

Distributive failure in the  
microcirculation of septic patients

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# Distributive failure in the microcirculation of septic patients

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‘Wie wat vindt heeft slecht gezocht’. Rutger Kopland

Voor Ruth, mijn grote liefde.



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# Introduction



## The context

Sepsis is a life-threatening complication of infection with a reported crude mortality between 20% and 60% [1,2]. The infection itself and a complex reaction of the body eventually leads to multiple organ failure, often despite adequate antibiotic treatment and (surgical) control of the infection site. By definition, sepsis in combination with organ dysfunction is called severe sepsis, arising the need for intensive treatment to prevent death. Mortality progressively increases with the number of organs that fail [3]. It is estimated that over 9000 patients with severe sepsis are annually admitted to an Intensive Care Unit in the Netherlands, which accounts for 11% of all ICU-admittances in the Netherlands [4]. Apart from high mortality rates, severe sepsis and its intensive treatment also lead to substantial morbidity: physical and psychological effects for patients are considerable and may be long-lasting [5]. With an average cost of €19.500 per treatment the overall annual direct medical costs of €168 million represent 0.5% of all health-care costs and 1.7% of the annual hospital budget in the Netherlands [6]. In summary, (severe) sepsis represents a large healthcare burden, both in costs and human suffering.

In order to reduce morbidity and mortality, for many decades extensive efforts have been exerted to correct the circulatory shock that accompanies organ failure in severe sepsis [7]. This condition occurs when oxygen supply cannot meet the needs of the tissue cells [8], and, if not corrected in time, may result in organ dysfunction. As opposed to other forms of shock, septic shock results from distributive alterations in tissue perfusion, caused by abnormal control of microvasculature with an abnormal distribution of a normal or increased cardiac output [9]. Many years ago, Weil and Shubin proposed a classification of shock with special reference to its distributive form [10]. The distributive defect in septic shock has been classically defined as a redistribution of volumes and reflects a defect in vascular regulation in the presence of normal or even supranormal oxygen supply. The complex nature of the pathophysiology of this syndrome has led to considerable controversy regarding patient management. Despite an increase in cardiac output and oxygen delivery to tissue, seemingly paradoxical regional dysoxia is evident, as indicated by high lactate levels, disturbed acid base balance, and enhanced levels of gastric CO<sub>2</sub> [11,12]. This situation is described as a deficit in oxygen extraction by peripheral tissues. Under conditions of distributive shock, the concept of heterogeneity of blood flow within the microcirculation could explain dysoxia, despite adequate upstream and downstream parameters of oxygen delivery [13,14]. Microcirculatory weak units are shunted at the regional level causing patchy hypoxic areas, whereas at the same time both arterial and venular pO<sub>2</sub> may appear normal.

However, until 1999 sepsis-specific animal models, such as direct in-vivo microscopy of the microcirculation after specific tissue preparation, could not be translated into human sepsis studies for practical reasons. In that year, orthogonal polarization spectral (OPS) imaging was introduced [15]. The OPS technique consists of a handheld device that illuminates an

area of interest with polarized light, while imaging the remitted light through a second polarizer (analyzer), oriented in a plane precisely orthogonal to the plane of illumination. If a wavelength within the haemoglobin absorption spectrum (e.g., 548 nm) is chosen, erythrocytes will appear dark and leukocytes may be visible as refringent bodies. The vessel walls itself are not visualized directly, although faint contours can be identified depending on the presence of intravascular erythrocytes. Visualization of the microcirculation by the technical successor of OPS imaging, sidestream dark field (SDF) imaging, is essentially based on the same physical principles [16]. As a result of direct in-vivo observation of the microcirculation in septic patients, microcirculatory abnormalities, and in particular heterogeneity of flow, are now being recognized as key characteristics in the pathogenesis of organ dysfunction during sepsis [17-19]. Presence and persistence of such abnormalities were found to be associated with prognosis of morbidity and mortality, in contrast to all the available systemic hemodynamic variables [17-19]. Combining OPS/SDF imaging with other techniques such as sublingual and buccal capnometry, has added to the understanding of the relation between microcirculatory abnormalities and metabolic tissue variables [20,21].

## Thesis

If heterogeneity of microcirculatory blood flow is a prominent characteristic in septic shock, not sensed by conventional systemic hemodynamic parameters, what are the consequences for the way doctors monitor the state and progression of the disease itself, as well as their therapeutic interventions? In chapter 1 a general introduction to this question is applied to the gut in sepsis.

Before scientific comparison between observations of the microcirculation under specific experimental conditions can be performed, quantification of observed microcirculatory alterations is needed. Apart from the technical complexity to transform visual images into concrete numbers, the proposed system of quantification should specifically be sensitive to detect microcirculatory alterations under these heterogeneous flow conditions. In chapter 2 we describe a study that aims to validate both intra- and interobserver variability of a semi-quantitative way for assessment of microcirculatory alterations in sepsis, in organs with different microvascular architecture.

In chapter 3 the discordance between systemic hemodynamic parameters and (sublingual) microcirculatory alterations is illustrated with a case report, in which the blood pressure of a patient with a septic shock is successfully restored with the potent vasopressor agent terlipressin, at the 'expense' of a complete shutdown of the microcirculation.

Given the fact that heterogeneity of blood flow can be observed within the microcirculation during sepsis, what does this imply for heterogeneity of flow between different organs and between different vascular compartments? In other words, is it conceivable that microcirculatory alterations in one particular organ may be different in a second organ at the same time? And will this relation be constant over time?

In chapter 4 an observational study is described, in which microcirculatory alterations in human sepsis are observed in two organs, the tongue and the intestinal tract, at 2 different time points. In chapter 5 the results of a comparable study are reported; under human septic conditions the relation between parameters of the peripheral vascular compartment and the microcirculatory compartment is elaborated.

An important issue up-to-date remains the specific meaning of the numerous observations that microcirculatory alterations occur under conditions of distributive- and other forms of shock. The absence of such alterations in several healthy control groups, its prognostic value in terms of morbidity and mortality in sepsis, as well as the relation with the occurrence of intracellular hypoxia and acidosis are strongly indicative for its relevance. However, this does not necessarily implicate that interventions to improve microvascular blood flow are beneficial to outcome of patients. The proof of the pudding is in the eating. In chapter 6 we describe the design and the results of a large single-centre double-blind randomised placebo controlled clinical trial, that aimed to improve microcirculatory flow in severe human sepsis with the organic nitrate nitroglycerin, in the setting of an initial strictly protocolised resuscitation.

Distributive shock is not restricted to sepsis, but may also occur under other conditions such as ischemia-reperfusion, systemic inflammatory response syndrome and CABG-procedures [13, 22]. During on-pump cardiac surgery patients are subject to a number of hemodynamic changes, associated with intestinal hypoperfusion [23]. In an observational study (chapter 7) we combined postoperative direct observation of the rectal microcirculation by means of SDF-imaging with rectal tonometry, to test the hypothesis that splanchnic ischemia is associated with (persistent) splanchnic microcirculatory alterations.

Chapter 8 aims to provide a 'state of the union' with regard to development of the microcirculation as a clinical concept and gives an overview over recent literature. The thesis ends with a summary in English and Dutch.

## References

1. Brun-Buisson C, Doyon F, Carlet J, Dellamonica P, Gouin F. Incidence, risk factors and outcome of severe sepsis and septic shock in adults; a multicenter prospective study in Intensive Care Units. *JAMA* 1995; 274:968-974.
2. Angus DC, Linde-Zwirble WT, Lidicker J, Clermont G, Carcillo J, Pinsky MR. Epidemiology of severe sepsis in the United States: Analysis of incidence, outcome, and associated costs of care. *Critical Care Medicine* 2001; 29(7):1303-1310.
3. Esper AM, Martin GS. Expanding international sepsis epidemiology: the impact of organ dysfunction. *Crit Care* 2009; 13:120.
4. Van Gestel A, Bakker J, Veraart CP, van Hout BA. Prevalence and incidence of severe sepsis in Dutch intensive care units. *Crit Care* 2004; 8:R153-R162.
5. Hofhuis JG, Spronk PE, van Stel HF, Schrijvers AJ, Rommes JH, Bakker J. The impact of severe sepsis on health-related quality of life: a long-term follow-up study. *Anesth Analg* 2008; 107:1957-1964.
6. Bakker J, Levi M, van Hout BA, van Gestel A. Sepsis, a complicated syndrome with major medical and social consequences. *Ned Tijdschr Geneesk* 2004; 148:975-978.
7. Magder S. Shock Physiology. In: Pinsky MR, Dhainaut JF, editors. *Pathophysiologic Foundations of Critical Care*. Baltimore, Maryland 21202, USA: Williams and Wilkins, 1993: 140-160.
8. Nelson DP, Samsel RW, Wood LD, Schumacker PT. Pathological supply dependence of systemic and intestinal O<sub>2</sub> uptake during endotoxemia. *J Appl Physiol*. 1988; 64:2410-2419.
9. Vincent JL; international sepsis forum. Hemodynamic support in septic shock. *Intensive Care Med* 2001; 27 Suppl 1:S80-92.
10. Weil MH, Shubin H. Proposed reclassification of shock states with special reference of distributive defects. *Adv Exp Med Biol* 1971; 23:13-23.
11. Bakker J, Coffernils M, Leon M, Gris P, Vincent JL. Blood lactate levels are superior to oxygen-derived variables in predicting outcome in human septic shock. *Chest* 1991; 99:956-962.
12. Gutierrez G, Palizas F, Doglio G, Wainsztein N, Gallesio A, Pacin J, Dubin A, Schiavi E, Jorge M, Pusajo J, et al. Gastric intramucosal pH as a therapeutic index of tissue oxygenation in critically ill patients. *Lancet*. 1992; 339:195-199.

13. Ashruf JF, Ince C, Bruining HA. Regional ischemia in hypertrophic Langendorff-perfused rat hearts. *Am J Physiol.* 1999; 277:H1532-H1539.
14. Ince C, Sinaasappel M. Microcirculatory oxygenation and shunting in sepsis and shock. *Critical Care Medicine* 1999; 27:1369-1377.
15. Groner W, Winkelmann JW, Harris AG, Ince C, Bouma GJ, Messmer K, Nadeau RG. Orthogonal polarization spectral imaging: A new method for study of the microcirculation. *Nat Med* 1999; 5:1209-1212.
16. Goedhart PT, Khalilzade M, Bezemer R, Merza J, Ince C. Sidestream dark field (SDF) imaging: a novel stroboscopic LED ring-based modality for clinical assessment of the microcirculation. *Optics express* 2007; 15:15101-14.
17. De Backer D, Creteur J, Preiser JC, Dubois MJ, Vincent JL. Microvascular blood flow is altered in patients with sepsis. *Am J Respir Crit Care Med* 2002; 166:98-104.
18. Sakr Y, Dubois MJ, De Backer D, Creteur J, Vincent JL. Persistent microcirculatory alterations are associated with organ failure and death in patients with septic shock. *Crit Care Med* 2004; 32:1825-31.
19. Trzeciak S, Dellinger RP, Parillo JE, Guglielmi M, Baja J, Abate NL, Arnold RC, Colilla S, Zanotti S, Hollenberg SM. Early microcirculatory perfusion derangements in patients with severe sepsis and septic shock: relationship to hemodynamics, oxygen transport and survival. *Ann Emerg Med* 2007; 49:88-98.
20. Weil MH, Nakagawa Y, Tang W, Sato Y, Ercoli F, Finegan R, Grayman G, Bisera J. Sublingual capnometry: A new non-invasive measurement for diagnosis and quantitation of severity of circulatory shock. *Crit Care Med* 1999; 27:1225-1229.
21. Creteur J, De Backer D, Sakr Y, Koch M, Vincent JL. Sublingual capnometry tracks microcirculatory changes in septic patients. *Intensive Care Med.* 2006; 32:516-523.
22. Elbers PW, Ince C. Mechanisms of critical illness--classifying microcirculatory flow abnormalities in distributive shock. *Crit Care* 2006; 10:221
23. Ascione R, Talpahewa S, Rajakaruna C, Reeves BC, Lovell AT, Cohen A, Angelini GD. Splanchnic organ injury during coronary surgery with or without cardiopulmonary bypass: a randomized controlled trial. *Ann Thorac Surgery* 2006; 81:97-103.



# Chapter 1

## The microcirculation of the gut in sepsis

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## Introduction

For many decades the specific vascular architecture of the gut and its hormonal response to shock has been studied. The discovery of extensive intestinal microvascular reactions in the absence of changes in systemic pressure variables [1,2] was a trigger to a long list of both preclinical and clinical research in this field. Today, microcirculatory distress in the gut is believed to be an early stage in the development of Multiple Organ Dysfunction Syndrome and has been hypothesised to be an important contributing factor in its development [3]. Dysregulation of the intestinal microcirculation by sepsis related mediators and inflammatory cells may lead to loss of barrier function, endothelial cell dysfunction and enhance clotting, red blood cell rigidity and leukocyte activation [4] and finally promotes cell death. However, the role of microcirculatory shutdown and concomitant impairment of oxygen delivery as a pathophysiologic entity in the development of cell dysoxia is still under debate. A number of different biochemical mechanisms, including reversible inhibition of cytochrome  $aa_3$  by nitric oxide, de-energization of the redox couple of mitochondrial nicotinamide adenine dinucleotide (NAD<sup>+</sup>/NADH) and irreversible inhibition of mitochondrial respiratory complexes by peroxynitrite have been described [5]. Whether this 'cytopathic hypoxia' in vivo may develop independently of insufficient oxygen supply, or must be seen as a cellular response to dysoxia, needs to be illuminated.

In this article we will focus on microvascular blood flow alterations in the gut during sepsis, and discuss recently published techniques with a promising potency to unravel existing controversies in septic shock and its treatment.

## Tissue oxygenation

Clinical observations on impairment of regional perfusion (e.g. big toe temperature) and its prognostic value have been made for many years [6,7]. However, bedside decisions were mainly made on the basis of gross systemic hemodynamic parameters. Moreover, in sepsis, where the alterations of microcirculatory blood flow have been described extensively, the use of vasopressors is still considered to be an important clinical tool [8]. On one hand, the introduction of regional measurement techniques has highlighted the inadequacy of global hemodynamic monitoring in regard to possible failure of microcirculatory oxygenation, but on the other hand it has also contributed to the controversy on what level of tissue oxygenation is considered 'adequate'. Ideally, 'adequate' should be defined on the level of cell function and energy utilisation. However, such parameters are not easily available and might not always reflect a situation of adequate oxygen supply. For example, returning blood pressure to normal levels, after tubular necrosis has happened, will not be followed by (immediate) return of renal function. If hibernation of the cell, due to a biochemical shutdown of energy utilisation within the mitochondrial respiratory chain exists [9], restoration of

oxygen supply by means of microvascular 'repair' after a long period of inadequate supply, will not automatically lead to revitalisation of such biochemical mechanisms. Whether mitochondrial failure is the culprit or not, ensuring 'adequate' oxygen supply to the microcirculation is an essential (rst) step in the resuscitation of vital cell functions.

## Regional techniques

Many techniques have been advocated to measure intestinal flow and adequacy of tissue oxygenation. Intestinal flow measurements include laser-Doppler and indocyanine green infusion [10], whereas assessment of the presence of dysoxia has been carried out by lactate measurements, gastric tonometry and oxygen electrodes. A rise in blood lactate is considered to reflect anaerobic cellular metabolism associated with tissue dysoxia, but direct sepsis mediated lactate production and changes in lactate clearance complicate the interpretation [11]. Gastric tonometry is a minimal invasive technique that detects intracellular dysoxia by measuring intraluminal  $p\text{CO}_2$  in relation to systemic  $p\text{CO}_2$ ; an increment in  $p\text{CO}_2$  gap reflects intracellular acidosis as a result of dysoxia. Reliable interpretation is possible after elimination of methodical errors like prepyloric feeding and lack of gastric suppression [12].

Direct measurement of tissue oxygen pressure with the use of polarographic oxygen electrodes has also been performed in the gut [13]. With a penetration depth of around 15  $\mu\text{m}$  the electrodes measure an average  $p\text{O}_2$  of tissue cells, capillaries and larger blood vessels around the probe. Conflicting results may be due to hidden hypoxic microcirculatory units next to well-perfused normoxic or hyperoxic units, since a pathologic heterogeneity of blood flow within one organ seems to be an important characteristic of the microcirculation during sepsis [14,15]. High tissue  $p\text{O}_2$  can be a result of cell death, mitochondrial injury, increased  $\text{O}_2$  delivery or microvascular arteriole-to-venule convective shunting. To address this problem, optical spectroscopy has been developed [16,17]. In these non-invasive techniques phosphorescence spectroscopy is used to measure oxygen pressures in the microcirculation, reflectance and absorbance spectrophotometry for haemoglobin saturation in microcirculatory blood, and fluorescence spectroscopy to measure cellular mitochondria energy state [18].

## Oxygenation of the microcirculation

Oxygen transport to the tissue is achieved by a combination of a heterogeneous convective mechanism (e.g. blood flow) and by diffusion, either directly from arterioles to aligning cells [19] or at the capillary interface, thus creating homogeneous oxygenation [20]. There is

evidence however, that during hypoxia the oxygenation might become more heterogeneous [21]; well-oxygenated microcirculatory units exist next to hypoxic units. In vitro NADH fluorescence imaging of the heart revealed persistence of dysoxic areas after a period of ischemia. These were called microcirculatory weak units, because they were also the first to become dysoxic during episodes of ischemia and were identified as composed of capillary vessels [21]. In a subsequent study [22], these vulnerable units were found to become the first dysoxic during nitric oxide (NO) inhibition or vasopressin administration in endotoxemic rat hearts, an effect not found in control hearts. The mucosal vascular architecture, with close proximity of the feeding arteriole to effluent venule at the base of the villi, might also be considered microcirculatory weak units, since they are prone to diffusional shunting from arteriole to venule, thereby bypassing villi microcirculation [3], although in other studies the serosal muscularis has been identified as being the compartment to remain shunted in resuscitated sepsis. In muscle tissue, however, no microcirculatory subunits could be identified [23], explaining the relative resistance of muscle to dysoxia [13].

## Microcirculatory shunting

If shunting mechanisms within the microcirculation exist during sepsis, one would expect to find higher venous  $pO_2$  values than  $pO_2$  values in the microcirculation itself, since venous blood is mixed with oxygen-rich arteriolar blood, leaving the microcirculation dysoxic behind. This  $pO_2$  gap could indeed be demonstrated in the intestine during hemorrhagic shock in a pig model [24]. During shock, venous  $pO_2$  decreased to a plateau level, whereas microcirculatory  $pO_2$  continued to decrease, causing a  $pO_2$  disparity. Resuscitation with crystalloid solutions or autologous blood was able to restore this gap. Interestingly, in the ileum a large difference in tissue  $pO_2$  of the serosa compared with the mucosa has been observed; mucosal  $pO_2$  levels were much lower than serosal  $pO_2$  [13,24,25]. Bohlen [25] also demonstrated that tissue  $pO_2$  at the tip of the villi was about half the value compared to  $pO_2$  levels at the base of the villi. Furthermore,  $pO_2$  levels as established by oxygen electrodes were significantly higher than those obtained by Pd-porphyrin phosphorescence, suggesting measurement of different microcirculatory compartments as a result of difference in penetration depth [26,27]

During sepsis the same widening of the  $pO_2$  gap was demonstrated in a porcine endotoxin infusion model [3,28]. The  $pO_2$  gap was more pronounced during endotoxemia as compared to haemorrhage with an equal depression of microcirculatory  $pO_2$ , suggesting a larger shunt fraction during endotoxemia than during haemorrhage.

There are four principal mechanisms that could cause shunting of the microcirculation. The first is convective arteriovenous shunting, by which blood flows directly from arterioles to venules through anatomical anastomosis. The existence of such shunts has been

demonstrated in previous studies [29,30]. The second mechanism is by direct diffusion of oxygen from arterioles to venules lying in close proximity to each other, as described in the heart at low coronary flows [31]. The third mechanism has been referred to as 'vascular steal', whereby certain microcirculatory units steal flow from disadvantaged microcirculatory units by selective vasodilatation [32]. During sepsis this heterogeneity of the microcirculation could be aggravated by a blockade of disadvantaged microcirculatory units through micro emboli, an increase in red blood cell rigidity, oedema formation and adherence of activated leukocytes to the vascular wall. A fourth theoretical mechanism of shunting involves the inability of haemoglobin to off-load oxygen fast enough to tissues as it passes through the microcirculation. Kinetics of oxygen release from erythrocytes to the serum are described by Gutierrez [33]. Especially at low saturation levels the oxygen off-load time can exceed the microcirculation transit time for the erythrocyte. When the off-load subsequently takes place in the venous pool, venous  $pO_2$  would increase above microcirculatory tissue levels. Under these circumstances haemoglobin saturation would be expected to be lower in the venous blood than haemoglobin saturation in the microcirculatory blood. Indeed, Lash and Bohlen [34] found a contradictory increase in microcirculatory haemoglobin saturation during muscle stimulation, whereas a previous study [35] had shown a depression of tissue  $pO_2$  under the same circumstances, suggesting oxygen off-load restriction as possible explanation.

## Orthogonal polarisation spectroscopy

Recent clinical publications [36,37], in which the microcirculation was studied in vivo by means of orthogonal polarisation spectral (OPS) imaging, have opened challenging new perspectives. OPS imaging, introduced by Slaaf and co-workers [38], uses green polarised light to observe the microcirculation in vivo. The technique, as described in detail elsewhere [39], consists of a hand-held microscope that illuminates an area of interest with polarised light, while imaging the remitted light through a second polarizer (analyser) oriented in a plane precisely orthogonal to that of the illumination. If a wavelength within the haemoglobin absorption spectrum (e.g., 548 nm) is chosen, red blood cells will appear dark and white blood cell may be visible as refringent bodies. The vessel walls are not visible directly and its imaging therefore depends on the presence of red blood cells. We validated OPS imaging by comparison with intravital microscopy in human volunteers, and introduced this technique clinically for the first time observations of the microcirculation of internal human organs [40-43].

De Backer and co-workers [37] used sublingual OPS imaging to 'peep' at the microcirculation in patients with sepsis in comparison to non-septic intensive care patients. In a semi-quantitative analyses of the video-stored images, flow was defined as continuous,

intermittent or absent; vessels were divided into small and large, using a diameter cut-off value of 20  $\mu\text{m}$ . Density of all blood vessels and perfusion of small blood vessels was almost reduced by half during sepsis compared to healthy volunteers and other ICU control subjects. Reduction of vessel diameter and flow were correlated with an increase in blood lactate levels, severity of illness and non-survivors, despite overall correction of systemic hemodynamic and oxygen delivery parameters. Topical application of acetylcholine totally reversed the observed alterations. Spronk et al. [36] resuscitated 8 sepsis patients with fluids, dopamine and ketanserin until pre-set systemic hemodynamic goals were achieved. After resuscitation, all patients had severely impaired sublingual microcirculatory flow, especially in the small micro vessels. Observation of no flow in the small vessels next to flow in the larger vessels confirm the shunting theory of sepsis [3,47] Within two minutes after a loading dose of 0.5 mg nitroglycerin and subsequent continuous infusion a marked increase in microvascular flow in all generation micro vessels was observed, confirming the hypothesis obtained from animal data [44, 45], that vasodilator therapy is able to recruit shunted micro vessels in sepsis.

## Future perspectives

Although the introduction of OPS opens promising new ways to study the microcirculation in vivo during sepsis in a minimal invasive way, many questions remain to be answered. Is the sublingual microcirculation a mirror of equally vulnerable microcirculatory units in the gut and other organs? The introduction of sublingual tonometry [46] and the demonstration that sublingual capnography [47] is correlated with gastric tonometry in septic patients suggest possibilities for the future, but further research is needed to clear this picture. De Backer and co-workers demonstrated a relation between a shutdown of micro vessels and severity of illness [37] and both topical and systemic vasodilators were able to open up previously shutdown small microcirculatory vessels [36,37]. But will pharmacological interference with this complex microvascular heterogeneity ultimately lead to better outcome? The same question from a different perspective also needs to be answered; could the use of vasopressors be detrimental in sepsis and septic shock? To solve this dilemma the meticulous search for understanding the pathogenesis of dysoxia must be continued. If hibernation of the cell during sepsis exists [5], due to a biochemical shut down of the energy supply by the respiratory chain, opening up the microcirculation alone will not be enough to restore cellular function. On the other hand, starting up cellular energy production without proper oxygen delivery seems illogical and has a potency to aggravate dysoxia.

## Therapeutic options

If reopening of the microcirculation in sepsis proves to be important, what pharmacological tools do we have to achieve this? Many vasodilators have been used in sepsis [48], but its application in sepsis treatment is controversial. Sodium nitroprusside and nitroglycerin are potent nitric oxide (NO) donors. Under physiologic circumstances, release of NO is tightly controlled by shear stress of flowing blood acting on arterial endothelial cells [49]. Unresponsive hypotension in sepsis is believed to occur from NO overproduction by inducible NO synthase (iNOS) [50], but experimental blocking of NO production has been associated with many deleterious effects on regional perfusion [51-54]. A clinical sepsis trial with the NO synthase inhibitor L-NMA was halt due to increase in mortality [55], despite earlier reports of improvement of global hemodynamic parameters [56]. Recently, selective iNOS inhibition was reported to blunt detrimental microcirculatory effects in porcine endotoxemia [51,57]. From a shunting point of view, providing additional NO may have beneficial effects; apart from its vasodilatory effect [36], it also diminishes leukocyte adhesion [58], improves erythrocyte deformity [59] and reduces oedema formation [60,61]

Prostacyclin (PGI<sub>2</sub>) also plays a physiological role in the regulation of local blood flow. Following vascular endothelial insults such as haemorrhage, reperfusion, hypoxia and sepsis the endogenous PGI<sub>2</sub> production is enhanced [62]. In addition to vasodilatation PGI<sub>2</sub> also inhibits leukocyte adhesion [63] and platelet aggregation. Amelioration of the decrease in arteriolar blood flow and an increment of arteriolar and venular diameters as well as perfused capillary density have been reported [64,65].

Other possible interventions include N-Acetyl-L-cysteine [66], pentoxifylline [67], dexamethason [68], selective gut microcirculatory control by topical vasodilators [69], phosphodiesterase inhibitors [70,71] and the use of blood substitutes [72,73]. Discussion of these possible therapeutic strategies is beyond the scope of this manuscript.

