels have been shown to correlate with clinical development of the systemic inflammatory response syndrome (SIRS) and with outcome in critically ill patients \(^{17,18}\).

To our knowledge, the effect of MB infusion on gastrointestinal perfusion and mucosal cell damage in septic shock patients has not been studied previously. We examined gastric tonometry and iFABP levels before, during and directly after a 4 hr infusion period of MB in septic shock patients.

**Patients and Methods**

**Patients**
The study was approved by the Central Committee on Research Involving Humans in the Netherlands (CCMO). Informed consent was obtained from the patient’s nearest relative/next of kin. Consecutive patients admitted to the intensive care unit (ICU) with a proven or suspected bacterial infection and 2 or more SIRS criteria \(^9\) were screened for inclusion in this study. Patients were recruited within 24 h of admission to the ICU when they met the criteria for septic shock \(^9\) and had mean arterial pressures (MAP) \(\leq 70\) mmHg despite adequate fluid replacement and a norepinephrine infusion at a rate of \(\geq 0.2\) \(\mu\)g/kg/min for at least an hour prior to recruitment. Patients were excluded if they were \(<18\) years, pregnant or lactating, or had proven myocardial ischemia or infarction \(<6\) months prior to the study. Throughout the study, all patients received standard conventional therapy for septic shock and received low-dose hydrocortisone (200 mg/24h). None of the patients received activated protein C.

A transpulmonary thermodilution catheter (PiCCO, Pulsion Medizintechnik, Munich, Germany) was inserted as per our routine practice for septic shock patients. Fluid resuscitation was guided by cardiac output and stroke volume variation as measured by pulse contour analysis (patients were considered to be fluid responsive if SVV \(>12\%\)) and per discretion of the treating intensivist.

**Interventions**
A gastric tonometry catheter (TonometricsTM-catheter, TONO-16F, Datex-Ohmeda Division, Helsinki, Finland) was inserted in the stomach. Calibration was performed according to the manufacturer’s guidelines. Enteral feeding was
discontinued and all patients received omeprazole 40 mg intravenously 1 h before the first measurements. Immediately after data collection at baseline, patients received a continuous central venous infusion of 1 mg/kg/hour MB (1% w/v) for 4 h, which was provided by the department of pharmacy of the VieCuri Medical Centre. Infusion of norepinephrine was only increased during the study period if MAP dropped below 60 mmHg despite additional fluid resuscitation. Norepinephrine infusion rate was decreased when MAP reached values > 80 mmHg. Data of all patients was analysed.

Measurements
We recorded demographic data as well as the severity of illness using APACHE II, number of failing organs, and SOFA-scores. Data on hospital mortality were collected after completion of the study.

Hourly transpulmonary thermodilution calibration measurements were performed (PiCCO) and global hemodynamic parameters including blood pressure, heart rhythm and rate, cardiac index, stroke volume variation (SVV), extravascular lung water index (EVLWI), and urine output were recorded. Carbon dioxide partial pressure was measured in the stomach (PgCO₂ in mmHg) by automated air tonometry using an equilibration time of 10 minutes. Arterial carbon dioxide partial pressure (PaCO₂ in mmHg) was measured simultaneously (blood gas analyzer, Bayer, Meijdrecht, The Netherlands) and P(g-a)CO₂ gap was calculated. Blood lactate levels were determined by routine clinical chemistry. Hematocrit and blood concentrations of hemoglobin, methemoglobin and bilirubin were measured to assess possible side effects of MB administration, such as hemolytic anemia and methemoglobinemia. Urinary excretion of iFABP was measured using a commercially available ELISA (Synbio, HK406) at t=0, 6, 24 h and calculated as ratio to urine creatinine concentration to correct for renal failure and urine dilution. Using this assay in 15 healthy controls, urinary iFABP concentration was 5.4±1.6 pg/ml (unpublished results).

Statistical analysis
Values are given as mean±SD or as median [25-75% range], depending on their distribution. Data analysis was performed using ANOVA repeated measures using SPSS 14.0 for Windows (SPSS Inc. Chicago, Il, USA). The Wilks' lambda F test of the multivariate analysis was used to investigate whether a difference over time was significant and dependent on the baseline value. We used the
Tukey-Kramer Multiple-Comparison test for post-hoc comparisons at different times.

The primary end point of this study was the effect of MB infusion on $P(g-a)CO_2$ gap in patients with refractory septic shock. A change of 10 mmHg in $P(g-a)CO_2$ gap was considered to be relevant. In a previous study using the same method, we found a standard deviation of 9 mmHg. With these data and a significance level alpha of 0.05, a sample size of 6-11 subjects was calculated to reach a power of 80-95%. Therefore, 10 patients were included in the study, which results in a power of 88% (PSPower V2.1.31).

### Results

**Patient characteristics**

The demographic and clinical characteristics of the patients are shown in Table 1. The effects of MB on the urinary excretion of NO-metabolites and markers of renal injury in 9 out of the current 10 patients were previously published24.

All patients had failure of at least 3 organs, which is reflected by a mean SOFA score of 11.3±2.5. Seven patients died in the ICU, one of refractory vasodilatory shock (within 12 h) and six patients because of progressive multiple organ failure. In the latter group, two patients died within 7 days and the remaining four patients within 28 days after the intervention. The mortality was high, but in

**Table 1: Demographic and clinical characteristics**

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Diagnosis</th>
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<td>3</td>
<td>Abdominal sepsis</td>
<td>Male</td>
<td>83</td>
<td>30</td>
<td>13</td>
<td>Survived</td>
</tr>
<tr>
<td>4</td>
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<td>83</td>
<td>21</td>
<td>15</td>
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<tr>
<td>5</td>
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<tr>
<td>6</td>
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<td>72</td>
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<tr>
<td>7</td>
<td>Necrotizing fasciitis</td>
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<tr>
<td>8</td>
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<td>Female</td>
<td>56</td>
<td>32</td>
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<td>Survived</td>
</tr>
<tr>
<td>9</td>
<td>Abdominal sepsis</td>
<td>Female</td>
<td>58</td>
<td>17</td>
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<td>Died</td>
</tr>
<tr>
<td>10</td>
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<td>57</td>
<td>29</td>
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<td>Survived</td>
</tr>
<tr>
<td>Mean ± SD</td>
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<td></td>
<td>70±10</td>
<td>26.8±6.5</td>
<td>11.1±2.5</td>
<td></td>
</tr>
</tbody>
</table>
keeping with the mean calculated predicted mortality rate (61%) and reflects the severity of illness in this selected group of patients. The median stay at the ICU was 17 days [8-28] and the three survivors stayed 64 days [56-122] in the hospital. Pathogenic organisms isolated by culture included Escherichia coli (n=2), Pseudomonas aeruginosa (n=3), Klebsiella pneumoniae (n=1), Enterobacter aerogenes (n=1). All patients received adequate and timely antibiotic treatment (data not shown). In 3 patients no positive cultures were obtained. Etiologies for infection included respiratory tract infection (n=2), abdominal sepsis following abdominal surgery (n=5), skin and soft tissue infection (n=2), and urosepsis (n=1).

**Global hemodynamic measurements**

All patients were adequately fluid resuscitated. In 4 patients, SVV could not be used to predict fluid responsiveness due to atrial fibrillation (n=3) or spontaneous breathing mode (pressure support ventilation, n=1)25. These 4 patients were volume challenged until no further increase in thermodilution cardiac output was noticed before commencement of the MB infusion. In the other 6 patients, median SVV [IQR] before MB infusion was 10.0% [6.3-10.8], indicating that further fluid administration would not result in an increase in stroke volume26. The effects of MB infusion on hemodynamic variables are shown in Figure 1. The median MAP [IQR] increased from 70 [69-71] mmHg at baseline to 77 [67-83] mmHg after 1 hour (p=.04). The other time points were not significantly different from baseline. The median norepinephrine infusion rate did not change from baseline during MB infusion. In 2 patients, the norepinephrine infusion rate could be decreased (by 67% and 25%, respectively) because MAP was >80 mmHg (as per protocol). The norepinephrine infusion rate was not changed in the other 8 patients.

The median cardiac index showed a decrease from 4.4 [3.2-5.5] l/min/m² at the start of MB infusion, to 3.6 [3.3-4.7] l/min/m² after 2 h of infusion and 3.9 [2.5-4.8] l/min/m² after 4 h, returning back to baseline values 4.7 [2-0.6] l/min/m² after MB infusion was stopped (p=.02). Values for EVLWI were ≤ 10 ml/kg and were not affected by MB infusion (p=.28), see Figure 1D. Lactate levels were elevated (4.0±3.6 mmol/l) and remained unchanged throughout the study period (p=.75).
Figure 1
Box plots of median arterial pressure (A), norepinephrine infusion rate (B), cardiac index (C) and extravascular lung water index (D) before, during and after MB infusion.

* p = .04
** p = .02
**Gastrointestinal variables**

For the total group of patients, the $P_{(g-a)}CO_2$ gap did not change significantly during or after MB infusion ($p=.16$, Figure 2). The median $P_{(g-a)}CO_2$ gap at baseline and following MB infusion in patients with a normal baseline $P_{(g-a)}CO_2$ gap ($< 20$ mmHg) and in patients with an elevated baseline $P_{(g-a)}CO_2$ gap ($\geq 20$ mmHg) is also shown in Figure 2 (post-hoc analysis). In 4 patients with an elevated baseline $P_{(g-a)}CO_2$ gradient, the median $P_{(g-a)}CO_2$ gradient decreased from 45 [41-56] mmHg before infusion to 41 [28-52] at the end of the 4 h infusion, and decreased further to 32 [26-36] mmHg 2 h after infusion ($p=.012$). No significant change was found in 6 patients with a normal baseline $P_{(g-a)}CO_2$ gradient prior to the MB infusion ($p=.57$). No significant correlation was found between the $P_{(g-a)}CO_2$ gradient and infused dose of norepinephrine ($r=0.18$, $p=.65$).

Compared to healthy volunteers, the median urinary iFABP concentration at baseline was significantly elevated in all patients (841 pg/ml, [525-2860]),
indicating important mucosal cellular damage. Corrected for urine creatinine concentrations, the median iFABP levels tended to decrease from 210 [79-437] pg/µmol creatinine at baseline to 116 [53-601] pg/µmol creatinine after 24 hours (p=0.15). No significant correlation was found between the P(g-a)CO₂ gradient and the iFABP levels (r=0.14, p=0.65).

Side effects
There were no signs of hyperbilirubinemia or methemoglobinemia. The mean methemoglobin level was 0.7 ± 0.2% at baseline, 0.7 ± 0.2% after 3 hrs, and 0.9 ± 0.1% after 6 hours. The hematocrit and levels of hemoglobin did not change after MB administration (data not shown). In addition, no significant deterioration in measures of oxygenation was found (data not shown). All MB-treated patients showed blue colouring of both urine and skin. No other side effects were observed.

Discussion
In the present study we examined the effects of methylene blue (MB) infusion in septic shock patients with advanced multi-organ failure on different measures of gastrointestinal perfusion and damage. Our results indicate that MB administration in septic shock patients may preserve gastro-intestinal perfusion and integrity. MB infusion at used dosages has no significant deleterious effects on the gastric mucosal metabolism perfusion rate as assessed by gastric tonometry despite a decrease in CI associated with MB-infusion. In the small subgroup of patients with an abnormal P(g-a)CO₂ gap (>20mmHg) at baseline, MB infusion resulted in a significant decrease in P(g-a)CO₂. Due to the small number of patients and the well-known limitations of post-hoc subgroup analyses, one should interpret these results with caution.

The urinary excretion of iFABP was highly elevated in all patients during this study, indicating significant mucosal cellular damage. A decreasing trend was shown after MB infusion, suggesting absence of further harm or possibly a protective effect of MB on enterocyte injury. These results should be interpreted with caution. The fall in corrected iFABP levels was not statistically significant. Other factors may have influenced the corrected iFABP levels such as: ongoing renal failure, which potentially could overestimate creatinine excretion; changes
caused by sepsis resuscitation unrelated to MB. In addition, the high baseline levels of iFABP in our study population may influence the ability to detect small changes induced by the treatment.

MB infusion decreased the CI in our patient population and transiently increased the MAP. This finding of increased MAP and SVR has been reported in all studies of MB in sepsis\textsuperscript{8,27}.

The effects of different vasopressors on gastrointestinal perfusion and mucosal cellular injury in septic shock is not straightforward and depends on the type of vasopressor used and the clinical situation of the patient\textsuperscript{8}. We have previously shown in a comparable group of septic shock patients, that vasopressin infusion in patients treated with high dose norepinephrine resulted in a decrease in gastrointestinal perfusion, reflected by a significant increase in gastric-arterial pCO\textsubscript{2} gap\textsuperscript{23}. Differences between various vasoconstrictive agents is further illustrated by the observation of LeDoux and co-workers, who showed that incremental doses of norepinephrine markedly increased MAP from 65 to 85 mmHg however, did not cause a significant change in gastrointestinal perfusion\textsuperscript{28}. The influence of several other vasoactive therapies on gastrointestinal perfusion in septic shock has been described in a recent review article\textsuperscript{29}. Adrenergic agents that increase blood pressure have variable effects on gastrointestinal perfusion. Our results suggest that, compared to other vasoactive drugs used in septic patients, MB may relatively preserve splanchnic mucosal perfusion.

An important mechanism that is potentially responsible for the effects of MB infusion is selective inhibition of production of NO and cGMP by MB. Induction of the NO system induced by inflammation can be regarded as one of the key mechanisms responsible for the mucosal microcirculatory defects observed\textsuperscript{8,12}. Animal studies have shown that NO acts as a final common pathway of mediators and neural pathways in the gastrointestinal tract and also is a major inhibitory component of gastrointestinal function\textsuperscript{30}. We have recently shown that MB infusion is associated with a decrease in NO production and an attenuation of the urinary excretion of renal tubular injury markers, indicating protection of renal function\textsuperscript{24}. MB also reduced NO overproduction in the lungs in an animal sepsis model, which resulted in the attenuation of lung injury\textsuperscript{31}. These results suggest that excessive concentrations of NO and free radicals may play a role in the development of lung and other organ injury. In our study however, no significant change in extravascular lung water was found during and after infusion of MB. This is consistent with the results of another study, where MB...
administration in septic patients did not change pulmonary vascular permeability as assessed by extravascular lung water. MB administration at used dosage was found to be safe in the present study. This is consistent with previous studies and experience. One case series reported the development of self-limiting postoperative encephalopathy in patients who received preoperative MB infusion for localization of parathyroid adenomas. The common factor in all five cases was female gender and preoperative use of serotonin-metabolism modifying agents. MB should not be used in patients with documented hypersensitivity to MB, and used cautiously in patients with glucose-6-phosphate dehydrogenase deficiency because of the risk of developing Heinz body anemia. Intensive care practitioners should also be aware of the fact that MB interferes with pulse oxymetry, resulting in falsely depressed oxygen saturation readings.

There are several limitations to this “proof of concept study”. Tonometry has known limitations and only provides information of the site of measurement. However, automated air tonometry has been well validated as a reproducible measure of mucosal metabolism perfusion ratio. Because of large inter-individual differences, we conducted a before-during-after design, as a large randomized placebo-controlled set-up was not feasible. In addition, the sample size is relatively small. It was however adequately powered to detect clinically significant changes in P(g-a)CO₂ gap over time as the patients served as their own controls. Furthermore, having used the same design in a previous study where we administered vasopressin to septic shock patients, we found that vasopressin resulted in an increase of P(g-a)CO₂ gap in all studied patients, independent of the baseline P(g-a)CO₂ gap, illustrating the high sensitivity to detect putative deleterious effects of a vasoactive compound on gastric mucosal perfusion metabolism ratio. The patients included in the current study were severely ill, suffered from advanced multiorgan failure and needed high doses of norepinephrine, which makes generalization of the results to other patients with less severe septic shock difficult. Finally, we only studied the short-term effects of MB infusion in septic shock patients. Whether the effects would be different during ongoing infusion of MB remains unknown. Future studies should investigate the effects of MB in early septic shock, before the occurrence of significant organ failure and address the question whether MB infusion may prevent or ameliorate progression to organ failure by preserving gut perfusion. From a pathophysiological point of view, early administration of MB in
Novel vasopressors

Hyperdynamic septic shock would be expected to be more efficacious than late in the course of the disease process\textsuperscript{35}. In addition, a large-scale randomized controlled study is necessary to test the effects of MB infusion on clinically relevant endpoints such as organ failure and mortality.

In conclusion, although MB infusion in patients with severe septic shock and advanced multi-organ failure increases MAP and decreases CI, this does not result in significant changes in gastric mucosal perfusion metabolism ratio and release of a mucosal cellular injury marker.

Acknowledgments

The authors would like to thank Gaelle Dutu, (biostatistician, Waikato Clinical School) for her valuable guidance in the statistical analysis of this study.
References


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Chapter 2.3

Short-term beneficial effects of methylene blue on kidney damage in septic shock patients

Suzanne Heemskerk, Frank M.P. van Haren, Norbert A. Foudraine, Wilbert H.M. Peters, Johannes G. van der Hoeven, Frans G.M. Russel, Rosalinde Masereeuw and Peter Pickkers

Abstract

Objective: We previously demonstrated that up-regulation of renal inducible nitric oxide synthase (iNOS) is associated with proximal tubule injury during systemic inflammation in humans. In this study, we investigated the short-term effect of methylene blue, an inhibitor of the NO pathway, on kidney damage and function in septic shock patients.

Design and setting: A prospective clinical study, conducted in an intensive care unit.