## References

1. Haglund U (1972) Vascular reactions in the small intestine of the cat during hemorrhage. *Acta Physiol Scand* 89:129-141
2. Gilmour DG, Aitkenhead AR, Hothersall AP et al (1980) The effect of hypovolaemia on colonic blood flow in the dog. *Br.J. Surg* 67:82-84
3. Ince C, Sinaasappel M (1999) Microcirculatory oxygenation and shunting in sepsis and shock. *Crit Care Med* 27:1369-1377

4. Hinshaw LB (1996) Sepsis/septic shock: participation of the microcirculation: an abbreviated review. *Crit Care Med* 24:1072-1078
5. Fink MP (2002) Bench-to-bedside review: Cytopathic hypoxia. *Crit Care* 6:491-499
6. Joly HR, Weil MH (1969) Temperature of the great toe as an indication of the severity of shock. *Circulation* 39:131-138
7. Kaplan LJ, McPartland K, Santora TA et al (2001) Start with subjective assessment of skin temperature to identify hypoperfusion in intensive care unit patients. *J Trauma* 50:620-628
8. Task force of the American College of Critical Care Medicine, Society of Critical Care Medicine (1999) Practice parameters for hemodynamic support of sepsis. *Crit Care Med* 27:639-660
9. Budinger GR, Chandel N, Shao ZH et al (1996) Cellular energy utilization and supply during hypoxia in embryonic cardiac myocytes. *Am J Physiol* 270:L44-L53
10. Ruokonen E, Takala J, Kari A et al (1993) Regional blood flow and oxygen transport in septic shock. *Crit Care Med* 21:1296-1303
11. Bakker J (2001) Lactate: may I have your votes please? *Intensive Care Med* 27:6-11
12. Marik P (1998) Gastric tonometry: the canary sings once again. *Crit Care Med* 26:809-810
13. Vallet B, Lund N, Curtis SE et al (1994) Gut and muscle tissue pO<sub>2</sub> in endotoxemic dogs during shock and resuscitation. *J Appl Physiol* 76: 793-800
14. Walley KR (1996) Heterogeneity of oxygen delivery impairs oxygen extraction by peripheral tissues: theory. *J Appl Physiol* 81:885-894
15. Humer MF, Phang PT, Friesen BP et al (1996) Heterogeneity of gut capillary transit times and impaired gut oxygen extraction in endotoxemic pigs. *J Appl Physiol* 81:895-904
16. Ince C, Bruining HA (1991) Optical spectroscopy for measurement of tissue hypoxia. In: Vincent JL (ed) *Update in Intensive Care and Emergency Medicine: update 1991*. Springer-Verlag, New York, pp 161-171
17. Ellis CG, Ellsworth ML, Pittman RN (1990) Determination of red blood cell oxygenation in vivo by dual video densitometric image analysis. *Am J Physiol* 258:H1216-H1223

18. Siegemund M, van Bommel J, Ince C (1999) Assessment of regional tissue oxygenation. *Intensive Care Med* 25:1044-1060
19. Ellsworth ML, Pittman RN (1990) Arterioles supply oxygen to capillaries by diffusion as well as by convection. *Am J Physiol* 258:H1240-H1243
20. Intaglietta M, Johnson PC, Winslow RM (1996) Microvascular and tissue oxygen distribution. *Cardiovasc Res* 32:632-643
21. Ince C, Ashruf JF, Avontuur JA et al (1993) Heterogeneity of the hypoxic state in rat heart is determined at the capillary level. *Am J Physiol* 264:H294-H301
22. Avontuur JAM, Bruining HA, Ince C (1995) Inhibition of nitric oxide synthesis causes myocardial ischemia in endotoxemic rats. *Circ Res* 76:418-425
23. Toth A, Pal M, Tischler ME et al (1996) Are there oxygen-deficient regions in resting skeletal muscle? *Am J Physiol* 270:H1933-H1939
24. Sinaasappel M, van Iterson M, Ince C (1999) Microvascular oxygen pressure in the pig intestine during hemorrhagic shock and resuscitation. *J Physiol* 514:245-253
25. Bohlen HG (1980) Intestinal tissue  $pO_2$  and microvascular response during glucose exposure. *Am J Physiol* 238:H164-H171
26. Germann R, Hasibeder W, Haisjackl M et al (1995) Dopamine-1 receptor stimulation and mucosal tissue oxygenation in the porcine jejunum. *Crit Care Med* 23:1560-1566
27. Sinaasappel M, Ince C (1996) Calibration of Pd-porphyrin phosphorescence for oxygen concentration measurements in vivo. *J Appl Physiol* 81:2297-2303
28. Sinaasappel M, Ince C (1999) Microvascular and venous  $pO_2$  diverge during endotoxemia. *Intensive Care Med* 25 (suppl 1):S9
29. Menger MD, Vollmar B (1996) In vivo documentation of an arteriovenous shunting rat pancreatic acinar tissue. *Pancreas* 13:125-129
30. Cronenwett JL, Lindenauer SM (1979) Direct measurement of arteriovenous anastomotic blood flow in the septic canine hindlimb. *Surgery* 85:275-282
31. Roth AC, Feigl EO (1981) Diffusional shunting in the canine myocardium. *Circ Res* 48:470-480

32. Shepherd AP, Kiel JW (1992) A model of countercurrent shunting of oxygen in the intestinal villus. *Am J Physiol* 262:H1136-H1142
33. Gutierrez G (1986) The rate of oxygen release and its effect on capillary O<sub>2</sub> tension: a mathematical analysis. *Respir Physiol* 63:79-96
34. Lash JM, Bohlen HG (1995) Excess oxygen delivery during muscle contractions in spontaneously hypertensive rats. *J Appl Physiol* 78:101-111
35. Lash JM (1995) Arterial and arteriolar contributions to skeletal functional hyperemia in spontaneously hypertensive rats. *J Appl Physiol* 78:93-100
36. Spronk PE, Ince C, Gardien MJ et al (2002) Nitroglycerin in septic shock after intravascular volume resuscitation. *Lancet* 360:1395-1396
37. De Backer D, Creteur J, Preiser J-C et al (2002) Microvascular blood flow is altered in patients with sepsis. *Am J Respir Crit Care Med* 166:98-104
38. Slaaf DW, Tangelder GJ, Reneman RS (1987) A versatile incident illuminator for intravital microscopy. *Int J Microcirc Clin Exp* 6:391-397
39. Groner W, Winkelmann JW, Harris AG et al (1999) Orthogonal polarization spectral imaging: A new method for study of the microcirculation. *Nat Med* 5:1209-1212
40. Harris AG, Sinitsina I, Messmer K (2000) The cytoscan™ model E-II, a new reflectance microscope for intravital microscopy: comparison with the standard fluorescence method. *J Vasc Res* 37:469-476
41. Mathura KR, Vollebregt KC, Boer K et al (2001) Comparison of OPS imaging and conventional capillary microscopy to study human microcirculation. *J Appl Physiol* 91:74-78
42. Mathura KR, Alić L, Ince C (2001) Initial clinical experience with OPS imaging for observation of the human microcirculation. In: Vincent JL (ed) *Yearbook of Intensive Care and Emergency Medicine* 2001. Springer-Verlag, New York, pp 233-245
43. Mathura KR, Bouma GJ, Ince C (2001) Abnormal microcirculation in brain tumours during surgery. *Lancet* 358:1698-1699
44. Ellis CG, Bateman RM, Sharpe MD et al (2002) Effect of maldistribution of microvascular blood flow on capillary O<sub>2</sub> extraction in sepsis. *Am J Physiol* 282:H156-H164

45. Siegemund M, Racovitza I, Ince C (2002) The rationale for vasodilator therapy in sepsis. In: Vincent JL (ed) Yearbook of Intensive Care and Emergency Medicine 2002. Springer-Verlag, New York, pp 221-231
46. Weil MH, Nakagawa Y, Tang W et al (1999) Sublingual capnometry: a new noninvasive measurement for diagnosis and quantitation of severity of circulatory shock. *Crit Care Med* 27:1225-1229
47. Marik PE (2001) Sublingual capnography: a clinical validation study. *Chest* 120:923-927
48. Buwalda M, Ince C (2002) Opening the microcirculation: can vasodilators be useful in sepsis? *Intensive Care Med* 28:1208-1217
49. Cooke JP, Stamler J, Andon N et al (1990) Flow stimulates endothelial cells to release a nitrovasodilator that is potentiated by reduced thiol. *Am J Physiol* 259:H804-H812
50. Evans T, Carpenter A, Kinderman H et al (1993) Evidence of increased nitric oxide production in patients with sepsis syndrome. *Circ Shock* 41:77-81
51. Siegemund M, van Bommel J, Schwarte LA et al (2001) Selective blockade of iNOS by 1400W but not by LNMA is beneficial to myocardial oxygenation after endotoxemia. *Intensive Care Med* 27(2, suppl):S178
52. Wang Y, Mathews WR, Guido DM et al (1995) Inhibition of nitric oxide synthesis aggravates reperfusion injury after hepatic ischemia and endotoxemia. *Shock* 4:282-288
53. Fukatsu K, Saito H, Fukushima R et al (1995) Detrimental effects of nitric oxide synthase inhibitor (N- $\omega$ -nitro-L-arginine-methyl-ester) in a murine sepsis model. *Arch Surg* 130:410-414
54. Werner J, Rivera J, Fernandez-del Castillo C et al (1997) Differing roles of nitric oxide in the pathogenesis of acute edematous versus necrotizing pancreatitis. *Surgery* 121:23-30
55. Grover R, Lopez A, Lorente J et al (1999) Multi-centre, randomized, placebo-controlled, double blind study of the nitric oxide synthase inhibitor 564C88: effect on survival in patients with septic shock. *Crit Care Med* 27(1, suppl):A33
56. Grover R, Bakker J, McLuckie A et al (1998) The nitric oxide synthase inhibitor 564C88 promotes the resolution of shock in patients with severe sepsis. *Crit Care Med* 26(1, suppl):A29

57. Pittner A, Nalos M, Asfar P et al (2003) Mechanisms of inducible nitric oxide synthase (iNOS) inhibition-related improvement of gut mucosal acidosis during hyperdynamic porcine endotoxemia. *Intensive Care Med* 29:312-316
58. Kubes P, Suzuki M, Grandner DN (1991) Nitric oxide: an endogenous modulator of leucocytes adhesion. *Proc Natl Acad Sci USA* 88:4651-4655
59. Starzyk D, Korbut R, Gryglewski RJ (1997) The role of nitric oxide in regulation of deformability of red blood cells in acute phase of endotoxaemia in rats. *J Physiol Pharmacol* 48:731-735
60. Payne D, Kubes P (1993) Nitric oxide donors reduce the rise in reperfusion-induced intestinal mucosal permeability. *Am J Physiol* 265:G189-G195
61. Oliver JA (1992) Endothelium-derived relaxing factor contributes to the regulation of endothelial permeability. *J Cell Physiol* 151:506-511
62. Scheeren T, Rademacher P (1997) Prostacyclin (PGI<sub>2</sub>): new aspects of an old substance in the treatment of critically ill patients. *Intensive Care Med* 23:146-158
63. Jones G, Hurly JV (1984) The effects of prostacyclin on adhesion of leucocytes to injured vascular endothelium. *J Pathol* 142:51-59
64. Muller B, Schmidtke M, Witt W (1987) Actions of the stable prostacyclin analogue iloprost on microvascular tone and -permeability in the hamster cheek pouch. *Prostaglandins Leukot Med* 29:187-198
65. Bouskela E, Rubanyi GM (1995) Effect of iloprost, a stable prostacyclin analog, and its combination with NW-nitro-L-arginine on early events following lipopolysaccharide injection: observations in the hamster cheek pouch microcirculation. *Int J Microcirc Clin Exp* 15:170-180
66. Bakker J, Zhang H, Depierreux M et al (1994) Effects of N-acetylcysteine in endotoxic shock. *J Crit Care* 9:236-243
67. Steeb GD, Wilson MA, Garrison RN (1992) Pentoxifylline preserves small-intestine microvascular blood flow during bacteremia. *Surgery*, 112:756-763
68. Tailor A, Tomlinson A, Salas A et al (1999) Dexamethason inhibition of leucocyte adhesion to rat mesenteric postcapillary venules: role of intercellular adhesion molecule 1 and KC. *Gut* 45:705-712

69. Hersh M, Madorin WS, Sibbald WJ et al (1998) Selective gut microcirculatory control (SGMC) in septic rats: a novel approach with a locally applied vasoactive drug. *Shock* 10:292-297
70. Pawlik WW, Hottenstein OD, Palen TE et al (1993) Adenosine modulates reactive hyperemia in rat gut. *J Physiol Pharmacol* 44:119-137
71. Loick HM, Möllhoff, Berendes E (1997) Influence of enoximone on systemic and splanchnic oxygen utilization and endotoxin release following cardiopulmonary bypass. *Intensive Care Med* 23:267-275
72. Jesch FH, Peters W, Hobbahn J et al (1982) Oxygen-transporting fluids and oxygen delivery with hemodilution. *Crit Care Med* 10:270-274
73. Intaglietta M (1997) Whitacker Lecture 1996: microcirculation, biomedical engineering, and artificial blood. *Ann Biomed Eng* 25:593-603

# Chapter 2

Quantifying bedside-derived imaging of microcirculatory abnormalities in septic patients: a prospective validation study

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## Abstract

### Introduction.

The introduction of orthogonal polarization spectral (OPS) imaging in clinical research has elucidated new perspectives in the role of microcirculatory flow abnormalities in the pathogenesis of sepsis. Essential to the process of understanding and reproducing these abnormalities is the method of quantification of flow scores.

### Method.

In a consensus meeting with collaborators from 6 research centres in different fields of experience with microcirculatory OPS imaging, premeditated qualifications for a simple, translucent and reproducible way of flow scoring were defined. Consecutively, a single-centre prospective observational validation study was performed in a group of 12 patients with an abdominal sepsis and a new stoma. Flow images of the microcirculation in vascular beds of the sublingual and stoma region were obtained, processed and analysed in a standardised way. We validated intra-observer and inter-observer reproducibility with kappa cross-tables for both types of microvascular beds.

### Results.

Agreement and kappa coefficients were  $> 85\%$  and  $> 0.75$  respectively for interrater and intrarater variability in quantification of flow abnormalities during sepsis, in different subsets of microvascular architecture.

### Conclusion.

Semi-quantitative analysis of microcirculatory flow, as described, provides a reproducible and transparent tool in clinical research to monitor and evaluate the microcirculation during sepsis.

## Introduction

Recent clinical investigations have identified microcirculatory abnormalities as a key component of the pathogenesis of sepsis [1,2]. These new insights have been mainly due to the introduction of orthogonal polarization spectral (OPS) imaging by Slaaf and co-workers [3], which uses green polarized light to observe the microcirculation in vivo. Implementing OPS imaging in a hand-held type of tool allowed us to observe the microcirculation of internal human organs for the first time [4,5]. The central role of microcirculatory abnormalities in sepsis was elucidated when OPS imaging was applied in critically ill patients. Microcirculatory abnormalities were found in septic patients by direct observation of the sublingual microcirculation by means of OPS imaging [6,7], and such abnormalities were found to be predictive in outcome [1].

An important issue in these investigations concerns the method of quantifying the OPS movies of microvascular structures, to identify flow abnormalities associated with sepsis, and evaluate its results. De Backer and co-workers introduced a semi-quantitative method, based on the number of perfused vessels crossing three equidistant horizontal and vertical lines [7,8]. We also developed a score, based on a slightly different principle [6]. Both methods require subjective assessment of flow to identify redistribution between different sized micro vessels, especially the capillaries. Although these methods have proven their worth in practice in identifying the nature of microcirculatory dysfunction in sepsis, neither method has yet been validated in terms of reproducibility. Furthermore there is a need for a more general method of analysis, applicable to other microvascular structures with different architecture than the usually investigated sublingual vascular bed.

In this study we present a consensus method of semi-quantitative analysis of OPS imaging that is suitable to quantify microcirculatory abnormalities in critically ill patients in different subsets of vascular beds, i.e. the sublingual region, villi of the small bowel and crypts of the colon. We validated this method for its interrater and intrarater variability and will discuss its potency for future automated analysis by means of software application.

## Materials and methods

### Specifications of the procedure.

We called together 6 collaborative centres involved in clinical microcirculation research in paediatric and adult intensive care units in the Netherlands, to come to a consensus about quantification of microcirculatory abnormalities in direct observations by means of OPS imaging. The 6 centres are involved in OPS studies in various human organ tissue such as the sublingual region, gut villi, rectal mucosa, skin, conjunctiva, gingival and brain tissue. This was important because we wished to reach a consensus regarding a method that is

applicable to the various microcirculatory beds. The aim of the process was to implement a systematic approach to the analysis of OPS derived microcirculatory flow imaging that would allow identification and quantification of microcirculatory abnormalities during critical illness. Preferably, the designed method should be able to analyse different microvascular structures that have variable vascular anatomy so as to avoid multiple scoring systems for the evaluation of flow imaging in specific organ oriented research. The scoring system should have clear definitions that are easy to teach and have acceptable interrater and intrarater variability. Storage of flow images should be possible at all times and performed in a structured way so that results can be discussed and (re)evaluated. Finally, its application should avoid time-consuming processing and its concept must be suitable for software analysis.

#### Definitions.

In order to meet these premeditated qualifications we designed a simple semi-quantitative judgement of microvascular flow, which distinguishes no flow (0), intermittent flow (1), sluggish flow (2) and continuous flow (3). In case a microvascular subunit contains different types of vessels with different diameters, e.g. the sublingual vascular bed, these quantifications of flow can be made per cohort of vessel diameter; small: 10-25  $\mu\text{m}$ , medium 26-50  $\mu\text{m}$  and large 51-100  $\mu\text{m}$  (Fig. 1 and 2).

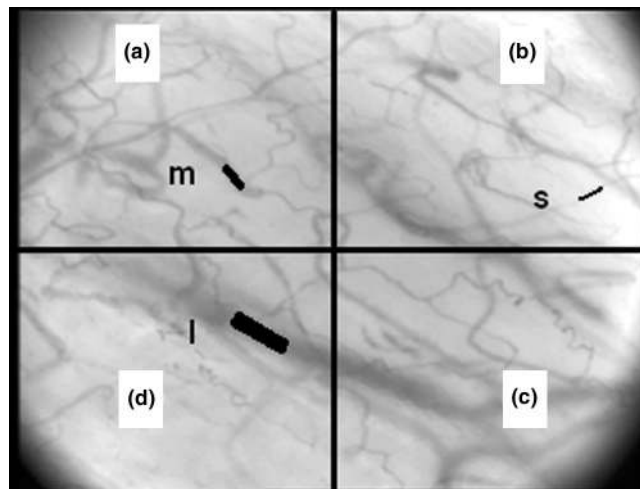


Figure 1. OPS imaging of a microvascular network, i.e. sublingual microvascular architecture. The image is divided in 4 quadrants (A,B,C,D) with examples of vessel classification: s small (10-25  $\mu\text{m}$ ), m medium (26-50  $\mu\text{m}$ ), l large (51-100  $\mu\text{m}$ ). Objective 5x, on screen 325x.

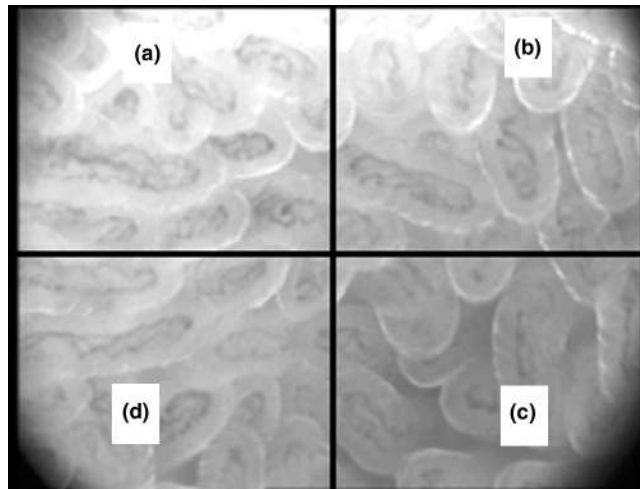


Figure 2. OPS imaging of a repeating vascular structure, i.e. villi of the small intestine. Objective 5x, on screen 325x.

#### Imaging technique.

The OPS technique, as described in detail elsewhere [9,10], consists of a hand-held device that illuminates an area of interest with polarized light, while imaging the remitted light through a second polarizer (analyser), oriented in a plane precisely orthogonal to the plane of illumination. If a wavelength within the haemoglobin absorption spectrum (e.g. 548 nm) is chosen, red blood cells will appear dark and white blood cells may be visible as refringent bodies. The vessel walls itself are not visualized directly and its imaging therefore depends on the presence of red blood cells.

#### Imaging and analysis procedure.

After gentle removal of saliva/faeces by an isotonic-saline-drenched gauze, steady images of at least 20 seconds are obtained and stored on digital videotape (SONY video walkman GV-D 1000E®), avoiding pressure artefacts. Subsequently the images are captured in 5-10-second representative video clips in avi format (sonyDVgate®). Video clips are analyzed blindly and at random to prevent coupling between images. Since heterogeneity of flow seems to be an important characteristic of microvascular alterations during sepsis [11], OPS images are obtained from three different regions within the site of interest and each image is divided into 4 equal quadrants (A,B,C and D). Quantification of flow is scored per quadrant, for each cohort of vessel diameter if applicable. The overall score, called microvascular flow index (MFI), is the sum of each quadrant-score, divided by the number of quadrants in which the vessel type is visible (Table 1 and 2).

Table 1. Example of Microvascular Flow Index (MFI) calculation for a (sublingual) microvascular network.

	Quadrant A	Quadrant B	Quadrant C	Quadrant D	MFI
Flow, small	2	3	3	2	$10/4=2.5$
Flow, medium	1	3	3	3	$10/4=2.5$
Flow, large	-	3	3	-	$6/2=3$

Table 2. Example of Microvascular Flow Index calculation for a repeating microvascular structure (gut villi).

	Quadrant A	Quadrant B	Quadrant C	Quadrant D	MFI
Flow villi	2	3	3	2	$10/4=2.5$

### Setting and patient selection.

To validate the above process of quantification we performed a single centre prospective observational validation study in a tertiary teaching-hospital with a 23 bed mixed ICU. During an 8-month period patients with a new stoma in the course of abdominal sepsis were included. Overt clinical necrosis of the stoma was a contraindication for OPS imaging. This particular model was chosen since a complete spectrum of microvascular flow abnormalities, ranging from no flow (0) to normal flow (3) was expected to be visualized in potentially 3 different microvascular subsets: the sublingual region, gut villi in an ileostomy and crypts in a colostomy. A local ethical and scientific committee waived the need for informed consent since the observations were considered non-invasive and no interventions were made.

### Statistical analysis.

Interrater and intrarater variability was calculated by kappa ( $\kappa$ ) cross tables for ordinal variables in Analyse-it® (Analyse-It Software, Leeds, United Kingdom), and presented with 95%-confidence intervals (CI). The advantage of  $\kappa$ -coefficient calculation above establishing agreement alone, lies in the fact that the  $\kappa$ -coefficient also takes into account the rule of chance [12,13]. The chance of agreement was estimated to be considerable with such a limited number of ordinal variables. A  $\kappa$ -coefficient  $> 0.6$  was considered good [13]. Weighted  $\kappa$ -coefficients ( $\kappa_w$ ) were additionally calculated in order to take into account the level of disagreement, giving weights to disagreement according to the magnitude of the discrepancy [14].

## Results

In an 8-month period 12 patients were included with a new stoma as part of treatment of an abdominal sepsis. OPS imaging was performed both in the sublingual region and in a stoma during the ICU stay on day 1, 3 and 7 after the surgical procedure. In 5 patients an ileostomy, and in 7 patients a colostomy was constructed. Mean APACHE II score of the included patients was 19.7 (SD  $\pm$  7.97) with an observed 45% ICU- and hospital mortality. All patients were ventilated.

For assessment of interrater variability each of 2 blinded investigators scored the flow in each sample independently. For the sublingual region there were 224 samples available. In 202 (90%) samples there was complete agreement; a scoring-difference of -1/+1 was found in 22(10%) cases (Table 3A). K-coefficient for interrater variability in the sublingual region was 0.85 (0.79-0.91; Table 4). Since agreement in this sample size appeared to be this good, further analysis was done in a reduced sample size (arbitrarily a 50% reduction of all available data was chosen). Stoma flow interrater agreement was complete in 85/96 (89%) cases; a -1/+1 difference consisted in 11/96 (11%) cases (Table 3B) with a  $\kappa$ -coefficient for the combined stoma site of 0.84 (95% CI 0.75-0.93; Table 4).

In order to assess intrarater variability, flow was scored 2 times independently by the same investigator. For sublingual flow complete intrarater agreement was found in 86/100 (86%) samples, a -1/+1 difference in 12/100 (12%) and a -2/+2 difference in 2 (2%) cases (Table 3C). Intrarater variability  $\kappa$ -coefficient was calculated to be 0.78 (0.67-0.89) for the sublingual region (Table 4). Stoma flow intrarater agreement was complete in 64/72 (89%), a -1/+1 difference consisted in 8/72 (11%) cases (Table 3D). K-coefficient for intrarater variability for the combined stoma sites was 0.83 (0.71-0.94) (Table 4).

Table 3A. Inter-observer agreement for flow score in the sublingual region.

Observer 2	Observer 1			
	Flow 0	Flow 1	Flow 2	Flow 3
Flow 0	16	2	0	0
Flow 1	2	22	3	0
Flow 2	0	4	65	8
Flow 3	0	0	3	99
			total	224

Table 3B. Inter-observer agreement for flow score in the combined stoma sites.

	Observation 1					
Observation 2	Flow 0	Flow 1	Flow 2	Flow 3		
Flow 0	9	3	0	0		
Flow 1	0	21	1	0		
Flow 2	0	6	29	1		
Flow 3	0	0	0	26		
					total	96

Table 3C. Intra-observer agreement for flow score in the sublingual region.

	Observation 1					
Observation 2	Flow 0	Flow 1	Flow 2	Flow 3		
Flow 0	4	0	0	0		
Flow 1	0	10	2	2		
Flow 2	0	1	30	7		
Flow 3	0	0	2	42		
					total	100

Table 3D. Intra-observer agreement for flow score in the combined stoma sites.

	Observation 1					
Observation 2	Flow 0	Flow 1	Flow 2	Flow 3		
Flow 0	9	0	0	0		
Flow 1	1	3	1	0		
Flow 2	0	1	32	1		
Flow 3	0	0	4	20		
					total	72

Table 4. Statistical data for semi-quantitative flow scoring in the sublingual region and in combined stoma sites. \*kappa plus 95% confidence intervals between brackets,  $\kappa_w$  = weighted kappa coefficient.

Reliability	agreement	chance	kappa*	$\kappa_w$
Interrater, sublingual	0.90	0.35	0.85 (0.79-0.91)	0.90
Intrarater, sublingual	0.86	0.37	0.78 (0.67-0.89)	0.81
Interrater, stoma	0.89	0.28	0.84 (0.75-0.93)	0.89
Intrarater, stoma	0.89	0.36	0.83 (0.71-0.94)	0.89

## Discussion

We have shown that inter- and intrarater agreement and  $\kappa$ -coefficient for our method of semi-quantitative analysis of OPS imaging of the microcirculation is high. This appears to be true for different microvascular structures. These results are important since the introduction of OPS flow imaging in the field of clinical research has gained new perspectives, unravelling the complex pathophysiology of microvascular dysfunction during sepsis.

For the first time alterations of human microcirculatory flow could be visualized in vivo [4,5]. In combination with sublingual capnometry [15,16] or near infrared spectroscopy, for measuring microcirculatory haemoglobin saturation [17,18], OPS imaging can be used to investigate the relationship between the microcirculation and metabolic state during sepsis. Persistent microvascular disturbances in the sublingual vascular bed during sepsis were associated with poor outcome, providing a tool for detecting distributive defects in sepsis, not achieved by conventional monitoring of systemic hemodynamic- or oxygen-derived variables [1]. Furthermore, therapeutic interventions, such as the use of volume resuscitation, vasopressors and vasodilators [6,19] can be monitored at their potential level of impact, in casu the microcirculation. However, these promises can only become true when obtained images are interpreted uniformly and quantification of microcirculatory flow abnormalities is reproducible.

To compare and evaluate OPS-derived flow imaging, it is essential to quantify the complete spectrum of flow disturbances during sepsis and other shock models. Although direct measurement of red blood cell velocity in a separate vessel is very well feasible, its application does not do justice to the complex microcirculatory flow patterns during sepsis, in which heterogeneity of flow seems to be a key characteristic [11]. It is therefore important to quantify a complete flow-pattern in a specific organ site, preferably in more than one location. Hence the choice not only to derive OPS images from 3 different locations within the organ site, but also to divide the image itself into four quadrants. The definitions of different flow patterns were kept simple (no flow (0), intermittent flow (1), sluggish flow (2) and continuous flow (3)) to avoid misconstruction. The overall good agreement in the quantification of flow, per group of vessel diameter if applicable, validates its transparency and reproducibility. Important for future implementation of this semi-quantitative flow score in clinical research or even clinical practice, is the fact that disagreement of flow quantification greater than +1/-1 was virtually absent, as expressed by the weighted  $\kappa$ -coefficients, thus eliminating the possibility of interchanging normal flow patterns with clearly pathologic flow patterns.

During sepsis a characteristic of microcirculatory flow might not only be a standstill, interruption or decrease of red blood cell velocity, but also hyperdynamic microcirculatory flow patterns have been observed. Since an increase in red blood cell velocity may also lead to shunting, by means of the inability of haemoglobin to off-load oxygen fast enough to tissues as it passes through the microcirculation [20], it seems important to distinguish normal flow from hyperdynamic flow as well. However, with the current OPS technique using 25 frames per second, it is not possible to detect these differences in flow adequately. In the future, these limitations might be overcome by a new imaging technique with a considerable better resolution: Sidestream Dark-Field (SDF) imaging [21]. Under these conditions a category 4 might be added to the flow variables.

The described type of analysis is especially suited for images derived from non-fixed positions of a hand-held device. Under these circumstances the exact length of the vessel can not be determined, preventing the exact quantification of red cell velocity and vessel diameter. However, the largely improved image quality of SDF imaging has now made it possible to apply process algorithms much more effectively. To date we develop image-processing software, designed for vessel identification in vascular images with a process known as segmentation. Velocity is determined semi-automatically after constructing space-time diagrams from the centreline intensity of vessels in subsequent video frames. It allows the user to query length, width and blood velocity of individual vessel segments, thus creating a detailed statistical report containing vascular parameters.

To avoid a complex set of non-comparable quantification systems for individual organ sites, the presented way of semi-quantitative analyses was not only designed for the evaluation of the behaviour of microcirculatory networks such as the sublingual region and the brain [6], but also for repeating vascular structures like those in the small intestine (villi), colon (crypts), rectum (crypts), liver (sinuses) and gingival tissue [22]. Intra- and interrater agreement and  $\kappa$ -coefficient for semi-quantitative flow analysis in stomas of the small intestine and colon were as good as for sublingual microcirculatory structures. This way of flow quantification seems therefore potentially applicable for the analysis of OPS imaging in many more microvascular structures not yet described in the literature.

## Conclusions

Semi-quantitative analysis of OPS derived flow imaging, as described, has a good intrarater and interrater reproducibility for the evaluation of microcirculatory flow patterns during sepsis, both for microcirculatory networks and for repeating microvascular structures. It provides a transparent and clinical applicable non-invasive tool to monitor and evaluate the microcirculation bedside.

### Key messages

- Semi-quantitative analysis of OPS derived flow imaging, as presented, has good interrater and intrarater reproducibility.
- The described way of analysis is applicable both for microcirculatory networks and for repeating microvascular structures.
- It provides a transparent, easy-to-use, clinical, non-invasive tool to monitor and evaluate the microcirculation bed-side.

#### List of abbreviations used

OPS = orthogonal polarization imaging  
CI = confidence interval  
SD = standard deviation

#### Competing interests

The authors declare that they have no competing interests

#### Authors' contributions

CB contributed to the design of the study, performed OPS imaging and analysis and drafted the manuscript. KM coordinated the consensus conference, provided technical support and revised the manuscript. PV performed statistical analysis and revised the manuscript critically. PS contributed to the design of OPS imaging analysis and revised the manuscript. CI conceived the study, participated in its design and coordination and helped to draft the manuscript. All authors read and approved the final manuscript.

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## References

1. Sakr Y, Dubois MJ, De Backer D, Creteur J, Vincent JL: Persistent microcirculatory alterations are associated with organ failure and death in patients with septic shock. *Crit Care Med* 2004, 2:1825-1831
2. Ince C: Microcirculation in distress: a new resuscitation end point. *Crit Care Med* 2004, 32:1963-1964.
3. Slaaf DW, Tangelder GJ, Reneman RS: A versatile incident illuminator for intravital microscopy. *Int J Microcirc Clin Exp* 1987, 6:391-397.