Patients: Nine patients (4M/5F, mean age 71 ± 3 yrs) with a proven or suspected bacterial infection and with refractory septic shock defined as a mean arterial pressure ≤70 mmHg despite norepinephrine infusion ≥0.2 μg/kg/min.

Intervention: A 4 hours continuous i.v. infusion of 1 mg/kg/h methylene blue.

Measurements and Results: The urinary excretion of NO metabolites decreased with (median [25-75% range]) 90% [75-95] from baseline to 6 hours after methylene blue administration (P<0.05). The first 24 hours, the creatinine clearance improved with 51% [18-173] after methylene blue treatment (P<0.05), but was still strongly impaired. During the first 6 hours after start of methylene blue treatment, both the urinary excretion of cytosolic glutathione S-transferaseA1-1 and P1-1, markers for proximal and distal tubule damage respectively, decreased with 45% [10-70, P<0.05] and 70% [40-85, P<0.05] compared to baseline. After termination of the methylene blue infusion, the NO metabolites and markers of tubular injury returned to pre-treatment levels.

Conclusion: In septic patients with refractory shock, short-term infusion of methylene blue is associated with a decrease in NO production and an attenuation of the urinary excretion of renal tubular injury markers.
Introduction

Refractory septic shock remains the major cause of death in noncoronary intensive care units, especially when accompanied by multiple organ failure, with an estimated mortality rate of 50-60%1. The incidence of acute renal failure in refractory septic shock is approximately 40-50%2.

Nitric oxide (NO) has emerged as an important contributory factor to the pathogenesis of septic shock. We previously demonstrated that induction of renal NO is associated with proximal tubule injury during systemic inflammation in humans3. NO stimulates soluble guanylate cyclase (sGC) by binding to its heme moiety, which generates cyclic guanosine monophosphate (cGMP)4. In the kidney, NO and cGMP production are associated with lipopolysaccharide-induced renal proximal tubular cell toxicity5. Selective sGC inhibition during septic shock in rats resulted in an attenuation of renal dysfunction6, indicating that blocking sGC may be a potential therapeutic strategy to treat septic shock-associated renal failure.

Methylene blue binds to sGC, blocks cGMP production, and has the ability to inhibit NO synthases and to scavenge NO7,8. Although several controlled and uncontrolled clinical studies showed beneficial effects of methylene blue on the hemodynamic instability during septic shock (reviewed in9), there are no studies on the putative protective renal effects. We examined the short-term effects of continuous infusion of low dose methylene blue (1 mg/kg/h) in patients with septic shock on urinary excretion of the acute kidney injury markers, cytosolic glutathione S-transferases (GSTs) present in proximal tubule (GSTA1-1) and distal tubule (GSTP1-1)10.

Materials and methods

Patients
The ethical committee of the VieCuri Medical Centre Venlo and the Dutch central committee on research involving human subjects approved this study, and informed consent was obtained from the patients’ legal representatives. Patients with a proven or suspected bacterial infection were included when they met the criteria for refractory septic shock11, defined as mean arterial pressure (MAP) ≤70 mmHg despite adequate fluid therapy and norepinephrine infusion at a rate ≥0.2

Effects on circulation, organ perfusion and immune system
μg/kg/min, existing for less than 24 hours. Patients were excluded if they were <18 years, pregnant or lactating, HIV seropositive or had acute ischemia or myocardial infarction <6 months prior to the study. Throughout the study, all patients received standard conventional therapy, including treatment with low-dose hydrocortisone (50 mg/24 hours). During the study period, aminoglycosides, drotrecogin alpha, and diuretics were not administered and no radiological examinations with potentially nephrotoxic contrast agents were performed. Eligible patients received a 4 hour continuous infusion of 1 mg/kg/hour methylene blue (1% w/v), provided by the VieCuri Medical Centre pharmacy. Arterial blood and catheterized urine were collected at several time points during the first 24 hours. Clinical parameters, the severity of illness using acute physiology and chronic health evaluation II (APACHE II) and sepsis-related organ failure assessment (SOFA) were recorded.

**Chemical assays**
Biochemical parameters were determined by routine clinical chemistry. Hemoglobin, methemoglobin and bilirubin were measured to assess possible side effects of methylene blue, such as hemolytic anemia and methemoglobinemia. Total amount of the stable NO metabolites, nitrate and nitrite, measure of NO radicals production, and the amounts of GSTA1-1 and GSTP1-1 in urine were assayed as described. The blue colour in urine due to methylene blue excretion, did not affect the chemical assays.

**Data analysis**
Values are given as mean ± SEM or median [25-75% range] depending on their distribution. Differences between experimental groups were tested by analysis of variances (ANOVA) for repeated measures. A two-sided P-value <0.05 was considered significantly different.

**Results**

**Patients**
Nine patients with septic shock were included (Table 4.1.). Seven patients ultimately died in the intensive care unit, one of refractory shock (within 12 hours) and six patients because of multiple organ failure. In the latter group, two patients
Table 4.1
Baseline characteristics

<table>
<thead>
<tr>
<th>Demographics</th>
<th>Methylene blue (n=9)</th>
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<tr>
<td>M/F</td>
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</tr>
<tr>
<td>Age (yrs)</td>
<td>70.9 ± 2.5</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>27.2 ± 1.9</td>
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<tr>
<td>APACHE II</td>
<td>26.6 ± 2.3</td>
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</table>

<table>
<thead>
<tr>
<th>SIRS criteria</th>
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</thead>
<tbody>
<tr>
<td>Temperature (°C)</td>
<td>35.9 [35.6-36.1]</td>
</tr>
<tr>
<td>&lt; 36.5 (n=5)</td>
<td>38.7 [38.6-38.8]</td>
</tr>
<tr>
<td>&gt; 38.5 (n=4)</td>
<td></td>
</tr>
<tr>
<td>Leukocytes (×10⁹/L)</td>
<td>2.7 [2.4-3.0]</td>
</tr>
<tr>
<td>&lt; 4.0 (n=3)</td>
<td>19.2 [17.3-21.1]</td>
</tr>
<tr>
<td>&gt; 12.0 (n=4)</td>
<td></td>
</tr>
<tr>
<td>Heart rate (bpm)</td>
<td>113 [100-122]</td>
</tr>
<tr>
<td>MAP (mmHg)</td>
<td>69 [65-70]</td>
</tr>
<tr>
<td>Norepinephrine infusion rate (µg/min/kg)</td>
<td>0.50 [0.30-1.00]</td>
</tr>
<tr>
<td>Respiratory rate (breaths/min)</td>
<td>19 [18-22]</td>
</tr>
<tr>
<td>Patients on ventilator †</td>
<td>9</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SEM or as median [25-75% range]. Demographics were assessed at inclusion of the patients and SIRS criteria at the start of the methylene blue treatment (< 24 hours). The high and low temperatures and leukocyte counts are gathered to demonstrate that patients included satisfied the SIRS criteria.

† Patients were ventilated with a FiO₂ of 54 ± 6% by Pressure Control (n=5) or Pressure Support (n=4). Ppeak 18 ± 2 cmH₂O, PEEP 14 ± 1 cmH₂O, leading to tidal volumes of 10.8 ± 0.7 ml/kg ideal body weight for females and 10.4 ± 0.5 ml/kg ideal body weight for males. M: male, F: female, BMI: body mass index, MAP: mean arterial pressure.

died within 7 days and the remaining four patients within 28 days after intervention. The mean calculated predicted mortality rate was 61% and all patients had at least three organ failures, reflecting a mean SOFA score of 11.1 ± 0.9. The median stay at the intensive care unit was 16 days [7-24], the two survivors stayed 89 days [52-121] in hospital. Pathogenic organisms isolated by culture and site of infection are illustrated in Table 4.2.

Median C-reactive protein was 178 mg/L [range 118-189], all patients had lactic acidemia (2.7 [2.1-3.7] mmol/L) and thrombocytopenia (68 [50-104] ×10⁹/L). The median MAP increased slightly with 5 mmHg [2-11] from 69 [65-70] at baseline to 74 [68-82] mmHg 3 hours after start of methylene blue (P<0.05), with no
change in norepinephrine infusion rate. Methemoglobinemia or hemolytic anemia did not develop after methylene blue (data not shown). All methylene blue-treated patients showed blue-colouring of urine and skin.

**Methylene blue attenuates NO formation**
The concentration NO metabolites in plasma was elevated in septic shock patients compared to healthy volunteers, but did not change after methylene blue administration (Fig. 4.1.A). In contrast, methylene blue significantly attenuated the urinary excretion of NO metabolites by 90% [75-95%, P<0.05] from baseline (233 [112-536] µmol/mmol creatinine) to 6 hours (37 [10-87] µmol/mmol creatinine) after the start of methylene blue (Fig. 4.1.B). At 4 hours, methylene blue infusion was stopped, after which the excretion of urinary NO metabolites increased by 135% [65-795%, P<0.05] within 6 to 24 hours after methylene blue treatment (Fig. 4.1.B).

**Methylene blue attenuates kidney damage**
All patients showed impaired renal function with oliguria and mild proteinuria (Table 4.3.). One patient suffered from anuria during the first day. Six patients required continuous veno-venous hemofiltration renal replacement therapy with a flow of 35 ml/kg (for 8.5 [4-13] days), of which 3 during the methylene blue infusion. The creatinine clearance improved by 51% [18-173%, P<0.05] during the first 24 hours after methylene blue, but was still strongly impaired (Table 4.3.).

### Table 4.2
**Causes of septic shock**

<table>
<thead>
<tr>
<th>Patient number</th>
<th>Type of organism</th>
<th>Site of infection</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>Escherichia coli</td>
<td>Abdomen</td>
</tr>
<tr>
<td>2</td>
<td>Escherichia coli</td>
<td>Abdomen</td>
</tr>
<tr>
<td>3</td>
<td>Enterobacter aerogenes</td>
<td>Lung</td>
</tr>
<tr>
<td>4</td>
<td>Klebsiella pneumonia</td>
<td>Lung</td>
</tr>
<tr>
<td>5</td>
<td>Pseudomonas aeruginosa</td>
<td>Lung</td>
</tr>
<tr>
<td>6</td>
<td>Escherichia coli</td>
<td>Abdomen</td>
</tr>
<tr>
<td>7</td>
<td>Escherichia coli</td>
<td>Urinary tract</td>
</tr>
<tr>
<td>8</td>
<td>Pseudomonas aeruginosa</td>
<td>Skin</td>
</tr>
<tr>
<td>9</td>
<td>Escherichia coli</td>
<td>Abdomen</td>
</tr>
</tbody>
</table>
**Figure 4.1**

NO metabolites in plasma and urine and the urinary excretion of tubular injury markers glutathione-S-transferase (GST)\textsubscript{A1-1} and \textsubscript{P1-1}.

NO metabolite levels in plasma (A, \(n=9\)) and urine (B, \(n=8\)) and levels of GST\textsubscript{A1-1} (C, proximal tubule, \(n=8\)) and GST\textsubscript{P1-1} (D, distal tubule, \(n=8\)) were measured in urine at various time intervals after methylene blue (MB) administration in septic shock patients. The urinary excretion of NO metabolites and GSTs was corrected for creatinine excretion. Data are expressed as median [25-75 % range] and analyzed by ANOVA with repeated measures over the two time periods.

\[\text{Plasma concentration NO} (\mu\text{M})\]

\[\text{Urinary NOx (mmol/mmol creatinine)}\]

\[\text{Urinary GSTA1} (\text{mg/mmol creatinine})\]

\[\text{Urinary GSTP1} (\text{mg/mmol creatinine})\]

\(* p<0.05\) vs. baseline

\(** p<0.05\) vs. 6 h after MB treatment
Table 4.3.
Kidney function parameters of septic shock patients (n=8) treated with methylene blue

<table>
<thead>
<tr>
<th>Kidney function parameter</th>
<th>Time</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Total urine volume (ml)</td>
<td>0-24 h</td>
<td>495 [169-885]</td>
</tr>
<tr>
<td>Protein excretion (mg/day)</td>
<td>0-24 h</td>
<td>342 [245-434]</td>
</tr>
<tr>
<td>Creatinine clearance (ml/min)</td>
<td>Baseline</td>
<td>8.2 [4.2-17.4]</td>
</tr>
<tr>
<td></td>
<td>24 h</td>
<td>10.6 [9.6-14.8] *</td>
</tr>
<tr>
<td>BUN (mmol/L)</td>
<td>Baseline</td>
<td>17.8 [10.8-20.0]</td>
</tr>
<tr>
<td></td>
<td>24 h</td>
<td>17.3 [10.4-22.5]</td>
</tr>
<tr>
<td>Fractional excretion of sodium</td>
<td>Baseline</td>
<td>(n=0)</td>
</tr>
<tr>
<td></td>
<td>24 h</td>
<td>3.1 [2.6-3.6] (n=3)</td>
</tr>
<tr>
<td>&gt; 2%</td>
<td>Baseline</td>
<td>0.6 [0.3-0.7] (n=8)</td>
</tr>
<tr>
<td></td>
<td>24 h</td>
<td>0.4 [0.2-0.5] (n=5)</td>
</tr>
</tbody>
</table>

Data are expressed as median [25-75% range]. * significantly different compared to the baseline, P<0.05.

The urinary excretion of both GSTA1-1 and GSTP1-1 was elevated in all septic shock patients, indicating both proximal and distal renal tubule damage. During the first 6 hours of methylene blue, urinary excretion of GSTA1-1 and GSTP1-1 was attenuated by 45% [10-70%] and 70% [40-85%] compared to baseline levels (Fig. 4.1.C+D, P<0.05). After ending methylene blue infusion, urinary excretion of GSTA1-1 and GSTP1-1 increased again, although not significantly (Fig. 4.1.C+D).

Discussion

Several clinical studies in septic shock patients have investigated the effects of methylene blue on the heart, vascular wall and lungs. This is the first report demonstrating that methylene blue attenuates kidney damage in human septic shock.

To determine the effect of NO-pathway inhibition on renal damage, we examined the urinary excretion of early tubular injury markers and found that methylene blue inhibited the NO-pathway and preserved the integrity of renal tubules. After termination of methylene blue infusion, these parameters returned to their
elevated pre-treatment levels. Both plasma concentrations (> 2-fold) and urinary NO metabolites levels in our septic shock patients were much higher compared to healthy volunteers, as demonstrated earlier. Hydrocortisone may have inhibited iNOS activation, however, the patients received a continuous infusion of hydrocortisone that started before methylene blue infusion and continued during the 24 hours period. Therefore, the observation that urinary NO metabolite excretion was only attenuated in the first 6 hours after the start of methylene blue suggests that this effect is not related to steroids. Although urinary NO metabolite excretion was attenuated, we did not find a reduction in plasma NO metabolites. This is in contrast with an earlier report, in which patients received a bolus injection of methylene blue prior to the continuous infusion.

Renal failure in septic shock patients is a complex and multifactorial disease process. During septic shock, the systemic vasodilation increases renal sympathetic activity and angiotensin concentration which results in intrarenal vasoconstriction with sodium and water retention and decreased glomerular filtration rate. We previously demonstrated that induction of renal iNOS, constitutively expressed in the kidney, is associated with proximal tubule injury during systemic inflammation in humans. As a result of its active secretory transport function and role in urine concentration, the proximal tubule is a susceptible target and often the first site of damage. Therefore, inhibition of peroxynitrite formation from excessively produced NO and superoxide by methylene blue may be beneficial for the kidney during septic shock, possibly explained by the local accumulation of methylene blue in renal proximal tubules. Global hemodynamic variables can influence renal function, however, only a small but statistically significant increase was found for mean arterial pressure, whereas other global hemodynamic parameters did not change during methylene blue treatment.

Since the detailed nature of our investigation, obviously these methods are not feasible in a large-scale clinical intervention study. The most elegant way to examine the effects of methylene blue would be in a randomized, placebo-controlled cross-over study. However, with such critically ill patients this design may be considered unethical. We deliberately chose a subgroup of severe septic shock patients with a high chance of sepsis-induced renal damage for two reasons: this is the group of patients in which methylene blue is used as a ‘last resort therapy’, and second, to demonstrate the putative beneficial effects of methylene blue on renal damage. With an estimated standard deviation of 36% in urinary GST excretion, 80 patients would be needed to demonstrate with 80% power a
10% reduction in renal injury. This number of patients was not feasible for our investigations. Therefore we decided to determine the parameters before, during and after methylene blue infusion during a 24 h period, which allows each patient to serve as own control.

Because of the observational nature and limited size of the present study, and the heterogeneity of the patient population, our findings warrant conformation on hard end points by a larger clinical trial. However, in our view, first a long-term study is necessary to assess the safety of chronic methylene blue administration in septic patients with refractory shock. Promising effects of methylene blue were found in a trial with vasoplegic patients after cardiac surgery treated with methylene blue, in which a reduction in both mortality and incidence of renal failure was observed.

In conclusion, short-term infusion of methylene blue in septic patients with refractory shock, is associated with a decrease in NO production and an attenuation of the urinary excretion of renal tubular injury markers.
References


Hypertonic fluid resuscitation
PART 3

NOVEL HEMODYNAMIC THERAPIES IN SEPTIC SHOCK;
Chapter 3.1

Hypertonic fluid administration in patients with septic shock: A prospective double-blind randomized placebo-controlled study

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Submitted for publication
Abstract

**Objectives:** Hypertonic fluid resuscitation is thought to exert effects on global and regional circulation as well as on cardiac function. We assessed the short-term effects of hypertonic fluid administration on hemodynamic parameters and cardiac function in septic shock patients.

**Design:** Double blind prospective randomized controlled trial.

**Setting:** Closed-format 15-bed mixed intensive care unit in a tertiary referral teaching hospital.

**Patients:** Patients ≥ 18 yrs who met international criteria for septic shock within 24 hrs of ICU admission were eligible for enrollment.

**Interventions:** Patients were randomized to receive 250ml 7.2% NaCl/6% hydroxyethylstarch (HT) or 500ml 6% hydroxyethylstarch (IT). This trial is registered with ANZCTR.org.au as ACTRN12607000259448.

**Measurements and Main Results:** The following measurements were taken before and after fluid administration at regular intervals: blood pressure, central venous pressure (CVP), doses of inotropic and vasopressor drugs, cardiac index (CI), stroke volume index (SVI), stroke volume variation (SVV), extravascular lung water index (EVLWI) and intrathoracic blood volume index (ITBVI). Systolic tissue Doppler velocities (TDI) of the medial mitral annulus were measured using echocardiography to assess left ventricular contractility. To quantify the effect on vascular tone we did a log transformation of the ratio mean arterial blood pressure divided by the norepinephrine infusion rate (log MAP/NE). Ongoing fluid requirements were recorded for 24 hours after the study fluid was administered. Compared to the IT group, HT treatment resulted in an improvement in log MAP/NA (p=.008), systolic TDI velocities (p=.03) and SVI (p=.017). No differences between the groups were found for preload parameters (CVP, SVV, ITBVI) or for afterload parameters (SVRI, MAP). HT treatment decreased the need for ongoing fluid resuscitation (2.8±1.5 liter/24 hours versus 4.1±1.6 liter/24 hours in the IT group, p=.046). Lactate levels tended to be higher in the HT group at baseline (p=.09) and this remained so after treatment (p=.04).

**Conclusions:** Compared to IT fluid administration, HT improved the vascular tone and cardiac contractility independent of changes in preload or afterload. The need for ongoing fluid resuscitation was also reduced.
Hypertonic fluid resuscitation

Introduction

Sepsis is the leading cause of death in critically ill patients. Recent definitions recognize the importance of sepsis-induced myocardial depression and include a low cardiac index or echocardiographic evidence of cardiac dysfunction as one of the criteria for diagnosis of severe sepsis. Although cardiac output is usually maintained or elevated in septic shock, intrinsic cardiac dysfunction is demonstrable in up to 40% of patients. The pathophysiology of sepsis-induced myocardial depression has not been fully elucidated but includes effects of cytokines and nitric oxide.

Tissue Doppler imaging is an easy and reproducible quantitative measurement of cardiac systolic and diastolic function. Recently, this technique has been shown to be useful as a way of determining septic cardiac dysfunction, and hence providing additional prognostic information in septic patients.

Fluid resuscitation, in order to restore intravascular volume and to ensure adequate tissue perfusion and oxygen delivery, is thought to be of key importance in the treatment of septic shock patients. Hypertonic fluid resuscitation has been successfully applied in the treatment of various shock states, especially in hemorrhagic shock. The macrocirculatory effects of hypertonic infusion in hemorrhagic shock are characterized by rapid plasma volume expansion and a decrease in afterload. Experimental evidence also suggests a possible improvement of myocardial contractility. Data in septic shock patients are scarce and currently there are reports regarding the direct effects of hypertonic fluids on cardiac function in septic patients.

In the present study we tested the hypothesis that the infusion of hypertonic fluid in patients with septic shock improves the cardiovascular function.

Materials and Methods

Patients
The study was performed between June 2007 and December 2008 in a closed-format 15-bed mixed intensive care unit (ICU) in a tertiary referral teaching hospital (Waikato Hospital, Hamilton, New Zealand). It was designed as a prospective, single-centre, randomized, controlled, double-blind clinical trial and is registered with ANZCTR.org.au as ACTRN12607000259448. Consecutive
adult patients with septic shock were screened for inclusion in the study. Septic shock was defined according to standardized criteria. Inclusion criteria were the need for fluid resuscitation guided by stroke volume variation (SVV) or as per discretion of the treating intensivist, and enrollment within 24 hours after ICU admission. Exclusion criteria were age <18 years, pregnancy, hyponatraemia as defined by a serum sodium level < 130 mmol/l, hypernatraemia as defined by a serum sodium level > 150 mmol/l, arrhythmias including atrial fibrillation and pacemaker rhythm, and myocardial ischemia or infarction < 1 month prior to the study. Patients fulfilling study criteria were randomized into two groups using sequentially numbered, opaque sealed envelopes. The study was approved by the Northern Y Regional Ethics Committee (NTY/06/08/070). We obtained written informed consent from patients or their surrogate decision makers, consistent with applicable laws.

**Interventions**

All patients had PICCO catheters in situ as per routine management of septic shock in our unit. The PICCO plus (software V7.1, Pulsion Medical Systems, Munich) was calibrated according to the guidelines of the manufacturer before the first measurement, then again after 1, 4 and 8 hours. After data collection at baseline, the treatment group received 250 ml of 6% hydroxyethylstarch (HES) 200 in 7.2% sodium chloride (Hyperhes®, Fresenius Kabi, Germany), the control group received 500 ml of 6% HES 200 in 0.9% sodium chloride (Starquin®, Biomed, New Zealand). The rationale behind the different bolus sizes was that hypertonic colloids have been shown to have a sustained intravascular volume effect approximately twice that of isotonic colloids. This way we aimed to minimize any differences in effect on plasma volume expansion and preload to achieve a better understanding of the effects of the hypertonic character of the study fluid per se. Vasopressor doses were titrated by the bedside nursing staff to achieve a target mean arterial blood pressure (MAP) as defined by the treating intensivist as per routine practice. Ongoing fluid resuscitation was also per discretion of the treating intensivist. Arterial blood samples were taken at baseline and after every 30 minutes for 4 hours and processed in a point of care analyzer (ABL 800 Flex, Radiometer, Copenhagen) to measure levels of lactate, sodium and chloride in addition to routine blood gases.
**Data collection**

Demographic data and severity of illness using APACHE II (acute physiology and chronic health evaluation II) and SOFA (sequential organ failure assessment) scores were recorded. Data on hospital mortality were collected after completion of the study.

The following hemodynamic variables were recorded every 15 minutes during 4 hours: arterial blood pressure (systolic, diastolic, and mean), heart rate, cardiac index (CI), stroke volume index (SVI), stroke volume variation (SVV), systemic vascular resistance index (SVRI), and central venous pressure (CVP). The extravascular lung water index (EVLWI) and intrathoracic blood volume index (ITBVI) were recorded when the PiCCO was calibrated (t=0, 1 and 4 hours). The urine output was recorded hourly. Fluid responsiveness was defined as an increase in CI > 10% measured 30 minutes after the fluid bolus. We performed a log transformation of the ratio mean arterial blood pressure divided by the norepinephrine infusion rate (log MAP/NE). This mathematical technique allows us to quantify the effect on vascular tone and correct for the beside adjustments of norepinephrine dose to achieve the target MAP.

The effect of the intervention on cardiac function was assessed by echocardiography. Echocardiography was performed before and 1 hour after the infusion of study fluid. All echo studies were performed by the same echocardiographer who was blinded to the treatment. The effect on diastolic function was assessed by measuring mitral valve inflow velocities using pulsed wave Doppler (E and A waves) and tissue Doppler imaging (TDI) of the medial mitral valve annulus during diastole. The early diastolic (E') velocity of the mitral annulus was assessed by TDI and the E/E' was calculated. LV diastolic dysfunction was defined as a composite of E'≤8cm/sec and/or mean E/ E'≥13. This was compared to E/A ratio to confirm presence or absence of diastolic dysfunction. The systolic (Sm) velocity of the mitral annulus was assessed by TDI and used as a measure for cardiac contractility in addition to stroke volume index as derived by PiCCO measurements. Doppler velocities measured 5 times were averaged.

**Statistical Analysis**

Data are presented as mean±SD or as median [25th - 75th percentile], depending on their distribution. Baseline characteristics were compared with a Student T-test or a Mann-Whitney-U test, as appropriate. Two way repeated measures
ANOVA was used to compare the effect of the treatments (between-group) and treatment-time interaction effects. All statistical analysis was done with NCSS 2007 (version 07.1.13, NCCS, Kaysville, Utah).

**Results**

*Patient Characteristics*

Demographic characteristics are shown in table 1. Patients were randomized to receive either a hypertonic fluid bolus (HT, n=12) or an isotonic fluid bolus (IT, n=12). The patients in the two groups had comparable demographic characteristics.

<table>
<thead>
<tr>
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<td>Lung</td>
<td>HT</td>
<td>7</td>
<td>37</td>
<td>Survived</td>
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</table>
characteristics and severity of illness as measured by APACHE II (IT 23.5±7.4 vs. HT 24.4±6.7, p=.75) and SOFA scores (IT 8.9±2.5 vs. HT 9.8±3.4, p=.50). The hospital mortality rate was 25% (3/12) in the hypertonic group and 30% (4/12) in the isotonic group (p=.75).

**Hemodynamic results**

The hemodynamic results are summarized in Table 2 and Fig 1. The CVP increased significantly in both groups to a comparable degree (Fig 1A, p=.86 between groups). Measures for preload ITBVI and SVV were not significantly affected by the treatment (data not shown). Only 4/12 patients in the HT group and 3/12 of patients in the IT group were found to be fluid responsive as defined by an increase in CI > 10% after 30 minutes. The norepinephrine infusion rate at baseline was nonsignificantly higher in the HT group compared to the IT group (15 [10.5-31.5] versus 8 [5.6-15] μg/min, p=.13). Compared to IT treatment, HT treatment resulted in a decrease in norepinephrine requirement (p=.04) and an improvement in vascular hyporeactivity as defined by log MAP/NE (p=.008 between groups, Fig 1B).

The urine output showed an increasing trend in the HT group and a decreasing trend in the IT group that did not reach statistical significance between

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**Table 2**

*Time course of hemodynamic parameters.*

Data are shown as mean±SD or median [IQR]. HT, hypertonic solution; IT, isotonic solution; MAP, mean arterial pressure; NE, norepinephrine infusion rate; CVP, central venous pressure; CI, cardiac index; HR, heart rate; SVV, stroke volume variation.

<table>
<thead>
<tr>
<th>Group</th>
<th>Baseline</th>
<th>30 min</th>
<th>60 min</th>
<th>120 min</th>
<th>180 min</th>
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<td>MAP (mmHg)</td>
<td>HT 72±5</td>
<td>76±10</td>
<td>78±9</td>
<td>79±11</td>
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<td>75±8</td>
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<tr>
<td></td>
<td>IT 72±5</td>
<td>78±9</td>
<td>76±6</td>
<td>74±7</td>
<td>78±7</td>
<td>73±4</td>
</tr>
<tr>
<td>CVP (cmH2O)</td>
<td>HT 13±5</td>
<td>15±6</td>
<td>14±4</td>
<td>13±4</td>
<td>15±5</td>
<td>16±7</td>
</tr>
<tr>
<td></td>
<td>IT 10±2</td>
<td>14±4</td>
<td>13±4</td>
<td>12±4</td>
<td>13±4</td>
<td>13±3</td>
</tr>
<tr>
<td>CI (l/min/m²)</td>
<td>HT 4.6±1.7</td>
<td>4.9±1.9</td>
<td>5±1.6</td>
<td>4.9±1.5</td>
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<tr>
<td></td>
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<td>HR (beats/min)</td>
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<td>99±15</td>
<td>103±19</td>
<td>107±24</td>
<td>111±24</td>
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<td></td>
<td>IT 93±17</td>
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<td>94±17</td>
<td>96±15</td>
<td>96±15</td>
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<tr>
<td>SVV (%)</td>
<td>HT 12±7</td>
<td>9±7</td>
<td>9±6</td>
<td>12±5</td>
<td>13±9</td>
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<td></td>
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<td>8±4</td>
<td>11±5</td>
<td>9±7</td>
<td>11±6</td>
<td>13±6</td>
</tr>
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</table>
Figure 1
Changes in cardiovascular variables over time before and following isotonic fluid (dashed lines, crosses) and hypertonic fluid administration (solid line, open circles).

1A: CVP, central venous pressure, \( p=0.86 \) between groups;
1B: \( \log (\text{MAP}/\text{NE}) \), logarithmic transformation of mean arterial blood pressure divided by norepinephrine infusion rate, \( p=0.008 \) between groups;
1C: SVI, stroke volume index, \( p=0.017 \) between groups
the groups (p=0.19). The HT group required less fluid resuscitation in the 24 hours following the administration of study fluid than the IT group (HT 2.8±1.5 liter/24 hours versus IT 4.1±1.6 liter/24 hours, p=0.046). The EVLWI did not change over time and was not different between groups (data not shown).

**Cardiac effects**

In the HT group the systolic velocity of the mitral annulus increased from 16±4 cm/s to 18±5 cm/s as compared to a decrease from 15±6 cm/s to 13±4 cm/s in the IT group (p=.03 between groups, Fig 2). The number of patients with diastolic dysfunction at baseline was 4/12 in the HT group and 6/12 in the IT group (p=.41). Following the fluid bolus these numbers were 3/12 in the HT group and 8/12 in the IT group (p=.04). All diastolic dysfunction was classified as mild (grade 1, impaired relaxation) with reversal of E/A ratio.

There was an instant increase in SVI in the HT group (from 42±13 ml/m² at baseline to 49±16 ml/m² after 30 minutes, p=.0012) that lasted for 2 hours. No change in SVI was observed in the IT group (p=0.017 between groups, Fig 1C). Because the change in SVI was accompanied by a decrease in heart rate in the HT group (p=0.0014 between groups, Table 2), the cardiac index was not affected significantly over time and by the treatment (p=0.38 between groups, Table 2).
Table 3

Metabolic variables over time in isotonic group (IT) and hypertonic group (HT).

Data reported as mean±SD. Hb, haemoglobin; PaO$_2$, arterial oxygen tension; P/F ratio, ratio arterial oxygen tension/inspired oxygen fraction; Bic, arterial bicarbonate level.

<table>
<thead>
<tr>
<th>Metabolic and respiratory variables</th>
</tr>
</thead>
</table>

The course of metabolic and respiratory variables is shown in Table 3. The lactate levels tended to be higher in the hypertonic group at baseline (p=.09) and this remained so after treatment (p=.04). The plasma sodium levels and the plasma chloride levels increased significantly in the HT group, but not in the IT group. This did not result in a significant change in the acid base status.

The P/F ratio decreased over time (Table 3, p=0.006) without being affected by the treatment group (difference between groups: p=0.12).

**Side effects**

Both treatments were well tolerated in all patients. No neurologic dysfunction, arrhythmias, coagulation abnormalities or any other adverse effects were noted.
Hypertonic fluid resuscitation

Discussion

We conducted a study comparing the effects of hypertonic and isotonic fluid administration in critically ill patients with septic shock within 24 hours of intensive care admission. We aimed to induce a comparable change in preload parameters by the two treatments and found a number of significant differences between the groups after the intervention.

Hypertonic fluid increased cardiac contractility as assessed by systolic tissue Doppler velocities, and decreased the proportion of patients with diastolic dysfunction. The improvement in cardiac contractility was confirmed by the observation that stroke volume index increased in the hypertonic group but not in the isotonic group. Importantly, the positive inotropic effect on the heart was not caused by a difference in preload or filling pressure as both treatments had similar effects on plasma volume expansion and preload as assessed by SVV, ITBVI and CVP. We specifically designed this study to eliminate differences in preload between the groups as caused by the fluid bolus by administering twice as much isotonic HES as hypertonic HES. Hypertonic fluids have been shown to increase intravascular volume more than isotonic fluids when given in the same quantity by mobilizing fluid from the intracellular compartment. In previous studies where this issue was not addressed, most of the effects found on cardiac function were influenced by changes in preload and/or afterload. No changes in inotropic drugs were made during the study period. The effects on cardiac contractility were also not caused by significant changes in afterload as measured by SVRI or MAP, as in both groups the MAP was kept constant by titrating norepinephrine infusion rate to a prescribed target MAP. Cardiac contractility improved in the HT group despite a reduction in the norepinephrine dose in that group.

This positive effect of hypertonic fluid on contractility in sepsis-induced myocardial depression has been described before in animal and human studies. Direct effects on myocardial cells could include a direct hyperosmolar effect that decreases myocardial edema and restores transmembrane potentials. Recently, a central mechanism involving cerebral periventricular sodium concentration and mediated by cerebral angiotensin II type 1 receptors has also been shown to contribute to the hemodynamic changes found after hypertonic fluid administration. Hypertonic fluid has not been shown to increase contractility in the absence of sepsis or a systemic inflammatory response.
We excluded patients with known and recently symptomatic coronary artery disease, because hypertonic fluids can potentially worsen coronary artery flow and cardiac contractility distal to coronary artery occlusion. In addition to the cardiac effects, the norepinephrine dose could be reduced in the hypertonic group while maintaining predefined MAP targets, while in the isotonic group the norepinephrine dose did not change. Again, this was not caused by differences in plasma volume expansion as discussed before. This finding is consistent with other studies. In a randomized controlled study in 50 children undergoing open-heart surgery, the hypertonic solution resulted in a decreased need for catecholamine support. The mechanism by which hypertonic fluid might reduce norepinephrine requirement is unclear, but may include effects on transmembrane potentials, altered sensitivity to vasopressor drugs and immunomodulatory effects.

The hemodynamic effects of hypertonic resuscitation are possibly dose dependent. For example, no hemodynamic changes were found after a small dose of hypertonic saline (5 ml/kg 3.5% sodium chloride) in patients with severe sepsis compared to normal saline. However, also no changes in sodium levels were found in this study raising the possibility that the dose used was insufficient to cause hemodynamic changes.