4. Mathura KR, Alić L, Ince C: Initial clinical experience with OPS imaging for observation of the human microcirculation. In *Yearbook of Intensive Care and Emergency Medicine*. Edited by Vincent JL. New York: Springer-Verlag; 2001:233-245.
5. Mathura KR, Bouma GJ, Ince C: Abnormal microcirculation in brain tumours during surgery. *Lancet* 2001, 358:1698-1699.
6. Spronk PE, Ince C, Gardien MJ, Mathura KR, Oudemans-van Straaten HM, Zandstra DF: Nitroglycerin in septic shock after intravascular volume resuscitation. *Lancet* 2002, 360:1395-1396.
7. De Backer D, Creteur J, Preiser JC, Dubois MJ, Vincent JL: Microvascular blood flow is altered in patients with sepsis. *Am J Respir Crit Care Med* 2002, 166:98-104.
8. De Backer, Creteur J, Vincent JL: Use of orthogonal polarization spectral imaging in intensive care. In *Orthogonal polarization spectral imaging*. Edited by Mesmer K. *Prog Appl Microcirc* 2000, 24:104-109.
9. Groner W, Winkelmann JW, Harris AG, Ince C, Bouma GJ, Messmer K, Nadeau RG: Orthogonal polarization spectral imaging: A new method for study of the microcirculation. *Nat Med* 1999, 5:1209-1212.
10. Harris AG, Sinitsina I, Messmer K: The cytoscan™ model E-II, a new reflectance microscope for intravital microscopy: comparison with the standard fluorescence method. *J Vasc Res* 2000, 37:469-476.
11. Ince C, Ashruf JF, Avontuur JA, Wieringa PA, Spaan JA, Bruining HA: Heterogeneity of the hypoxic state in rat heart is determined at the capillary level. *Am J Physiol* 1993, 264:H294-H301.
12. Kundel HL, Polansky M: Measurement of observer agreement. *Radiology* 2003, 228: 303-308.
13. Cohen J: A coefficient of agreement for nominal scales. *Educ Psychol Meas* 1960, 20:37-46.
14. Cohen J: Weighted kappa: nominal scale agreement with provision for scale disagreement or partial credit. *Psychol Bull* 1968, 70:213-220.
15. Weil MH, Nakagawa Y, Tang W: Sublingual capnometry: a new non-invasive measurement for diagnosis and quantitation of severity of circulatory shock. *Crit Care Med* 1999, 27:1225-1229.
16. Marik PE. Sublingual capnography: a clinical validation study. *Chest* 2001, 20:923-927.

17. Schwarte LA, Fournell A, van Bommel J, Ince C: Redistribution of intestinal microcirculatory oxygenation during acute hemodilution in pigs. *J Appl Physiol* 2005, 98:1070-1075.
18. Buise MP, Ince C, Tilanus HW, Gommers D, van Bommel J: The effect of nitroglycerin on microvascular perfusion and oxygenation during gastric tube reconstruction. *Anesth Analg* 2005, 100:1107-1111.
19. Boerma EC, van der Voort PHJ, Ince C: Sublingual microcirculatory flow is impaired by the vasopressin-analogue terlipressin in a patient with catecholamine-resistant septic shock. *Acta Anaesth Scand* 2005, 49:1387-1390.
20. Gutierrez G: The rate of oxygen release and its effect on capillary O<sub>2</sub> tension: a mathematical analysis. *Respir Physiol* 1986, 63:79-96.
21. Ince C: The microcirculation is the motor of sepsis. *Critical Care* 2005, 9(suppl 4):S13-S19.
22. Lindeboom JAH, Mathura KR: Microvascular changes in alveolar distraction osteogenesis. *J Vasc Res* 2004, 41(suppl 2):3.1.



# Chapter 3

Sublingual microcirculatory flow is impaired by the vasopressin-analogue terlipressin in a patient with catecholamine-resistant septic shock

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## Abstract

For many decades arterial blood pressure regulation has been an important issue in the treatment of septic shock. The pathogenesis of this persistent hypotension is complex and multifactorial, but inability of vascular smooth muscle to contract in the presence of vasoconstrictive agents, seems to be a key factor. Many mechanisms have been proposed to account for this failure, including nitric oxide (NO) overproduction and vasopressin deficiency (1). However, improvement of outcome due to intervention in these mechanisms fails to be reported, despite restoration of blood pressure.

Recent studies of the microcirculation in humans by means of orthogonal polarization spectral (OPS) imaging have opened challenging new perspectives to study the microcirculation (2,3). We report a case in which sublingual OPS imaging was performed upon administration of terlipressin in a patient with catecholamine-resistant septic shock. It indicates that much caution should be taken when considering such potent vasoconstrictor when correcting blood pressure during shock.

## Case report

An 80-year old woman was presented with fever (40,7°C), a declining level of consciousness and a few petechiae, all developing within a few hours. Positive cultures with *Neisseria meningitidis* of both spinal fluid and blood confirmed the diagnosis meningococcal meningitis/sepsis. Prompt antibiotic treatment and dexamethason 10 mg iv 4 times daily was started.

Within the first hour of admittance mechanical ventilation, aggressive fluid resuscitation and the use of catecholamines was needed. No overt purpura fulminans developed. A decreased P/F ratio (37 kPa), an elevated creatinine level (132  $\mu\text{mol/L}$ ) and thrombocytopenia ( $81.10^9$ ) indicated multiple organ dysfunction. Despite increasing doses of norepinephrine a mean arterial pressure of 60 mm Hg could not be maintained and the patient became oliguric. Sublingual OPS imaging was performed and analyzed semi-quantitatively as described elsewhere (3).

At baseline overall microcirculatory flow was well preserved, without evidence of heterogeneity. The light guide of the OPS device was fixed in a steady position, avoiding pressure artefacts, and a single bolus of 1 mg terlipressin was given intravenously. Over a 60-minute period OPS imaging was performed real-time and recorded at 20 minute intervals. Hemodynamic parameters were measured at the same time points, extended with  $t = 2$  and 3 hours (Table).

Within 10 minutes a rise in mean arterial pressure and urinary output occurred. The patient developed an intense pallor and the peripheral perfusion index (PFI), derived from the pulse oxymetry signal (Intellivue MP70, Phillips Medical Systems), fell to an undetectable low range with a strong rise of the central-to-toe temperature difference ( $\Delta T$ ). OPS-imaging samples after three consecutive periods of twenty minutes each showed a dramatic decrease in small-vessel (10-25  $\mu\text{m}$ ) numbers and eventually a complete stand-still of flow (fig. 1 and 2).

Six hours after the administration of terlipressin the blood pressure declined again and the patient died within 24 hours as a result of irreversible shock.

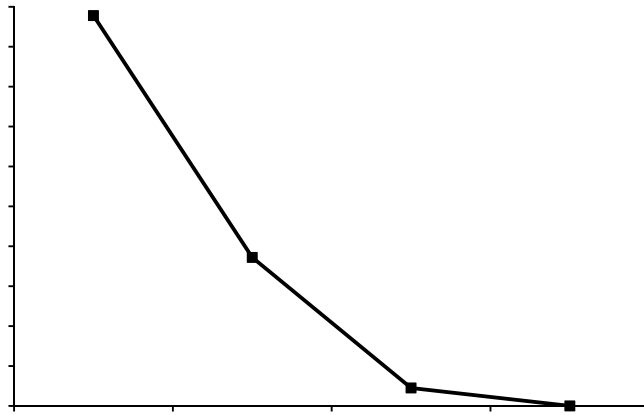


Fig. 1 Proportion of perfused capillaries using orthogonal polarization spectral imaging before (base) and 20, 40, 60 minutes respectively after a bolus of 1 mg terlipressin iv.

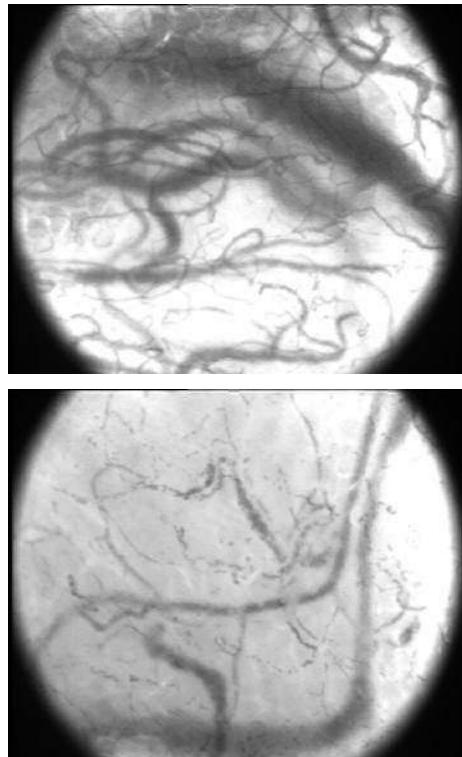


Fig. 2 Representative orthogonal spectral images obtained before (upper panel) and 60 minutes after (lower panel) administration of 1 mg terlipressin iv. (objective 5x, on screen 325x)

## Discussion

Vasopressin is a vasoactive hormone with a range of vasomodulatory activities. The vasopressin analogue terlipressin has a higher V1a/V2 receptor ratio and is long-acting as compared to vasopressin (4,5). The complexity of its physiologic effects could be explained by four known mechanisms of action: activation of V1 vascular receptors, modulation of ATP-dependent K<sup>+</sup> channels, modulation of NO and potentiation of adrenergic agents. Whether vasopressin causes vasodilation or vasoconstriction depends on the vascular bed, model, dose, and duration of exposure (4).

Up to date the role of vasopressin on survival in patients with septic shock remains to be established. No randomised controlled trials in humans with mortality as primary endpoint were performed. In two studies with different endpoints, mortality was reported (6,7). A recent Cochrane review calculated a relative risk on survival of vasopressin versus placebo in these 2 studies (N=58) to be 1.04 (95% CI 0.06-19.33) (8).

Data on the effects of vasopressin administration on splanchnic microvasculature seem to be conflicting. Low-dose vasopressin in a sepsis model limited the increase in ileal pCO<sub>2</sub>-gap, as compared to norepinephrine (9). In humans low-dose vasopressin was demonstrated to improve macrohemodynamics with preservation of the gastric tonometry Pr-aCO<sub>2</sub> gap (6), but the same group reported high incidences of ischemic skin and tongue lesions. High-dose vasopressin in norepinephrine-dependent patients with septic shock caused a significant rise in gastric pCO<sub>2</sub>-gap, indicating splanchnic hypoperfusion (10). Low-dose terlipressin increased ileal microcirculation in endotoxic rats (4). However, high-dose terlipressin has been reported to increase portal hypertension, to reduce splanchnic blood flow in several animal models (4) and to increase L-lactate concentrations in the human rectal mucosa (11).

This case report outlines the potential contradictive effects of a vasopressin-analogue within a patient with septic shock. On one hand mean arterial blood pressure, with an expected rise in cerebral perfusion pressure (12), is restored. We also observed a rise in urine production. This might be the result of an indirect effect via rise of perfusion pressure, or a possible direct effect of vasopressin, either due to increased water permeability in the collecting duct by stimulation of V2 receptors, or by counter-acting sepsis-induced V2-receptor and aquaporin-2 content down-regulation in the kidney (13). On the other hand the perfusion of the skin is markedly reduced, base deficit continues to rise and sublingual microcirculatory flow comes to a stand still (table, fig. 1). Thus, OPS imaging might provide a substrate for earlier observations of skin necrosis (14) and a decline of gut mucosal circulation (11,15).

Table Hemodynamic, tissue perfusion and acid-base parameters (MAP mean arterial pressure, HR heart rate, CVP central venous pressure,  $\Delta T$  central-to-toe temperature difference, PFI peripheral perfusion index, BE base excess)

	Baseline	20 min	40 min	1 hour	2 hours	3 hours
MAP (mmHg)	58	78	80	80	100	105
HR (beats/min)	98	96	96	98	108	119
CVP (mmHg)	13	14	13	12	11	8
$\Delta T$ ( $^{\circ}C$ )	1,7	2,3	8	12,5	12,8	13,4
PFI	6,3	4,8	<0,3	<0,3	<0,3	<0,3
Urine output (ml/h)	20	-	-	110	165	500
Dopamine ( $\mu g/kg/min$ )	17	17	17	17	17	17
Noradrenaline ( $\mu g/kg/min$ )	4	4	4	3,7	2,3	1,7
Fluid balance (ml)	+6149	-	-	+6284	+6390	+6145
BE (mmol/L)	-11,8	-	-	-12,7	-	-15,9

With the same technique Dubois et al reported little or no effect of low dose vasopressin on sublingual microcirculation in a patient with distributive shock (3). That different effects on the microcirculation with the same class of vasopressor agents can be observed is an important observation, especially in the light of the finding that persistent depressed sublingual microcirculation predicts non-survival (16). Associated with the marked decrease of capillary perfusion an increment of  $\Delta T$  and drop in PFI was found. Both indicators of microcirculatory impairment are associated with poor clinical outcome (17,18). It is therefore important to notice that in our patient considerable doses of the vasoconstrictor norepinephrine were used to maintain a minimum level of mean arterial pressure. Although equivalent to doses in other OPS-studies (2,3) we observed in this setting no hampering of flow in the absence of terlipressin.

From our and Dubois' study (3) it is clear that generalized conclusions about the action of vasopressin on the microcirculation should be made with caution. Dosage, the vasopressin-analogue used, circulating volume, severity of illness and genetic repertoire contribute to its effects. With all its limitations, including the absence of cardiac output- and SvO<sub>2</sub> measurements, our study shows that, although effective in correcting severe hypotension, terlipressin can be deleterious to microcirculatory perfusion. Monitoring the microcirculation may provide a useful tool to titrate vasopressin in critically ill septic patients.

## References

1. Landry DW, Oliver JA. The pathogenesis of vasodilatory shock. *N Engl J Med* 2001; 345:588-595.
2. Spronk PE, Ince C, Gardien MJ, Mathura KR, Oudemans-van Straaten HM, Zandstra DF. Nitroglycerin in septic shock after intravascular volume resuscitation. *Lancet* 2002; 360:1395-1396.

3. Dubois MJ, de Backer D, Creteur J, Anane S, Vincent JL . Effect of vasopressin on sublingual microcirculation in a patient with distributive shock . *Intensive Care Med* 2003; 29:1020-1023.
4. Asfar P. Terlipressin in chronic hyperdynamic endotoxic shock: is it safe? *Intensive Care Med* 2003; 29:154-155.
5. Kam PC, Williams S, Yoong FF. Vasopressin and terlipressin: pharmacology and its clinical relevance. *Anaesthesia* 2004; 59:993-1001.
6. Dünser MW, Mayr AJ, Ulmer H, Knotzer H, Sumann G, Pajk W, Friesenecker B, Hasibeder WR. Arginine vasopressin in advanced vasodilatory shock . *Circulation* 2003; 107:2313-2319.
7. Malay MB, Ashton RC Jr, Landry DW, Townsend RN. Low-dose vasopressin in the treatment of vasodilatory septic shock . *J Trauma* 1999; 47:699-703.
8. Mullner M, Urbanek B, Havel C, Losert H, Waechter F, Gamper G. Vasopressors for shock . *Cochrane Database Syst Rev* 2004; 3:CD003709.
9. Sun Q, Dimopoulos G, Nguyen DC, Tu Z, Nagy N, Hoang AD, Rogiers P, De Backer D, Vincent JL. Low-dose vasopressin in the treatment of septic shock sheep . *Am J Respir Crit Care Med* 2003; 168:481-486.
10. Van Haren FMP, Rozendaal FW, van der Hoeven JG. The effect of vasopressin on gastric perfusion in catecholamine-dependent patients in septic shock . *Chest* 2003; 124:2256-2260.
11. Perner A, Jorgensen VL, Waldau T. Terlipressin increased the concentration of L-lactate in the rectal lumen in a patient with septic shock . *Acta Anaesthesiol Scand* 2004; 48:1054-1057.
12. Bradley PG, Allen EK, Menon DK. Terlipressin for cerebral perfusion pressure support in a patient with septic shock . *Anaesthesia* 2004; 59:619.
13. Grinevich V, Knepper MA, Verbalis J, Reyes I, Aguilera G. Acute endotoxemia in rats induces down-regulation of V2 vasopressin receptors and aquaporin-2 content in the kidney medulla . *Kidney Int* 2004; 65:54-62.
14. Dünser MW, Mayr AJ, Tur A, Pajk W, Barbara F, Knotzer H, Ulmer H, Hasibeder WR. Ischaemic skin lesions as a complication of continuous vasopressin infusion in catecholamine-resistant vasodilatory shock: incidence and risk factors . *Crit Care Med* 2003; 31:1394-1398.
15. Westphal M, Freise H, Kehrel BE, Bone HG, Van Aken H, Sielenkamper AW. Arginine vasopressin comprises gut microcirculation in septic rats . *Crit Care Med* 2004; 32:194-200.

16. Sakr Y, Dubois MJ, De Backer D, Creteur J, Vincent JL. Persistent microcirculatory alterations are associated with organ failure and death in patients with septic shock. *Crit Care Med* 2004; 32:1825-1831.
17. Lima AP, Beelen P, Bakker J. Use of peripheral perfusion index derived from the pulse oxymetry signal as a noninvasive indicator of perfusion. *Crit Care Med* 2002; 30:1210-1213.
18. Kaplan LJ, McPartland K, Santora TA, Trooskin SZ. Start with subjective assessment of skin temperature to identify hypoperfusion in intensive care unit patients. *J Trauma* 2001; 50:620-628.

# Chapter 4

Relationship between sublingual and  
intestinal microcirculatory perfusion in  
patients with abdominal sepsis

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## Abstract

### Objective:

To evaluate the relation between sublingual and intestinal microcirculatory alterations in patients with an abdominal sepsis.

### Design:

Prospective observational study

### Setting:

A 23-bed mixed intensive care unit of a tertiary teaching hospital

### Patients:

Twenty-three patients with an abdominal sepsis and a newly constructed intestinal stoma in the study group. Nineteen outpatient healthy individuals with an intestinal stoma and 10 non-sepsis patients with a less-than-24-hours-old intestinal stoma were included as controls.

### Interventions:

none

### Measurements and Main Results:

Orthogonal polarization spectral imaging of the sublingual and intestinal microcirculation was performed on day 1 and 3. In addition, parameters of systemic hemodynamics, such as cardiac index, heart rate, blood pressure, central venous pressure and dosages of vasopressor and inotropic agents were obtained. On day 1 there was no correlation of the microvascular flow index (MFI) between the sublingual and intestinal microcirculatory beds (Spearman's rho ( $r_s$ ) = 0.12, 95% CI -0.51-0.31,  $p = 0.59$ ). Furthermore, there was no significant correlation between microcirculatory alterations and parameters of systemic circulation ( $r_s \leq 0.25$ ). On day 3 however, a correlation between sublingual and intestinal microcirculatory flow appears to be restored ( $r_s = 0.74$ , 95% CI 0.28-0.92,  $p = 0.006$ ), mainly due to a normalisation of flow in both regions.

### Conclusions:

On day 1 of an abdominal sepsis there is a complete dispersion of flow, not only between hemodynamic compartments of a different order, but also between the sublingual and intestinal microcirculation. Over time, both sublingual and intestinal MFI tended to normal values.

## Introduction

Many years ago Weil and Shubin proposed a classification of shock with special reference to its distributive forms (1). The distributive defect in septic shock has been classically defined as a redistribution of volumes and reflects a defect in vascular regulation in the presence of normal or even supernormal oxygen delivery. However, the precise nature of these distributive alterations has been largely unknown for many decades. In the previous years, research, using Orthogonal Polarization Spectral (OPS) imaging, has revealed that microcirculatory alterations may explain the distributive defects seen in sepsis. Application of this technique (2) in a hand-held device made direct observation of the human microcirculation during sepsis possible. This technique is not only applicable in the sublingual region, but also in other organs like the intestinal tract, for instance when a stoma is available. Microcirculatory abnormalities, and in particular, heterogeneity of flow, are now being recognized as key characteristics in the pathogenesis of organ dysfunction during sepsis (3,4). Combining OPS imaging with other techniques such as sublingual capnometry added to the understanding of the relation between microcirculatory abnormalities and metabolic tissue parameters (5,6). Persistence of such abnormalities were found to be associated with prognosis, in contrast to all the available systemic hemodynamic parameters (7). An important question to be addressed in this approach is to what extent the sublingual region reflects microcirculatory abnormalities in other organs. This is of particular importance, since an inability to extract oxygen as a result of local tissue factors, is considered to be a distinctive quality of septic shock in comparison to other shock models (8,9). In other words, during sepsis there is a local inability to regulate oxygen delivery despite adequate oxygen supply, resulting in tissue hypoxia. Since local, rather than systemic factors seem to determine oxygen consumption, it is imaginable that sublingual microcirculatory abnormalities during septic shock do not reflect alterations in other microvascular beds, nor will it be likely that these microcirculatory abnormalities are correlated with systemic hemodynamic parameters. In this study we tested the hypothesis that septic shock in humans is characterized by a dissociation not only between the microcirculation and the systemic circulation, but also that each microcirculatory organ system starts to behave individually as loss of integrative control by the systemic circulation and local factors start to dominate (micro)circulatory defects. We tested this hypothesis in patients with an abdominal septic shock and, apart from the sublingual region, a surgical intestinal stoma as a second organ site accessible for OPS microcirculatory imaging.

## Materials and methods

### Setting and patient selection.

A single center prospective observational study was performed in a tertiary teaching-hospital with a 23-bed mixed ICU. Between January and September 2004 patients with a new intestinal stoma in the course of abdominal sepsis were included. Patients were only included when the source of the sepsis was confirmed by faecal spill in the abdominal cavity, as observed during the surgical procedure. Cultures of abdominal fluid samples were performed for confirmation. Overt clinical necrosis of the stoma and an age <18 years were contraindications for enrolment.

Furthermore, 2 subsets of patients were included as controls for comparison with the study group on day one. One group consisted of healthy individuals from the surgical outpatient department, with a stoma of at least 3 months old. The other group was formed by non-ICU patients with a less-than-24-hours-old intestinal stoma in the absence of sepsis, according to the Bone criteria (10).

A local ethical and scientific committee approved the study protocol and written informed consent was obtained from the patients or their next of kin, according to Dutch and European legislation.

### Protocol and data collection.

After the initial surgical procedure with the construction of an intestinal stoma, patients were admitted to the ICU. By protocol, none of the patients received vasodilatory therapy or steroids before the first OPS images were obtained; hereafter such therapy was to the discretion of the attending physician, who was blinded for the OPS-imaging results. None of the patients received activated protein C during the protocol. Before baseline measurements, hypovolemia was excluded by repeated volume challenges up to the point where stroke volume (SV) did not increase any further, or when central venous pressure (CVP) reached 15 mm Hg. Mean arterial pressure (MAP) was maintained at a minimum level of 65 mm Hg with dopamine up to 15  $\mu\text{g}/\text{kg} \cdot \text{min}$ , in case peak flow velocity in the descending aorta was below 70 cm/s. In case peak flow velocity exceeded 70 cm/s, together with a MAP < 65 mm Hg, norepinephrine was added. Hereafter, routine macro-hemodynamic parameters. Cardiac index (CI), SV and peak flow velocity were measured by oesophageal Doppler technology (CardioQ<sup>®</sup>, Deltex Medical, West Sussex, UK) within ten minutes from the time of OPS imaging. Age, gender, length of stay (LOS), Acute Physiology And Chronic Health Evaluation (APACHE) II and Sequential Organ Failure Assessment (SOFA) scores were documented (11,12). During the ICU stay sequential OPS imaging was performed on day 1 and day 3, with a lag time between sublingual and stoma region of less than 10 minutes.

### Imaging technique.

The OPS technique, as described in detail elsewhere (2,13), consists of a hand-held device that illuminates an area of interest with polarized light, while imaging the remitted light through a second polarizer (analyser), oriented in a plane precisely orthogonal to the plane of illumination. If a wavelength within the haemoglobin absorption spectrum (e.g. 548 nm) is chosen, erythrocytes will appear dark and leukocytes may be visible as refringent bodies. The vessel

walls itself are not visualized directly, although faint contours can be identified depending on the presence of intravascular erythrocytes.

### OPS imaging and analysis procedure.

OPS imaging and semi-quantitative analysis, was performed as described in detail elsewhere (14). The inter-observer and intra-observer agreement for individual flow scores was validated both for microcirculatory networks and repeating vascular structures (14). In short, steady images of at least 20 seconds were obtained after gentle removal of saliva/faeces by an isotonic-saline-drenched gauze, avoiding pressure artefacts, and stored on digital videotape (SONY video walkman GV-D 1000E®, Sony, Tokyo, Japan). Intestinal stomas were penetrated with the OPS device 5-10 cm, beyond the abdominal wall. Subsequently, the images were captured in 5-10-second representative video clips in avi format (sony DVgate®, Sony, Tokyo, Japan). Video clips were analyzed blindly by an investigator not involved in data collection and in random order to prevent coupling between images.,

OPS images were obtained from three different regions within the site of interest and each image is divided into 4 equal quadrants. Quantification of flow (no flow: 0, intermittent flow: 1, sluggish flow: 2 and continuous flow: 3) is scored per quadrant, for each cohort of vessel diameter (small: 10-25 µm, medium 26-50 µm and large 51-100 µm), if applicable. The overall score, called microvascular flow index (MFI), is the sum of each quadrant-score, divided by the number of quadrants in which the vessel type is visible. The net result (MFI) is an average score over 12 quadrants (3 regions times 4 quadrants per region) derived from the overall flow impression of all vessels with a particular range of diameter in a given quadrant. Anatomically, microvascular beds of villi (small intestine) and crypts (colon) only consist of small vessels.

### Statistical analysis.

Data are presented in medians and interquartile ranges (IQR). For comparison of groups non-parametrical tests are used; for comparison of two groups a Mann-Whitney test is applied and for comparison of more than 2 groups a Kruskal-Wallis test. Evolution over time was assessed by a non parametric test (Wilcoxon signed-rank test) for paired data. Non-parametric rank correlation is expressed as Spearman's rho ( $r_s$ ). A two-sided p value of <0.05 is considered statistically significant.

## Results

### Patients, day 1.

Twenty-three ICU patients with a median APACHEII score of 20 (14-24) were enrolled in the study. All of them fulfilled the entry-criteria; cultures of the abdominal cavity revealed a mixed intestinal flora in all patients. Baseline characteristics and hemodynamic parameters are summarized in Table 1. ICU and in-hospital mortality was 26.1 and 34.7% respectively, with an ICU LOS of 15.3 (IQR 3-14.5) days and an in-hospital LOS of 29.8 (IQR 11.5-47) days. The first control group consisted of 19 healthy outpatient individuals with a median age of 59 (IQR 51-74) and a male:female ratio of 12:7; 9 patients had an ileostomy, 10 patients a colostomy. In the second control groups 10 non-sepsis patients with a less-than-24-hours-old intestinal stoma were included. Their median age was 61 (IQR 55-75) and their male:female ratio 6:4; 5 patients had an ileostomy, 5 patients a colostomy. It was possible to obtain good OPS images of both microvascular beds in all patients and no adverse events, such as bleeding or perforation of the stoma site, were reported. No re-operations for stoma necrosis or abdominal compartment syndrome had to be performed during the study.

Table 1. Characteristics study population day 1 (n = 23)

Gender, male/female, n	17/6
Age, years	70 (56-77)
APACHEII score	20 (14-24)
SOFA score	7 (5-10)
Ventilator, use of, n	22
CVVH, use of, n	4
Norepinephrine, n (dose in $\mu\text{g}/\text{kg} \cdot \text{min}$ )	3; 0 (0-0)
Dopamine, n (dose in $\mu\text{g}/\text{kg} \cdot \text{min}$ )	23; 7 (4-14)
Stoma site, n	
small intestine	5
colon	18
Surgical diagnosis, n	
ischemia, vascular	1
ischemia, mechanical (strangulation)	4
blow out (carcinoma)	2
blow out (Ogilvie)	4
diverticulitis	6
anastomotic leakage	6

APACHE, acute physiology and chronic health evaluation; SOFA, sepsis-related organ failure assessment; CVVH, continuous veno venous hemofiltration. Data are presented as medians (25th-75th percentiles), unless stated otherwise.

### Patients, day 3.

Twelve patients were evaluable; 7 were discharged and 2 died before day 3. Follow up was not available for 2 other patients. Evolution of systemic hemodynamic parameters, dosages of inotropic and vasopressor agents, lactate and pH, haemoglobin, PEEP settings and SOFA scores from day 1 to day 3 is shown in Table 2. Interestingly, three patients with persistent reduction in microcirculatory flow (sublingual MFI 1.92, 2.0 and 2.08 respectively) on day 3 survived to hospital discharge, whereas 2 patients with a normal flow (sublingual MFI 2.66 and 3.0 respectively) on day 3 died.

Table 2. Evolution over time of systemic hemodynamic parameters, PEEP settings and metabolic variables in the study population

	Day 1 (n=23)	Day 3 (n=12)	P Value
Heart rate, beats/min	97 (78-114)	98 (87-103)	0.03
Mean arterial pressure, mm Hg	70 (66-87)	79 (68-90)	0.33
Central venous pressure, mm Hg	9 (7-14)	12 (9-16)	0.15
Cardiac index, L/min · m <sup>2</sup>	4.5 (3.5-5.1)	4.8 (3.6-6)	0.06
Dopamine dose, µg/kg · min	7 (4-14)	5 (2-8)	0.04
PEEP, cm H <sub>2</sub> O	12 (8-14)	11 (10-15)	0.48
Lactate, mmol/L	2.9 (1.4-5)	3.0 (2.1-3.8)	0.18
pH	7.37 (7.34-7.39)	7.38 (7.35-7.4)	0.69
Haemoglobin, mmol/L	5.2 (5-5.9)	5.3 (4.5-5.5)	0.25

PEEP, positive end-expiratory pressure. Data are presented as medians (25th-75th percentiles). P values are calculated for paired data (n=12) by a non-parametric Wilcoxon signed-rank test.

### Microcirculatory flow index day 1.

#### Intestinal stoma versus controls.

On day 1, MFI at the stoma site was significantly lower in the sepsis group (n = 23) as compared to the non-sepsis control group (n = 10) (median 2.08, IQR 1.25-2.42 and 3, IQR 3-3 respectively, p = 0.001). At the same time MFI at the stoma site of the non-sepsis group did not differ from the outpatient group (n = 19) (median 3, IQR 3-3 and 3, IQR 3-3 respectively, p = 0.29, g.1).