Finally, hypertonic fluid administration significantly decreased the need for ongoing fluid resuscitation in the 24 hours following administration. Because all the significant differences found in the measured hemodynamic parameters lasted less than 8 hours, this would suggest a different mechanism. Explanations could include a reduction in the severity of capillary leak syndrome; an improvement in splanchic perfusion resulting in an improved gut barrier and reduced inflammatory activity; immunomodulatory effects as have been previously described. In a recent clinical study, equivalent fluid balances were found between the group receiving hypertonic/dextran solution and the control group receiving normal saline. This study was different from ours in many aspects. Different study fluids were used and our patients were more severely ill indicated by higher APACHE II scores. All our patients were per definition on vasopressor support, compared to approximately half of the patients in Oliveira’s study, suggesting a more severe inflammatory syndrome with a more profound vasodilatory shock in our patients.

In our study and numerous other studies, hypertonic fluid administration in shock states has been shown to be safe. We found a transient increase in plasma
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sodium and chloride levels without evidence of harm and without a significant effect on the acid-base equilibrium. This is consistent with previous reports. Our study has several limitations. We studied physiological end points in a “proof-of-concept” design in a relatively small number of patients. The study was not designed nor powered to investigate important clinical endpoints such as organ failure and mortality. Future studies should look at timing, dose-response, and the effects of repeated or continuous administration of hypertonic fluids in septic patients. Ideally this should be followed by a large PRCT testing the hypothesis that early hypertonic fluid resuscitation improves outcome in sepsis.

In summary, our results demonstrate that in septic shock patients, hypertonic fluid can be safely administered and results in improved cardiac and hemodynamic function independent of and in addition to the effects on plasma volume expansion.
References


Chapter 3.2

The effects of hypertonic versus isotonic fluid administration on the sublingual and gastric mucosal microcirculation in septic shock patients

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Abstract

**Objective:** Sustained abnormalities in the sublingual and intestinal mucosal microcirculation are associated with morbidity and mortality in septic patients. Hypertonic fluids improve microcirculatory blood flow in other shock states. We tested whether hypertonic versus isotonic fluid administration exerts different effects on the gastric mucosal metabolism perfusion ratio, as assessed by tonometry, and on the sublingual microcirculation, as assessed by Sidestream Dark Field (SDF) imaging, in fluid responsive and non-responsive septic shock patients.

**Design:** Prospective randomized placebo-controlled double blind clinical trial.

**Setting:** Closed-format 15-bed mixed intensive care unit in a tertiary teaching hospital.

**Patients:** Adult patients fulfilling criteria for septic shock within 24 hours of ICU admission.

**Interventions:** Patients were randomly assigned to receive 250ml 7.2% NaCl /6% hydroxyethylstarch (HT group, n=12) or 500ml 6% hydroxyethylstarch (IT group, n=12) over 15 minutes.

**Measurements and Main results:** Primary outcome was P[g-a]CO₂ gap as measured by gastric tonometry at baseline and every 30 min during 4 hours; and sublingual microcirculatory flow of small vessels as assessed by SDF imaging before and 1 hour after fluid administration. Fluid responsiveness was defined as an increase in cardiac index >10% after fluid administration. The median [IQR] P[g-a]CO₂ gap at baseline was elevated both in the HT group (21 [13-24] mmHg) and in the IT group (26 [20-51] mmHg, p=.17), indicating gastrointestinal hypoperfusion. The median [IQR] microvascular flow index was decreased in both groups (HT 2.25 [1.83-2.67], IT 2.39 [1.39-2.53], p=.93). Following fluid administration, no differences were observed in the course of the P[g-a]CO₂ gap or in any of the sublingual microvascular variables between the HT and the IT group. No difference was observed in the course of the P[g-a]CO₂ gap between fluid responders and non-responders. The sublingual microcirculatory flow index significantly improved in fluid responders from 2.32 [1.63-2.79] at baseline to 2.90 [2.43-2.98], while no change was observed in non-responders (from 2.30 [1.25-2.63], to 2.38 [1.33-2.55], p=.04), independent of the type of fluid administered.

**Conclusions:** In fluid responsive patients, fluid administration promotes sublingual microcirculatory blood flow, independent of fluid type. In septic shock patients, hypertonic fluid administration does not promote gastrointestinal mucosal perfusion or sublingual microcirculatory blood flow in comparison to isotonic fluid.
Introduction

Adequate fluid resuscitation is an important element in the treatment of septic shock patients. Traditionally, fluid resuscitation is guided by global hemodynamic parameters such as blood pressure, cardiac output and oxygen-derived variables. Microcirculatory abnormalities in septic patients have been well recognized, classified and quantified over recent years. Mucosal microcirculatory abnormalities in the gastrointestinal tract are reflected by an increased P[g-a]CO$_2$ gap that can be detected by gastric tonometry. Persisting sublingual as well as gastrointestinal microcirculatory alterations are associated with organ failure and mortality in septic shock, irrespective of normalization of systemic parameters. Recent studies suggest that the effects of isotonic fluid resuscitation result in an improved sublingual microcirculatory blood flow, both in non-septic and septic patients, whereas treatment strategies aimed to improve gastrointestinal hypoperfusion, including fluid resuscitation, have failed to correct mucosal perfusion abnormalities.

Hypertonic fluid resuscitation has been studied extensively in experimental and human traumatic hemorrhagic shock. Compared to isotonic fluids, hypertonic fluids have been shown to cause hemodilution and to reduce shock-induced endothelial and red blood cells edema, increasing blood flow at the capillary level by reducing viscosity and hydraulic resistance to a greater extent. In addition to immediate blood volume expansion and restoration of cardiac output in animal models of hemorrhagic shock, hypertonic fluid administration has been shown to correct microcirculatory alterations as assessed by intravital microscopy. Data concerning the effects of hypertonic fluids in septic patients are scarce. Although several previous studies in patients with sepsis have shown transient improvements of global perfusion, its effect on microcirculatory abnormalities is currently unknown.

We evaluated the effects of hypertonic versus isotonic fluid administration on gastric mucosal perfusion and sublingual microcirculation in septic shock patients. We hypothesized that hypertonic fluid would improve both gastric mucosal perfusion and sublingual microcirculation, compared to isotonic fluid.
Materials and Methods

Patients
The study was designed as a prospective, randomized, controlled, double blind clinical trial and is registered with ANZCTR.org.au as ACTRN12607000259448. The study was approved by the Northern Y Regional Ethics Committee (NTY/06/08/070). We obtained written informed consent from patients or their surrogate decision makers. The study protocol and the systemic cardiovascular effects of hypertonic versus isotonic fluid administration in the same group of patients will be described separately. Briefly, consecutive patients who fulfilled standard criteria for septic shock within 24 hours of ICU admission were randomized to receive hypertonic fluid (HT group) or isotonic fluid (IT group).

Interventions
A gastric tonometry catheter (TonometricsTM-catheter, TONO-i6F) was inserted in the stomach. Calibration of the tonometry module was performed according to the guidelines of the manufacturer (Datex-Ohmeda Division, Helsinki, Finland). Enteral feeding was discontinued, and omeprazole (40 mg iv) administered 1 hour before the first tonometry measurements to decrease interindividual variability.

Following collection of baseline data, the HT group received 250 ml of 6% hydroxyethylstarch 200/7.2% sodiumchloride (Hyperhes®, Fresenius Kabi, Germany) and the IT group received 500 ml 6% hydroxyethylstarch 200/0.9% sodiumchloride (Starquin®, Biomed, New Zealand), infused over 15 minutes. Because hypertonic fluids have been shown to increase plasma volume to a greater extent than isotonic fluids, we aimed to minimize this difference by administering twice the amount of isotonic fluid compared to hypertonic fluid. Differences or treatment effects observed would therefore more likely be the result of hypertonicity than caused by a difference in plasma volume expansion.

Measurements

Gastric tonometry
The partial pressure of CO₂ in the stomach was measured by automated air tonometry using an equilibration time of 10 minutes. PaCO₂ was measured...
simultaneously with a point-of-care blood gas analyzer (ABL 800 Flex, Radiometer, Copenhagen), and the difference between gastric and partial CO₂ pressure (P[g-a]CO₂ gap) was calculated every 30 minutes during 4 hours.

**Sublingual microcirculation**
The microcirculation was studied with Sidestream Dark Field (SDF) imaging using the Microscan® (Microvision Medical, Amsterdam, the Netherlands). SDF imaging is a stroboscopic light emitting diode ring-based imaging modality that is incorporated in a handheld device. The device illuminates an area of interest for clinical observation of the microcirculation. If a wavelength within the hemoglobin absorption spectrum (e.g., 530 nm) is chosen, red blood cells will appear dark and white blood cells may be visible as refringent bodies. The vessel walls are not visualized directly and imaging therefore depends on the presence of red blood cells. After the removal of saliva and other secretions, the device was applied on the lateral side of the tongue. Five sequences of 10 seconds each from different areas were recorded by a research investigator who was blinded to the treatment and stored in AVI-format on a Macintosh Powerbook (Apple computers, California). The SDF movies were recorded at baseline and 1 hour after the administration of study fluid. The video clips were blindly analysed offline by another investigator who had no involvement with the data collection. The images were presented in a random order to prevent inter-image coupling.

Semiquantitative analysis was performed as described in detail elsewhere. SDF images were divided into four equal quadrants. Quantification of flow (no flow: 0, intermittent flow: 1, sluggish flow: 2, and continuous flow: 3) was scored per quadrant for each vessel diameter cohort (small: 10–25 μm, medium 26–50μm, and large 51–100 μm). The microvascular flow index (MFI) was calculated as the sum of each quadrant score divided by the number of quadrants in which the vessel type was visible. The final MFI was averaged over a maximum of 12 quadrants (three regions, four quadrants per region) derived from the overall flow impressions of all vessels with a particular range of diameter in a given quadrant. The heterogeneity index was calculated, following the method of Trzeciak and colleagues, as the difference between the highest and lowest MFI, divided by the mean MFI of all sublingual sites at a single time point. Calculation of total (small) vessel density was performed with the AVA 3.0 software package (MicroVision Medical, Amsterdam, The Netherlands), as described and validated recently using a cut-off diameter for small vessels of <20 μm. After stabilization of the
images using the AVA 3.0 software, we defined the perfused (small) vessel density and the proportion of perfused (small) vessels (PPVs) in terms of the number and percentage of crossings with perfused (small) vessels per total length of three equidistant horizontal and three equidistant vertical lines.19

Statistical analysis
We anticipated a mean \( P[g-a]CO_2 \) gap of 10 mmHg at baseline with a standard deviation (SD) of 9 mmHg, based on an earlier study20. We calculated a sample size of 24 patients to detect an absolute difference in \( P[g-a]CO_2 \) gap of 12 mmHg in a two-sided test with an alpha level of 0.05 and power >80%. The Statistical Package for Social Sciences (SPSS 15.1 for Windows, Chicago IL) and the NCSS 2007 (version 07.1.13, Kaysville, Utah) were used for statistical analysis. Data are presented as mean ± SD or median [interquartile range, IQR] depending on the distribution. The effects on non-normally distributed parameters were compared using the Mann–Whitney test and the Wilcoxon signed rank test for paired measurements. Two way repeated measures ANOVA was used to compare the effect of the treatment on the \( P[g-a]CO_2 \) gap by examining the interaction between treatment group and time. We considered a p value of < 0.05 to be statistically significant.

Results

Patient characteristics
Baseline characteristics of the 2 groups of patients are presented in Table 1. Overall the baseline characteristics were well balanced between the groups, although the norepinephrine dose, SOFA score and plasma lactate level in the HT group tended to be higher (all p-values NS). A total of 8 patients, 4 in each treatment group, were found to be fluid responsive, defined as an increase in cardiac index >10% measured 30 minutes after the fluid administration as compared to baseline. The hospital mortality rate was 25% (3/12) in the HT group and 30% (4/12) in the IT group (p=NS). More detailed cardiovascular and metabolic data from this study will be described in a separate paper. Briefly, following fluid administration, no differences were observed between the treatment groups with regards to preload variables (central venous pressure, stroke volume variation, intrathoracic blood volume index), mean arterial blood pressure or cardiac index.
**Hypertonic fluid resuscitation**

Table 1

**Baseline characteristics.**

APACHE, Acute Physiology and Chronic Health Evaluation; SOFA, Sequential Organ Failure Assessment.

Data are presented as mean ± SD, as numbers (%) or as median [interquartile range].

<table>
<thead>
<tr>
<th>Variables</th>
<th>IT group (n=12)</th>
<th>HT group (n=12)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>61±13</td>
<td>56±16</td>
<td>0.45</td>
</tr>
<tr>
<td>Men</td>
<td>6 (50%)</td>
<td>7 (58%)</td>
<td>0.68</td>
</tr>
<tr>
<td>APACHE II</td>
<td>23.5±7.4</td>
<td>24.4±6.7</td>
<td>0.75</td>
</tr>
<tr>
<td>SOFA</td>
<td>8.9±2.5</td>
<td>9.8±3.4</td>
<td>0.5</td>
</tr>
<tr>
<td>Mean arterial pressure, mmHg</td>
<td>73±5</td>
<td>72±5</td>
<td>0.75</td>
</tr>
<tr>
<td>Cardiac index, L/min/m²</td>
<td>4.8±1.4</td>
<td>4.6±1.7</td>
<td>0.42</td>
</tr>
<tr>
<td>Norepinephrine dose, µg/min</td>
<td>11 [7.5-20]</td>
<td>20 [14-42]</td>
<td>0.13</td>
</tr>
<tr>
<td>Lactate, mmol/l</td>
<td>2±0.9</td>
<td>3.5±2.7</td>
<td>0.09</td>
</tr>
</tbody>
</table>

**Tonometry**

The median [IQR] P[g-a]CO₂ gap at baseline was 26 [20-51] mmHg in the IT group and 21 [13-24] mmHg in the HT group (p=.17), indicating gastrointestinal hypoperfusion. The time course of P[g-a]CO₂ gap in the two groups is shown in Figure 1. The P[g-a]CO₂ gap did not change significantly over time (p=.39) and there was no significant difference between the treatment groups (p=.31).

Patients who were found to be fluid responsive had similar baseline P[g-a]CO₂ gap compared to patients who were not responsive to fluid (23 [9-34] mmHg versus 22 [17-54], p=.54). The change in P[g-a]CO₂ gap over time was not different between fluid responsive and fluid unresponsive patients (p=.64).

**Sublingual microcirculation**

The microvascular variables measured before and one hour after the fluid bolus are summarized in Table 2. At baseline, all variables were similar between the two treatment groups. The median [IQR] microvascular flow index was decreased in both groups (HT 2.25 [1.83-2.67], IT 2.39 [1.39-2.53], p=.93). Following the fluid bolus, no significant changes were found in any of the microcirculatory measurements in either group compared to baseline or between the groups. In fluid responsive patients, small vessel MFI increased from 2.32 [1.63-2.79] at baseline to 2.90 [2.43-2.98], while no change was observed in non-responders (from 2.30 [1.25-2.63], to 2.38 [1.33-2.55]), p=.04 between groups (Fig 2).
Figure 1
Box plot demonstrating the time course of P(g-a)CO₂ gap.
Dots represent outliers. Black boxes IT group, gray boxes HT group.

Figure 2
Box plot of microvascular flow index (MFI) in small vessels (<20 μm) before and after fluid administration.
Black boxes fluid responsive patients, gray boxes fluid non-responders.
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Table 2

Microvascular variables.

MFI, microcirculatory blood flow index; TVD, total vessel density of (small) vessels; PPV, proportion of perfused (small) vessels; PVD, perfused (small) vessel density; p before, p value for difference in baseline measurements using Mann-Whitney U test; p treatment, p value for difference caused by treatment using Sign test. Cut-off for small vessels <20μm. All data are presented as median [interquartile range].

<table>
<thead>
<tr>
<th>Variables</th>
<th>Group</th>
<th>Before</th>
<th>After</th>
<th>p before</th>
<th>p treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>MFI small vessels</td>
<td>IT</td>
<td>2.39 [1.39-2.53]</td>
<td>2.4 [2.29-2.56]</td>
<td>0.93</td>
<td>0.41</td>
</tr>
<tr>
<td></td>
<td>HT</td>
<td>2.25 [1.83-2.67]</td>
<td>2.67 [1.29-2.9]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MFI medium vessels</td>
<td>IT</td>
<td>2.63 [2.05-2.91]</td>
<td>2.78 [2.68-2.89]</td>
<td>0.79</td>
<td>0.65</td>
</tr>
<tr>
<td></td>
<td>HT</td>
<td>2.65 [2.42-2.95]</td>
<td>2.88 [1.59-3]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MFI large vessels</td>
<td>IT</td>
<td>3 [2.85-3]</td>
<td>3 [2.83-3]</td>
<td>0.83</td>
<td>0.58</td>
</tr>
<tr>
<td></td>
<td>HT</td>
<td>3 [2.85-3]</td>
<td>3 [2.69-3]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TVD, mm/mm²</td>
<td>IT</td>
<td>15.2 [13.3-14.7]</td>
<td>12.8 [12.2-14]</td>
<td>0.45</td>
<td>0.68</td>
</tr>
<tr>
<td></td>
<td>HT</td>
<td>15.7 [12.9-14.6]</td>
<td>13.9 [12.16-5]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PPV, %</td>
<td>IT</td>
<td>96 [91-100]</td>
<td>96 [92-98]</td>
<td>0.88</td>
<td>0.82</td>
</tr>
<tr>
<td></td>
<td>HT</td>
<td>97 [84-100]</td>
<td>99 [71-100]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PVD, 1/mm</td>
<td>IT</td>
<td>8.67 [7.76-9.8]</td>
<td>8.5 [8.01-8.98]</td>
<td>0.74</td>
<td>1</td>
</tr>
<tr>
<td>Heterogeneity index</td>
<td>IT</td>
<td>0.47 [0.35-1.61]</td>
<td>0.62 [0.35-1.02]</td>
<td>0.60</td>
<td>0.66</td>
</tr>
<tr>
<td></td>
<td>HT</td>
<td>0.89 [0.5-0.93]</td>
<td>0.47 [0.17-1.57]</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Discussion

In the present study, hypertonic fluid administration did not have a significant effect on the gastrointestinal mucosal metabolism perfusion ratio, as assessed by gastric tonometry, and on any of the measured sublingual microvascular variables in comparison to isotonic fluid. To our knowledge, no other human data are currently available on the microcirculatory effects of hypertonic fluid administration in sepsis.