#### Sublingual versus intestinal stoma.

On day 1, MFI of small vessels in the sublingual region did not show a significant correlation with MFI at the stoma site ( $r_s = 0.12$ , 95% CI -0.51-0.31, p = 0.59), in which, by anatomy, only vessels from the small type are present (g. 2A).

#### Sublingual versus systemic hemodynamic parameters.

Correlation coefficients between MFI sublingual and macro-hemodynamic parameters such as heart rate (HR), CI, MAP, CVP, lactate and use of inotropic and vasopressors agents

were all insignificant (Table 3). All correlation coefficients between MFI sublingual site and parameters of morbidity (SOFA, LOS) were insignificant ( $r_s = 0.27$  and  $0.28$  respectively).

#### Intestinal stoma versus systemic hemodynamic parameters.

Similar non-significant correlation coefficients were found for MFI intestinal stoma and macro-hemodynamic parameters (Table 3), as well as for correlation coefficients between MFI intestinal stoma and parameters of morbidity (SOFA, LOS) ( $r_s = 0.33$  and  $-0.12$  respectively).

Table 3. Correlation between microvascular flow index and systemic hemodynamic parameters in study population, day 1 (n = 23)

	MFI sl	MFI stoma
Heart rate	0.004	-0.04
Mean arterial pressure	0.10	-0.08
Cardiac index	0.07	-0.02
Central venous pressure	0.25	-0.32
Norepinephrine dose	0.21	0.11
Dopamine dose	0.06	-0.21
Lactate	0.04	-0.23
MFI sublingual	-	0.12
MFI intestinal stoma	0.12	-

MFI, microvascular flow index (small vessels;  $< 20 \mu\text{m}$ ); sl, sublingual. Data are presented as Spearman rank correlation ( $r_s$ ).

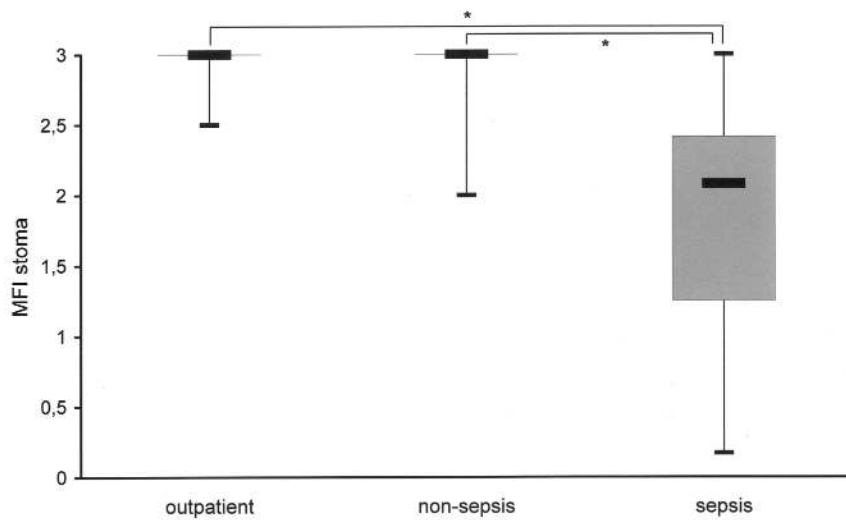


Figure 1. Boxplot of microvascular flow index (MFI) of small vessels ( $< 20 \mu\text{m}$ ) at the intestinal stoma site of the outpatient group, non-sepsis group (day 1) and sepsis group day 1. \* $p < 0.05$ .

**Microcirculatory flow index day 3.**

**Intestinal stoma in relation to day 1.**

Intestinal stoma MFI increased from day 1 to day 3 significantly (median 1.83, IQR 1.33-2.46 and 2.66, IQR 2.34-2.83 respectively,  $p = 0.02$ ) (fig. 3).

**Sublingual in relation to day 1.**

In comparison to day 1, sublingual MFI on day 3 also increased significantly (median 2.08, IQR 1.25-2.42 and 2.66, IQR 2.5-2.92 respectively,  $p = 0.01$ ) (fig. 3). MFI-changes over time from day 1 to day 3 for individuals are depicted in fig. 2C.

**Sublingual versus intestinal stoma.**

In contrast to the situation on day 1, there was a significant correlation between MFI of the stoma and sublingual region ( $r_s = 0.74$ , 95% CI 0.28-0.92,  $p = 0.006$ ) (fig. 2B, Table 4) on day 3.

**Sublingual versus systemic hemodynamic parameters.**

On day 3 MFI sublingual showed a significant correlation with CI ( $r_s = 0.65$ ,  $p < 0.05$ ), whereas correlations with HR, MAP, CVP, lactate and use of inotropic and vasopressors agents remained insignificant (Table 4). Correlation coefficients between MFI sublingual and parameters of morbidity (SOFA, LOS) were insignificant ( $r_s = 0.28$  and  $-0.26$  respectively).

**Intestinal stoma versus systemic hemodynamic parameters.**

On day 3 MFI stoma showed a significant correlation with HR ( $r_s = 0.79$ ,  $p < 0.01$ ). Correlations with CI, MAP, CVP, lactate and use of inotropic and vasopressors agents remained insignificant (Table 4). Correlation coefficients between MFI intestinal stoma and parameters of morbidity (SOFA, LOS) were insignificant ( $r_s = -0.13$  and  $-0.46$  respectively).

Table 4. Correlation between microvascular flow index and systemic hemodynamic parameters in study population, day 3 (n = 12).

	MFI sl	MFI stoma
Heart rate	0.57	0.79**
Mean arterial pressure	-0.07	0.13
Cardiac index	0.65*	0.51
Central venous pressure	-0.37	-0.37
Norepinephrine dose	0.13	0.36
Dopamine dose	0.46	0.13
Lactate	NA	NA
MFI sublingual	-	0.74**
MFI intestinal stoma	0.74**	-

MFI, microvascular flow index (small vessels;  $< 20 \mu\text{m}$ ); sl, sublingual; NA, not available. Data are presented as Spearman rank correlation ( $r_s$ ). \*\* $p < 0.01$ , \* $p < 0.05$

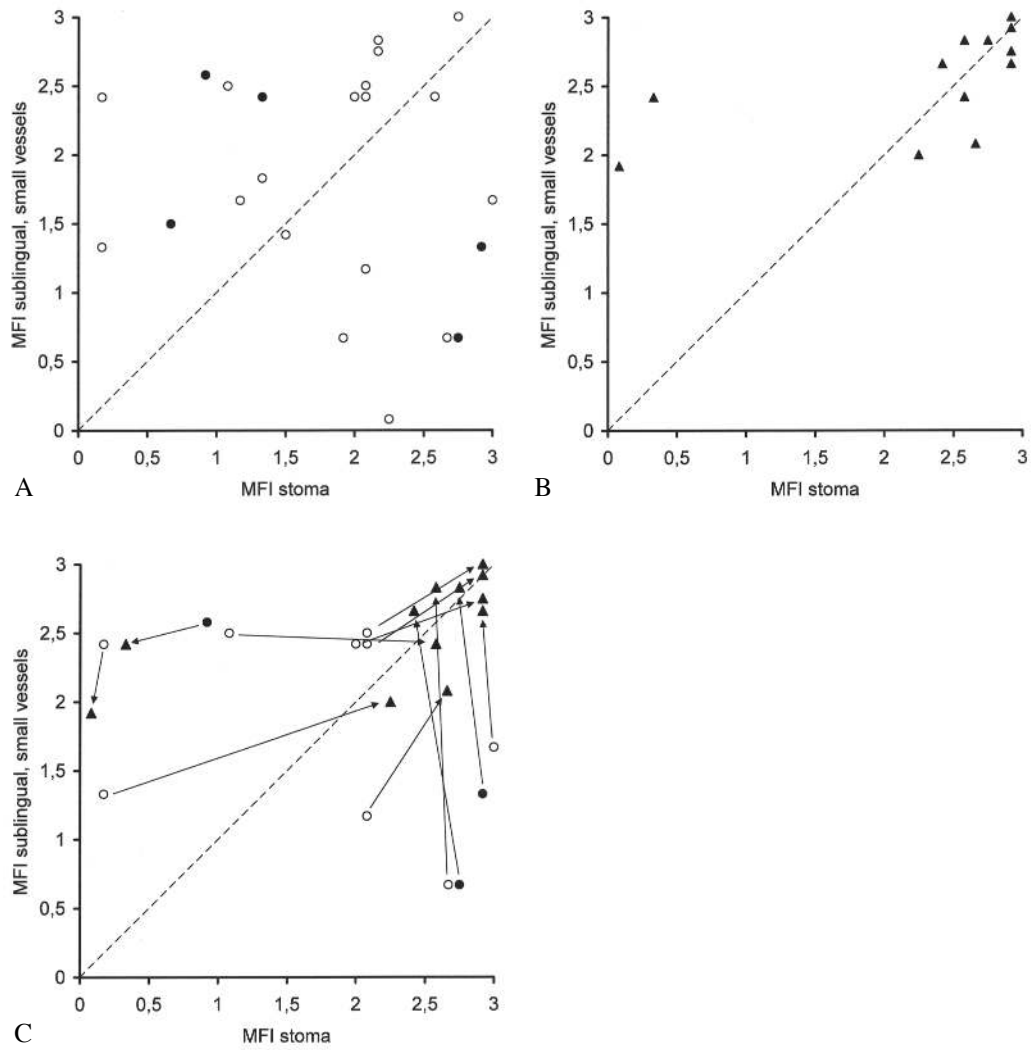


Figure 2. Scatter of microvascular flow index (MFI) of small vessels ( $< 20 \mu\text{m}$ ) in the sublingual region versus the MFI in the intestinal stoma region. On day 1 each individual is represented by a circle ( $\circ$ ) (colostomy,  $\bullet$  ileostomy);  $r_s \text{ day 1} = 0.12$  (Fig.2A). On day 3 individuals are represented by a triangle ( $\blacktriangle$ );  $r_s \text{ day 3} = 0.74$  (Fig.2B). Arrows depict the movement over time from MFI day 1 to day 3 for each individual in relation to the identity line (---) (Fig.2C).

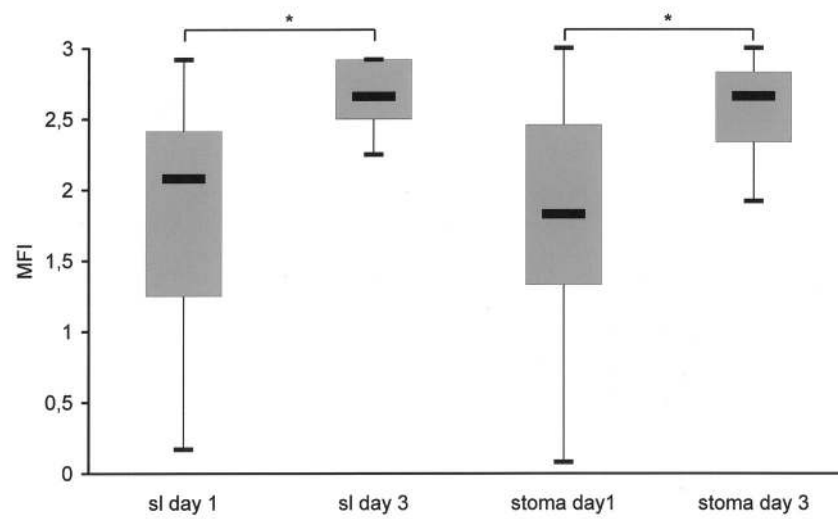


Figure 3. Boxplot of microvascular flow index (MFI) of small vessels (<20 μm) of the study population on day 1 and day 3 in the sublingual (sl) region and at the intestinal stoma site. \*p < 0.05.

## Discussion

The results in the presented study are consistent with the hypothesis that microcirculatory abnormalities rather than systemic hemodynamic parameters are the predominant factor during sepsis and septic shock (15,16). In accordance with earlier reports (3,17), we could not find a significant correlation on day 1 between the commonly used systemic hemodynamic parameters (HR, MAP, CVP and CI) on the one hand, and abnormalities in microcirculatory blood flow, as established with OPS imaging, on the other hand. A recent report about the effect of dobutamine in septic shock on microcirculatory alterations, independent of systemic hemodynamic changes (18) seems to be in accordance with our presented data. On day 3 the correlation between microcirculatory blood flow and systemic hemodynamics seems, in part, to be restored. Whether this is a true effect due to the evolution over time of sepsis itself, or a net result of a complex of therapeutic interventions, remains to be established.

The most downstream 'hemodynamic compartment' consists of microcirculatory units. During sepsis, heterogeneity of flow between and within these microcirculatory units seems to be a characteristic finding, thus creating the concept of microcirculatory weak units (15). In this study, at a specific time point day 1, no correlation between the microvascular flow of two different microvascular beds (sublingual and intestinal region) could be established. In other words: if one looks at OPS images of the sublingual microvascular bed during sepsis, it is impossible to predict what these images will be at the same time in an

intestinal microvascular bed and vice versa. The complete absence of correlation on day 1 between hemodynamic parameters of systemic and microcirculatory compartments, as well as between equal types of compartments, enhances the idea of a dispersive nature of flow during the initial phase of sepsis. A combination of local microcirculatory factors, such as activated coagulation, shedding of the glycocalyx during endothelial cell activation (19), reduced red blood cell deformability (9), iNOS expression (20) and loss of autoregulation (8) is likely to influence microcirculatory flow, rather than systemic hemodynamic parameters. On day 3 this dispersive nature of microcirculatory flow has ceased and a correlation between MFI sublingual and intestinal stoma could be established, mainly by an overall movement of MFI in both regions towards normal values around the identity line (Fig. 2). Once again the evolution over time of sepsis itself and influences of therapeutic strategies may play a role, as expressed in the model of microcirculatory and mitochondrial distress syndrome (MMDS) (16). It seems therefore important to take these time dependent relations into account by the design of further sepsis studies.

Time-dependency might explain why, in contrast to our present data, an earlier pilot study reported a correlation between microvascular flow in stomas and the sublingual region (21). In this study of Spronk et al. time of inclusion was not defined and almost all patients were included after the first 24 hours of sepsis, thus potentially eliminating the initial dispersion of flow in both microcirculatory beds.

Recently, others demonstrated a good correlation between sublingual microcirculatory perfusion and gastric mucosal  $PCO_2$  (6,22), suggesting a good correlation between sublingual/buccal and gastric perfusion. However, in human studies no direct observations of the stomach microcirculation are made and in the animal study of Fries et al. direct microcirculatory OPS observations are made of the serosa of the stomach as opposed to the mucosa of the small and large intestine in this study. Furthermore, like in all animal cecal-ligation-and-puncture sepsis-models, observations were made a few hours before death. The possibility that such terminal model of sepsis could lead to a final common pathway of microcirculatory failure, and therefore to a correlation between different microcirculatory beds, cannot be ruled out.

It is of note that in this study all 3 patients with persistent reduction in microcirculatory flow (sublingual MFI 1.92, 2.0 and 2.08 respectively) on day 3 survived to hospital discharge, whereas 2 out of 9 patients with a normal flow (sublingual MFI 2.66 and 3.0 respectively) on day 3 died. However, this observational study was not designed to detect differences between survivors and non-survivors. To address the issue of relation between microcirculatory alterations and parameters of morbidity and mortality, Sakr et al. (7) performed a study, in which was demonstrated that persistence of microcirculatory alterations during sepsis was associated with organ dysfunction and non-survival. In a paper of De Backer and co-workers the proportion of perfused small vessels was higher in survivors than in non-survivors, but there was no correlation between microcirculatory alterations and MAP, CI and mixed-venous oxygen saturation (3).

With regard to the use of OPS imaging in intestinal stomas, as a model of intestinal microcirculation, several limitations of the study have to be taken into account. Since the surgical procedure itself might influence the microcirculation of the intestinal mucosa, we included a second control group of non-sepsis patients with a newly constructed stoma. MFI of this non-sepsis group differed significantly from the sepsis group during the initial phase of the abdominal sepsis (Fig. 1), whereas comparison of MFI between the non-sepsis group and the outpatient group showed no differences. Typical heterogeneity of flow only was observed during sepsis, both in the sublingual and intestinal microvascular beds and was absent in both control groups. This suggests that sepsis, and not the surgical procedure, is the main determinant for the observed microcirculatory flow alterations.

A second confounding factor on intestinal stoma microcirculation could have been the influence of the intra-abdominal pressure. Routine measurement of intra-abdominal pressure was not part of the protocol, but only performed in case of clinical suspicion of an abdominal compartment syndrome. However, none of the patients underwent a relaparotomy to relieve abdominal pressure during the protocol and an expected left-shift of the relation between MFI stoma and MFI sublingual (since elevated intra-abdominal pressure would theoretically diminish only intestinal flow and not sublingual flow) (Fig. 2) was not observed.

Thirdly, not only the influence of local inflammation with concomitant vasoconstriction and vasodilation as a result of generalized peritonitis might have been of influence, but it is also well known that the release of systemic pro- and anti-inflammatory mediators differs strongly between the various sepsis models (23). Furthermore, variation in local iNOS expression may influence microcirculatory blood flow (20).

Finally, the approach used, to score microcirculatory flow in a semi-quantitative way has its limitations. Although the method was specifically designed to differentiate between heterogeneous flow patterns during sepsis, it is still possible to underscore subtle microcirculatory abnormalities of individual vessels, since the score is derived from an overall impression of a specific vessel type in a particular quadrant. Using other scores or direct measurement of flow in each individual vessel may have yielded different results.

## Conclusions

In conclusion, no correlation between the microvascular flow in the sublingual region and the mucosa of an intestinal stoma could be found during day 1 of an abdominal sepsis in humans. Together with the absence of correlation between systemic hemodynamic parameters and microcirculatory flow, these data suggest a complete dispersion of blood flow in the different hemodynamic compartments during abdominal sepsis. The observed microcirculatory abnormalities during sepsis in an intestinal stoma seem to be determined by the sepsis itself, rather than by the surgical procedure. On day 3 however, correlation

between sublingual and intestinal microcirculatory flow appears to be restored, mainly due to a normalization of flow in both regions. It seems important to take this time-related dispersion between different microcirculatory beds into account for the design of future sepsis studies and to extend the research on correlation between sublingual and intestinal microcirculatory abnormalities to other human models of sepsis.

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## References

1. Weil MH and Shubin H: Proposed reclassification of shock states with special reference of distributive defects. *Adv Exp Med Biol* 1971; 23:13-23
2. Groner W, Winkelman JW, Harris AG, et al: Orthogonal polarization spectral imaging: A new method for study of the microcirculation. *Nat Med* 1999; 5:1209-1212
3. De Backer D, Creteur J, Preiser JC, et al: Microvascular blood flow is altered in patients with sepsis. *Am J Respir Crit Care Med* 2002; 166:98-104
4. Trzeciak S, Rivers EP: Clinical manifestations of disordered microcirculatory perfusion in severe sepsis. *Crit Care* 2005; 9(Suppl 4):S20-S26
5. Weil MH, Nakagawa Y, Tang W, et al: Sublingual capnometry: a new non-invasive measurement for diagnosis and quantitation of severity of circulatory shock. *Crit Care Med* 1999; 27:1225-1229
6. Creteur J, De Backer D, Sakr J, et al: Sublingual capnometry tracks microcirculatory changes in septic patients. *Intensive Care Med* 2006;32:516-523
7. Sakr Y, Dubois MJ, De Backer D, et al: Persistent microcirculatory alterations are associated with organ failure and death in patients with septic shock. *Crit Care Med* 2004; 2:1825-1831
8. Ellis CG, Jagger J, Sharp M: The microcirculation as a functional system. *Critical Care* 2005; 9(suppl 4):S3-S8

9. Baskurt OK, Gelmont D and Meiselman HJ: Red blood cell deformability in sepsis. *Am J Respir Crit Care Med* 1998; 157:421-427
10. Bone RC, Balk RA, Cerra FB, et al: Definitions for sepsis and organ failure and guidelines for the use of innovative therapies in sepsis. The ACCP/SCCM Consensus Conference Committee. American College of Chest Physicians/Society of Critical Care Medicine. *Chest* 1992; 101:1644-1655
11. Knaus WA, Draper EA, Wagner DP, et al. APACHE II: a severity of disease classification system. *Crit Care Med* 1985; 13:818-829
12. Vincent JL, de Mendonca A, Cantraine F, et al: Use of the SOFA score to assess the incidence of organ dysfunction/failure in intensive care units: results of a multicenter, prospective study. Working group on "sepsis-related problems" of the European Society of Intensive Care Medicine. *Crit Care Med* 1998; 26:1793-1800
13. Harris AG, Sinitsina I, Messmer K, et al: The cytoscan™ model E-II, a new reflectance microscope for intravital microscopy: comparison with the standard fluorescence method. *J Vasc Res* 2000; 37:469-476
14. Boerma EC, Mathura KR, van der Voort PHJ, et al: Quantifying bedside-derived imaging of microcirculatory abnormalities in septic patients: a prospective validation study. *Critical Care* 2005; 9:R601-R606
15. Ince C, Ashruf JF, Avontuur JA, et al: Heterogeneity of the hypoxic state in rat heart is determined at the capillary level. *Am J Physiol* 1993; 264:H294-H301
16. Ince C: The microcirculation is the motor of sepsis. *Crit Care* 2005; 9(suppl4):S13-S19
17. LeDoux D, Astiz ME, Carpati CM, et al: Effects of perfusion pressure on tissue perfusion in septic shock. *Crit Care Med* 2000; 28:2729-2732
18. De Backer D, Creteur J, Dubois MJ, et al: The effects of dobutamine on microcirculatory alterations in patients with septic shock are independent of its systemic effects. *Crit Care Med* 2006; 34:403-408
19. Mulivor AW, Lipowsky HH: Role of glycocalyx in leucocyte-endothelial cell adhesion. *Am J Physiol Heart Circ Physiol* 2002; 283:1282-1291

20. Almac E, Siegemund M, Demirci C, et al: Microcirculatory recruitment manoeuvres correct tissue CO<sub>2</sub> abnormalities in sepsis. *Minerva Anesthesiol* 2006; 72: 509-519
21. Spronk PE, Rommes JH, Hesselink EJ, et al: Comparison of sublingual and intestinal microvascular flow in critically ill patients. *Intensive Care Med* 2003; 29 (Suppl. 1):S179
22. Fries M, Weil MH, Sun S, et al: Increases in tissue PCO<sub>2</sub> during circulatory shock reflect selective decrease in capillary flow. *Crit Care Med* 2006; 34:446-452
23. Opal SM, DePalo VA: Anti-inflammatory cytokines. *Chest* 2000; 117:1162-1172

# Chapter 5

Disparity between skin perfusion and  
sublingual microcirculatory alterations in  
severe sepsis and septic shock: a prospective  
observational study

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## Abstract

### Objective.

Measurement of central-to-toe temperature difference has been advocated as an index of severity of shock and as a guide for circulatory therapy in critically ill patients. However, septic shock, in contrast to other forms of shock, is associated with a distributive malfunction resulting in a disparity between vascular compartments. Although this disparity has been established between systemic and microcirculatory parameters, it is unclear whether such disparity exists between skin perfusion and microcirculation. To test this hypothesis of disparity, we simultaneously measured parameters of the two vascular compartments, in the early phase of sepsis.

### Design.

Prospective observational study in patients with severe sepsis/septic shock in the first 6 hours of ICU admittance. Simultaneous measurements of central-to-toe temperature difference and sublingual microcirculatory orthogonal polarisation spectral imaging, together with parameters of systemic hemodynamics.

Setting. 22 bed mixed-ICU in a tertiary teaching hospital

Patients. 35 consecutive patients in a 12-month period

Interventions: none

### Measurements and results.

In 35 septic patients and a median APACHE II score of 20, no correlation between central-to-toe temperature gradient and microvascular flow index was observed ( $r_s = -0.08$ ,  $p = 0.65$ ). Also no significant correlation between temperature gradient/microvascular flow index and systemic hemodynamic parameters could be demonstrated.

### Conclusion.

During the early phase of resuscitated severe sepsis and septic shock there appears to be no correlation between sublingual microcirculatory alterations and the central-to-toe temperature difference. This finding adds to the concept of a dispersive nature of blood flow under conditions of sepsis between microcirculatory and systemic hemodynamics.

Keywords: Orthogonal Polarization Spectral (OPS) imaging, microcirculation, peripheral circulation, temperature gradient, skin perfusion, sepsis.

## Introduction

Over the last decades it has become clear, that despite correction of systemic hemodynamics, the incidence of organ dysfunction and mortality remains high in sepsis. Already in 1969 Joly and Weil identified the cold toe as a new and easily accessible parameter of severity of circulatory shock [1]. The authors observed a correlation between an increment in central-toe temperature difference ( $\Delta T$ ) and adverse outcome in a mixed ICU population. 30 years later this was confirmed with a subjective assessment of skin temperature [2]. In a mixed surgical population cool skin temperature was associated with lower cardiac output and central venous oxygen saturation and higher lactate levels as opposed to warm skin temperature, thus using skin perfusion as a marker for systemic hypoperfusion.

However, Weil had earlier reclassified circulatory shock to identify distributive shock, including septic shock, as a different entity, in which there is an inability of blood to reach the exchange sites [3]. This concept was confirmed by microcirculatory measurements made in septic patients after the introduction of sublingual Orthogonal Polarization Spectral (OPS) imaging [4]. It has become clear that the discordance between systemic hemodynamic parameters and the microcirculatory alterations is most prominent during sepsis [5], as opposed to other forms of shock. These alterations have also been identified as markers for morbidity and mortality [6] whereas systemic hemodynamic parameters failed to do so under septic conditions [7].

However, no investigations exist as to what extent skin perfusion correlates with microcirculatory abnormalities during sepsis. Since  $\Delta T$  is easily obtainable in the clinical setting, we conducted an observational study [8] in human sepsis to answer the question: is there a relationship between  $\Delta T$  and microcirculatory alterations during sepsis? Based on our understanding of distributive shock we expected a disparity between these two parameters.

## Materials and methods

### Imaging technique.

The OPS technique, as described in detail elsewhere [4], consists of a hand-held device that illuminates an area of interest with polarized light, while imaging the remitted light through a second polarizer. If a wavelength within the haemoglobin absorption spectrum (e.g. 548 nm) is chosen, red blood cells will appear dark.

### Imaging and analysis procedure.

OPS imaging and semi-quantitative analysis was performed as described in detail elsewhere [9]. The overall microvascular flow index (MFI) is an average score over a maximum of 12 quadrants (3 regions times 4 quadrants per region) derived from the overall flow impression of all vessels with a particular range of diameter in a given quadrant.

### Setting and patient selection.

We performed a single centre prospective observational study in a tertiary teaching-hospital with a 22 bed mixed ICU. During a 12-month period, patients with severe sepsis/septic shock, according to international criteria [10], were included. Patients were included only when the source of the sepsis was suspected or confirmed (e.g. infiltrate on chest X-ray plus positive sputum gram stain/culture, faecal spill in the abdominal cavity observed during surgical procedure). Age <18 years, (diabetic) peripheral vascular disease and a body mass index > 35 were contraindications for enrolment. A local ethical and scientific committee approved of the study protocol and written informed consent was obtained from the patients or their surrogate decision makers, according to applicable laws.

### Protocol and data collection.

Patients were admitted to the ICU directly from the emergency department or operation room. All patients were ventilated and sedated with morphine/midazolam. By protocol, none of the patients received vasodilatory therapy, steroids or activated protein C before the OPS images were obtained. Before measurement, fluid resuscitation was applied until repeated volume challenges did not increase stroke volume (SV) 10% or more, or when central venous pressure (CVP) reached 15 mm Hg. Mean arterial pressure (MAP) was maintained at a minimum level of 60 mm Hg with dopamine up to 10 µg/kg · min and additional norepinephrine. Cardiac index (CI) and SV were measured by oesophageal Doppler technology (CardioQ®, Deltex Medical, West Sussex, UK).  $\Delta T$  was calculated as the difference between rectal- and skin temperature; skin temperature was measured by a probe on the dorsum of the foot (Philips Medical Systems 21078A, Eindhoven, the Netherlands) under constant room temperature.  $SvO_2$  was not measured routinely. Age, gender, length of stay (LOS), Acute Physiology And Chronic Health Evaluation (APACHE) II and Sequential Organ Failure Assessment (SOFA) scores were calculated after 24 hours [11,12].

### Statistical analysis.

The statistical package for the social sciences (SPSS 12.0.1 for Windows, Chicago Illinois, USA) was used for statistical analysis. Data are presented in medians and interquartile ranges (IQR). Non-parametric rank correlation is expressed as Spearman's rho ( $r_s$ ). For subgroup analysis a Bonferroni correction was applied. A two-sided p value of < 0.05 is considered statistically significant.

## Results

Thirty-five ICU sepsis patients with a median APACHE II score of 20 (14-23) were enrolled; 20 patients also engaged in a previous reported study [13]. All patients fulfilled the entry-criteria; cultures confirmed the source of sepsis in all cases. Baseline characteristics and

hemodynamic parameters are summarized in Table 1. ICU and in-hospital mortality were 25.7% and 32.4% respectively, with an ICU LOS of 7 (IQR 3-13) days and an in-hospital LOS of 20 (IQR 11.8-35.3) days. All measurements were obtained in the first 6 hours of ICU admittance.

Primary outcome of the study, the relation between MFI and  $\Delta T$ , appeared to be absent; non-parametric rank correlation ( $r_s$ ) was -0.08 ( $p=0.65$ ,  $g.1$ ). After subgroup analysis  $r_s$  in severe sepsis was -0.04 ( $n=16$ ,  $p=0.87$ ) and in septic shock -0.23 ( $n=19$ ,  $p 0.35$ ).