With regards to the effects of fluid resuscitation on gastrointestinal perfusion, experimental studies have shown conflicting results. Hypertonic saline resuscitation improved measures of splanchnic perfusion to a greater extent compared to isotonic fluids in some animal studies of hemorrhagic shock. However, in other animal studies of hemorrhagic shock, hypertonic saline improved cardiac output but muscle, intestinal and renal microvascular blood flow remained significantly depressed. In an animal model of septic shock, large volume...
isotonic fluid resuscitation was unable to restore the P\([g-a]CO_2\) gap\(^{26}\). Hypertonic fluid administration in a porcine model of acute hyperdynamic endotoxemia resulted in a persistently high oxygen delivery to splanchnic organs\(^{27}\). In a more recent animal study, hypertonic fluid administration failed to restore splanchnic perfusion variables, but reduced the degree of gut epithelial cells apoptosis\(^{28}\).

Gastrointestinal mucosal hypoperfusion is thought to be a contributing factor in the pathophysiology of multi-organ failure. Failure to improve microcirculatory abnormalities present in the gastrointestinal system detected by an increased P\([g-a]CO_2\) gap during tonometry is associated with a poor outcome\(^5,8\). Nevertheless, treatment strategies aimed at improving the gastrointestinal hypoperfusion in human studies have so far failed to correct mucosal perfusion abnormalities and hence have not shown to improve outcome\(^5,29,31\). Our study was not designed nor powered to examine the effects of the fluid administration on endpoints such as survival, or to correlate survival to the P\([g-a]CO_2\) gap.

In addition, we also did not find any improvement or recruitment of the sublingual microcirculation regardless of the fluid administered, indicating that hypertonic fluid administration exerts no immediate intrinsic effects on the sublingual microcirculation in septic shock patients. This finding is in contrast with previous studies in hemorrhagic shock models and patients. Several reasons may be put forward to explain these differences. First, septic shock is essentially a different disease process with specific distributive microcirculatory abnormalities as compared to hemorrhagic shock. Second, fluid resuscitation in hemorrhagic shock studies is likely to result in an improvement in global perfusion, as most subjects would be expected to be fluid responsive. The different effects on microvascular variables between hypertonic and isotonic fluids in these studies may simply be explained by a more profound macrocirculatory effect (plasma volume expansion) of hypertonic fluids. Indeed, in all studies that compared hypertonic with isotonic fluid administration, hypertonic fluid invariably increased preload, cardiac output and blood pressure significantly more than isotonic fluids. Our study was specifically designed to investigate the effects of hypertonic fluids per se while preventing a different effect on preload or volume status, and this aim was achieved as measures of preload and afterload were not different between the groups before or after fluid administration (data not shown). Finally, different methods have been used to measure and describe the microcirculation in the past. In the present study we used a relatively new and
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well-validated instrument, and the analysis was performed in accordance with the recommendations of a recent round table conference consensus\textsuperscript{19}.

Interestingly, in the subgroup of patients that were defined as fluid responsive, fluid administration resulted in a significant improvement in the sublingual microvascular variable MFI, independent of the type of fluid administered. The proportion of fluid responders was similar in the HT group and the IT group. This improvement in microvascular flow therefore seems to be the result of an overall improvement in preload and global perfusion (cardiac index) and again no intrinsic difference between the two fluids was found. In the non-responders, fluid resuscitation did not increase cardiac index and did not improve the microcirculation. These findings are consistent with a previous report in which fluid resuscitation improved the sublingual microcirculation in 20 septic shock patients who were predicted and found to be fluid responsive\textsuperscript{10}. Despite the improvement observed in sublingual microcirculation, fluid responders did not show a significant improvement in P[g-a]CO\textsubscript{2} gap compared to non-responders. This finding illustrates that the relationship between sublingual and gastrointestinal microcirculation is not straightforward. For example, in a model of endotoxemia, fluid resuscitation corrected both serosal intestinal and sublingual microcirculation, but was unable to restore intestinal mucosal perfusion\textsuperscript{32}. In a different study, orthogonal polarization spectral imaging was performed of the sublingual and intestinal microcirculation in 23 patients with abdominal sepsis and a newly constructed intestinal stoma. On day 1 of sepsis, a complete dispersion of microcirculatory flow between the different microvascular beds was observed\textsuperscript{33}. When dobutamine was administered to septic patients, resulting in an increased cardiac index, a significant correlation between sublingual and gastric mucosal CO\textsubscript{2} pressures as well as with the proportion of well-perfused sublingual capillaries was found\textsuperscript{34}. It is difficult to compare this study with our study as we examined the effects of fluid resuscitation as opposed to dobutamine infusion. Conceivably, different interventions may have different effects on microcirculatory variables.

Our study has several limitations. The HT group tended to be sicker with higher SOFA scores, higher norepinephrine infusion rate and higher lactate levels, although none of these differences reached statistical significance. In addition, tonometry only provides information of the site of measurement, and mucosal
blood flow in sepsis has been shown to be heterogeneous. However, it is considered to be a well validated and reproducible measure of mucosal metabolism perfusion ratio\(^5\). Because the sublingual microcirculation was only measured before and 1 hour after fluid administration, we cannot rule out that differences between the treatment groups may have occurred later in time. For example, in a recent randomized controlled study investigating the effects of nitroglycerin on the sublingual microcirculation in septic patients, protocollized resuscitation improved small vessel MFI gradually over 24 hours\(^{35}\).

In conclusion, administration of hypertonic fluid in early septic shock is not superior to isotonic fluid with regards to gastric mucosal metabolism perfusion ratio and sublingual microcirculation. In fluid responsive patients with septic shock, fluid resuscitation improves the sublingual microvascular flow, but not the gastric mucosal metabolism perfusion ratio, irrespective of the type of fluid used. The use of hypertonic fluids instead of isotonic fluids to improve the microcirculation and splanchnic perfusion in septic shock patients is not warranted.
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References


PART 3

NOVEL HEMODYNAMIC THERAPIES IN SEPTIC SHOCK;
Chapter 3.3

THE EFFECTS OF HYPERTONIC FLUID ADMINISTRATION ON THE GENE EXPRESSION OF INFLAMMATORY MEDIATORS IN CIRCULATING LEUCOCYTES IN SEPTIC SHOCK PATIENTS

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Abstract

Objective: To investigate the effect of hypertonic fluid administration on inflammatory mediator gene expression in septic patients.

Design and Setting: Prospective, randomized, controlled, double-blind clinical study in a 15-bed mixed intensive care unit in a tertiary referral teaching hospital.

Interventions: Twenty-four patients with septic shock were randomized to receive a bolus of hypertonic fluid (HT, 6% HES/7.2% NaCl) or isotonic fluid (IT, 6% HES/0.9% NaCl). This trial is registered with ANZCTR.org.au as ACTRN12607000259448.

Measurements and Main Results: Blood samples were taken before and at regular time intervals after the fluid administration. Real-time reverse transcriptase polymerase chain reaction (RT rtPCR) was used to quantify mRNA expression of different inflammatory mediators in peripheral leukocytes. In the HT group as compared to the IT group, levels of gene expression of MMP9 and L-selectin were significantly suppressed (p=.0002 and p=.007, respectively), and CD11b gene expression was elevated (p=.02). No differences were found in the other mediators examined.

Conclusions: In septic shock patients, hypertonic fluid administration as compared to isotonic fluid modulates expression of genes that are implicated in leukocyte-endothelial interaction and capillary leakage. This difference could have beneficial clinical consequences on the course of sepsis and development of multi-organ failure.
Introduction

Small volume hypertonic fluid resuscitation has been investigated extensively, especially in hemorrhagic shock. The immediate effects include intravascular volume expansion, restoration of cardiac output and blood pressure, and possibly improvement of regional and microcirculatory blood flow. Hypertonic resuscitation also exerts immunologic and anti-inflammatory effects, which may be of potential benefit in the early resuscitation and management of septic shock. Different conventional hemodynamic optimization strategies in septic patients result in distinct biomarker patterns. In addition to the anti-inflammatory effects of resuscitation with isotonic fluids, volume resuscitation with hypertonic fluids appears to exert intrinsic beneficial effects, modulating the inflammatory response and apoptosis in trauma and sepsis. Amongst these effects are the attenuation of neutrophil cytotoxicity and inhibition of the interaction between neutrophils, platelets and endothelium. Hypertonic saline alters neutrophil cell shape resulting in cytoskeleton remodelling, which has implications for signal transduction and the cytotoxic response. The anti-inflammatory effects on neutrophils, oxidative burst and cytokine release are mediated through the signalling molecule mitogen-activated protein kinase (MAPK) p38, and suggest the existence of an osmolarity sensing system in immune cells of humans.

The immune response during inflammation and sepsis is complex and involves a network of control elements that includes pathogen associated molecular patterns, cell adhesion molecules, pro- and anti-inflammatory mediators released by activated macrophages and complement activation. Plasma levels of inflammatory mediators in sepsis reflect the overflow of these mediators into the bloodstream and may give limited insight into the actual activation of the leucocytes and the innate immune system. In a previous study, we have described the use of real time reverse transcriptase polymerase chain reaction (RT rtPCR) to quantify inflammatory mediator expression in circulating leukocytes of septic patients.

The aim of the present study was to quantify the changes in inflammatory mediator expression in circulating leukocytes, obtained from septic shock patients who were randomly assigned to receive a bolus of hypertonic or isotonic fluid.
Methods

Following approval by the Northern Y Regional Ethics Committee (NTY/06/08/070) we conducted a single-centre double blind prospective randomized controlled study in the Intensive Care Unit of a tertiary referral teaching hospital. Informed consent was obtained from patients or their nearest relative. This study is part of a trial investigating the cardiovascular effects and the effects on gastric and sublingual microcirculation of hypertonic and isotonic resuscitation, which will be published separately. The trial is registered with ANZCTR.org.au as ACTRN12607000259448.

Study protocol
Consecutive adult patients with septic shock were screened for inclusion in the study. Septic shock was defined according to standardized criteria12. The study protocol is described in detail elsewhere. In short, patients were randomized to receive 250ml NaCl 7.2%/6% hydroxyethylstarch (hypertonic group, HT) or 500ml 6% HES (isotonic group, IT) to achieve a comparable increase in preload. Blood samples were taken from the arterial catheter at baseline and after 4, 8, 12 and 24 hours after fluid infusion for further analyses.

Laboratory methods
Real-time reverse transcriptase polymerase chain reaction (RT rtPCR) was used to quantify mRNA expression of different inflammatory mediators in peripheral leukocytes. Based on their importance in the immune response and pathology of sepsis, we chose 10 representative genes from a variety of different groups of inflammatory mediators: inflammatory cytokine interleukin-6 (IL-6), anti-inflammatory cytokine interleukin-10 (IL-10), chemokine interleukin-8 (IL-8), intercellular adhesion molecule-1 (ICAM-1), monocyte chemoattractive protein-1 (MCP-1), tissue factor (TF), integrin cluster of differentiation molecule CD11b, L-selectin, matrix metalloproteinase-9 (MMP9) and a housekeeper gene β2 microglobulin (B2M). Table 1 shows the abbreviation, major activity and the source of expression of the investigated mRNA transcripts. A housekeeper gene was used to correct for the absolute amounts of total mRNA variations between different samples. To quantify the level of hypertonicity that was achieved, plasma sodium levels [Na+] were measured every 30 min using a point-of-care blood gas analyzer (ABL 800 Flex, Radiometer, Copenhagen).
**Table 1**

**Sources and biological effect of investigated inflammatory mediators**

<table>
<thead>
<tr>
<th>Inflammatory Mediator</th>
<th>Abbreviation</th>
<th>Major cell sources</th>
<th>Major activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Interleukin 6</td>
<td>IL-6</td>
<td>T cells, Macrophages</td>
<td>Mediator of fever and acute phase response. Has both pro- and anti-inflammatory properties</td>
</tr>
<tr>
<td>Interleukin 8</td>
<td>IL-8</td>
<td>Macrophages, Epithelium, Endothelium</td>
<td>Mediator inflammatory response. Chemotactic mainly for neutrophils</td>
</tr>
<tr>
<td>Interleukin 10</td>
<td>IL-10</td>
<td>Monocytes, Lymphocytes</td>
<td>Anti-inflammatory, inhibits synthesis various pro-inflammatory cytokines</td>
</tr>
<tr>
<td>Intercellular adhesion molecule 1</td>
<td>ICAM-1</td>
<td>Leucocytes, Endothelium</td>
<td>Facilitates leucocyte endothelial transmigration, signal transduction pro-inflammatory pathways</td>
</tr>
<tr>
<td>Monocyte chemoattractant protein 1</td>
<td>MCP-1</td>
<td>Monocytes, Endothelium, Smooth muscle cells</td>
<td>Chemotactic mainly for monocytes</td>
</tr>
<tr>
<td>Tissue factor</td>
<td>TF</td>
<td>Subendothelial tissue, Platelets, Leucocytes</td>
<td>Initiation coagulation cascade, intracellular signalling (angiogenesis, apoptosis)</td>
</tr>
<tr>
<td>Cluster of differentiation molecule 11b</td>
<td>CD11b</td>
<td>Monocytes, Neutrophils, Macrophages, Natural killer cells</td>
<td>Regulates leucocyte adhesion and migration, implicated in phagocytosis and cell mediated cytotoxicity</td>
</tr>
<tr>
<td>L-selectin</td>
<td>L-selectin</td>
<td>Leucocytes</td>
<td>Adhesion and homing receptor for leucocytes to enter secondary lymphoid tissues</td>
</tr>
<tr>
<td>Matrix metalloproteinase 9</td>
<td>MMP9</td>
<td>Macrophages, Neutrophils, Endothelium</td>
<td>Breakdown extracellular matrix, invasion of inflammatory cells</td>
</tr>
<tr>
<td>β2 microglobulin</td>
<td></td>
<td></td>
<td>Housekeeping Gene</td>
</tr>
</tbody>
</table>

**Laboratory protocol**

One ml of blood was added to 4ml of 5M guanidine thiocyanate (GITC) solution to preserve the RNA. Total cellular RNA was isolated from the cell samples using the following method. 0.5 ml 2M sodium acetate (pH 4), and 2.0 ml of 100% ethanol were added to the GITC-lysed blood and the sample mixed and allowed to stand in an ice bucket for 10 mins before being centrifuged for 15 mins at 15000 (g) at 4°C. The supernatant was carefully decanted so as not to disturb the pellet, which was resuspended in 0.5 ml of GITC solution and then mixed. When the pellet was dissolved, 50μl of 2M sodium acetate was added followed by 0.5ml of water- saturated phenol. The solution was placed on ice for 10mins and then 200μl of chloroform added and the tube vortexed before being centrifuged at 16,000g for 10 mins. The top layer was removed to new tube and an equal volume
of 100% Analar Isopropanol added and mixed by inversion, following which the sample was placed on ice for 10-15 mins to precipitate the total RNA. The tube was recentrifuged at 16,000g for 10 mins, the supernatant removed and the pellet resuspended in 1 ml 70% ethanol, then centrifuged at 16000 rpm or g for 5 mins. The ethanol was removed and the pellet briefly air dried. The pellet was resuspended in 18 µl of Tris/Mn RNA buffer and 2µl of Promega DNase solution was added and the sample incubated, shaking at 37°C for 30 min to digest any contaminating DNA. 2 µl Stop solution was added, heated and shaken at 65°C for 10 minutes, with the samples then put on ice. Quality and quantity of RNA checked on a Nanodrop instrument by measuring absorbance at 260-,280 and 203-nm wavelength.

A cDNA copy of total RNA was prepared using the SuperScript III reverse transcriptase first strand cDNA synthesis kit (Invitrogen, Carlsbad, CA) according to the manufacturers instructions, using oligo(dT)15 (Roche Molecular Systems, Pleasanton, CA) to prime the reactions. Briefly Reverse transcription reactions were performed using a PTC200 DNA engine,( BioRad, Hercules, USA) in tubes using 1.0 to 1.5 µg RNA, 1µL of 50 µM Oligo dT (Roche, Auckland, NZ) and sterile MQ water to achieve the desired volume. The tube was then heated to 70°C for 5 min to destroy any RNA secondary structures. The tubes were cooled on ice before the reverse transcriptase components were added. The enzyme mix for each sample contained 2.5 µL sterile MQ water, 4 µL 5X first strand buffer, 1 µL 0.1 M DTT, 1 µL dNTP mix (10 mM), to which was added 0.5 µL SuperScriptIII (Invitrogen). This was added to the 0.2 mL tubes containing the RNA and Oligo dT mixture using an electronic dispenser, mixed, then spun down (5 k rpm for 20 seconds) and left at 25°C for 5 minutes before incubating at 50°C for 1 hour. The reaction was then halted by heating at 70°C for 15 minutes.