Secondary outcome was the relationship between MFI and  $\Delta T$  on the one hand and systemic hemodynamic parameters and parameters of morbidity/mortality on the other hand. Correlation coefficients between MFI/ $\Delta T$  and macro-hemodynamic parameters such as heart rate (HR), CI, MAP, CVP, lactate and use of inotropic and vasopressors agents or parameters of morbidity (APACHE II and SOFA) were all statistically insignificant (Table 2). There was no differences between median MFI/ $\Delta T$  of survivors and non-survivors (2.42 and 2.42; 3.3 and 3 respectively)

Table 1. Characteristics study population (n = 35)

Gender, male/ female, n	21/ 14
Age, years	65 (56-77)
APACHE II score	17 (14-23)
SOFA score	7 (6-9)
Ventilator, use of, n	35
PEEP level, cm H <sub>2</sub> O	12 (10-15)
CVVH, use of, n	2
Norepinephrine, n (dose in $\mu\text{g}/\text{kg} \cdot \text{min}$ )	8; 0.02 (0-0.17)
Dopamine, n (dose in $\mu\text{g}/\text{kg} \cdot \text{min}$ )	23; 6 (4-10)
Source of sepsis, n	
• Abdominal	28
• Pneumonia	7
Heart rate, beats/min	107 (92-119)
Mean blood pressure, mmHg	71 (66-81)
Central venous pressure, mmHg	11 (8-14)
Cardiac index, L/min $\cdot$ M	4.5 (3.5-5.3)
Central-to-toe temperature difference, °C	3.2 (2.2-6)
Lactate, mmol/L	2.5 (1.3-3.4)

APACHE, acute physiology and chronic health evaluation; SOFA, sepsis-related organ failure assessment; CVVH, continuous veno venous hemo ltration. Data are presented as medians (25th-75th percentiles), unless stated otherwise.

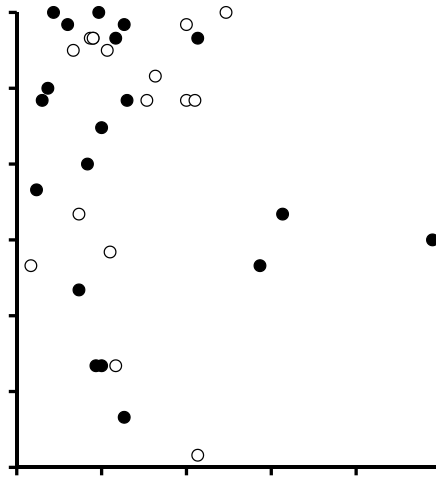


Figure 1. Scatter of microvascular flow index (MFI) of small vessels ( $< 20 \mu\text{m}$ ) in the sublingual region versus the central-to-toe temperature gradient ( $\Delta T$ ). Spearman rank correlation =  $-0.08$ ,  $p 0.65$ . ( severe sepsis, septic shock)

Table 2. Correlation between microvascular flow index,  $\Delta T$  and systemic hemodynamic parameters / parameters of morbidity in study population ( $n = 35$ )

	MFI		$\Delta T$	
Heart rate	0.12	$p=0.50$	0.03	$p=0.85$
Mean arterial pressure	0.17	$p=0.33$	0.38	$p=0.18$
Cardiac index	-0.06	$p=0.74$	-0.15	$p=0.39$
Central venous pressure	0.13	$p=0.49$	0.1	$p=0.59$
Norepinephrine dose	0.17	$p=0.34$	0.04	$p=0.82$
Dopamine dose	0.10	$p=0.58$	0.10	$p=0.58$
Lactate	-0.17	$p=0.37$	0.1	$p=0.59$
SOFA	0.18	$p=0.29$	-0.08	$p=0.64$
APACHE II	-0.2	$p=0.24$	0.05	$p=0.76$

MFI, microvascular flow index (small vessels;  $< 20 \mu\text{m}$ );  $\Delta T$ , central-to-toe temperature difference; SOFA, sepsis-related organ failure assessment; APACHE, acute physiology and chronic health evaluation. Data are presented as Spearman rank correlation ( $r_s$ ).

## Discussion

The presented study demonstrates a lack of correlation between  $\Delta T$  and OPS-derived sublingual microcirculatory alterations during sepsis, after initial resuscitation. Although one may consider  $\Delta T$  as an index of skin perfusion, this gradient has also been associated with systemic hemodynamic variables [14]. Previous studies demonstrated a good relationship between central-to-toe temperature difference and severity of shock [1,2]. In patients with circulatory shock  $\Delta T$  during therapy was associated with outcome, predicted fluid responsiveness in correlation with plasma arginine vasopressin concentrations in preterm infants, and discriminated between circulatory and non-circulatory causes of dyspnoea [14].

However, during sepsis and septic shock, microcirculatory abnormalities rather than systemic hemodynamic parameters seem to be the predominant factor [5] and heterogeneity of flow between and within microcirculatory units seems to be a characteristic finding. In the previous years, research, using OPS imaging, has added to the understanding of the patho-physiologic role of microcirculatory alterations in the distributive defects seen in sepsis. Persistence of OPS derived microcirculatory abnormalities was found to be associated with prognosis, in contrast to all available systemic hemodynamic parameters [6]. The observed lack of correlation between  $\Delta T$  and MFI therefore adds to these previous data on the dispersion between systemic and microcirculatory alterations in sepsis, after initial resuscitation. Alternatively, skin perfusion itself might not reflect systemic hemodynamics in sepsis, as suggested by insignificant correlations between  $\Delta T$  and systemic hemodynamics in our study (table 2). Interestingly, Vincent and co-workers also reported a poor correlation between  $\Delta T$  and cardiac output during septic shock, as opposed to other forms of shock [15].

Limitations of the study are enclosed in the method of semi/quantitative analysis used. Whether or not the flow score from 0 to 3 is linear or non-linear remains to be established; up till now it is technically impossible to measure exact red blood cell flow velocities in individual vessels in OPS-derived images. In case of a complete non-linear relationship between the semi-quantitative flow score and exact flow speed the observed relationship between MFI and  $\Delta T$  would be influenced considerably.

Using  $\Delta T$  as the single parameter of skin perfusion is another limitation of the study. Laser-Doppler has the ability to detect altered vascular reactivity, especially in conditions of ischemia-reperfusion. Under non-septic conditions laser-Doppler imaging of the dorsum of the foot showed a linear relationship with skin temperature [16]. Recently, transcutaneous  $pO_2$  measurement of the skin with near infrared spectroscopy has become available. However, up till now its use is poorly validated in the ICU setting.

Finally, thermoregulatory effects of opioids in general can not be ruled out, whereas the influence of midazolam has been reported to be futile [17].

## Conclusions

During the early phase of resuscitated severe sepsis and septic shock there appears to be no correlation between sublingual microcirculatory alterations and the central-to-toe temperature difference. This finding adds to the concept of a dispersive nature of blood flow under conditions of sepsis between microcirculatory and systemic hemodynamics.

## References

1. Joly HR, Weil MH (1969) Temperature of the great toe as an indication of the severity of shock. *Circulation* 39:131-138
2. Kaplan LJ, McPartland K, Santora TA, Trooskin SZ (2001) Start with subjective assessment of skin temperature to identify hypoperfusion in intensive care unit patients. *J Trauma* 50:620-628
3. Weil MH, Shubin H (1971) Proposed reclassification of shock states with special reference of distributive defects. *Adv Exp Med Biol* 23:13-23
4. Groner W, Winkelmann JW, Harris AG, Ince C, Bouma GJ, Messmer K, Nadeau RG (1999) Orthogonal polarization spectral imaging: A new method for study of the microcirculation. *Nat Med* 5:1209-1212
5. De Backer D, Creteur J, Preiser JC, Dubois MJ, Vincent JL (2002) Microvascular blood flow is altered in patients with sepsis. *Am J Respir Crit Care Med* 166:98-104
6. Sakr Y, Dubois MJ, De Backer D, Creteur J, Vincent JL (2004) Persistent microcirculatory alterations are associated with organ failure and death in patients with septic shock. *Crit Care Med* 32:1825-1831
7. De Backer D, Creteur J, Dubois MJ, Sakr Y, Koch M, Verdant C, Vincent JL (2006) The effects of dobutamine on microcirculatory alterations in patients with septic shock are independent of its systemic effects. *Crit Care Med* 34:403-408
8. Boerma EC, Konijn AJM, Kuiper MA, Gerritsen RT, Kingma WP, Ince C (2007) Disparity between microcirculatory and skin perfusion in sepsis. *Intensive Care Med* 33(suppl2):S168
9. Boerma EC, Mathura KR, van der Voort PHJ, Spronk PE, Ince C (2005) Quantifying bedside-derived imaging of microcirculatory abnormalities in septic patients: a prospective validation study. *Critical Care* 9:R601-R606

10. Levy MM, Fink MP, Marshall JC, Abraham E, Angus D, Cook D, Jonathan C, Opal SM, Vincent JL, Ramsay G for the international sepsis definitions conference (2003) 2001 SCCM/ESICM/ACCP/ATS/SIS International Sepsis Definitions Conference. *Intensive Care Med* 29:530-538
11. Knaus WA, Draper EA, Wagner DP, Zimmerman JE (1985) APACHE II: a severity of disease classification system. *Crit Care Med* 13:818-829
12. Vincent JL, de Mendonca A, Cantraine F, Moreno R, Takala J, Suter PM, Sprung CL, Colardyn F, Blecher S (1998) Use of the SOFA score to assess the incidence of organ dysfunction/failure in intensive care units: results of a multicenter, prospective study. Working group on "sepsis-related problems" of the European Society of Intensive Care Medicine. *Crit Care Med* 26:1793-1800
13. Boerma EC, van der Voort PHJ, Spronk PE, Ince C (2007) Relationship between sublingual and intestinal microcirculatory perfusion in patients with abdominal sepsis. *Crit Care Med* 35:1055-1060
14. Lima A, Bakker J (2005) Noninvasive monitoring of peripheral perfusion. *Intensive Care Med* 31:1316-1326
15. Vincent JL, Moraine JJ, van der Linden P (1988) Toe temperature versus transcutaneous oxygen tension monitoring during acute circulatory failure. *Intensive Care Med* 14:64-68
16. Yvonne-Tee GB, Rasool AH, Halim AS, Rahman AR (2006) Noninvasive assessment of cutaneous vascular function in vivo using capillaroscopy, plethysmography and laser-Doppler instruments: its strengths and weaknesses. *Clin Hemorheol Microcirc* 34:457-473
17. Kurz A, Sessler DI, Annadata R, Dechert M, Christensen R, Bjorksten AR (1995) Midazolam minimally impairs thermoregulatory control. *Anesth Analg* 81:393-398



# Chapter 6

Effects of nitroglycerin on sublingual microcirculatory blood flow in patients with severe sepsis/ septic shock in the setting of early goal-directed therapy and dynamic exclusion of fluid-responsiveness: a double-blind randomised placebo controlled trial

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Submitted

## Abstract

### Objective.

The persistence of sublingual microcirculatory alterations has been associated with morbidity and mortality in human sepsis. Such alterations occur despite pressure-guided resuscitation from severe sepsis/septic shock. Earlier data suggested that such impaired microcirculatory blood flow could be corrected with intravenous nitroglycerin in these patients. We tested this concept in the setting of early goal-directed therapy and dynamic exclusion of fluid-responsiveness.

**Design.** Prospective, single centre, randomised, placebo-controlled, double-blind clinical trial.

**Setting.** Closed-format 22-bed mixed ICU in a tertiary teaching-hospital.

### Patients.

All patients of at least 18 years of age with sepsis, according to international criteria, and at least one sign of organ dysfunction lasting less than 48 hours, as the principal reason for ICU admission were eligible for enrolment.

### Interventions.

Patients were randomly assigned to receive nitroglycerin (n=35) or placebo (n=35) after fulfilment of resuscitation endpoints, as confirmed by strict protocolised treatment. This trial is registered with ClinicalTrials.gov, number NCT00493415.

### Measurements and main results.

The primary outcome was sublingual microcirculatory blood flow, as assessed by direct observation using side-stream dark field imaging. After 24 hours of protocolised resuscitation, we observed the recruitment of sublingual microcirculation in both groups, as indicated by a significant improvement in the microcirculatory flow index as compared with baseline. However, no difference in the sublingual microvascular flow index was observed between groups. The median microvascular flow index in sublingual small-sized vessels was 2.71(1.85-3) in the nitroglycerin group and 2.71(1.27-3),  $p = 0.80$  in the placebo group. In medium-sized vessels, the respective values were 3(2.75-3) versus 2.86(2.19-3),  $p = 0.21$ ; and in large-sized vessels, 3(3-3) versus 3(2.89-3),  $p = 0.06$ .

### Conclusions.

In the context of early goal-directed therapy and dynamic exclusion of fluid-responsiveness in patients with severe sepsis or septic shock, we conclude that intravenous nitroglycerin does not promote sublingual microcirculatory blood flow.

## Introduction

For many decades, sepsis has been classified as a distributive form of shock (1). As opposed to all other forms of shock, it is not characterised by a reduction in cardiac output, but by a distributive inability of blood to effectively reach the main exchange site, namely the microcirculation. This view of distributive shock was confirmed by direct microcirculatory observations in septic patients after the introduction of sublingual Orthogonal Polarization Spectral imaging (2) and its technical successor Sidestream Dark Field (SDF) imaging (3). It has become clear that the discordance between systemic haemodynamic parameters and microcirculatory alterations is most prominent during sepsis, in contrast to other forms of shock (4). Under septic conditions, these alterations have also been identified as markers for morbidity and mortality (5,6), irrespective of the correction of systemic haemodynamic parameters and oxygen-derived variables (5-7). The view that resuscitated sepsis is persistently unresponsive to improvement led to the direction of therapeutic modalities toward improvement of microcirculatory function. It was within this context that the paper by Spronk et al. (8) gave credence to a new concept: the instant improvement of microcirculatory flow by a bolus of the vasodilatory substance nitroglycerin (NTG). NTG was indicated following persistence of impaired microcirculatory flow despite fulfilment of the traditional resuscitation endpoints. However, this study failed to adequately assess the influence of other factors, such as the evolution of microcirculatory alterations over time. Specifically, an early goal-directed therapy (EGDT) approach to sepsis resuscitation (9) was outside the scope of their effort. We therefore designed a clinical trial in patients with severe sepsis or septic shock, and incorporated a strict EGDT-based resuscitation protocol and careful exclusion of fluid-responsiveness. Our goal was to compare: 1. Sublingual SDF-derived microcirculatory effects of NTG with those of placebo over time, and 2. The effects of NTG on systemic haemodynamic parameters with those of placebo over time.

## Materials and Methods

### Patients

The study was performed between January 2007 and June 2008 in a closed-format 22-bed mixed ICU in a tertiary teaching-hospital. It was designed as a phase III prospective, single centre, randomised, placebo-controlled, double-blind clinical trial and is registered with ClinicalTrials.gov, number NCT00493415 and with EudraCT, number 2006-004298-88. All patients of at least 18 years of age with suspected sepsis as the principal reason for ICU admission at the study site were eligible for assessment. Inclusion criteria were the presence of sepsis, according to international criteria (10), and at least one sign of organ dysfunction lasting less than 48 hours, as summarised in Table 1. Reasons for exclusion were pregnancy, use of nitrate-derivatives within 24 hours prior to admission, strict indication

for intravenous nitroglycerin (unstable coronary syndrome) or therapeutic restrictions (do not resuscitate orders excluded). A local ethical and scientific committee approved the study protocol, and written informed consent was obtained from the patients or their surrogate decision makers, consistent with applicable laws.

Table 1. Inclusion criteria: fulfillment of at least one item in each list is needed for enrolment in the study

Suspected source of infection:
New infiltrate on X-ray plus positive gram stain or culture
Positive blood culture
Confirmed bowel perforation
Positive urine gram stain or culture
Positive spinal fluid gram stain or culture
Necrotising fasciitis plus positive gram stain or culture
Arthritis plus positive gram stain or culture
Mediastinitis plus positive gram stain or culture
Pancreatitis plus positive fine needle aspiration
Signs of organ failure:
Oliguria: urine output <0.5 mg/kg/h for at least 2 consecutive hours after adequate volume resuscitation, or serum creatinine >177 mmol/l in the absence of chronic renal failure (creatinine >177 mmol/l or haemodialysis)
Metabolic acidosis: pH <7.35 and lactate >2.5 mmol/L
Respiratory failure: PaO <sub>2</sub> /FiO <sub>2</sub> <26.6 kPa
Coagulation disorders: platelet count <100 x 10 <sup>9</sup> /l or PT/INR >1.5 in the absence of coumarine derivatives
Cardiovascular failure: persistent hypotension (RR systolic <90 mm Hg) despite adequate volume supply, resulting in the use of vasopressors (dopamine >5 mcg/kg/min, norepinephrine any dose)

### Protocol

Prior to randomisation, all patients followed a strict protocol to optimise systemic haemodynamic parameters in accordance with the basic principles of early goal-directed therapy (9). Systemic haemodynamic assessment was achieved through continuous invasive monitoring of arterial blood pressure and right heart catheterisation with continuous cardiac output and central venous oxygen saturation (Vigilance®, Edwards Lifesciences, Saint-Prex, Switzerland). Until a pulmonary artery catheter was in place, the use of fluids and vasoactive agents was at the discretion of the attending physician, whose goal was to maintain a minimal mean arterial pressure (MAP) of 60 mm Hg. After calibration, treatment of circulatory failure was performed using the following strict hierarchical order: 1. Establishment of fluid-responsiveness by repeated infusions of at least 250 ml crystalloids, colloids or blood products, until the increase in left ventricular stroke volume was less than 10%, or until the pulmonary artery wedge pressure exceeded 18 mm Hg. 2. Treatment of heart

failure, defined as a cardiac index  $< 2.5 \text{ l/m}^2/\text{min}$  or central venous oxygen saturation  $< 70\%$ , with dopamine administered at up to  $10 \mu\text{g/kg/min}$  and additional enoximone in the event of an inadequate response to dopamine. 3. Reversal of hypotension with norepinephrine in case of MAP  $< 60 \text{ mmHg}$  despite the aforementioned steps.

During the 24-hour study medication infusion period, these therapeutic goals remained unchanged. The use of hydrocortisone up to a maximum of  $100 \text{ mg iv 3 q.d.}$  was permitted for shock reversal in case of vasopressor dependency; the general red blood cell transfusion trigger was haematocrit  $< 25\%$ . After fulfilment of all systemic haemodynamic endpoints, and additional repeat exclusion of fluid-responsiveness, we recorded the baseline systemic and microcirculatory parameters. Treatment with NTG ( $1 \text{ mg/ml}$ ) or placebo (isotonic saline) was randomly assigned. The drug and placebo were prepared in identical syringes and were then delivered for use by the pharmacist. During the first 30 minutes of administration, a front load of  $2 \text{ ml}$  was given continuously ( $4 \text{ ml/h}$ ); during the next 23.5 hours, the infusion rate was kept constant at  $2 \text{ ml/h}$ . In cases of patient body weight  $< 50 \text{ kg}$ , infusion rates were reduced by  $50\%$ . For safety reasons, a sustained MAP  $< 60 \text{ mmHg}$  during infusion despite protocolised treatment constituted a reason to immediately and permanently stop the infusion therapy.

#### Imaging and analysis

SDF imaging is a stroboscopic light emitting diode ring-based imaging modality that is incorporated in a hand-held device. The device illuminates an area of interest for clinical observation of the microcirculation. It has been successfully validated against its technical predecessor, namely orthogonal polarization spectral imaging (3). If a wavelength within the haemoglobin absorption spectrum (e.g.  $530 \text{ nm}$ ) is chosen, red blood cells will appear dark and white blood cells may be visible as refringent bodies. The vessel walls are not visualised directly and imaging therefore depends on the presence of red blood cells. Semi-quantitative analysis was performed as described in detail elsewhere (11). In short, a minimum of 3 steady images of at least 20 seconds in duration were obtained from the sublingual region by a research investigator other than the attending physician. A specially trained group of 4 individuals was available during the study period on a 24/7 basis, with the exception of a total of 7 days. After gentle removal of saliva by an isotonic-saline-drenched gauze and avoiding pressure artefacts, images were acquired and stored on a digital videotape (SONY Video Walkman GV-D 1000E<sup>®</sup>, Sony, Tokyo, Japan). Subsequently, the images were captured in 5-10 second representative AVI format video clips (sonyDVgate<sup>®</sup>, Sony, Tokyo, Japan). Video clips were blindly analysed offline by an investigator who had no involvement in the data collection. The images were presented in random order so as to prevent inter-image coupling. SDF images were obtained from three different locations within the sublingual region, and each image was divided into 4 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  $\mu\text{m}$ , medium 26-50  $\mu\text{m}$  and large 51-100  $\mu\text{m}$ ). 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 (3 regions, 4 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 (6), 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 (TVD) was performed with the AVA 3.0® software package (MicroVision Medical, Amsterdam, The Netherlands), as described and validated recently (12) using a cut-off diameter for small vessels of < 20  $\mu\text{m}$ . After stabilisation of the images using the AVA 3.0 software, we defined the perfused (small) vessel density (PVD) and the proportion of perfused (small) vessels (PPV) in terms of the number and percentage of crossings with perfused (small) vessels per total length of 3 equidistant horizontal and 3 equidistant vertical lines. This method has been described elsewhere by de Backer et al. and is in accordance with reports of a round table conference (13).

#### Data collection

The following data were recorded at baseline: general characteristics; severity of illness and predicted mortality consistent with APACHE IV (14), SOFA (15) (calculated over the first 24 hours following ICU admission) and RIFLE (16) scores; systemic haemodynamic parameters; sublingual SDF images; and results of standard laboratory tests, including blood gases and arterial lactate concentrations, blood cultures and cultures of specimens sampled from each presumed site of infection. Systemic haemodynamics, SDF images, arterial lactate concentrations and blood gases were recorded at 30 minutes, and 2, 12 and 24 hours after the start of the study medication and the SOFA plus RIFLE score was calculated daily during each patient's ICU stay. Survival status was confirmed for each subject at the end of their hospitalisation. The primary endpoint was MFI within the 24-hour study medication period. Secondary endpoints were the SOFA score, systemic haemodynamics, dose of dopamine/norepinephrine and survival distribution from randomisation to hospital discharge.

#### Statistical analysis

We anticipated a mean MFI at baseline of 1.6 with a standard deviation (SD) of 0.83, based on earlier observations (17). We calculated a sample size of 70 patients to detect an absolute difference in MFI of 0.6 in a two-sided test with a 0.05 type I error and an 80% probability. The Statistical Package for Social Sciences (SPSS 15.1 for Windows, Chicago Illinois, USA) was used for statistical analyses. For continuous variables, all data are presented as mean  $\pm$  SD, or as medians and interquartile ranges (IQR) in case of non-normal distributions. The effects of treatment on systemic and microcirculatory parameters were compared between groups using an independent sample t-test. The effects on non-normally distributed parameters (MFI, TVD, PPV, PVD, LOS, cumulative SOFA's and maximum RIFLE score) were

compared using the non-parametric Mann-Whitney test. Comparison of mortality rates across different treatment strategies was performed using the  $\chi^2$  test. Cumulative event curves (censored endpoint at day 60) were estimated with the Kaplan-Meier procedure and the effect of treatment on survival probability was compared between groups with a log rank-test. Comparison against baseline of systemic and microcirculatory haemodynamic parameters after 24 hours was performed with a paired t-test, or with a Wilcoxon signed rank test in case of variables that were not normally distributed. A two-sided p value of < 0.05 was considered statistically significant.

## Results

Out of 133 patients who were screened for eligibility, 70 patients were randomly assigned to receive treatment (Figure 1). Baseline characteristics were well balanced between the groups (Table 2). Exceptions were an insignificantly higher age and RIFLE score in the placebo group and more prominent abdominal sepsis in the NTG group. Causative pathogens were identified in 60 (86%) patients and blood cultures were positive for 16 (22.8%) patients. Nine patients in the NTG group and seven patients in the placebo group received enoximone during the study period; two patients in the placebo group received activated Protein C. Three patients in the NTG group and one patient in the placebo group died within 24 hours of admission due to progressive cardiac failure or cardiac arrest. Withdrawal from the study because of sustained hypotension was not reported.

In both groups, MFI in small vessels significantly increased over a 24-hour period of protocolised resuscitation, in comparison to baseline. In the NTG group, the change was from 1.67(0.67-2.42) to 2.71 (1.85-3),  $p < 0.0001$  and in the placebo group from 1.42(0.83-2.63) to 2.71(1.27-3),  $p = 0.006$  (Table 3, Figure 2). MFI in medium and large-sized vessels increased in a small but significant way in the NTG group, and not in the placebo group (Table 3).

Despite this overall increment in small vessel MFI over time, there was no significant difference in primary outcome between the NTG and placebo groups at 24 hours after the administration of study medication (Table 3). Median MFI in sublingual small vessels was 2.71 (1.85-3) versus 2.71 (1.27-3),  $p = 0.80$ ; in medium-sized vessels 3 (2.75-3) versus 2.86 (2.19-3),  $p = 0.21$ ; and in large-sized vessels 3 (3-3) versus 3 (2.89-3),  $p = 0.06$  (Table 3, Figure 2). The number of responders, defined as any MFI increment for small vessels between baseline and 24 hours was similar in both the NTG and placebo groups: 24 (68.5%) versus 20 (57.1%),  $p = 0.46$ . The heterogeneity index and parameters of (perfused) vessel density (TVD, PPV and PVD) did not differ between groups, except for a significant but small difference in PPV after 30 minutes and after 24 hours (Table 3).

The heterogeneity index decreased significantly over time in both groups, but parameters of (perfused) vessel density remained unaltered.

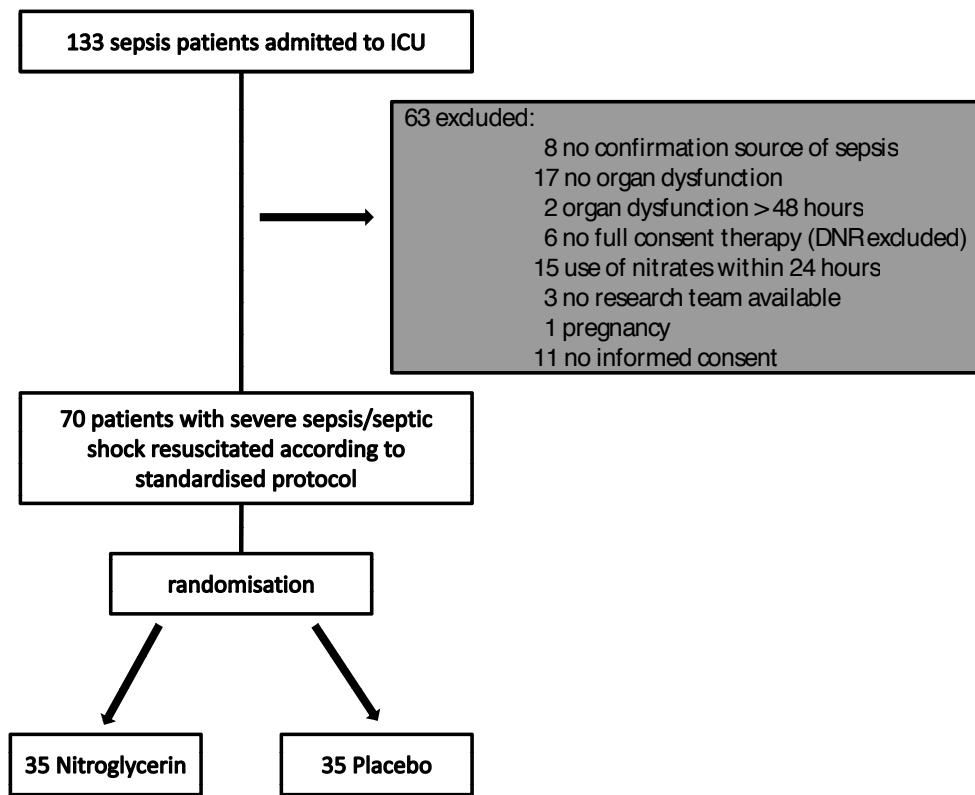


Figure 1. Trial protocol

Table 2. Baseline characteristics

Variables	Nitroglycerin(n=35)	Placebo(n=35)	p Value
Men	21(60)	22(62.9)	0.81
Age	64±15.8	59(15.4)	0.2
APACHE IV	88±24	94±62	0.59
Predicted mortality APACHE IV (%)	43±21	39±16	0.36
SOFA	9±3	10±3	0.66
Source of infection			
Lung	12(34)	12(34)	
Abdominal	19(54)	12(34)	
Urinary tract	2(6)	2(6)	
Other	2(6)	9(26)	
Mean arterial pressure, mm Hg	72±11.5	71±12.0	0.97
Heart rate, beats/min	109±16.7	112±17.8	0.52
Central venous pressure, mm Hg	12±4.0	13±5.5	0.26
Cardiac index, l/min . m <sup>2</sup>	4.1±1.3	4.3±1.3	0.63
Pulmonary artery wedge pressure, mm Hg	15±6.0	16±5.9	0.51
Mixed venous oxygen saturation, %	71±8.4	71±6.3	0.87
Oxygen consumption, ml/min . m <sup>2</sup>	153±46	165±75	0.41
Oxygen delivery, ml/min . m <sup>2</sup>	581±168	608±214	0.55
Oxygen extraction, %	28±8.8	27±7.1	0.78
Dopamine dose, n, µg/kg . min	30, 5.4±3.8	30, 6.0±3.5	0.53
Norepinephrine dose, n, µg/kg . min	11, 0.37±0.52	12, 0.27±0.43	0.37
Central-to-toe temperature gradient, °C	5.5±2.6	5.4±2.5	0.87
Ventilation-perfusion ratio, %	30±10	32±10	0.31
Ventilator, use of	34(97.1)	35(100)	0.32
PEEP, cm H <sub>2</sub> O	12±3	13±3	0.28
Lactate, baseline, mmol/l	2.8±2.3	2.4±2.1	0.5
Lactate, highest before baseline, mmol/l	3.5±2.4	3.2±2.9	0.63
pH	7.31±0.9	7.31±1.0	0.96
Base excess, mmol/l	-5.8±5.3	-6.2±6.0	0.26
Haematocrit, %	31±4	32±6	0.80
ARF RIFLE score on admission	0.51±0.98	0.89±1.08	0.14

APACHE Acute Physiology And Chronic Health Evaluation, SOFA Sequential Organ Failure Assessment, MFI Microvascular Flow Index, PEEP Positive End Expiratory Pressure, ARF Acute Renal Failure, RIFLE Risk Injury Failure Loss and Endstage. All data are presented as mean±SD or as numbers (%).

Table 3. Microvascular variables over time

Variables	Baseline		30 minutes	
	NTG(n=35)	Placebo(n=35)	NTG(n=35)	Placebo(n=35)
MFI small vessels	1.67 (0.67-2.42)	1.42 (0.83-2.63)	1.83 (1.08-2.75)	1.83 (0.83-2.83)
MFI medium vessels	2.33 (1.83-2.83)	2.33 (2-2.83)	2.67 (2.25-2.83)	2.42 (2.17-2.92)
MFI large vessels	2.92 (2.75-3)	2.92 (2.75-3)	3 (2.83-3)	3 (2.75-3)
TVD, mm/mm <sup>2</sup>	14 (12.8-15.6)	15 (12.3-16.1)	13.9 (12.2-15)	14.1 (12.8-15.9)
PPV, %	98 (93-100)	97 (89-100)	100 (96-100)	97 (90-99) *
PVD, 1/mm	9.1 (8.3-10.5)	9.8 (8.4-10.8)	9.7 (8.7-10.5)	9.7 (8-10.5)
Heterogeneity index	1.76 (0.88-2.84)	1.96 (0.66-3)	1.71 (0.36-2.17)	1.53 (0.36-2.75)

MFI microcirculatory flow index, NTG nitroglycerin, TVD total vessel density of (small) vessels, PPV proportion of perfused (small) vessels, PVD perfused (small) vessel density. Cut-off value for small vessels < 20  $\mu\text{m}$ . All data are presented as medians (IQR). \*P Value < 0.05 between groups, non-parametric test for independent samples. † P value < 0.05, ‡ < 0.0001 after 24 hours in comparison to baseline, non-parametric test for dependent samples.

(Table 3. Microvascular variables over time)

2 hours		12 hours		24 hours	
NTG(n=35)	Placebo(n=35)	NTG(n=34)	Placebo(n=35)	NTG(n=32)	Placebo(n=34)
2.25 (1.42-2.75)	2.25 (1.25-2.92)	2.34 (0.83-3)	2.08 (1.5-2.83)	2.71 (1.85-3)‡	2.71 (1.27-3)†
2.83 (2.42-3)	2.75 (2.33-3)	2.79 (2.08-3)	2.67 (2.5-2.92)	3 (2.75-3)‡	2.86 (2.19-3)
3 (3-3)	3 (3-3)	3 (2.81-3)	3 (2.92-3)	3 (3-3)†	2.89 (3-3)
14.3 (13.2-15.1)	14 (12.9-16)	14 (13.1-15.7)	13.9 (13.2-15.5)	13.9 (12.5-15.7)	14.7 (13.1-16.1)
99 (96-100)	98 (93-100)	99 (93-100)	99 (93-100)	100 (98-100)	98 (86-100)*
9.7 (8.4-10.7)	9.5 (8.7-11.3)	10 (8.3-10.8)	9.1 (8.2-10.5)	10.2 (8.7-11.2)	10.1 (8.5-10.7)
0.82 (0.26-2.11)	1.24 (0.34-2.4)	1.22 (0-2.89)	1.44 (0.35-2)	0.74 (0-1.62)‡	0.54 (0-1.76)†

Table 4. Systemic haemodynamic variables over time

Variables	Baseline		30 minutes	
	NTG(n=35)	Placebo(n=35)	NTG(n=35)	Placebo(n=35)
Mean arterial pressure, mm Hg	72±11.5	71±12.0	66±14.7	70±11.8
Heart rate, beats/min	109±16.7	112±17.8	109±18.1	111±20.3
Central venous pressure, mm Hg	12±4	13±5.5	11±4	12±4.7
Cardiac index, l/min . m <sup>2</sup>	4.1±1.3	4.3±1.3	4.1±1.3	4.1±1.2
Pulmonary artery wedge pressure, mm Hg	15±6	16±6	15±6	16±6
Mixed venous oxygen saturation, %	71±8.4	71±6.3	69±8.6	71±8.2
Oxygen consumption, ml/min . m <sup>2</sup>	153±46	165±75	147±49	160±173
Oxygen delivery, ml/min . m <sup>2</sup>	581±168	608±214	554±160	588±173
Oxygen extraction, %	28±8.8	27±7.1	28±8.9	28±7.3
Dopamine dose, n, µg/kg . min	30, 5.4±3.8	30, 6.0±3.5	31, 5.6±3.9	30, 5.8±3.3
Norepinephrine dose, n, µg/kg . min	11, 0.07±0.1	12, 0.05±0.09	17, 0.07±0.1	18, 0.07±0.7
Central-to-toe temperature gradient, °C	5.5±2.6	5.4±2.5	5.2±2.3	5.2±3
Ventilation-perfusion ratio, %	30±10	32±10	33±11	32±10
Lactate, mmol/l	2.8±2.3	2.4±2.1	2.8±2.5	2.3±2
Haematocrit, %	31±4.2	32±6.0	30±4.1	32±5.7
Fluid balance, l	-	-	-	-

NTG nitroglycerin. All data are presented as mean±SD. \*P value <0.05, non-parametric test for independent samples between treatment groups.

(Table 4. Systemic haemodynamic variables over time)

	2 hours		12 hours		24 hours	
	NTG(n=35)	Placebo(n=35)	NTG(n=34)	Placebo(n=35)	NTG(n=32)	Placebo(n=34)
	66±12.2	67±8.9	64±11.7	66±10.8	69±10.7	67±10.7
	108±19.7	106±17.3	108±17.4	105±18.9	109±20.0	108±17.2
	11±4.3	12±4.3	11±4	12±5.5	12±4	12±5.1
	4.1±1.3	3.9±1	4.0±1.2	3.7±0.8	4.2±1.0	4.1±0.87
	14±5	16±5	15±5	16±5	14±6	15±6
	70±8.6	69±7.7	71±10.4	72±5.9	73±7.1	75±6.1
	145±50	151±38	141±37	142±43	147±43	135±33
	542±155	563±158	544±161	540±117	588±159	583±140
	29±9.4	29±7.5	28±11.1	26±6.2	26±6.9	24±7.0
	31,6.1±3.9	32,6.3±3.2	32,6.7±4.0	32,7.0±3.3	31,6.0±4.2	30,7.1±3.6
	18,0.08±0.1	20,0.08±0.2	25,0.12±0.09	25,0.09±0.11	17,0.12±0.12	18,0.12±0.20
	4.9±2.9	6.5±6.1	4.4±1.9*	6.6±3.8	4.3±2.1	5.4±2.5
	29±8	30±8	28±8	28±10	27±8	30±10
	2.6±2.2	2.1±1.7	2.0±1.9	1.8±2.0	1.9±1.6	2.69±4.2
	30±3.8	32±5.3	30±3.9	33±4.8	30±3.9	31±3.8
	-	-	-	-	5.8±2.7	6.5±2.3

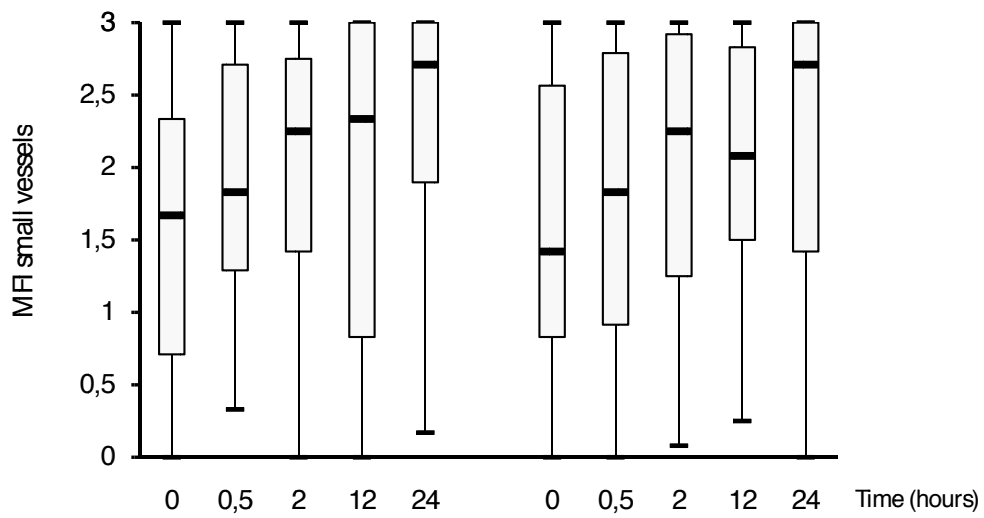
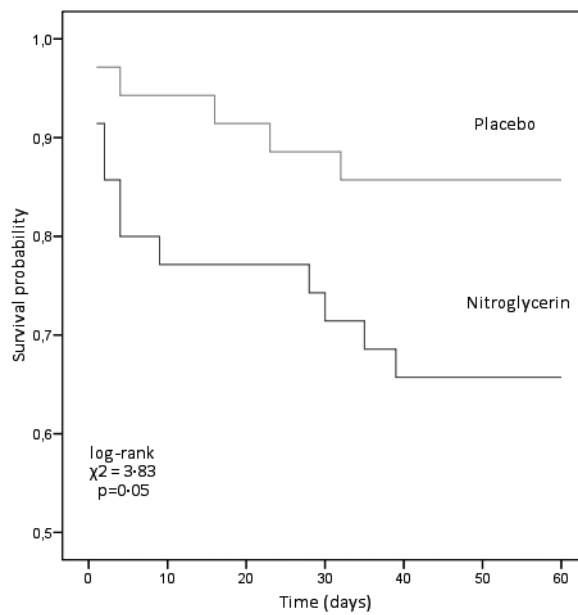


Figure 2. Box plots of sublingual microvascular flow index (MFI) in small vessels (< 20 μm) during the 24-hour study period



Number of survivors							
Placebo	35	33	32	31	30	30	30
NTG	35	27	25	23	23	23	23

Figure 3. Kaplan-Meier plot of survival from randomization to day 60

Table 5. Morbidity and mortality outcome variables

Variables	Nitroglycerin (n=35)	Placebo(n=35)	P Value
ICU mortality, n (%)	11(31.4)	4(11.4)	0.08
Hospital mortality, n (%)	12(34.3)	5(14.2)	0.09
LOS ICU, median (IQR)	8(4-12)	12(7-16)	0.03
LOShospital, median (IQR)	21(8-35)	29(17-48)	0.04
Cumulative SOFA day 1-5	30(22-41)	46(32-53)	0.003
Cumulative SOFA ICU	46(28-80)	66(49-121)	0.02
ARF RIFLE score maximum, median (IQR)	3(0-3)	1(0-3)	0.37
CVVH, use of, n (%)	12(34.3)	11(31.4)	0.87

LOS Length of Stay, SOFA Sequential Organ Failure Assessment, MFI Microvascular Flow Index, ARF Acute Renal Failure, RIFLE Risk Injury Failure Loss and Endstage.

In terms of secondary outcomes, there was no difference in systemichaemodynamic variables between treatment groups, with the exception of a lower central-to-toe temperature gradient in the NTG group in comparison to placebo ( $4.4 \pm 1.9$  and  $6.6 \pm 3.8$  respectively,  $p=0.004$ ) after 12 hours (Table 4). Mean blood pressure, oxygen consumption and arterial lactate levels did not differ at any time. Both groups were administered equivalent doses of dopamine/norepinephrine and no significant difference was recorded in terms of fluid balance after 24 hours (Table 4).

Both for ICU and hospital LOS, as well as for cumulative SOFA scores over the first 5 days of ICU admission, there was a significant reduction in favour of the NTG group (Table 5). It is important to note that the NTG group presented a substantially higher ICU mortality rate, although the difference between the groups remained insignificant (31.4% in the NTG group and 11.4% in the placebo group,  $p=0.08$ ). In addition, the NTG group displayed a higher in-hospital mortality rate, although the difference was insignificant (34.3% in the NTG group and 14.2% in the placebo group,  $p=0.09$ ) (Table 5, Figure 3).

## Discussion

In the present study, we found that protocolised resuscitation resulted in recruitment of sublingual microcirculation, as indicated by a significant improvement in the MFI in comparison to baseline. However, we did not find any additional MFI improvement as a result of treatment with NTG in comparison to placebo in terms of sublingual microcirculatory alterations in patients with severe sepsis or septic shock, with the exception of a very small but significant increment in the proportion of perfused small vessels in the NTG group. In addition, we did not detect any significant differences in systemichaemodynamic parameters between the two treatment strategies, with the exception of a transient significantly lower central-to-toe temperature gradient evidenced in the NTG group.

Our findings are not consistent with a previous report in which 8 patients responded promptly to an intravenous bolus of 0.5 mg NTG after pressure-guided resuscitation with a significant increase in sublingual MFI (8). However, despite use of an equivalent NTG dose, there are marked differences in respect to the study designs. To our knowledge, the present study is the first randomized placebo-controlled trial that has aimed to improve microcirculatory blood flow in patients with severe sepsis/septic shock, after a rigorous resuscitation protocol based upon the principles of early goal-directed therapy and repetitive dynamic testing of fluid-responsiveness. Resuscitation endpoints (minimal mean blood pressure of > 60 mmHg, central venous pressure > 12 mmHg at the lowest dopamine dose) in the previous study did not rule out fluid-responsiveness, nor persistence of heart failure. The fact that MFI of large vessels in the study by Spronk et al. was below 2.5 in the majority of patients and that it responded to NTG to the same extent as in small vessels is consistent with persistent fluid-responsiveness. Specifically, flow in large vessels is maintained despite gross flow alterations in small vessels (13), as was the case in our study. In a recent report, septic patients who had been characterized as responders to fluid expansion in terms of classical systemic haemodynamic parameters demonstrated concomitant improvement of sublingual microcirculatory blood flow (18). Furthermore, treatment of (relative) heart failure by targeting a central venous oxygen saturation  $\geq 70\%$  has been proven to lead to improved outcomes (9). However, despite a reported relation between improvement of the SOFA score and sublingual microcirculatory blood flow (19), a causative relation between optimization of venous oxygen saturation and microcirculatory blood flow remains to be established. Besides these differences in resuscitation endpoints, other factors, such as timing and concomitant medication, may have played a role. The previously reported immediate response to NTG after 5 minutes was outside the scope of our observations and use of the  $5\text{-HT}_2$ -serotonergic receptor blocker ketanserin was not part of our protocol. Indeed, under conditions of local hypoxia, red blood cell scavenging of nitric oxide has been demonstrated to evoke a situation-dependent vasoconstrictive effect in the context of serotonin administration (20).

An important assumption that underpinned our study design was the notion that NTG would act as a nitric oxide (NO) donor. NTG and other organic nitrates are believed to use the same signalling pathway as NO generated by NO synthases (NOS, 21). In this manner, NO-related promotion of vascular smooth muscle relaxation, attenuation of leukocyte-endothelium and leukocyte-platelet interaction (22) and reduction of oedema formation (23) were all considered potential mechanisms to promote microcirculatory blood flow. However, in contrast to isosorbide dinitrate, NTG showed a striking dissociation of its vascular activity and NO-donor properties (24). This dissociation together with a diminished suppression of activity by the NO-scavenger carboxy-PTIO (25) challenges the widely accepted NTG/NO hypothesis.

Apart from the discussed mechanism of action, the net effect of NTG on the delicate balance between vasodilatation and vasoconstriction also depends on mechanisms referred to as tolerance and pseudotolerance. Pseudotolerance involves the dose-dependent, non-NTG specific neurohormonal counter-regulation due to the use of vasodilatory substances and has been reported to occur within 48 hours of administration (23). It includes increases in plasma catecholamine, vasopressin and aldosterone levels, as well as enhanced plasma renin activity (25). NTG-specific hypercontractile responses to angiotensin II and phenylephrine as a result of NTG-induced endothelial dysfunction following chronic NTG exposure is termed nitrate tolerance (26), and is believed to be elicited by oxidative stress (21). NTG treatment has previously been shown to stimulate vascular peroxynitrite formation as a reaction product of NO and superoxide (27,28). Insufficient concentrations of the NOS III cofactor tetrahydrobiopterin may lead to further uncoupling of NOS III, with subsequent superoxide release (29). It is conceivable that, due to previous endothelial dysfunction and a relative lack of substrates in sepsis, situation-dependent formation of peroxynitrite and superoxide instead of NO may occur, even within the timeframe of the present study. The observed absence of differences in both microcirculatory and systemic parameters between NTG and placebo treatment does not disallow the potential absence of NO-mediated vascular relaxation and warrants further study.

Although not designed to detect differences in survival between the NTG and placebo groups, this study revealed an insignificant but substantial difference in absolute numbers with regard to ICU and in-hospital mortality, in favor of the placebo group. However, the observed lower cumulative SOFA scores in the NTG group, do not create an unequivocal picture. Due to our relatively small sample size, an imbalance between the two groups cannot be ruled out. We observed no differences in circulatory parameters that could explain the difference in mortality. The overall in-hospital mortality in both groups was lower than that predicted by the APACHE IV scores: we observed an in-hospital mortality rate of 34.1% in the NTG group, as compared with a predicted  $43\pm 21\%$  rate. In the placebo group, the 14.2% predicted value was lower than the  $39\pm 16\%$  observed metric. Nevertheless, the potential deleterious mechanism of peroxynitrite and superoxide formation due to NTG treatment in these septic patients raises questions about its use. Further scientific examination of this finding is exigent.

Limitations of the present study are largely related to the trial design. Specifically, the complete blinding between the attending physician and the investigator involved the inclusion of all eligible patients, regardless of the extent of their baseline sublingual microcirculatory alterations. Although the MFI at baseline was consistent with our power calculations across both groups, we note our study's consequent loss of discriminative ability due to the inclusion of patients without a significant decrease in baseline MFI. Furthermore, the (predominantly) semi-quantitative methodology used in our analysis of flow and

capillary density may have resulted in the loss of detailed information that was beyond the recording range of our instruments. In this respect, we note that both MFI and heterogeneity index evolved over time, consistent with other reports in the literature (6,17), whereas PPV remained unaltered over time and was considerably higher than that in previous reports (4,5). This discrepancy might be due to differences in resuscitation endpoints. The use of software in this study to accurately measure vessel diameter, instead of discriminating small from large vessels by eye, might also lead to the inclusion of generally better perfused larger vessels in PPV.

In conclusion, our presented data do not support the hypothesis that treatment of patients with severe sepsis or septic shock with intravenous nitroglycerin promotes sublingual microcirculatory blood flow and perfusion. Our results are of note in the context of rigorous resuscitation based on EGDT-derived principles and the dynamic exclusion of fluid-responsiveness.

#### Conflict of interest statement

Professor Ince is CEO of MicroVisionMedical, a university-based company that develops optical spectroscopic tools, such as the SDF imaging methodology used in the present study. He holds patents and shares of relevance to this role. The other authors state that they have no conflicts of interest.

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## References

1. Weil MH, Shubin H: Proposed reclassification of shock states with special reference of distributive defects. *Adv Exp Med Biol* 1971; 23:13-23
2. Groner W, Winkelmann JW, Harris AG, et al: Orthogonal polarization spectral imaging: A new method for study of the microcirculation. *Nat Med* 1999; 5:1209-1212
3. Goedhart PT, Khalilzade M, Bezemer R, et al: Sidestream dark field (SDF) imaging: a novel stroboscopic LED ring-based modality for clinical assessment of the microcirculation. *Optics express* 2007; 15:15101-15114
4. De Backer D, Creteur J, Preiser JC, et al: Microvascular blood flow is altered in patients with sepsis. *Am J Respir Crit Care Med* 2002; 166:98-104

5. Sakr Y, Dubois MJ, De Backer D, et al: Persistent microcirculatory alterations are associated with organ failure and death in patients with septic shock. *Crit Care Med* 2004; 32:1825-1831
6. Trzeciak S, Dellinger PD, Parillo JE, et al: Early microcirculatory perfusion derangements in patients with severe sepsis and septic shock: relationship to hemodynamics, oxygen transport and survival. *Ann Emerg Med* 2007; 49:88-98
7. De Backer D, Creteur J, Dubois MJ, et al: The effects of dobutamine on microcirculatory alterations in patients with septic shock are independent of its systemic effects. *Crit Care Med* 2006; 34:403-8
8. Spronk PE, Ince C, Gardien MJ, et al: Nitroglycerin in septic shock after intravascular volume resuscitation. *Lancet* 2002; 360:1395-1396
9. Rivers E, Nguyen B, Havstad S, et al: Early goal-directed therapy in the treatment of severe sepsis and septic shock. *N Engl J Med* 2001; 345:1368-1377
10. Levy MM, Fink MP, Marshall JC, et al: International Sepsis Definitions Conference. *Intensive Care Med* 2003; 29:530-538
11. Boerma EC, Mathura KR, van der Voort PHJ, et al: Quantifying bedside-derived imaging of microcirculatory abnormalities in septic patients: a prospective validation study. *Critical Care* 2005; 9:R601-606
12. Dobbe JGG, Streekstra GJ, Atasever B, et al: Measurement of functional microcirculatory geometry and velocity distributions using automated image analysis. *Med Biol Eng Comput* 2008; 46:659-670
13. De Backer D, Hollenberg S, Boerma EC, et al: How to evaluate the microcirculation: report of a round table conference. *Critical Care* 2007; 11:R101
14. Zimmerman JE, Kramer AA, McNair DS, et al: APACHE IV: hospital mortality assessment for today's critically ill patients. *Crit Care Med* 2006; 34:1297-1310
15. Vincent JL, de Mendonca A, Cantraine F, et al: Use of the SOFA score to assess the incidence of organ dysfunction/failure in intensive care units: results of a multicenter, prospective study. Working group on "sepsis-related problems" of the European Society of Intensive Care Medicine. *Crit Care Med* 1998; 26:1793-1800

16. Bellomo R, Ronco C, Kellum JA, et al: Acute renal failure - definition, outcome measures, animal models, fluid therapy and information technology needs: the second international consensus conference of the acute dialysis quality initiative (ADQI) group. *Critical Care* 2004; 8:R204
17. Boerma EC, van der Voort PHJ, Spronk PE, Ince C. Relationship between sublingual and intestinal microcirculatory perfusion in patients with abdominal sepsis. *Crit Care Med* 2007; 35:1055-1060
18. Pottecher J, Deruddre S, Georger JF, et al: Both passive leg raising and volume expansion improve sublingual microcirculation in preload-dependent septic patients. *Intensive Care Med* 2008; 34(suppl 1):S103
19. Trzeciak S, McCoy JV, Dellinger PR, et al: Early increases in microcirculatory perfusion during protocol-directed resuscitation are associated with reduced multi-organ failure at 24 h in patients with sepsis. *Intensive Care Med* 2008; 34:2210-2217
20. Han TH, Qamirani E, Nelson AG, et al: Regulation of nitric oxide consumption by hypoxic red blood cells. *Proc Natl Acad Sci U S A* 2003; 100:12504-12509
21. Münzel T, Daiber A, Mülsch A: Explaining the phenomenon of nitrate tolerance. *Circ Res* 2005; 97:618-628
22. Kurose I, Wolf R, Grisham MB, Granger DN. Modulation of ischemia/reperfusion-induced microvascular dysfunction by nitric oxide. *Circ Res* 1994; 74:376-382
23. Kleschyov AL, Oelze M, Daiber A, et al: Does nitric oxide mediate the vasodilatory activity of nitroglycerin? *Circ Res* 2003; 93:e104-112
24. Pieper GM, Siebeneich W: Use of nitronyl nitroxide to discriminate the contribution of nitric oxide radical in endothelium-dependent relaxation of control and diabetic blood vessels. *J Pharmacol Exp Ther* 1997; 283:138-147
25. Parker JD, Farrell B, Fenton T, et al: Counter-regulatory responses to continuous and intermittent therapy with nitroglycerin. *Circulation* 1991; 84:2336-2345
26. Heitzer T, Just H, Brockhoff C, et al: Long-term nitroglycerin treatment is associated with supersensitivity to vasoconstrictors in men with stable coronary artery disease: prevention by concomitant treatment with captopril. *J Am Coll Cardiol* 1998; 31:83-88

27. Mihm MJ, Coyle CM, Jing L, Bauer JA: Vascular peroxynitrite formation during organic nitrate tolerance. *J Pharmacol Exp Ther* 1999; 291:194-198
28. Hink U, Oelze M, Kolb Ph, et al: Role for peroxynitrite in the inhibition of prostacyclin synthase in nitrate tolerance. *J Am Coll Cardiol* 2003; 42:1826-1834
29. Stroes ES, van Faassen EE, Yo M, et al: Folic acid reverts dysfunction of endothelial nitric oxide synthase. *Circ Res* 2000; 86:1129-1134



# Chapter 7

## Low incidence of rectal microcirculatory alterations after elective on-pump cardiac surgery

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Submitted

## Abstract

### Introduction.

Hemodynamic changes, related to on-pump cardiac surgery, have been reported to impair intestinal perfusion. Intestinal tonometry has been the only clinical available monitoring reported to detect splanchnic ischemia. We tested the hypothesis that splanchnic ischemia may be associated with splanchnic microcirculatory alterations. To this end we used Sidestream Dark Field (SDF) imaging in combination with rectal tonometry at the same time-point to investigate if rectal microcirculatory alterations occur with splanchnic ischemia as detected by rectal tonometry following elective cardiac surgery.

### Methods.

A single-centre prospective observational study was conducted in postoperative elective cardiac surgery patients, within 30 minutes after ICU admittance. In group 1 (n=28) simultaneously SDF imaging and automated gas tonometry was performed in the rectal pouch. In group 2 (n=10) sublingual SDF was performed.

### Results.

In the rectal pouch median Microvascular Flow Index (MFI) was 3(3-3) and proportion of perfused vessels (PPV) 85% (72-93). Median rectal-to-arterial partial carbon dioxide pressure difference was 0.2 (-0.2-1.1) kPa; 6 (21%) patients had a  $\Delta P_{\text{CO}_2} > 1.1$  kPa, among them 2 (7%)  $> 1.4$  kPa. In the sublingual region median MFI was 3(3-3); median PPV was 99% (97-100) and higher in comparison to rectal PPV,  $p = 0.001$ .

### Conclusions.

Simultaneous SDF-imaging and automated gas tonometry in the rectal pouch seems feasible in postoperative sedated patients. Despite many hemodynamic changes during elective on-pump cardiac surgery, the observed incidence of SDF-derived microcirculatory alterations in combination with elevated rectal-to-arterial  $p\text{CO}_2$  gap, was found to be low in the early postoperative ICU-period in this patient group. Extending microcirculatory in-vivo observation to the sublingual region did not disclose marked discordance of microcirculatory blood flow between the different microvascular beds.

### Trial registration.

ClinicalTrials.gov NCT00547859

## Introduction

During on-pump cardiac surgery patients are subject to a number of hemodynamic changes, associated with intestinal hypoperfusion [1]. With the exception of controlled hypotension [2], hemodilution [3], hypothermia [4], shifts from pulsatile to continuous blood flow [5], systemic inflammation [6,7] and use of vasopressors [8] have all been reported to impair intestinal perfusion, as a result of distributive flow alterations. Until recently, intestinal tonometry was the only clinical available monitoring device to detect splanchnic ischemia. Application of this technique in stomach and rectal pouch [9,10] and combination with intestinal endoluminal microdialysis [11] appeared to be feasible. Although the presence of splanchnic ischemia is of predictive value for postoperative complications in cardiac surgery [12-14], practical drawbacks as well as the need for a more fundamental understanding of gastro-intestinal pathophysiology, have hampered the use of tonometry as routine monitoring tool [15].

Over the last year's direct in-vivo observation of the microcirculation, by means of Orthogonal Polarization Spectral imaging and its technical successor Sidestream Dark Field (SDF) imaging, has become available at the bedside [16,17]. Application of these techniques in rectal pouch [18] and surgical intestinal stomas [19], as well as combination with gastric and intestinal capnometry [20,21] has been described. SDF-derived detection of heterogeneous microcirculatory blood flow abnormalities, as a result of distributive flow alterations in sepsis, is now well established [22]. However, little is known about the incidence of such intestinal flow alterations in relation to cardiac surgery. Several reports indicate the (transient) presence of sublingual microcirculatory alterations during cardiopulmonary bypass [23-25]. Whether these data also apply for the intestinal tract is unknown.

We tested the hypothesis that splanchnic ischemia may be associated with splanchnic microcirculatory alterations after cardiopulmonary bypass. To this end we used rectal Sidestream Dark Field (SDF) imaging in combination with rectal tonometry at the same time-point to investigate if rectal microcirculatory alterations occurs with splanchnic ischemia as detected by rectal tonometry following elective on-pump cardiac surgery.

## Material and methods

### Setting and patient selection.

The study was performed between November 2007 and April 2008 in a closed-format 22-bed mixed ICU in a tertiary teaching-hospital. It was designed as a prospective, single centre, observational, clinical trial and registered with ClinicalTrials.gov NCT00547859. Group 1 consisted of patients of at least 18 years of age scheduled for coronary artery bypass grafting and/or cardiac valve surgery with cardiopulmonary bypass (CPB). Patients with a medical history of potential influence on the vascularisation of the recto-sigmoid area (operation, local radiotherapy, inflammation) were excluded. Patients with considerable

fecal contamination of the rectal pouch at the time of measurement were also excluded. After analysis of the initial results group 2 was added to the study, in order to compare rectal SDF imaging with the more commonly reported sublingual SDF imaging. For this second, post-hoc group, the same inclusion criteria were applicable, with the exception of the rectal-pouch-specific contraindications. Availability of a member of a group of 2 investigators, trained in SDF imaging and tonometry, was a leading premise during the study period. A local ethical and scientific committee approved of the study protocol and written informed consent was obtained from patients preoperatively, according to applicable laws.