A check of cDNA production was performed by amplification of the housekeeping gene β2 M using 0.5 µL cDNA samples with negative controls. The cycle time and temperature settings were initially 95 °C, 2 minutes; then 40 repeating cycles of 94 °C, 20 seconds; 55 °C, 20 seconds, 68 °C, 30 seconds; before a final step of 68 °C for 5 minutes. The cDNA samples were stored at -70°C until used in reverse transcriptase polymerase chain reaction (rtPCR).

**Real-time rtPCR quantification**

PCR products were labelled with SYBR® 82 (Invitrogen). RT rtPCR was performed in 100 µL thin-walled tubes (Corbett Research) and monitored in a
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Rotor-Gene™ 6000 (Corbett Research). Each 20 μL reaction mixture contained Real-time PCR Mastermix (i0x Thermostart® Reaction Buffer (AB Ltd.), 1/20,000 dilution of SYBR® 82, 5mM MgCl2 pH 8.5, 0.5 U of ABGene Thermostart® DNA polymerase (AB Ltd.) and 5 pmol of forward and reverse primers) and approximately 1μL of cDNA.

Following an initial denaturation step at 95°C for 15 minutes, 40 cycles were performed using 94°C for 20 seconds, annealing at 55°C for 20 seconds, extension at 68°C for 30 seconds, and fluorescence acquisition at 80°C for 10 seconds using the yellow channel (excitation at 530 nm, detection at 555 nm).

Following amplification in each run, a dissociation melt curve was determined. PCR products were heated from 75°C to 99°C in 0.5°C increments every 5 seconds. All melt curves showed a single peak consistent with the presence of a single amplicon. Each reaction was run in duplicate, and the Ct values (Roto-Gene software, version 1.7) and PCR efficiencies averaged 10. The mean Ct and PCR efficiency values were used to estimate the initial copy number (ICN) of mRNA transcripts of each particular gene, including the house-keeping gene (B2M) 14.

Specimens in which the RNA yield, quality or amplification efficiency were compromised, were rejected for analysis.

**Data analysis**

The level of gene expression was quantified using the initial copy number. We did a logarithmic transformation on this number to achieve a normal distribution of the data, and hence to allow the use of repeated measures analysis of variance (ANOVA). ‘Treatment-group’ was the between-subject variable, and ‘time’ was the within-subject variable. The ‘timextreatment-group’ interaction term was the indication of the evolution of different responses between the two treatment groups. We used the Tukey-Kramer Multiple-Comparison test for post-hoc comparisons at different times. The effects on nonnormally distributed parameters were compared using the Mann-Whitney test and the Wilcoxon signed rank test for paired measurements. Because of the explorative nature of this study, correction for multiple testing was not performed. All statistical calculations were performed using NCSS 2007 (version 07.1.13, NCCS, Kaysville, Utah).
Results

Baseline characteristics are shown in Table 2. The treatment groups had similar severity of disease, as expressed by APACHE II and SOFA scores. No differences in baseline counts of white blood cells (WBC) and polymorphonuclear cells (PMN) were present (Table 2).

Gene expression at baseline
The expression at baseline of all measured mediators was comparable between the two groups (Table 3). The genes IL-6 and TF were insufficiently expressed to use for further data analysis. Patients with abdominal sepsis had significantly more variability in the baseline gene expression as compared with the other sepsis patients (SD 4.1 ± 0.8 versus 2.8 ± 0.8, p=0.03). The expression of MMP9 in patients with abdominal sepsis tended to be higher compared to patients with pulmonary or other sepsis (16.5 ± 3.5, versus 13.9 ± 2.6 and 15.2 ± 3.7), but this difference did not reach statistical significance (p=.21 and p=.57, respectively).

Treatment effects
In the HT group, [Na+] increased from 135 ± 5 mmol/l at baseline to 143 ± 7 mmol/l after 30 mins (p<.0001). This corresponds with a plasma osmolality of approximately 300 mOsm/kg. No significant change in [Na+] was found in the IT group. The WBC count following study fluid administration did not change significantly from baseline (IT 11 [8-17] x109/l, p=.54; HT 17 [11-25] x109/l, p=.52) and was not different between the treatment groups (p=.15). The PMN count after treatment was also not different from baseline (IT 10 [7-16] x109/l, p=.44; HT 15 [8-22] x109/l, p=.98) or between groups (p=.28).

The expression of the investigated genes over time in both treatment groups is shown in Table 3. MMP9 showed a significant effect over time (ANOVA, p=0.001, expression at 24hr different from expression at 8hrs and 12hrs (post-hoc test)) and the interaction term (ANOVA, p=0.0002). This indicates that the MMP9 expression at 24hrs decreased in the HT group, whereas in the IT group the MMP9 expression was still elevated (Figure 1A). L-selectin expression was also more suppressed after more than 4hrs in the HT group, as compared to the IT group (ANOVA, p=0.007, Figure 1B). CD11b showed a significant increase in expression over the first 8hrs (time ANOVA, p=0.04), an effect that was more pronounced in the HT compared to the IT group (ANOVA, p=0.02). However,
Hypertonic fluid resuscitation

Table 2
Baseline characteristics.

APACHE, Acute Physiology and Chronic Health Evaluation; SOFA, Sequential Organ Failure Assessment; WBC, white blood cell count; PMN, polymorphonuclear leucocytes. Data are presented as mean ± SD, as numbers (%) or as median [interquartile range].

<table>
<thead>
<tr>
<th>Variables</th>
<th>IT group (n=12)</th>
<th>HT group (n=12)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>61±13</td>
<td>56±16</td>
<td>0.45</td>
</tr>
<tr>
<td>Men</td>
<td>6 (50%)</td>
<td>7 (58%)</td>
<td>0.68</td>
</tr>
<tr>
<td>APACHE II</td>
<td>23.5±7.4</td>
<td>24.4±6.7</td>
<td>0.75</td>
</tr>
<tr>
<td>SOFA</td>
<td>8.9±2.5</td>
<td>9.8±3.4</td>
<td>0.5</td>
</tr>
<tr>
<td>WBC (10⁹/l)</td>
<td>10.7 [7.4-14.5]</td>
<td>14.9 [6.7-35.6]</td>
<td>0.30</td>
</tr>
<tr>
<td>PMN (10⁹/l)</td>
<td>9.7 [6.4-12.9]</td>
<td>13.1 [9.9-28.3]</td>
<td>0.28</td>
</tr>
<tr>
<td>Source of sepsis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Abdominal, n=10</td>
<td>5</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Pneumonia, n=8</td>
<td>5</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Soft tissue, n=3</td>
<td>1</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Other, n=3</td>
<td>1</td>
<td>2</td>
<td></td>
</tr>
</tbody>
</table>

Table 3
Expression of genes expressed as log initial copy number at different time points.

Data are shown as mean ± SD. HT, hypertonic group. IT, isotonic group.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Group</th>
<th>0 hours</th>
<th>4 hours</th>
<th>8 hours</th>
<th>12 hours</th>
<th>24 hours</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-8</td>
<td>HT</td>
<td>15.1 ± 6.5</td>
<td>12.0 ± 4</td>
<td>12.8 ± 0.8</td>
<td>13.1 ± 3.5</td>
<td>10 ± 4.6</td>
</tr>
<tr>
<td></td>
<td>IT</td>
<td>11.7 ± 1.7</td>
<td>9.6 ± 4</td>
<td>11.1 ± 4.2</td>
<td>11.9 ± 5.8</td>
<td>10.1 ± 3.1</td>
</tr>
<tr>
<td>IL-10</td>
<td>HT</td>
<td>7.0 ± 2.6</td>
<td>7.6 ± 5.9</td>
<td>6.7 ± 3.8</td>
<td>9.2 ± 2.9</td>
<td>7.3 ± 4.3</td>
</tr>
<tr>
<td></td>
<td>IT</td>
<td>6.1 ± 3.5</td>
<td>11.2 ± 4.9</td>
<td>5.9 ± 2.8</td>
<td>6.5 ± 2.8</td>
<td>7.4 ± 4.2</td>
</tr>
<tr>
<td>ICAM-1</td>
<td>HT</td>
<td>6.9 ± 3.8</td>
<td>8 ± 2.4</td>
<td>6.2 ± 3.4</td>
<td>7.1 ± 1.2</td>
<td>10.1 ± 8.7</td>
</tr>
<tr>
<td></td>
<td>IT</td>
<td>3.3 ± 3.8</td>
<td>12.6 ± 6.3</td>
<td>8.1 ± 2.8</td>
<td>5.8 ± 1.3</td>
<td>6.9 ± 2</td>
</tr>
<tr>
<td>MCP-1</td>
<td>HT</td>
<td>9 ± 8.8</td>
<td>10.6 ± 1.6</td>
<td>10.4 ± 1.9</td>
<td>11.4 ± 1.9</td>
<td>16.5 ± 8.7</td>
</tr>
<tr>
<td></td>
<td>IT</td>
<td>6.4 ± 3.7</td>
<td>12.5 ± 4.8</td>
<td>11 ± 6.2</td>
<td>10.3 ± 8</td>
<td>9.9 ± 7.2</td>
</tr>
<tr>
<td>CD11b</td>
<td>HT</td>
<td>4.1 ± 2.8</td>
<td>11.6 ± 3.8</td>
<td>12.2 ± 3.9</td>
<td>17.0 ± 5.0</td>
<td>14.9 ± 3.6</td>
</tr>
<tr>
<td></td>
<td>IT</td>
<td>8.8 ± 4.7</td>
<td>10.9 ± 3.6</td>
<td>12.6 ± 1.6</td>
<td>7.1 ± 5.0</td>
<td>6.6 ± 2.0</td>
</tr>
<tr>
<td>L selektin</td>
<td>HT</td>
<td>13.1 ± 6.9</td>
<td>14.9 ± 4.6</td>
<td>10.2 ± 4.3</td>
<td>12.0 ± 3.5</td>
<td>10.2 ± 4.4</td>
</tr>
<tr>
<td></td>
<td>IT</td>
<td>15.2 ± 4.9</td>
<td>15.9 ± 5.0</td>
<td>15.1 ± 4.6</td>
<td>14.6 ± 4.1</td>
<td>15.5 ± 3.8</td>
</tr>
<tr>
<td>MMP9</td>
<td>HT</td>
<td>15.7 ± 3.5</td>
<td>15.7 ± 4.4</td>
<td>17.1 ± 2.9</td>
<td>18.8 ± 2.4</td>
<td>12.8 ± 4.5</td>
</tr>
<tr>
<td></td>
<td>IT</td>
<td>15.5 ± 3.1</td>
<td>16.2 ± 4.1</td>
<td>18 ± 3.4</td>
<td>16.3 ± 3.6</td>
<td>17.9 ± 3.4</td>
</tr>
</tbody>
</table>
Figure 1
Changes in inflammatory mediators over time for the two treatment groups.

HT, hypertonic group; IT, isotonic group. Data are expressed as mean (SD) of the logarithm of the initial copy number.
after 12 hrs the levels returned to time=0 levels in the IT group, but remained elevated in the HT group (Figure 1C). The other mediators ICAM, IL8, IL-10 and MCP-1 did not show any statistically significant changes over time or between treatment groups.

Discussion

In this study we examined the effects of hypertonic versus isotonic fluid administration on circulating leukocyte expression of important inflammatory mediators in septic shock patients. To our knowledge this has not been studied before in this group of patients.

Hypertonic fluid administration resulted in a different gene expression pattern compared to isotonic fluid. In the HT group, the expression of MMP9 and L-selectin was suppressed as compared to the IT group. CD11b remained elevated after 12 hours in the HT group while returning to baseline in the IT group. These differences may have clinical relevance.

MMP9 is released from granules of neutrophils and induces capillary leakage by degrading endothelial membranes. High plasma levels of this inflammatory marker as well as high mRNA expression in septic patients have been reported previously 11, 15, 16. Both plasma MMP9 concentrations and monocyte MMP9 mRNA levels were significantly higher in non-survivors than in survivors of septic shock 16. Hypertonic fluid administration has been shown to reduce capillary leakage and improve capillary blood flow in several studies 5, 17. This effect has been attributed mainly to the direct osmotic effects on endothelial cell swelling and luminal narrowing 18, 19. Our finding suggests that suppression of MMP9 could offer an additional explanation by which hypertonic fluids reduce capillary leakage and oedema formation. Although we did not specifically investigate the degree of capillary leakage in our study, we did find that patients treated with hypertonic fluid needed significantly less fluid in the following 24 hours compared to patients in the IT arm (HT 2.8±1.5 liter/24 hours versus IT 4.1±1.6 liter/24 hours, p=0.046) (unpublished data).

L-selectin is a transmembrane glycoprotein expressed on leucocytes, involved in rolling and adhesion of leucocytes along vessel walls adjacent to the site of injury. The strength of the binding is enhanced by shear stress 20. In our study, expression of L-selectin was depressed in the HT group. This finding is consistent
with previous findings and suggests that hypertonic fluid modulates the immune response by preventing neutrophil adhesion to the endothelium. In several animal models of shock, intravital microscopy was used to visualize neutrophil rolling and adhesion to the endothelium in a real-time fashion. Hypertonic resuscitation decreased neutrophil rolling and adherence in these studies.

The mediator CD11b is member of the integrin family, which is responsible for adhesion of leucocytes to endothelial cells. These integrins are expressed constitutively and kept largely in an inactive state to undergo in situ activation upon leukocyte-endothelial contact by both biochemical and mechanical signals. This activation process takes place within fractions of seconds by in situ signals transduced to the rolling leukocyte as it encounters specialized endothelial-displayed chemoattractants. Our finding of elevated gene expression of CD11b after 12 hours in the HT group compared to control is not easy to interpret. Because we did multiple comparisons in our study, we cannot rule out that the difference in CD11b expression is caused by chance. Presuming the difference is not caused by chance, one could hypothesize that hypertonic fluid reduces activation of integrins either directly or by inhibiting activation stimulating factors. This in turn could stimulate a feedback loop resulting in increased gene expression, but this is highly speculative as the process of leukocyte adhesion is complex and regulated by multiple factors. Rizoli and co-workers showed in animal models of hemorrhagic shock that hypertonic fluid prevents LPS-stimulated expression and activation of CD11b in the lung. In a randomized controlled study by the same group in patients with traumatic hemorrhagic shock hypertonic fluid abolished shock-induced CD11b up-regulation. There are important differences between these studies and ours that could account for the different findings. Hemorrhagic shock and septic shock are distinctly different disease processes with important differences in immune response. Furthermore, timing of the intervention may be important. In the animal experiments described, hypertonic fluid was given before the LPS challenge, which is obviously unachievable in patients already in septic shock.

We were unable to measure sufficient expression of the inflammatory genes for IL-6 and TF to include them in our analysis. A possible explanation could be, that our measurements essentially targeted gene expression in neutrophils, while IL-6 is mainly expressed in monocytes and TF in (sub) endothelium. One could also speculate that high blood levels of inflammatory peptides may result in homeostatic suppression of the associated genes.
Similar to our previous study”, there was a trend towards increased expression of MMP9 in patients with abdominal sepsis compared to other forms of sepsis, although in the present study this difference did not reach statistical significance. This observation reiterates that the inflammatory response in sepsis is heterogeneous depending on the source and the infecting organism.

Our study has several limitations. Septic shock patients are not a homogenous population and the expression of inflammatory mediators is highly variable and not only dependent on the source of sepsis but also on the genetic make up of the host, which defines the immune response. We did not directly measure inflammatory mediator peptide levels in the peripheral blood, which is the more common way to study the immune response to sepsis. The levels and dynamics of these mediators correlate with outcome. One of the main problems when measuring inflammatory mediator peptide levels in the peripheral blood is that only the endocrine overflow is measured, not the local autocrine and paracrine receptor binding effects. On the other hand, measuring expression of the inflammatory mediator genes may not reflect the functional activity of the end­ protein, as this also depends on translation and various post-translational modifications that determine whether the protein becomes active. Currently there are no methods to reliably measure functional protein activity. In addition, inflammatory gene activation tends to be a slow process and can take many hours depending on the gene measured. This is in contrast to the immediate and short­ term changes observed in inflammatory mediator peptide levels in peripheral blood, and could account for the time course of changes found in our study. In addition, our methodology does not allow us to distinguish between direct effects and indirect effects, e.g., downstream in a cascade of events, or induced by a change in the level of inhibition. Finally, the level of hypertonicity that was achieved in the HT group may not have been optimal to significantly influence immune function. It has been proposed that the level of hypertonicity should probably exceed 330 mOsm/kg in order to benefit patients in terms of immune function.

In conclusion, we have shown that in septic shock patients, hypertonic fluid as compared to isotonic fluid modulates expression of several, but not all measured genes that are implicated in neutrophil-endothelial interaction and capillary leakage. This difference could have beneficial clinical consequences pertaining the course of sepsis and development of multi-organ failure. To our knowledge,
this is the first study to report on the effects of hypertonic resuscitation on inflammatory gene expression in septic shock patients.
Hypertonic fluid resuscitation

References


NOVEL HEMODYNAMIC THERAPIES IN SEPTIC SHOCK;
Hypertonic fluid resuscitation


NOVEL HEMODYNAMIC THERAPIES IN SEPTIC SHOCK;
Summary

Context

Sepsis places a significant burden on healthcare resources and presents a major challenge to both clinicians and researchers. Despite best efforts, approximately half the patients that develop septic shock, the most severe form of sepsis, progress to multi-organ failure and death. Improved understanding of the pathophysiology of sepsis and, consequently, better treatment modalities are urgently needed.