#### Anesthesia, CPB and peri-operative management.

Anesthesia was intravenously induced with sufentanil 1-2  $\mu\text{g}/\text{kg}$ , midazolam 0.1 mg/kg and rocuronium 0.1-0.5 mg/kg. In case of poor left ventricle function enoximone 0.25 mg/kg was added. Dexamethasone 1 mg/kg was administered to all patients. An 8.5 Fr four-lumen central venous catheter (BD Careflow, BD medical Systems, Singapore, Singapore) was inserted into the right internal jugular vein. In case of a poor right or left ventricle function or cardiac valve operation a pulmonary artery catheter (Vigilance®, Edwards Lifesciences, Saint-Prex, Switzerland) was inserted. During the operation intranasal, rectal and skin temperature was monitored. Maintenance of anesthesia during the operation was achieved with sufentanil 1  $\mu\text{g}/\text{kg}$ , sevoflurane (1.5-3%) during pre-bypass period and propofol during CPB period. After standard median sternotomy, aortic root and right atrial cannulation, non-pulsatile CPB with a heart-lung machine (HL-30, Maquet Jostra Medizintechnik AG, Hirrlingen, Germany) was established at a target systemic blood flow of 2.7 litre  $\text{min}^{-1} \text{m}^{-2}$  and adjusted at a minimum central venous saturation of 65%. Surgery was performed under mild hypothermia (32-34 °C). After aortic cross-clamping crystalloid or blood cardioplegia was administered depending on the attending surgeon. Arterial perfusion pressure was kept between 50 and 70 mmHg, using phenylephrine if necessary. Anticoagulation was established with heparin (300-400 IE/kg) intravenously, 10 minutes before initiation of CPB. Target activated clotting time was 450 sec. At the end of CPB, anticoagulation was antagonized with protamine intravenously (1.33 x initial heparin dose). Hematocrit was kept above 0.20. Postoperative patients received routine institutional ICU-treatment including tight glucose control and low tidal volume during initial pressure control ventilation, aiming at early extubation unless clinically unwarranted.

#### Tonometry.

Within 30 minutes after ICU admittance data were obtained. For measurement of regional carbon dioxide production in the intestinal wall an 18F tonometric catheter (Tonometrics®, Datex-Ohmeda, Helsinki, Finland) was placed into the rectal pouch blindly, after manual exclusion of considerable fecal contamination. Use of enema and lubricating gel was avoided. Following insertion of the catheter into the rectal pouch a sampling line was connected to the automated air tonometry system (Tonocap®, Datex-Ohmeda, Helsinki, Finland). After 10

minutes air-filled equilibration, rectal  $p\text{CO}_2$  ( $\text{Pr}_{\text{CO}_2}$ ) was sampled and analyzed automatically, using infrared spectroscopy. At the same time arterial blood gas analysis was performed for arterial  $p\text{CO}_2$  ( $\text{Pa}_{\text{CO}_2}$ ) measurement.  $\text{CO}_2$  gap ( $\Delta\text{P}_{\text{CO}_2}$ ) was calculated as  $\text{Pr}_{\text{CO}_2} - \text{Pa}_{\text{CO}_2}$ .

#### SDF-imaging and analysis procedure.

Directly after measurement of  $\text{Pr}_{\text{CO}_2}$  SDF imaging was performed. SDF is a stroboscopic light emitting diode ring-based imaging modality, incorporated in a hand-held device, that illuminates an area of interest for the clinical observation of the microcirculation and is validated in comparison with its technical predecessor orthogonal polarization spectral imaging [17]. If a wavelength within the haemoglobin 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 its imaging therefore depends on the presence of red blood cells. Semi-quantitative analysis was performed as described in detail elsewhere [26]. In short, a minimum of 3 steady images of at least 20 seconds were obtained from the sublingual region by a separate research investigator, other than the attending physician. After gentle removal of saliva/faeces by irrigation with isotonic saline and avoiding pressure artefacts, images were obtained and stored on digital videotape (SONY video walkman GV-D 1000E<sup>®</sup>, Sony, Tokyo, Japan). Subsequently, images were captured in 5-10 second representative video clips in avi format (sonyDVgate<sup>®</sup>, Sony, Tokyo, Japan). Video clips were analyzed off-line blindly by an investigator not involved in data collection and in random order to prevent coupling between images. SDF images were obtained from three different locations within the region of interest, and each image is divided into 4 equal quadrants. Quantification of flow (no flow: 0, intermittent flow: 1, sluggish flow: 2 and continuous flow: 3) was scored per quadrant. In the sublingual region this was performed for small vessels (< 20  $\mu\text{m}$ ); due to its anatomy (crypts), the microvascular bed in the rectal pouch only consists of small vessels. The score, called microvascular flow index (MFI), is the sum of each quadrant-score, divided by the number of quadrants in which the vessel type is visible. The overall MFI is an average score over a maximum of 12 quadrants (3 regions times 4 quadrants per region) derived from the overall flow impression of all vessels with a particular range of diameter in a given quadrant. Proportion of perfused small vessels (PPV) in the sublingual region was defined as percentage of crossings with perfused small vessels per total length of 3 equidistant horizontal and 3 equidistant vertical lines, in accordance with reports of a round table conference [27]. Since crypts are well defined microvascular units, as opposed to a microcirculatory network in the sublingual region, PPV in the rectal pouch was adapted as the percentage of perfused crypts, divided by the total number of crypts  $\times 100\%$ . Vessels density parameters were considered inapplicable to the rectal microvasculature.

### Protocol and data collection.

Demographic data, CPB and aortic cross-clamp duration, lowest hematocrit and use of vasopressor/inotropic agents during operation were documented. In-hospital mortality was predicted using the European System for Cardiac Operative Risk Evaluation (Euro SCORE) [28]. Systemic hemodynamic assessment was obtained by continuous invasive monitoring of arterial blood pressure and central venous catheterisation. During the time of tonometry and SDF-imaging blood for standard laboratory tests, including blood gases, arterial lactate concentrations and central venous oxygen saturation was sampled. Number of days of mechanical ventilation, ICU- and hospital length of stay as well as in-hospital mortality were documented.

### Statistical analysis.

Data are presented as median and interquartile ranges (IQR). The statistical package for social sciences (SPSS 15.1 for Windows, Chicago Illinois, USA) was used for statistical analysis. For comparison between groups a non-parametrical test (Mann-Whitney) was used because of a small number of patients. A two-sided p value of  $<0.05$  was considered statistically significant.

## Results

In group 1 (rectal SDF/tonometry, n=28) 50 patients were screened for eligibility; 3 patients withheld informed consent, 2 patients had a history of rectocolitis and 17 were excluded, due to faecal contamination of the rectal pouch. In group 2 (sublingual SDF, n=10) all eligible patients were enrolled. Baseline characteristics and clinical data of both groups are summarized in table 1. In group 1 six patients were mechanically ventilated for more than 1 day; 4 because of primary respiratory failure, 2 as a consequence of heart failure. In group 2 one patient developed multiple organ failure after re-sternotomy for cardiac tamponade. Abdominal complications were not reported and all patients survived to hospital discharge.

Results are summarized in table 2. In the rectal pouch incidence of microcirculatory flow alterations was almost absent: median MFI was 3(3-3). Median PPV, as parameter of microcirculatory perfusion, was 85% (72-93). Rectal tonometry detected elevated intestinal  $\text{CO}_2$  production in a minority of patients. Median  $\Delta P_{\text{CO}_2}$  was 0.2 (-0.2-1.1) kPa; 6 (21%) patients had a  $\Delta P_{\text{CO}_2} > 1.1$  kPa, among them 2 (7%)  $> 1.4$  kPa.

In the sublingual region SDF-imaging did not detect microcirculatory flow alterations: median MFI 3(3-3). Median PPV in the sublingual region was 99% (97-100); this was significantly higher in comparison to median rectal PPV,  $p = 0.001$ .

Table 1. Baseline characteristics and clinical data.

Variables	Group 1(n=28)	Group 2(n=10)	p Value
Men, n(%)	25(89)	8(80)	0.46
Age	64(59-72)	71(64-71)	0.14
EuroSCORE	3.5(2-5)	5(2-8)	0.42
APACHE II	12(10-16)	12(9-14)	0.51
SOFA	4(3-5)	4(3-4)	0.49
CPB duration, min	122(87-142)	103(72-123)	0.25
Aorta cross-clamp time, min	81(58-90)	77(53-85)	0.59
Lowest hematocrit during CPB, %	25(23-27)	23(22-26)	0.33
Type of operation, n(%)			
CABG alone	25(89)	7(70)	-
Heart valve surgery	2(7)	1(10)	-
CABG + heart valve surgery	1(4)	2(20)	-
Mechanical ventilation, days	1(1-1)	1(1-1)	0.66
ICU stay, days	1(1-2)	1(1-1)	0.42
Postoperative in-hospital stay, days	6(5-9)	7(4-14)	0.68
Hospital mortality, n(%)	0(0)	0(0)	-

EuroSCORE, European System for Cardiac Operative Risk Evaluation; APACHE, Acute Physiology And Chronic Health Evaluation; SOFA, Sequential Organ Failure Assessment; CPB, CardioPulmonary Bypass; CABG, Coronary Arterial Bypass Grafting. Data are presented as median (IQR) unless stated otherwise. Group 1, rectal SDF/tonometry; group 2, sublingual SDF.

Table 2. SDF imaging/tonometric parameters and simultaneous hemodynamic variables.

Variables	Group 1(n=28)	Group 2(n=10)	p Value
Mean arterial pressure, mmHg	82(75-90)	79(70-90)	0.33
Heart rate, beats/min	90(90-93)	98(90-104)	0.02
Central venous pressure, mm Hg	10(7-12)	7(6-10)	0.04
Central venous oxygen saturation, %	72(66-75)	71(61-74)	0.73
Use of inotropes, n(%)	4(14)	1(10)	-
Use of vasopressors, n(%)	7(25)	1(10)	-
Use of intravenous nitroglycerin, n(%)	16(57)	8(80)	-
Central (oesophagus) temperature, °C	36(35-36)	36(36-37)	0.002
Hematocrit, %	28(26-29)	29(25-33)	1.0
Arterial lactate concentration, mmol/L	2.4(1.7-3.2)	2.2(1.7-3.9)	0.69
Microvascular Flow Index	3(3-3)	3(3-3)	0.37
Proportion of Perfused Vessels, %	85(72-93)	99(97-100)	0.001
$\Delta P_{CO_2}$ , kPa	0.2(-0.2+1.1)	-	-

SDF, Sidestream Dark Field;  $\Delta P_{CO_2}$ , rectal - arterial carbon dioxide partial pressure. Data are presented as median (IQR) unless stated otherwise. Group 1, rectal SDF/tonometry; Group 2, sublingual SDF.

## Discussion

The incidence of microcirculatory flow alterations in the rectal pouch of on-pump postoperative cardiac surgery patients was very low. The observed MFI is in line with previously reported intestinal microcirculatory blood flow in healthy controls [19]. Rectal PPV, as parameter of perfusion, was significantly lower in comparison to sublingual PPV. Previously reported sublingual PPV for healthy controls was within the same range as in this study [29]. This might indicate that, after CPB-supported cardiac surgery, intestinal microcirculatory perfusion is more susceptible to alterations than microcirculatory blood flow. However, the observed absence of elevated  $\Delta P_{\text{CO}_2}$  seems to indicate that critical perfusion deficits, leading to tissue dysoxia, are unlikely to persist in the postoperative period. Post-hoc addition of group 2, in which sublingual SDF-derived microcirculatory alterations were almost absent as well, makes gross heterogeneity between different microvascular beds, as observed in sepsis [19], less probable.

The observed rectal tonometry results seem to be in agreement with an earlier report. Fisher et al. observed a mean rectal-to-arterial  $p\text{CO}_2$  gap of 2 kPa within a 4 hour postoperative period in cardiac surgery patients [10]. In a comparable patient group the median postoperative gastric-to-arterial  $p\text{CO}_2$  gap was 0.8 (0.13-1.46) kPa [14]. This seems to be in discordance with an earlier reported 70% incidence of elevated  $\Delta P_{\text{CO}_2}$  ( $> 1.1$  kPa), detected by gastric tonometry in postoperative elective cardiac surgery, with a predictive value for postoperative complications [13]. However, direct comparison between rectal and gastric tonometry revealed poor agreement, both in cardiac [10] and general pediatric surgery [9]. Local elevated  $p\text{CO}_2$  levels, as a result of enteral luminal gas production have been suggested as a major source for this disagreement. In the presented study, performance of simultaneous rectal tonometry and direct in-vivo observation of the microcirculation with SDF in the same vascular compartment, appeared to be feasible and adds to the understanding that the observed low incidence of increased  $\Delta P_{\text{CO}_2}$  truly reflects adequate rectal microcirculatory blood flow. It is of note that this does not rule out intestinal organ failure as a result of hypoxic hypoxia, as both tonometry [30] and SDF-imaging may fail to detect this. Hypoxic hypoxia, as a result of hemodilution, may therefore explain a previously observed increase of rectal endoluminal lactate levels during and after routine cardiac surgery [11], rather than microcirculatory flow alterations.

There are several limitations to the study. Semi-quantitative assessment of microcirculatory blood flow and perfusion entails the risk of losing delicate details. Furthermore, measurement of SDF-derived parameters related to capillary density, as suggested for optimal microcirculatory assessment [27], has not yet been validated for rectal mucosa. We specifically designed the study to perform SDF-imaging and tonometry simultaneously in the same compartment, but this also limits the scope of the data, both in space and time. We tried to extend this by sublingual SDF-imaging in a second group, with the risk of introducing imbalances between groups. However, simultaneous data acquisition in both

compartments is practically impossible and devices for clinical sublingual tonometry are not commercially available. Rectal SDF-imaging during CPB was considered practically infeasible. Due to lack of extremes, both in SDF and tonometry parameters, correlations between the two techniques and/or clinical outcome could not be determined.

## Conclusions

Despite many hemodynamic changes during elective on-pump cardiac surgery, the observed incidence of SDF-derived microcirculatory alterations in combination with elevated rectal-to-arterial  $p\text{CO}_2$  gap, appeared to be low in the early postoperative ICU-period in this patient group. Extending microcirculatory in-vivo observation to the sublingual region did not disclose marked discordance of microcirculatory blood flow between the different microvascular beds. Simultaneous SDF-imaging and automated air tonometry in the rectal pouch seems feasible in postoperative sedated patients.

### Key messages

- Incidence of intestinal microvascular alterations, as detected by combined rectal SDF-imaging and -tonometry, is low in postoperative elective on-pump cardiac surgery patients
- Under these specific circumstances, supplementary direct observation of the sublingual microcirculation did not reveal marked heterogeneity between the intestinal and sublingual microvascular compartment
- Simultaneous performance of SDF-imaging and tonometry in the rectal pouch is feasible

### List of abbreviations

SDF	Sidestream Dark Field
CPB	CardioPulmonary Bypass
$\text{Pr}_{\text{CO}_2}$	rectal partial carbon dioxide pressure
$\text{Pa}_{\text{CO}_2}$	arterial partial carbon dioxide pressure
$\Delta\text{P}_{\text{CO}_2}$	rectal-to-arterial partial carbon dioxide pressure difference
MFI	Microvascular Flow Index
PPV	Proportion of Perfused Vessels
Euro SCORE	European System for Cardiac Operative Risk Evaluation
IQR	InterQuartile Range
APACHE	Acute Physiology And Chronic Health Evaluation
SOFA	Sequential Organ Failure Assessment
CABG	Coronary Arterial Bypass Grafting

### Competing interests

CI is CEO of MicroVision Medical, a university based company that develops optical spectroscopic tools, such as the SDF imaging methodology used in the present study. He holds patents and shares of relevance to this role. The other authors state that they have no conflicts of interest.

### Author's contribution

CB and CI designed the trial

KK and AK performed SDF imaging and tonometry

CB, AG, JV, HB and CI analyzed the data

CB wrote the draft

All authors read and approved the final manuscript.

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## References

1. Ascione R, Talpahewa S, Rajakaruna C, Reeves BC, Lovell AT, Cohen A, Angelini GD: Splanchnic organ injury during coronary surgery with or without cardiopulmonary bypass: a randomized controlled trial. *Ann Thorac Surg* 2006, 81:97-103.
2. Fukusaki M, Nakamura T, Hara T, Fukushima H, Hasuo H, Sumikawa K: Splanchnic perfusion during controlled hypotension with haemodilution under iso urane anaesthesia in elderly patients. *Eur J Anaesthesiol* 1999, 16:519-525.
3. Thorén A, Nygren A, Houtz E, Ricksten SE: Cardiopulmonary bypass in humans--jejunal mucosal perfusion increases in parallel with well-maintained microvascular hematocrit. *Acta Anaesthesiol Scand*. 2005, 49:502-9.
4. Croughwell ND, Newman MF, Lowry E, Davis RD Jr, Landolfo KP, White WD, Kirchner JL, Mythen MG: Effect of temperature during cardiopulmonary bypass on gastric mucosal perfusion. *Br J Anaesth* 1997, 78:34-38.
5. Nagaoka H, Innami R, Watanabe M, Satoh M, Murayama F, Funakoshi N: Preservation of pancreatic beta cell function with pulsatile cardiopulmonary bypass. *Ann Thorac Surg* 1989, 48:798-802.
6. Tofukuji M, Stahl GL, Metais C, Tomita M, Agah A, Bianchi C, Fink MP, Sellke FW: Mesenteric dysfunction after cardiopulmonary bypass: role of complement C5a. *Ann Thorac Surg* 2000, 69:799-807.

7. Wan S, LeClerc JL, Vincent JL: Inflammatory response to cardiopulmonary bypass: mechanisms involved and possible therapeutic strategies. *Chest* 1997, 112:676-92.
8. O'Dwyer C, Woodson LC, Conroy BP, Lin CY, Deyo DJ, Uchida T, Johnston WE: Regional perfusion abnormalities with phenylephrine during normothermic bypass. *Ann Thorac Surgery* 1997, 63:728-735.
9. Weiss M, Schmitz A, Salgo B, Dullenkopf A: Rectal luminal Pr(CO<sub>2</sub>), measured by automated air tonometry, does not reflect gastric luminal Pr(CO<sub>2</sub>) in children. *J Anesth* 2006, 20:243-246.
10. Fisher EM, Kerr ME, Hoffman LA, Steiner RP, Baranek RA: A comparison of gastric and rectal CO<sub>2</sub> in cardiac surgery patients. *Biol Res Nurs* 2005, 6:268-280.
11. Solligård E, Wahba A, Skogvoll E, Stenseth R, Grønbech JE, Aadahl P: Rectal lactate levels in endoluminal microdialysate during routine coronary surgery. *Anaesthesia* 2007, 62:250-258.
12. Fiddian-Green RG, Baker S: Predictive value of the stomach wall pH for complications after cardiac operations: comparison with other monitoring. *Crit Care Med* 1987, 15:153-156.
13. Bennett-Guerrero E, Panah MH, Bodian CA, Methiakalm BJ, Alfarone JR, DePerio M, Mythen MG: Automated detection of gastric luminal partial pressure of carbon dioxide during cardiovascular surgery using the tonocap. *Anesthesiology* 2000, 92:38-45.
14. Kavarana MN, Frumento RJ, Hirsch AL, Oz MC, Lee DC, Bennett-Guerrero E: Gastric hypercarbia and adverse outcome after cardiac surgery. *Intensive Care Med* 2003, 29:742-748.
15. Hamilton MA, Mythen MG: Gastric tonometry: where do we stand? *Curr Opin Crit Care* 2001, 7:122-127.
16. Groner W, Winkelman JW, Harris AG, Ince C, Bouma GJ, Messmer K, Nadeau RG: Orthogonal polarization spectral imaging: A new method for study of the microcirculation. *Nat Med* 1999, 5:1209-1212.
17. Goedhart PT, Khalilzada M, Bezemer R, Merza J, Ince C: Sidestream Dark Field (SDF) imaging: a novel stroboscopic LED ring-based imaging modality for clinical assessment of the microcirculation. *Optics Express* 2007, 15:15101-15114.
18. Dondorp AM, Ince C, Charunwatthana P, Hanson J, van Kuijen A, Faiz MA, Rahman MR, Hasan M, Bin Yunus E, Ghose A, Ruangveerayut R, Limmathurotsakul D, Mathura K, White NJ, Day NP: Direct in vivo assessment of microcirculatory dysfunction in severe falciparum malaria. *J Infect Dis* 2008, 197:79-84.

19. Boerma EC, van der Voort PHJ, Spronk PE, Ince C: Relationship between sublingual and intestinal microcirculatory perfusion in patients with abdominal sepsis. *Crit Care Med* 2007, 35:1055-1060.
20. Creteur J, De Backer D, Sakr Y, Koch M, Vincent JL: Sublingual capnometry tracks microcirculatory changes in septic patients. *Intensive Care Med* 2006, 32:516-23.
21. Dubin A, Edul VS, Pozo MO, Murias G, Canullán CM, Martins EF, Ferrara G, Canales HS, Laporte M, Estenssoro E, Ince C: Persistent villi hypoperfusion explains intramucosal acidosis in sheep endotoxemia. *Crit Care Med* 2008, 36:535-42.
22. Ince C: The microcirculation is the motor of sepsis. *Critical Care* 2005, 9(suppl 4):S13-S19.
23. Bauer A, Koer S, Thiel M, Eifert S, Christ F: Monitoring of sublingual microcirculation in cardiac surgery using orthogonal polarization spectral imaging: preliminary results. *Anesthesiology* 2007, 107:939-45.
24. Den Uil CA, Lagrand WK, Spronk PE, van Domburg RT, Hoand J, Lüthen C, Brugts JJ, van der Ent M, Simoons ML: Impaired sublingual microvascular perfusion during surgery with cardiopulmonary bypass: a pilot study. *J Thorac Cardiovasc Surg* 2008, 136:129-34.
25. Maier S, Hasibeder WR, Hengl C, Pajk W, Schwarz B, Margreiter J, Ulmer H, Engl J, Knotzer H: Effects of phenylephrine on the sublingual microcirculation during cardiopulmonary bypass. *Br J Anaesth* 2009, e-pub ahead of publishing. Doi:10.1093/bja/eap018
26. Boerma EC, Mathura KR, van der Voort PHJ, Spronk PE, Ince C: Quantifying bedside-derived imaging of microcirculatory abnormalities in septic patients: a prospective validation study. *Critical Care* 2005, 9:R601-R606.
27. De Backer D, Hollenberg S, Boerma C, Goedhart P, Ospina-Tascon G, Dobbe I, Ince C: How to evaluate the microcirculation: report of a round table conference. *Critical Care* 2007, 11:R101.
28. Roques F, Nashef SA, Michael P, Gauducheau E, de Vincentiis C, Baudet E, Cortina J, David M, Faichney A, Gabrielle F, Gams E, Harjula A, Jones MT, Pintor PP, Salamon R, Thulin L: Risk factors and outcome in European cardiac surgery: analysis of the EuroSCORE multination database of 19030 patients. *Eur J Cardiothorac Surg* 1999, 15:816-823.
29. De Backer D, Creteur J, Dubois MJ, Sakr Y, Vincent JL: Microvascular alterations in patients with acute severe heart failure and cardiogenic shock. *Am Heart J* 2004, 147:91-97.

30. Dubin A, Murias G, Estenssoro E, Canales H, Badie J, Pozo M, Sottile JP, Barán M, Pálizas F, Laporte M: Intramucosal-arterial PCO<sub>2</sub> gap fails to reflect intestinal dysoxia in hypoxic hypoxia. *Critical Care* 2002, 6:514-520.



# Chapter 8

The microcirculation as a clinical concept;  
work in progress

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## Abstract

### Purpose of review.

The present review discusses the evolution of the microcirculation from a theoretical idea to a clinical concept, as a result of the introduction of direct in-vivo observation techniques.

### Recent findings.

Technical proceedings in the acquisition and assessment of microcirculatory imaging are described, as well as the first report of in-vivo mitochondrial  $pO_2$  measurements. Experimental data on immune-tolerance, leukocyte dynamics, vascular permeability and regional hypoxia have contributed to unravel the complex origin of microcirculatory alterations. Several reports have highlighted the concept of heterogeneity of microcirculatory blood flow, observed within and between different microvascular beds. The previously reported prognostic value of microcirculatory alterations has now been expanded to the early phase of sepsis and in postoperative patients. Finally, a list of interventions in experimental and clinical settings is discussed with regard to their potency to influence microcirculatory changes in shock.

### Summary.

Direct in-vivo observation of the microcirculation has enabled the construction of microcirculatory failure as a clinical concept in the critically ill. Aiming for pro-microcirculatory recruitment strategies in order to improve outcome will be the challenge for the near future.

### Keywords.

Microcirculation, side-stream dark field, sepsis, heterogeneity.

## The microcirculation as a clinical concept; work in progress

### Introduction

The Platonistic idea of the microcirculation as a physiological compartment, in which life-conditional processes such as the exchange of gasses, nutrients and waste products take place, exists from the days of Harvey and Malpighi. In clinical practice however, the microcirculation remained a black box, which physiologic activities at best could be estimated from more easily accessible upstream and downstream circulatory parameters. This changed after the introduction of the Orthogonal Polarization Spectral (OPS) imaging technique, that enabled direct in-vivo observation of the microcirculation in clinical research [1]. Under different conditions, microcirculatory alterations could now be observed in critically ill patients [2,3] and the persistence of such alterations appeared to be predictive for morbidity and mortality, irrespective of systemic hemodynamics [4,5]. Furthermore, OPS-derived microcirculatory perfusion abnormalities could be linked to hypoxic metabolic responses and therapeutic interventions [6], thus providing a powerful tool to unravel the potential pathophysiological role of the microcirculation in shock. The present review will focus on recent proceedings in microcirculatory research as a clinical concept and discuss its potential influence in future management of the critically ill.

#### Techniques and quantification of images

A high level of sensibility to potential methodological errors in the acquisition and assessment of microcirculatory images is of utmost importance, as touched upon in an animal study, comparing laser-Doppler and OPS to detect mesenteric hypoperfusion [7]. It was with this intention that a round table conference was held to discuss a whole array of practical and theoretical issues that need to be addressed adequately before conclusions can be drawn from OPS-derived data [8]. Among many topics avoidance of pressure-artefacts seemed paramount to all participants and recommendations were provided. Furthermore, drawbacks from semi-quantitative assessment of both parameters of convexity and diffusion were discussed. At that time, the ideal quantification of microcirculatory perfusion by a parameter, equal to the classically used functional capillary density, was still hampered by lack of reliable automated software analysis in combination with the need for further hardware development. Since then several developments seem to meet these drawbacks in partial. Side stream dark-field (SDF) has become the technical successor of OPS and is now validated against it [9]. It consists of a stroboscopic LED ring-based imaging modality, based upon the same physical principle as OPS: the absorption of green light by the hemoglobin content of red blood cells. Overall measurement of diameter demonstrated excellent correlation between the two techniques; at the same time SDF-imaging improved capillary sharpness and contrast in comparison to OPS, allowing better density measurements in

the most crucial part of the microcirculation. Secondly, a software package has become available, that enables automated measurement of functional microcirculatory geometry and velocity distributions [10]. Although still time-consuming, exact pixel-by-pixel density quantification and red blood cell velocity measurements by means of space-time diagrams have now come within reach.

Apart from the aforementioned methodological limitations, practical applicability of semi-quantitative microcirculatory blood flow assessment, expressed as microcirculatory flow index (MFI), was subject to two papers. Comparison in 149 paired samples in 11 ICU patients between real-time point-of-care assessment of MFI and conventional off-line analysis showed good agreement; sensitivity was 90% and specificity 91% for the detection of a MFI < 2.5 [11]. This ability to discriminate between normal and abnormal microcirculatory blood flow at the bedside has potential practical implications for future trials. Dubin and co-workers performed a comparative study in a sheep model of hemorrhagic shock [12]. Red blood cell velocity was measured continuously over a wide range during step-by-step blood flow reduction and appeared to correlate well with the semi-quantitative MFI.

Over the last years there has been a debate about the causal role of the observed microcirculatory alterations; are they culprit or bystander? Apart from the idea that impaired microcirculatory blood flow is the primary event that leads to cellular dysfunction, there is also an adaptive theory, in which shut down of microcirculatory blood flow is explained as a result of reduced oxygen demand in mitochondrial dysfunction. Although this important issue is beyond the scope of this article, the development of a novel technology has the potential for a breakthrough in this respect [13]. Microcirculatory  $pO_2$  measurements have been performed with Pd-porphyrin phosphorescence quenching. This technique is based upon the principle that, once struck by light, the time-constant of the excited phosphorescence decay-curve of this exogenous compound, is  $pO_2$  dependent, allowing quantitative measurements of microcirculatory  $pO_2$  in-vivo in experimental models. Mik and co-workers extended this principle to measure mitochondrial  $pO_2$  using delayed fluorescence of mitochondrial endogenous protoporphyrin IX. They were the first to measure in this way in-vivo mitochondrial  $pO_2$  in rat liver, without affecting mitochondrial respiration [14]. Since the reported technology is scalable and relies on an endogenous compound, it has promising potential to link the field of microcirculatory and mitochondrial clinical research in circulatory failure.