The hemodynamic changes observed during septic shock include severe vasodilation, myocardial depression, capillary leakage and impaired microcirculatory blood flow, resulting in redistribution of regional blood flow. Microcirculatory abnormalities in septic patients have been well recognized, classified and quantified over recent years. Persisting microcirculatory alterations are associated with organ failure and mortality in septic shock, irrespective of normalization of systemic parameters. Gastrointestinal microcirculatory abnormalities are thought to be important in septic shock patients, both as an indicator of inadequate resuscitation and potentially as a mechanism by which multi-organ failure may occur. Mucosal hypoperfusion in the gastrointestinal tract is reflected by an increased gastric to arterial CO₂ pressure gradient (P[g-a]CO₂ gap) that can be detected by gastric tonometry.

Conventional septic shock therapy includes supportive treatment such as fluid resuscitation, administration of vasopressors (adrenergic and nonadrenergic drugs), respiratory and renal supportive measures, and the administration of antibiotics and other forms of source control. New vasopressors have been proposed, as well as alternative agents for fluid resuscitation. These new therapies may have beneficial or detrimental effects not only on systemic hemodynamics, but also on the (gastrointestinal) microcirculation and on the immune system.
Conventional vasopressors and fluids are often ineffective to completely reverse the circulatory abnormalities in septic shock patients. Therefore, the aim of this thesis was to investigate new vasopressors (vasopressin and methylene blue), as well as a different approach to fluid resuscitation (hypertonic fluids). We simultaneously examined the effects of these treatments on the macrocirculation and the microcirculation, as there is often a disjunction between the two.

Thesis

In part i of this thesis, the various issues concerning gastrointestinal perfusion in septic shock were reviewed. We discuss gastrointestinal mucosal hypoperfusion as to the underlying mechanism resulting in multiple organ dysfunction, as well as its role as a prognostic marker. The different available measurement techniques (tonometry, laser Doppler flowmetry, reflectance spectrophotometry, near-infrared spectroscopy, orthogonal polarisation spectral imaging, indocyanine green clearance, hepatic vein catheterisation and measurements of plasma D-lactate) are described, including advantages and disadvantages for their use in everyday clinical practice. Finally, we present an overview of studies targeting correction of the perfusion abnormalities and the effects of these interventions on patient outcome. Summarizing the data, we concluded that normal regulation of gastrointestinal blood flow is impaired in septic shock patients, resulting in mucosal hypoperfusion. Gastrointestinal mucosal hypoperfusion is an important marker, and probably also a cause, of poor prognosis in septic patients. Impaired gut barrier function could play a role in amplification of systemic inflammation, causing distant multiple organ dysfunction. The aim of monitoring splanchic perfusion is to detect and thereby to be able to prevent and reverse tissue hypoperfusion. Several techniques have been developed to measure gastrointestinal perfusion. These methods share the ability to predict outcome in septic shock patients. Gastric tonometry is the most widely used technique because of its non-invasiveness and ease of use. However, as treatment strategies aimed at improving gastrointestinal perfusion have not been able to correct mucosal perfusion abnormalities and hence have not shown to improve outcome, the use of these tools in clinical decision-making is currently limited. New treatment strategies for septic shock should be tested for their effects on gastrointestinal perfusion, to further clarify its role in patient
management, and to prevent therapies detrimental to gastrointestinal perfusion being implemented.

In part 2, we examined the effects of the vasoconstrictive agents vasopressin and methylene blue in septic shock patients.

In chapter 2.1, we investigated the effects of vasopressin infusion on global hemodynamics and on the gastrointestinal perfusion as assessed with gastric tonometry in septic shock patients. Previous studies have reported that vasopressin deficiency in septic shock is an important pathway contributing to vasodilation and hence trials investigating the risks and benefits of vasopressin administration in septic shock need to be conducted. Vasopressin is known to exert vasoconstrictive properties throughout the gastrointestinal system and as such, is being used for treatment of oesophageal varices bleeding. We demonstrated in our study that low dose vasopressin infusion (0.04 U/min) in septic shock patients increased plasma vasopressin levels from 17 pg/ml at baseline to 230 pg/ml after 4 hours of infusion, and increased mean arterial blood pressure from 61 mmHg at baseline to 68 mmHg after 4 hours of vasopressin infusion. This was accompanied by an increase in the median P[g-a]CO$_2$ gap from 5 mmHg at baseline to 19 mmHg after 4 hours, indicating development of splanchnic hypoperfusion. We recommended that, in the view of this potential detrimental effect, vasopressin treatment of septic shock patients should be limited to controlled clinical trials until its effect on clinical outcome such as organ failure and mortality has been clarified. Recently, the results of a large clinical trial have been published, comparing vasopressin versus norepinephrine infusion in patients with septic shock. The investigators showed that low-dose vasopressin did not reduce mortality rates as compared with norepinephrine among patients with septic shock who were treated with catecholamine vasopressors. However, in the prospectively defined stratum of less severe septic shock, the mortality rate was lower in the vasopressin group than in the norepinephrine group. Based on these and our observations, we recommend now that vasopressin should not be administered to patients with severe septic shock with a norepinephrine requirement of $\geq 15 \mu g/min$.

In chapter 2.2, we examined the effect of methylene blue (MB) infusion on gastric tonometry and intestinal fatty acid binding protein (iFABP) levels in septic shock
patients. Previous studies have shown that iFABP is a useful biochemical marker of enterocyte injury and gut ischemia in experimental models and in humans. In addition, iFABP levels correlate with clinical development of the systemic inflammatory response syndrome (SIRS) and with outcome in critically ill patients. Excessive nitric oxide (NO) production is a key factor in the pathophysiology of septic shock and contributes to pathological vasodilation and the development of multiple organ dysfunction. MB inhibits several steps in the NO pathway. In several clinical studies, MB administration in septic patients improved hemodynamic parameters. In our study in patients with septic shock and advanced multi-organ failure, MB infusion at a dose of 1 mg/kg/hr increased the median arterial blood pressure from 70 to 77 mmHg, and decreased the cardiac index (CI) from 4.4 to 3.6 l/min/m². Importantly, these hemodynamic changes were not associated with deterioration of the gastric mucosal perfusion metabolism ratio as indicated by tonometry, and by the release of iFABP. In the subgroup of patients with an elevated baseline P[g-a]CO₂ gradient, defined as ≥20 mmHg, the median P[g-a]CO₂ gradient decreased from 45 mmHg before infusion to 41 at the end of the 4 h infusion and decreased further to 32 mmHg 2 hours after cessation of MB infusion. We concluded that MB infusion in patients with severe septic shock and advanced multi-organ failure increased MAP and decreased CI, without compromising and potentially even protecting the gastrointestinal mucosa.

In chapter 2.3, we investigated the effects of MB infusion on NO production and kidney damage and function in the same group of septic shock patients. We found that the urinary excretion of NO metabolites decreased with 90% from baseline to 6 hours after MB administration. The first 24 hours, the creatinine clearance improved with 51% after MB treatment. During the first 6 hours after start of MB treatment, both the urinary excretion of cytosolic glutathione S-transferase A1-1 and P1-1, markers for proximal and distal tubule damage respectively, decreased with 45% and 70% compared to baseline. After termination of the MB infusion, the NO metabolites and markers of tubular injury returned to pre-treatment levels. In conclusion, MB induced modulation of the NO pathways and exerted short-term beneficial effects on kidney function.

In part 3, we studied the effects of hypertonic fluid administration. Fluid resuscitation to improve and restore oxygen delivery is an important element of
the treatment of septic shock. Different resuscitation solutions have different effects on the global, regional and microcirculation, as well as different intrinsic effects on the immune system. Hypertonic solutions have been studied predominantly in traumatic hemorrhagic shock. Specific effects on the circulation and the immune system have been identified in these studies, which could theoretically be beneficial in septic shock. However, studies in septic shock patients are scarce. We conducted a prospective, randomized, controlled, double blind, single centre, clinical trial to compare the effects of hypertonic and isotonic fluid administration in septic shock patients.

In chapter 3.1 we report the effects of hypertonic and isotonic fluid administration on the global cardiovascular function. Our results showed that in septic shock patients, hypertonic fluid can be safely administered and results in improved systolic and diastolic cardiac function as assessed with echocardiography and with pulse contour analysis derived stroke volume index. We also demonstrated an improvement of the vascular tone, resulting in a decreased requirement for norepinephrine. Finally, the need for ongoing fluid resuscitation was reduced in the hypertonic group (2.8 liter/24 hours versus 4.1 liter/24 hours in the isotonic group), indicating an improvement of the sepsis-induced capillary leakage. All these effects were independent of and in addition to the effects on plasma volume expansion, evidenced by similar values for central venous pressure, stroke volume variation and intrathoracic blood volume index in both groups.

In chapter 3.2, we examined the effects on gastrointestinal perfusion as assessed with tonometry, as well as the effects on the sublingual microcirculation as assessed by Sidestream Dark Field (SDF) imaging. The median P\([g-a]\)CO\(_2\) gap at baseline was elevated both in the hypertonic group and in the isotonic group (21 and 26 mmHg, respectively), indicating gastrointestinal hypoperfusion. The median sublingual microvascular flow index was also decreased in both groups (hypertonic 2.25, isotonic 2.39). Following fluid administration, no differences were observed in the course of the P\([g-a]\)CO\(_2\) gap or in any of the sublingual microvascular variables between the hypertonic and the isotonic group. Our study showed that administration of hypertonic fluid in early septic shock was not superior to isotonic fluid with regards to gastric mucosal metabolism perfusion ratio and sublingual microcirculation. In addition, we found that in fluid responsive patients, fluid resuscitation improved the sublingual microvascular
flow, but not the gastric mucosal metabolism perfusion ratio, irrespective of the type of fluid used. We concluded that the administration of hypertonic fluids to improve the microcirculation and splanchnic perfusion in septic shock patients is not warranted.

Finally, in chapter 3.3 we investigated whether hypertonic fluid had specific immunomodulatory effects in septic shock patients. Hypertonic fluid administration resulted in a different gene expression pattern compared to isotonic fluid. In the hypertonic group, the expression of MMP9 and L-selectin was suppressed as compared to the isotonic group. CD11b remained elevated after 12 hours in the hypertonic group while returning to baseline in the isotonic group. MMP9 induces capillary leakage by degrading endothelial membranes. L-selectin is a transmembrane glycoprotein expressed on leukocytes, involved in rolling and adhesion of leukocytes along vessel walls adjacent to the site of injury. The mediator CD11b is member of the integrin family, which is responsible for adhesion of leukocytes to endothelial cells. Our results showed that in septic shock patients, hypertonic fluid as compared to isotonic fluid modulated the expression of genes that are implicated in neutrophil-endothelial interaction and capillary leakage. These findings could offer an alternative explanation for the observed decreased need for ongoing fluid resuscitation in the hypertonic group, described in chapter 3.1. Immunomodulation with hypertonic fluids could have useful clinical consequences on the course of sepsis and development of multi-organ failure.

Conclusions

The research presented in this thesis has contributed to a better understanding of potential beneficial as well as detrimental effects exerted by selected new hemodynamic treatments for patients with septic shock.

Our studies confirmed that in septic shock, gastrointestinal mucosal perfusion is highly abnormal. Attempts to improve this parameter with nonconventional vasopressors and fluid resuscitation were not successful, despite an improvement in global hemodynamic parameters such as blood pressure. This lack of effective treatments is consistent with the literature and also reiterates that the routine use
of tonometry as therapeutic target for sepsis treatment is not warranted. However, tonometry and other measurement techniques of gastrointestinal mucosal perfusion continue to be important research tools to investigate potential beneficial or detrimental effects of new sepsis treatment strategies.

Vasopressin carries the potential for worsening the splanchnic hypoperfusion and should be avoided in patients who require high doses of norepinephrine, and be used with caution in other patients. Methylene blue infusion selectively inhibits the nitric oxide pathway in septic patients and has potential protective effects on gastrointestinal and kidney perfusion and function. Further studies should be conducted to investigate the effects of methylene blue treatment on relevant clinical outcome parameters such as organ failure and mortality.

Hypertonic resuscitation does not promote the sublingual and gastric microcirculatory flow in sepsis, contrary to what was expected from preclinical research and studies conducted in other shock states. In the subgroup of fluid responsive patients, fluid administration improves the sublingual microcirculation irrespective of the tonicity of the fluid. We were the first to show that hypertonic fluid administration improves septic cardiac systolic and diastolic dysfunction as well as the global hemodynamic situation independent of the degree of plasma volume expansion. This intrinsic effect of induced hypertonicity also modulates expression of genes that are implicated in capillary leakage and the need for ongoing fluid resuscitation is reduced. These results warrant further investigation. Future research should concentrate on the effects of repeated or ongoing hypertonic fluid administration, as well as the timing and the optimal dose, and ultimately the effects on hard clinical endpoints such as patient outcome.
Novel hemodynamic therapies in septic shock;
SAMENVATTING

Achtergrond

Het ziektebeeld sepsis trekt een zware wissel op het gezondheidszorgbudget en vormt een grote uitdaging voor zowel artsen als onderzoekers. Ondanks alle inspanningen ontwikkelt de helft van de patiënten met septische shock – de ernstigste vorm van sepsis – multiorgaan falen en komt te overlijden. Het is daarom van uitermate groot belang om de pathofysiologie van sepsis beter te leren begrijpen, zodat zodoende betere behandelstrategieën ontwikkeld kunnen worden.

De hemodynamische veranderingen waarmee septische shock gepaard gaat zijn ernstige vaatverwijding, verminderde hartfunctie, capillaire lekkage en gestoorde doorbloeding van de haarvaten (microcirculatie), hetgeen resulteert in redistributie van de regionale doorbloeding. De afwijkingen van de microcirculatie die worden waargenomen bij patiënten met sepsis, zijn recent geclasseerd en gekwantificeerd. Indien de waargenomen afwijkingen van de microcirculatie persisteren ondanks behandeling, is dit geassocieerd met een grotere kans op orgaanfalen en overlijden. Dit fenomeen is onafhankelijk van de effecten van de behandeling op de globale bloedsomloop. De microcirculatie van het maagdarmstelsel is ook afwijkend bij patiënten met septische shock. Dit zou zowel een uiting kunnen zijn van inadequate behandeling, alsmede een mechanisme waardoor multiorgaan falen zou kunnen ontstaan. Verminderde doorbloeding van het slijmvlies van het maagdarmstelsel kan worden vastgesteld aan de hand van een toegenomen verschil tussen de CO₂ druk in de maag en in het bloed (P[g-a]CO₂ gradiënt), hetgeen kan worden gemeten met behulp van tonometrie.

De conventionele behandeling van septische shock omvat ondermeer het toedienen van vocht, toediening van bloeddrukverhogende medicijnen (vaatverdunners), ondersteuning van de ademhaling en de nierfunctie, naast het onder controle krijgen van de infectie door toediening van antibiotica en met
andere maatregelen. Er zijn nieuwe vaatvernauwers en infuusvloeistoffen beschikbaar, welke zowel gunstige als ongunstige effecten zouden kunnen hebben op de globale bloedsomloop, op de microcirculatie en op het immuunsysteem.

Conventionele vaatvernauwers en infuusvloeistoffen zijn vaak niet in staat om de afwijkende bloedsomloop bij patiënten met septische shock volledig te herstellen. Daarom was het doel van dit proefschrift, om nieuwe vaatvernauwers (vasopressine en methyleen blauw), alsmede een alternatieve vloeistofstrategie (hypertone infuusvloeistof) te onderzoeken. We hebben de effecten van deze behandelingen onderzocht op de globale bloedsomloop en op de microcirculatie, omdat deze effecten vaak niet met elkaar overeenstemmen.

Proefschrift

In het eerste deel van dit proefschrift werden verschillende zaken met betrekking tot de doorbloeding van het maagdarmstelsel bij septische shock patiënten belicht. We bediscussiëren in hoeverre een verminderde doorbloeding van het slijmvlies van het maagdarmstelsel zou kunnen leiden tot multiorgaan falen, en welke rol deze afwijkingen zouden kunnen spelen bij het voorspellen van de prognose van de patiënt. Vervolgens bespreken we de achtergrond van de verschillende beschikbare meetmethoden (tonometrie, laser Doppler fluxmetrie, reflectance spectrophotometrie, near-infrared spectroscopy, orthogonale polarisatie spectrale imaging, indocyanine groen klaring, vena hepatica katheterisatie en meting van de plasmaconcentratie van D-lactaat), alsook voordelen en beperkingen van deze technieken voor klinisch gebruik. Tenslotte presenteren we een overzicht van studies waarin onderzocht werd of behandeling, gericht op verbetering van de gestoorde doorbloeding van het maagdarmstelsel, ook een effect heeft op de uitkomst van patiënten. Samenvattend concluderen we dat de normale regulatie van de bloeddoorstroming van het maagdarmstelsel verstoord is bij patiënten met septische shock, resulterend in verminderde doorbloeding van het slijmvlies. Deze abnormale doorbloeding is geassocieerd met een slechte prognose. Een gestoorde darmbarrière functie zou kunnen leiden tot amplificatie van systemische inflammatie respons, resulterend in orgaanfalen. Het doel van het meten van de doorbloeding van het maagdarmstelsel is het opsporen, en zo het
voorkomen of behandelen van verstoorde doorbloeding. Verschillende
meetmethoden zijn ontwikkeld die het vermogen delen om de uitkomst van
patiënten met septische shock te voorspellen. De meest gebruikte methode is
tonometrie van de maag, omdat deze minimaal invasieve techniek eenvoudig toe
te passen en goed reproduceerbaar is. Studies waarin getracht werd de abnormale
doorbloeding van het maagdarmstelsel te verbeteren, faalden in deze opzet en
lieten dus ook geen positieve invloed zien op de uitkomst van patiënten. Dit is de
reden dat in de alledaagse praktijk, het meten van de doorbloeding van het
maagdarmstelsel slechts een beperkte rol heeft. Het effect van nieuwe
behandelmethodevoor septische shock op de doorbloeding van het
maagdarmstelsel dient echter wel getest te worden, opdat het belang voor de
behandeling van patiënten beter kan worden begrepen, en ook om te voorkomen
dat een behandeling met nadelige effecten wordt geïmplementeerd.

In het tweede deel onderzochten we de effecten van de vaatverdunnende
middelen vasopressine en methyleen blauw bij patiënten met septische shock.

In hoofdstuk 2.1 bestudeerden we de effecten van vasopressine toediening aan
septische shock patiënten, op de globale bloedsomloop en op de doorbloeding
van het maagdarmstelsel met behulp van tonometrie. Eerdere studies hebben
aangetoond dat een tekort aan vasopressine bij septische shock patiënten een
belangrijke oorzaak is van de geobserveerde vaatverwijding. De positieve en
negatieve effecten van toediening van vasopressine aan septische shock patiënten
dienen aldus onderzocht te worden. Vasopressine wordt ook gebruikt bij de
behandeling van spataderen in de slokdarm die bloeden, vanwege het
vaatverdunnende effect in het maagdarmstelsel.

In onze studie toonden we aan, dat een 4 uur durende infusie van een lage
dosis vasopressine (0.04 U/min) aan patiënten met septische shock resulteerde
in een toename van de plasma concentratie van vasopressine van 17 tot 230 pg/ml
en in een toename van de gemiddelde bloeddruk van 61 tot 68 mmHg. Dit ging
echter gepaard met een toename in de P[g-a]CO₂ gradiënt van 5 naar 19 mmHg,
hetgeen een afname van de doorbloeding van het maagdarmstelsel impliceert.
We concludeerden dat, vanwege dit potentiële nadelige effect, behandeling van
septische shock patiënten met vasopressine gelimiteerd dient te blijven tot
gecontroleerde klinische studies, totdat het effect op uitkomstparameters zoals
orgaanfalen en sterfte is opgehelderd. Recent zijn de resultaten van een grote
studie gepubliceerd, waarin vasopressine vergeleken werd met noradrenaline bij patiënten met septische shock. De onderzoekers toonden aan dat behandeling met een lage dosis vasopressine niet resulteerde in een daling van de mortaliteit vergeleken met noradrenaline. In de vooraf gedefinieerde subgroep van patiënten met een minder ernstige vorm van septische shock was de mortaliteit in de vasopressine groep wel lager dan in de noradrenaline groep. Gebaseerd op deze en onze resultaten is onze aanbeveling, dat vasopressine niet dient te worden gebruikt bij patiënten met septische shock die $\geq 15 \mu g/min$ noradrenaline toegediend krijgen.

In hoofdstuk 2.2 onderzochten we de effecten van methyleen blauw (MB) infusie op de P[g-a]CO$_2$ gradiënt en op de concentratie van intestinaal vetzuur bindend eiwit (intestinal fatty acid binding protein, iFABP) bij patiënten met septische shock. Eerder werd aangetoond, zowel in experimentele modellen als bij mensen, dat iFABP een biochemische marker is voor schade aan darmepitheelcellen en van zuurstoftekort van de darm. Bovendien correleert de iFABP concentratie met de klinische ontwikkeling van het systemische inflammatoire respons syndroom, alsnmede met de prognose van kritisch zieke patiënten. Overmatige stikstomoxonoxide (nitric oxide, NO) productie speelt een centrale rol bij de pathofysiologie van septische shock en draagt bij aan het ontstaan van pathologische vaatverwijding en de ontwikkeling van multiorgaan falen. MB remt diverse stappen van het NO metabolisme. In verschillende klinische studies werd aangetoond dat MB verschillende hemodynamische parameters verbetert.

In onze studie bij patiënten met septische shock en gevorderd multiorgaan falen, resulteerde MB infusie (dosis 1 mg/kg/uur) tot een toename van de gemiddelde bloeddruk van 70 naar 77 mmHg en tot een afname van het hartminuutvolume (geïndexeerd, CI) van 4.4 naar 3.6 l/min/m$^2$. Deze hemodynamische veranderingen gingen niet gepaard met een toename van de P[g-a]CO$_2$ gradiënt of van de iFABP concentratie. In de subgroep van patiënten met een verhoogde P[g-a]CO$_2$ gradiënt aan het begin van de studie ($\geq 20$ mmHg) nam de P[g-a]CO$_2$ gradiënt af van 45 tot 41 mmHg na 4 uur MB infusie, en verder tot 32 mmHg 2 uren na het staken van de MB toediening. We concludeerden dat MB infusie aan patiënten met ernstige septische shock en multiorgaan falen de bloeddruk verhoogt en het hartminuutvolume verlaagt, zonder een nadelig effect uit te oefenen en mogelijk met een beschermend effect op het slijmvlies van het maagdarmstelsel.
In hoofdstuk 2.3 onderzochten we de effecten van MB infusie op de productie van NO en op nierfunctie en nierschade in dezelfde groep patiënten. We toonden aan dat tijdens behandeling met MB de uitscheiding van NO metabolieten in de urine afnam met 90%. De creatinineklaring (maat voor de nierfunctie) verbeterde met 51% in de eerste 24 uren na behandeling met MB. De uitscheiding in de urine van de cytosolic glutathion S-transferase A1-1 en P1-1, markers voor celschade aan respectievelijk de proximale en distale tubulus, nam af met 45% en 70%. Na het staken van de MB infusie bereikten de concentraties van NO metabolieten en de markers voor schade aan de tubuluscellen weer hun uitgangswaarde. We concludeerden dat MB infusie het metabolisme van NO moduleerde en in ieder geval kortdurend de nierschade die door sepsis was ontstaan verminderde.

In het derde deel van dit proefschrift onderzochten we de effecten van toediening van hypertone infuusvloeistof. Vloeistoediening (volumeresuscitatie) is een belangrijk element van de behandeling van septische shock, bedoeld om het zuurstoftransport naar cellen en organen te herstellen of verbeteren. Verschillende infuusmiddelen hebben verschillende effecten op de globale en regionale bloedsomloop, op de microcirculatie, alsmede op de werking van het immuunsysteem. Hypertone vloeistoffen zijn voornamelijk bestudeerd in de setting van verbloedingsschok, bijvoorbeeld na een trauma. Deze studies hebben laten zien dat hypertone vloeistoffen specifieke effecten op de bloedsomloop en op het immuunsysteem uitoefenen, welke op theoretische gronden nuttig zouden kunnen zijn bij de behandeling van septische shock. Studies bij patiënten met septische shock zijn echter schaars. Wij voerden een prospectieve, gerandomiseerde, gecontroleerde, dubbelblinde, klinische studie uit om de effecten van hypertone en isotone infuusvloeistof te vergelijken bij patiënten met septische shock.

In hoofdstuk 3.1 vergeleken we de effecten van toediening van hypertone en isotone infuusvloeistoffen op de globale bloedsomloop en de hartfunctie. Onze resultaten toonden aan dat het veilig is om hypertone vloeistof toe te dienen aan patiënten met septische shock. Zowel de systolische als de diastolische hartfunctie verbeterde, gemeten met behulp van echocardiografie en met pols contour analyse. Verder verbeterde ook de vaattonus, zodat de noradrenaline toediening kon worden verminderd. Tenslotte hadden de patiënten in de hypertone groep
minder vloeistofinfusie nodig in de 24 uur na de studie (2,8 liter versus 4,1 liter in de isotone groep), hetgeen een verbetering van het capillaire lek syndroom suggereert. Al deze effecten waren onafhankelijk van de mate van plasmavolume expansie, aannemelijk gemaakt door vergelijkbare waarden van centraal veneuze druk, slagvolume variatie en intrathoracaal bloedvolume in beide groepen.

In hoofdstuk 3.2 onderzochten we de effecten op de doorbloeding van het maagdarmstelsel met behulp van tonometrie van de maag, alsmede de effecten op de microcirculatie onder de tong, gemeten met Sidestream Dark Field imaging (SDF). De $P[g-a]CO_2$ gradiënt voor het begin van de studie was verhoogd in beide groepen (21 mmHg in de hypertone groep, 26 mmHg in de isotone groep), hetgeen een gestoorde doorbloeding van het maagdarmstelsel suggereert. De microcirculatie onder de tong was ook gestoord in beide groepen (microvascular flow index 2.25 in de hypertone groep en 2.39 in de isotone groep). Na de vloeistoftoediening werden geen verschillen waargenomen tussen beide groepen wat betreft de $P[g-a]CO_2$ gradiënt en de metingen van de microcirculatie onder de tong. We toonden aldus aan dat hypertone vloeistof toediening niet beter is dan isotone vloeistof wat betreft de doorbloeding van het maagdarmstelsel en de microcirculatie onder de tong. Bij patiënten die vloeistofresponsief waren, gedefinieerd als een toename van het hartminuutvolume >10% na vloeistoftoediening, verbeterde vloeistoftoediening de microcirculatie onder de tong, maar niet de $P[g-a]CO_2$ gradiënt. Dit effect was onafhankelijk van het type vloeistof dat werd toegediend. We concludeerden dat de toediening van hypertone infusvloeistoffen aan patiënten met septische shock, met als doel verbetering van de microcirculatie en de doorbloeding van het maagdarmstelsel, niet gerechtvaardigd is.

In hoofdstuk 3.3 tenslotte, onderzochten we of hypertone infusvloeistof specifieke effecten op het immuunsysteem van septische shock patiënten heeft. Toediening van hypertone vloeistof resulteerde in een ander patroon van expressie van genen die coderen voor ontstekingsmediatoren, dan isotone vloeistof. De expressie van MMP9 en L-selektine was onderdrukt in de hypertone groep vergeleken met de isotone groep. CD11b was verhoogd na 12 uren in de hypertone groep maar niet in de isotone groep. MMP9 induceert capillaire lekkage door degradatie van de vaatwandmembraan. L-selektine is een transmembraan glycoproteïne dat tot expressie komt op de membraan van witte bloedcellen. Het
is betrokken bij het rollen van witte bloedcellen en het tot stand komen van de verbinding tussen witte bloedcellen en de vaatwand ter plaatse van schade of infectie. CD11b behoort tot de familie van integrines, welke verantwoordelijk is voor het plakken van witte bloedcellen aan vaatwandcellen. Onze resultaten toonden aan, dat hypertone vloeistof in vergelijking met isotope vloeistof een modulerende werking uitoefent op de expressie van genen die betrokken zijn bij de interactie tussen witte bloedcellen en vaatwandcellen, en bij het ontstaan van capillaire lekkage. Deze observaties zouden een alternatieve verklaring kunnen geven voor de afgenomen vloeistofbehoefte in de hypertone groep, zoals beschreven in hoofdstuk 3.1. Modulatie van het immuunsysteem door toediening van hypertone vloeistof zou gunstige effecten kunnen hebben op het beloop van sepsis en het ontstaan van multiorgaan falen.

Conclusies

Het onderzoek dat gepresenteerd wordt in dit proefschrift heeft bijgedragen tot een beter begrip van potentieel gunstige zowel als ongunstige effecten van geselecteerde nieuwe hemodynamische behandelingen voor patiënten met septische shock.

Onze studies bevestigen dat de doorbloeding van het maagdarmstelsel bij septische shock patiënten ernstig verstoord is. Pogingen om deze afwijkingen te corrigeren met behulp van onconventionele vaatvernauwers en vloeistof-toediening waren niet succesvol, ondanks de waargenomen verbetering van de globale bloedsomloop. Deze observatie komt overeen met de literatuur. Het gebrek aan effectieve behandelmethoden om de doorbloeding van het maagdarmstelsel te verbeteren betekent ook, dat routine gebruik van meetmethoden zoals tonometrie ter optimalisatie van de behandeling niet aangeraden kan worden. Tonometrie en andere meetmethoden om de doorbloeding van het maagdarmstelsel te meten zijn echter wel van groot belang als onderzoeksinstrumenten waarmee potentiële gunstige en ongunstige effecten van nieuwe behandelmethoden voor sepsis kunnen worden vastgesteld.

Vasopressine vermindert de doorbloeding van het maagdarmstelsel en dient te worden vermeden bij septische patiënten die een hoge noradrenalinebehoefte
hebben, en met beleid te worden gebruikt in alle andere patiënten. Methyleen blauwtoediening remt het NO-metabolisme bij patiënten met septische shock op een selectieve manier, en oefent een potentiell beschermend effect uit op het maagdarmstelsel en op de nieren. Verder onderzoek dient te worden verricht om te onderzoeken welke effecten MB behandeling heeft op belangrijke klinische parameters zoals orgaanfalen en sterfte.

Toediening van hypertone infuusvloeistof leidt niet tot een verbetering van de doorbloeding van het maagdarmstelsel en van de microcirculatie onder de tong, ondanks dat dit werd verwacht op basis van experimentele studies en studies bij verbloedingsshock. Vloeistoftoediening leidt wel tot verbetering van de microcirculatie onder de tong bij patiënten die vloeistofresponsief zijn, ongeacht de toniciteit van de toegediende vloeistof. Wij hebben bovendien als eerste aangetoond, dat toediening van hypertone vloeistof de hartfunctie en de globale bloedsomloop verbetert bij patiënten met septische shock, onafhankelijk van de mate van plasmavolume expansie. Dit intrinsieke effect van het induceren van een hypertone status leidt ook tot modulatie van genen die betrokken zijn bij het capillair lek syndroom en resulteert in een afgenomen vloeistofbehoefte. Deze resultaten geven aanleiding tot verder onderzoek. Dit onderzoek dient gericht te worden op de effecten van herhaalde of continue toediening van hypertone vloeistof, alsook op de relatie tussen dosis en timing op het effect. Uiteindelijk dient onderzocht te worden, welke effecten hypertone vloeistofresuscitatie heeft op harde klinische eindpunten zoals sterfte.
Novel hemodynamic therapies in septic shock;
Acknowledgments

I have always skipped this section when reading other peoples’ theses: too predictable, too politically correct, too commonplace and cliché. However, now it is my turn to thank many people and I finally understand that this section is actually the most important part of every doctoral thesis. Obtaining a PhD is not something one can do on its own. It requires a tight and strong team; working in close cooperation, joint action resulting in the ultimate synergism; a combined victory!

Gladly and proudly I introduce to you my accomplices. If anyone feels left out, I am happy to refer that person to my wife who is a therapist specialised in loss and grief management.

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My dear parents, you have always supported me despite the fact that some decisions I made in life were difficult for you. This thesis may seem a bit abstract and technical to you, but it is because of you that I managed to achieve...
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Last but most definitely not least, my beautiful family. Son Luka, full of passion and emotion, strong yet vulnerable, always asking profound questions about life and the universe. You could not believe that your dad was going to be one of the wisest persons in the world. And who am I to contradict that? Daughter Rifka, determined enough to walk at the age of 9 months. Always dancing and singing, full of radiant energy, a ray of sunshine in my life. My beautiful wife Marquita, full of love and patience. Thank you for always being there for me. I promise I will never attempt to obtain another PhD!
CURRICULUM VITAE

Frank van Haren was born on the 20th of September 1968 in Hengelo, the Netherlands. He finished his secondary school (Atheneum) at the Twickel College in Hengelo in 1986. He started his medical training that same year at the University of Nijmegen, receiving his Doctoraal Certificate in 1991 and his Arts Certificate in 1993. His research project: “The role of ornithine-decarboxylase activity to detect colorectal malignancies” was nominated for the Hippocrates Award for Medicine and for the Parke-Davis Award for Gastroenterology.

Specialist training in the field of Internal Medicine (“Internist”) was undertaken at the St. Joseph Hospital in Veldhoven (Supervisor of Training Dr P.G.G. Gerlach) and the University Nijmegen Medical Centre (Prof. Dr J.W.M. van der Meer) and finished at the turn of the century. Later in the year 2000, the requirements were met for specialist registration in Intensive Care Medicine (“Intensivist”), completed at the University Nijmegen Medical Centre (Dr F.W. Santman). In 2001 Frank passed the European Diploma in Intensive Care Medicine in Rome, Italy.

He worked as Intensive Care specialist in the Jeroen Bosch Hospital in ‘s-Hertogenbosch from 2001-2003 and in the VieCuri Medical Centre in Venlo from 2003-2005. Educational activities in the Netherlands included: Instructor Fundamental Critical Care Support (FCCS); Instructor Advanced Trauma Life Support (ATLS); Instructor Advanced Paediatric Life Support (APLS); Lecturer on “Ethics in the ICU” and “Nutritional support in the ICU” for the Dutch national intensive care training programme; and Lecturer for the Dutch Society of Transplantation Medicine. As a member of the Working Group on Nutrition and Metabolism of the Dutch Society of Intensive Care Medicine, he co-authored the Dutch guidelines for Nutrition in the Intensive Care Unit. He presented several research and state-of-the-art topics at national and international conferences.

Frank moved to Hamilton, New Zealand in 2005, together with his wife Marquita, son Luka and daughter Rifka. Following completion of the Overseas Trained

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Specialist assessment, he fulfilled requirements for Australian and New Zealand registration in Intensive Care Medicine in 2006 and received his Fellowship of the Joint Faculty of Intensive Care Medicine (now College of Intensive Care Medicine, CICM). In 2008 he completed the Postgraduate Diploma in Perioperative and Critical Care Echocardiography at the University of Melbourne, Australia.

Currently Frank works as Intensive Care Specialist in the Waikato Hospital, a tertiary referral and teaching hospital in Hamilton, New Zealand. He is Supervisor of Training for the CICM Intensive Care C24 core-training programme. In addition, Frank is Medical Director of the Intensive Care Department at the Southern Cross Hospital, a large private hospital in Hamilton. He has been appointed Honorary Clinical Senior Lecturer of the Waikato Clinical School, University of Auckland.