### Pathophysiology

Sepsis is a syndrome that lacks strict definitions, based on specific aetiology. As a result, many insults may contribute to microcirculatory flow alterations, without the ability to discriminate between each cause-effect relation separately. One of the crucial factors seems to be timing. In a model of human endotoxemia, a single dose of LPS (2ng/kg) for 5 consecutive days in healthy volunteers, provoked clinical signs of systemic inflammation, as well as a significant rise in cytokines on day one [15]. At the same time SDF-derived

microcirculatory blood flow decreased significantly after 2 hours, with a concomitant decrease of the recovery slope in near-infrared spectroscopy of forearm blood flow, after a standardized occlusion procedure. On day 5 however, clinical symptoms, cytokine response, microcirculatory alterations and vascular reactivity remained unaltered after LPS infusion, indicating that immune-tolerance may play an important aetiological role in differences of microcirculatory alterations over time.

Increased vascular permeability may be another culprit in the development of microcirculatory alterations in sepsis. In an animal sepsis model Hollenberg and co-workers tested the hypothesis that abrogation of inducible nitric oxide synthase (iNOS) would decrease leukocyte rolling, leukocyte adhesion and vascular permeability [16]. However, observations with fluorescence intravital microscopy revealed that neither genetic deletion, nor pharmacologic blockade of iNOS affected the sepsis-related aggravation of leukocyte rolling and adhesion, whereas iNOS inhibition attenuated microvascular permeability. The authors conclude that iNOS-induced vascular permeability during sepsis seems to be independent of its action on leukocytes.

The impact of local hypoxia on organ failure was studied in an experimental model of sepsis-induced acute renal failure [17]. LPS-induced reduction of renal blood flow was associated with a significant decrease in cortical microcirculatory  $pO_2$  and anuria, whereas the equivalent mechanical reduction in renal blood flow did not provoke a decrease in cortical microcirculatory  $pO_2$ , with partial preservation of creatinin clearance, despite impaired global renal oxygen uptake capacity. An overview article places oxygen pathways' alterations as a potential central player in the pathogenesis of acute kidney injury, with a pivotal role for nitric oxide [18]. Furthermore, persistent tissue hypoxia is believed to contribute to the development of chronic renal dysfunction following acute ischemic renal injury.

### Heterogeneity

In distributive shock, such as in sepsis, the concept of microcirculatory weak units and heterogeneity of microcirculatory blood flow has been introduced [19]. The increase of the  $pO_2$  gap between microcirculatory and venous  $pO_2$  in sepsis, as opposed to other forms of shock, was hypothesized to be compatible with enhanced shunting as a result of an inhomogeneous blood flow distribution. Direct observation of the microcirculation with OPS-imaging confirmed the relationship between heterogeneity of microcirculatory blood flow and severity of sepsis, expressed as a stepwise increase in heterogeneity index for each cohort of severity of illness [5]. In several papers the idea of heterogeneity within and between various vascular compartments was tested. In human abdominal sepsis there was complete dispersion between OPS-derived sublingual and intestinal microcirculatory alterations on day one [20]. On day 3 however, microcirculatory blood flow almost normalized and demonstrated a movement towards the identity line in both vascular beds, underlining the time-dependency of microcirculatory alterations and heterogeneity in sepsis. The

same group investigated in sepsis the correlation between sublingual microcirculatory alterations and the central-to-toe temperature gradient, as an easily accessible parameter of combined peripheral and central circulation [21]. Again, a significant correlation could not be demonstrated, illustrating the specific role of the microcirculation in sepsis.

In an experimental model of endotoxic shock, blood flow diminished concomitantly in three microvascular beds (sublingual, intestinal mucosa and intestinal serosa) in combination with a rise in tonometry-derived  $p\text{CO}_2$  gap [22]. However, after fluid resuscitation, sublingual and serosal microvascular blood flow as well as the  $p\text{CO}_2$  gap restored to baseline, whereas mucosal blood flow and  $p\text{CO}_2$  gap remained markedly altered in comparison to controls. Furthermore, intramucosal  $p\text{CO}_2$  gap was inversely related to the percentage of perfused villi, but not to global intestinal blood flow. These observations add to the understanding of the heterogeneous character of microcirculatory blood flow and its response to insults and therapy in sepsis. As a consequence, extrapolating data from observations in one microvascular bed to another should be done with great restraint, in general, but especially under heterogenic septic conditions.

### Prognosis

Microcirculatory alterations have previously been demonstrated to contain independent prognostic value with respect to morbidity and mortality in sepsis [4,5]. This same prognostic value was tested within the specific setting of early goal-directed therapy for patients with sepsis, in a time-dependent manner [23]. After initial protocolized resuscitation, sequential organ failure assessment (SOFA) was performed in combination with sublingual SDF-imaging. A second set of SDF images was obtained within 3-6 hours after the first assessment whilst maintaining the same macrohemodynamic resuscitation endpoints. An a priori set decrease of SOFA score  $\geq 2$  (responders) between baseline and 24 hours was associated with a small, but significant positive change in median MFI. In case of a decrease in SOFA score  $< 2$  points (non-responders) after 24 hours, median change in MFI was negative. Although absolute difference in change of MFI over time between responders and non-responders were very small, it significantly discriminated in severity of multi-organ failure. The conclusion of the authors, that these data support the hypothesis that targeting the microcirculation in addition to the macrocirculatory resuscitation endpoints, warrants further study.

The prognostic value of microcirculatory alterations has also been subject to investigation in the postoperative setting [24]. In a group of patients following major abdominal surgery, sublingual microvascular blood flow abnormalities were more frequent in patients who developed complications in comparison to a group without complications, resulting in a significant increase in hospital length of stay. The observed microvascular alterations were mild and transient within 8 hours after surgery; cutaneous laser-Doppler flowmetry at the abdominal wall could not discriminate between groups.

In sepsis, measurements of muscle tissue oxygenation by means of near-infrared spectroscopy were also investigated on its ability to predict outcome [25]. In three cohorts of severity of illness, altered recovery of tissue oxygenation after a standardized ischemic occlusion period, occurred more frequently in sepsis patients as compared to hemodynamically stable ICU patients and healthy volunteers. Within the sepsis group the presence and persistence of this altered vascular reactivity after 24 and 48 hours, was associated with non-survival.

### Interventions

Over the last years the influence of many (therapeutic) interventions on microcirculatory alterations have become a topic of research. Many of these interventions are used in practical critical care on a routine bases. In an open label study the short-term infusion of propofol in otherwise healthy surgery patients was associated with a reversible reduction in sublingual perfused small vessel density [26]. Red blood cell transfusion in septic patients did not markedly alter overall sublingual microcirculatory perfusion [27]. In patients with a reduced capillary perfusion at baseline however, red blood cell transfusion was associated with improved microvascular perfusion.

Recent reports have highlighted the topic of fluid resuscitation from the perspective of the microcirculation. Pottecher and co-workers demonstrated in septic patients a relation between systemic fluid responsiveness and the microcirculatory response [28]. In case of a respiratory variation in arterial pulse pressure above 13%, both passive leg-raising and fluid infusion increased the proportion of perfused sublingual vessels significantly. In addition to these observations, Luengo demonstrated, also in septic patients, a further improvement of sublingual microcirculatory blood flow, irrespective of systemic hemodynamics, including cardiac output [29]. At first glance these data may seem conflicting. However, from the perspective of the clinical concept of the microcirculation, these observations add to the understanding, that in sepsis intact systemic hemodynamics remain the first essential step to provide adequate microcirculatory perfusion. On top of that, microcirculatory perfusion may be impaired by local factors, due to the additional distributive nature of microcirculatory flow alterations. Ideally, future trials that focus on microcirculatory recruitment strategies should incorporate both perspectives.

In an experimental sepsis model the effect of the calcium sensitizer levosimendan on microcirculatory perfusion and oxygenation was tested [30]. Microvascular oxygenation, assessed by oxygen-dependent Pd-porphyrin phosphorescence quenching, improved significantly in comparison to fluid and/or norepinephrine resuscitation. Microcirculatory blood flow remained unaltered between groups, suggesting a mechanism for improvement of microcirculatory oxygenation, independent from microcirculatory perfusion. In an animal model of endotoxemia, iNOS blockade by low-dose dexamethason, in addition to fluid resuscitation, was reported to prevent the appearance of renal cortical microvascular

hypoxia [31]. In a pig-model of endotoxemia, the use of the nitric oxide donor SIN-1, added to fluid resuscitation, was associated with an increase in ileal serosal microvascular  $pO_2$  and a concomitant decrease in ileal-arterial  $pCO_2$  gap [32]. These data seemed to be conflicting with a previous report, in which an iNOS-inhibitor W1400 in the same animal model was also reported to blunt the otherwise progressive rise in ileal-arterial  $pCO_2$  gap [33]. However, this apparent contradiction is explained by a selective mucosal expression of iNOS. In case of iNOS inhibition preferential mucosal blood flow is redirected towards the serosa; if a nitric oxide donor is administered, the serosal microcirculation is enabled to open up as a result of an exogenous vasodilatory compound [34]. Direct observation of microcirculatory blood flow in serosa and mucosa, in combination with tonometry, helped to solve the puzzle.

## Conclusions

As a result of the ability to observe microcirculatory blood flow with OPS or SDF-imaging, in a direct and clinically applicable manner, the concept of microcirculatory dysfunction in the evolvement from shock to multi-organ failure can now be tested. Many recent reports have added to the understanding of the heterogeneous character of microcirculatory blood flow in sepsis. Pathophysiological insults, as well as routinely used interventions in the management of critically ill patients, appear to be associated with a change in microvascular perfusion. Aiming for pro-microcirculatory recruitment strategies in order to improve outcome will be the challenge for the near future.

### Acknowledgements:

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## References

1. Groner W, Winkelmann JW, Harris AG, et al. Orthogonal polarization spectral imaging: A new method for study of the microcirculation. *Nat Med* 1999; 5:1209-1212.
2. De Backer D, Creteur J, Preiser JC, et al. Microvascular blood flow is altered in patients with sepsis. *Am J Respir Crit Care Med* 2002; 166:98-104.
3. De Backer D, Creteur J, Dubois MJ, et al. Microvascular alterations in patients with acute severe heart failure and cardiogenic shock. *Am Heart J* 2004; 147:91-99.
4. Sakr Y, Dubois MJ, De Backer D, et al. Persistent microcirculatory alterations are associated with organ failure and death in patients with septic shock. *Crit Care Med* 2004; 32:1825-1831.

5. Trzeciak S, Dellinger RP, Parrillo JE, et al. Early microcirculatory perfusion derangements in patients with severe sepsis and septic shock: relationship to hemodynamics, oxygen transport and survival. *Ann Emerg Med* 2007; 49:88-98.
6. Büchele GL, Ospina-Tascon GA, De Backer D. How microcirculation data have changed my clinical practice. *Curr Opin Crit Care* 2007; 13:324-331.
7. Bracht H, Krejci V, Hildebrand L, et al. Orthogonal polarization spectroscopy to detect mesenteric hypoperfusion. *Intensive Care Med* 2008; 34:1883-1890.
8. De Backer D, Hollenberg S, Boerma EC, et al. How to evaluate the microcirculation: report of a round table conference. *Critical Care* 2007; 11:R101.
9. Goedhart PT, Khalilzade M, Bezemer R, et al. Sidestream dark field (SDF) imaging: a novel stroboscopic LED ring-based modality for clinical assessment of the microcirculation. *Optics express* 2007; 15:15101-15114.
10. Dobbe JG, Streekstra GJ, Atasever B, et al. Measurement of functional microcirculatory geometry and velocity distributions using automated image analysis. *Med Biol Eng Comput* 2008; 46:659-670.
11. Arnold R, Trzeciak S, Dellinger PR, et al. Real-Time Point-Of-Care Assessment of Microvascular Flow in Critically Ill Patients. *Acad Emerg Med* 2008; 15(suppl 1):S30.
12. Dubin A, Pozo MO, Ferrara G, et al. Systemic and macrocirculatory responses to progressive hemorrhage. *Intensive Care Med* 2009; 35:556-564.
13. Mik EG, Stap J, Sinaasappel M, et al. Mitochondrial PO<sub>2</sub> measured by delayed fluorescence of endogenous protoporphyrin IX. *Nat Methods* 2006; 3:939-945.
14. Mik EG, Johannes T, Zuurbier CJ, et al. In vivo mitochondrial oxygen tension measured by a delayed fluorescence lifetime technique. *Biophys J* 2008; 95:3977-3990.
15. Draisma A, Bemelmans R, van der Hoeven JG, et al. Microcirculation and vascular reactivity during endotoxemia and endotoxin tolerance in humans. *Shock* 2008, epub ahead of print. DOI 10.1097/SHK.0b013e318193e187
16. Hollenberg SM, Guglielmi M, Parrillo JE. Discordance between microvascular permeability and leukocyte dynamics in septic inducible nitric oxide synthase deficient mice. *Crit Care* 2007; 11:R125.

17. Johannes T, Mik EG, Ince C. Nonresuscitated endotoxemia induces microcirculatory hypoxic areas in the renal cortex in the rat. *Shock* 2009; 31:97-103.
18. Legrand M, Mik EG, Johannes T, et al. Renal hypoxia and dysoxia after reperfusion of the ischemic kidney. *Mol Med* 2008; 14:502-516.
19. Ince C, Sinaasappel M. Microcirculatory oxygenation and shunting in sepsis and shock. *Crit Care Med* 1999; 27:1369-1377.
20. Boerma EC, van der Voort PHJ, Spronk PE, Ince C. Relationship between sublingual and intestinal microcirculatory perfusion in patients with abdominal sepsis. *Crit Care Med* 2007; 35:1055-1060.
21. Boerma EC, Kuiper MA, Kingma WP, et al. Disparity between skin perfusion and sublingual microcirculatory alterations in severe sepsis and septic shock: a prospective observational study. *Intensive Care Med* 2008; 34:1294-1298.
22. Dubin A, Edul VS, Pozo MO, et al. Persistent villi hypoperfusion explains intramucosal acidosis in sheep endotoxemia. *Crit Care Med* 2008; 36:535-542.
23. Trzeciak S, McCoy JV, Dellinger RP, et al. Early increases in microcirculatory perfusion during protocol-directed resuscitation are associated with reduced multi-organ failure at 24 h in patients with sepsis. *Intensive Care Med* 2008; 34:2210-2217.
24. Jhanji S, Lee C, Watson D, et al. Microvascular flow and tissue oxygenation after major abdominal surgery: association with post-operative complications. *Intensive Care Med* 2009; 35:671-676.
25. Creteur J, Carollo T, Soldati G, et al. The prognostic value of muscle StO<sub>2</sub> in septic patients. *Intensive Care Med* 2007; 33:1549-1556.
26. Koch M, De Backer D, Vincent JL, et al. Effects of propofol on human microcirculation. *Br J Anaesth* 2008; 101:473-478.
27. Sakr Y, Chierigo M, Piagnerelli M, et al. Microvascular response to red blood cell transfusion in patients with severe sepsis. *Crit Care Med* 2007; 35:1639-1644.
28. Pottecher J, Derudder S, Georger F, et al. Both passive leg raising and volume expansion improve sublingual microcirculation in preload-dependent septic patients. *Intensive Care Med* 2008; 34(suppl 1):S103.

29. Luengo C, Losser MR, Legrand M, et al. Fluid resuscitation improves microcirculatory flow in septic shock patients. *Intensive Care Med* 2008; 34(suppl 1):S103.
30. Fries M, Ince C, Rossaint R, et al. Levosimendan but not norepinephrine improves microvascular oxygenation during experimental septic shock. *Crit Care Med* 2008; 36:1886-1891.
31. Johannes T, Mik EG, Klingel K, et al. Low-dose dexamethasone supplemented fluid resuscitation reverses endotoxin-induced acute renal failure and prevents cortical microvascular hypoxia. *Shock* 2008, epub ahead of print. DOI:10.1097/SHK.0b013e318188d198.
32. Siegemund M, Van Bommel J, Sinaasappel M, et al. The NO donor SIN-1 improves intestinal-arterial PCO<sub>2</sub> gap in experimental endotoxemia: an animal study. *Acta Anaesthesiol Scand* 2007; 51:693-700.
33. Pittner A, Nalos M, Asfar P, et al. Mechanisms of inducible nitric oxide synthase (iNOS) inhibition-related improvement of gut mucosal acidosis during hyperdynamic porcine endotoxemia. *Intensive Care Med* 2003; 29:312-316.
34. Almac E, Siegemund M, Demirci C, Ince C. Microcirculatory recruitment maneuvers correct tissue CO<sub>2</sub> abnormalities in sepsis. *Minerva Anesthesiol* 2006; 72:507-519.



# Summary

Summary

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Treatment of sepsis and its life-threatening complications as a result of shock and multiple organ failure remain a persisting challenge for many healthcare workers, with considerable (long-lasting) morbidity and mortality for patients, despite strenuous efforts. Since sepsis is a syndrome, that is ill-defined on the basis of frequently observed clinical symptoms that overlap numerous other disease states, and not delimited as a clear patho-physiological entity, improvement of clinical outcome is conceived to be influenced by many factors. In that respect, the infection itself, the complex reaction of the body and the set of therapeutic interventions are all subject to further investigation. On top of that, the distributive nature of circulatory failure during sepsis has clear distinct characteristics in comparison to other forms of shock. Hypovolemic, cardiogenic and obstructive shock occur as a result of an inadequate cardiac output in relation to tissue demand, whereas septic shock is the result of distributive alterations in tissue perfusion, even in the presence of a normal or elevated cardiac output. Microcirculatory weak units become shunted, explaining patchy cellular dysoxia in the presence of apparently adequate upstream and downstream parameters of oxygen delivery. The concept of heterogeneity of blood flow under conditions of distributive shock, as in sepsis, is subject to research in this thesis. The clinical implication of this concept is clear: despite aggressive monitoring of many parameters of systemic hemodynamics in the intensive care unit, the potential culprit lesion in sepsis remains unrevealed.

In chapter 1 the concept of heterogeneity of blood flow in sepsis is elaborated in more detail with a focus on the gut. Due to the specific vascular architecture of the small intestine, the tip of the villus is more susceptible to hypoxia as a result of convexional and diffusional forms of shunting, thus becoming a microcirculatory weak unit. The ability to observe the microcirculation in-vivo with the new technique orthogonal polarization spectral (OPS) imaging, incorporated in a hand-held device, is discussed.

Before structured research with in-vivo imaging of the microcirculation is possible, the problem of quantification of the observed microcirculatory alterations has to be dealt with. Apart from the technical problem to convert complex visual images into numbers, the method must particularly be sensitive to detect heterogeneity of flow within the microcirculation. Since an automated analysis, based on software contour analysis of vessels and subsequent measurement of surface area and red blood cell velocity, was not feasible at the time, we proposed a semi-quantitative assessment of microcirculatory flow in chapter 2. After dividing the image into quadrants, flow can be expressed as a number between 0 and 3, ranging from complete stand-still to continuous. Although expressing data in a non-continuous way is a considerable disadvantage, scoring a minimum of 12 quadrants enables us to detect heterogeneity of flow. The overall microvascular flow index, an average of all the individual quadrant scores, can be obtained within a reasonable timeframe and processing this variable as (pseudo)continuous may be performed, according to statistical laws. Inter- and intraobserver variability appeared to be good for different vessels sizes in both capillary networks and repeating vascular structures, with potential applicability for the assessment of microcirculatory alterations in different organs.

In chapter 3 the dilemma of the apparent discordance between systemic hemodynamic variables and microvascular blood flow is illustrated with a clinical case-report. A patient with meningococcal meningitis and sepsis remained persistently hypotensive, despite aggressive volume therapy, inotropes and the use of the vasopressor norepinephrine. Despite low blood pressure and oliguria, OPS-derived sublingual microcirculatory blood flow appeared to be normal. After a single bolus of terlipressin, a potent vasopressor, blood pressure and urine production were successfully restored. However, a complete shutdown of the microcirculation was observed, and the patient died with progressive metabolic acidosis.

In chapter 4 the concept of heterogeneity of blood flow, as observed within the microcirculation, is expanded to the relation between 2 microvascular beds in different organs, at different time points. In 23 patients with an abdominal sepsis and a surgical stoma the microcirculation is simultaneously observed with OPS imaging, both in the sublingual region and the intestinal region (via the stoma). Within 24 hours after the initial surgical procedure a complete discordance of flow between the 2 vascular beds was observed. After 48 hours however, a normalisation of microcirculatory flow in both microvascular beds 'restored' the correlation. These observations underline the idea that, especially under conditions of distributive shock, observed abnormalities in one organ, cannot be translated one-on-one to another organ. Even more, such correlation might be time-dependent.

If heterogeneity of flow within and between microvascular beds exists, what does this implicate for the relation between 2 different vascular compartments. In previous chapters the discordance between systemic and microcirculatory hemodynamic parameters was already subject of research. In chapter 5 the relation between a parameter of peripheral circulation, the 'big-toe-temperature', situated between the systemic circulation and the microcirculation, and sublingual microcirculatory perfusion is elaborated in 35 ICU-patients with severe sepsis. Again, a discordance between the 2 vascular compartments was observed. Although under conditions of other forms of shock a decrease in 'big-toe-temperature' is reported to be associated with severity of shock, the distributive failure in sepsis seems to blunt such correlation. Under these conditions an integrative approach of parameters to information from all relevant vascular compartments seem necessary to unravel the complete picture.

In contrast to many other parameters, microcirculatory alterations in sepsis are associated with morbidity and mortality. However, this does not automatically implicate that pro-microcirculatory strategies will lead to better patient outcome. In chapter 6 results of a prospective single centre double-blind randomised placebo controlled trial are discussed. Aim of the study was to add a specific pro-microcirculatory strategy on top of a sepsis resuscitation protocol, that incorporates reported beneficial hemodynamic interventions,

such as early goal-directed therapy and dynamic exclusion of fluid-responsiveness. Based on earlier reports we used nitroglycerin to promote microcirculatory blood flow. Seventy patients with organ failure as a result of sepsis were included. Despite fulfillment of preset systemic hemodynamic resuscitation endpoints, sublingual microcirculatory blood flow was considerably altered at the start of the study medication. Within 24 hours microcirculatory blood flow improved significantly. However, no significant difference between placebo and nitroglycerin was observed. These unexpected findings warrant further studies that focus on situation-dependent differences in effects of nitroglycerin in sepsis and even question the presumed mechanism of action as a nitric oxide donor.

In chapter 7 another clinical model of distributive failure was explored. During cardiopulmonary bypass patients are subject to a considerable number of hemodynamic changes, associated with sublingual distributive alterations. Our aim was to investigate the relation between intestinal microcirculatory alterations and cellular dysoxia. To this extent direct observation of the rectal microcirculation in combination with rectal tonometry was performed in 26 low-risk on-pump cardiac surgery patients. In previous reports splanchnic ischemia, as detected with tonometry, was identified as a risk factor for postoperative complications in these patients. In the specific setting of this single-centre study the observed incidence of postoperative intestinal microcirculatory alterations, as well as splanchnic ischemia, was low. Extending microcirculatory in-vivo observations to the sublingual area did not disclose marked discordance of microcirculatory blood flow between the different microvascular beds.

To end this thesis chapter 8 provides an overview of the recent literature that has added to the understanding of the microcirculation as a clinical concept.

Summary

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# Samenvatting



De behandeling van ernstige infecties (sepsis), de daarmee gepaard gaande levensbedreigende complicaties als gevolg van orgaan falen en het niet in stand kunnen houden van een adequate bloedsomloop (shock), zijn een blijvende uitdaging voor gezondheidszorg medewerkers, met aanzienlijke (langdurige) schade en sterfte voor patiënten, ondanks niet aatende inspanningen van alle betrokkenen. Sepsis is een slecht omschreven syndroom, waarvan de definities gebaseerd zijn op veel voorkomende algemene klinische verschijnselen, die overlap vertonen met andere ziekten, en is niet duidelijk afgebakend als een ziektebeeld met een eenduidig oorzakelijk mechanisme. Verbetering van klinische uitkomst is dan ook een multifactorieel proces; de infectie zelf, de complexe reactie van het lichaam hierop en de set van therapeutische interventies zijn wat dat betreft alle onderwerp van verdere studie.

Bovendien heeft de aard van het onderliggende falen van de bloedsomloop bij sepsis duidelijk onderscheidende kenmerken ten opzichte van andere vormen van shock. Hypovolemische shock (verbloeding), cardiogene shock (hartfalen) en obstructieve shock (blokkade in de bloedsomloop) zijn alle het gevolg van een te laag hart minuutvolume in relatie tot de weefsel behoefte, maar septische shock is het gevolg van veranderingen in de verdeling van weefsel doorbloeding (distributieve shock), in de aanwezigheid van een normaal of zelfs verhoogd hart minuutvolume. De bloeddorstrooming van kwetsbare plaatsen ('weak units') in de haarvaatjes (microcirculatie) wordt kortgesloten (geschunt) met een onregelmatige verdeling van cellen met zuurstofschuld tot gevolg, bij schijnbaar normale waarden van zuurstofaanbod stroomopwaarts en stroomafwaarts. Het concept van een heterogene verdeling van de bloedstroom bij een distributieve shock, zoals in sepsis, is onderwerp van het onderzoek voor deze thesis. De klinische implicaties van dit concept zijn duidelijk: ondanks agressieve bewaking en bijstelling van allerlei parameters van de globale bloedsomloop op de intensive care, blijft de plaats waar de mogelijk verantwoordelijke afwijkingen plaats vinden aan het oog onttrokken.

In hoofdstuk 1 wordt het concept van een heterogene verdeling van de bloedstroom verder uitgewerkt aan de hand van het voorbeeld van de darm. Als gevolg van de specifieke vaatarchitectuur in de dunne darm is het uiteinde van een darmvlok (villus) gevoelig voor zuurstof tekort; het wordt gemakkelijk gepasseerd door de bloedstroom zonder contact te maken (geschunt) en is zo een concreet voorbeeld van zo'n 'weak unit' in de microcirculatie. De mogelijkheid om de microcirculatie direct te observeren in levende mensen en dieren (in-vivo) door middel van een nieuwe techniek die is ingebouwd in een kleine camera, orthogonal polarization spectral (OPS) imaging genaamd, wordt toegelicht.

Voordat gestructureerd onderzoek gedaan kan worden met het visualiseren van de microcirculatie moet er eerst een belangrijk probleem opgelost worden: het uitdrukken in maat en getal van de zichtbare afwijkingen in de microcirculatie. Naast het feit dat dit

kwantificeren van complexe visuele informatie een technisch lastig probleem is, moet zo'n methode ook recht doen aan het feit dat bloedstroom snelheid van de microcirculatie onregelmatig verdeeld kan zijn. Omdat automatische analyse met behulp van software ondersteunde contourherkenning van de bloedvaten en het daarna berekenen van vaatoppervlak en bloedstroom snelheid op dat moment nog niet mogelijk was, beschrijven wij in hoofdstuk 2 een door ons voorgestelde methode, waarbij de doorstroming van de microcirculatie kan worden uitgedrukt in verschillende categorieën. Het beeld wordt in kwadranten verdeeld en de bloedstroom krijgt een heel getal toegekend tussen 0 en 3, variërend van complete stilstand tot een continue beweging. Alhoewel het kwantificeren met hele getallen per categorie duidelijke nadelen in zich bergt, stelt het scoren van tenminste 12 kwadranten ons in staat het heterogene karakter van de doorbloeding te detecteren. De totaal score, als gemiddelde van alle individuele kwadrant scores, kan worden vastgesteld binnen een redelijke tijdspanne en mag volgens de wetten van de statistiek verrekend worden als een continue variabele. De reproduceerbaarheid van de methode bleek goed te zijn voor vaten van verschillende grootte en was toepasbaar voor zowel vaatnetwerken als repeterende vaatstructuren in verschillende organen.

In hoofdstuk 3 wordt de ogenschijnlijke tegenstelling tussen parameters van de globale bloedsomloop en de microcirculatie geïllustreerd aan de hand van een case report. Een patiënt met een (meningococcal) hersenvliesontsteking en – sepsis hield een te lage bloeddruk, ook na toediening van veel vocht, middelen die de pompkracht van het hart bevorderen (inotropica) en het bloeddruk verhogende medicijn noradrenaline. Ondanks deze lage bloeddruk en een verminderde urineproductie bleek de, met de OPS camera bekeken, bloedstroom in de microcirculatie onder de tong ongestoord te zijn. Na het toedienen van een krachtige bloeddruk verhogend medicijn, terlipressine, herstelden de bloeddruk en de urineproductie zich, maar de microcirculatie kwam geheel tot stilstand en de patiënt overleed onder het beeld van een voortschrijdende verzuring (metabole acidose).

In hoofdstuk 4 wordt het concept van een heterogene verdeling van de bloedstroom nader uitgewerkt met de vraag wat dit betekent voor de relatie tussen de microcirculatie van 2 verschillende organen, en op verschillende tijdstippen. Bij 23 patiënten met een ernstige sepsis vanuit de buik en de noodzaak voor het aanleggen van een kunstmatige darmuitgang (stoma) werd gelijktijdig met de OPS camera onder de tong en in de darm (stoma) gekeken. Binnen 24 uur na de chirurgische ingreep was er geen relatie aantoonbaar tussen de 2 vaatbedden. Na 48 uur echter herstelde de bloedstroom zich in de microcirculatie van beide 'organen', waarmee ogenschijnlijk de relatie 'hersteld' werd. Deze observaties benadrukken de gedachte dat waarnemingen in het ene orgaan niet zomaar geprojecteerd mogen worden op het andere orgaan, met name onder omstandigheden van een heterogene verdeling van de bloedstroom. Bovendien kan zo'n relatie veranderen in de tijd.

Als er een verschil van doorstroming tussen de microcirculatie van 2 organen kan worden waargenomen, wat voor implicaties heeft dit dan voor de relatie tussen 2 verschillende vaatcompartimenten. In eerdere hoofdstukken waren de verschillen tussen de globale bloedsomloop en de microcirculatie al onderwerp van onderzoek. In hoofdstuk 5 wordt in 35 patiënten met ernstige sepsis de relatie nader uitgewerkt tussen de microcirculatie en de temperatuur van de grote teen, als maat voor de doorbloeding van het perifere vaatcompartiment, dat gelegen is tussen het centrale vaatcompartiment en de microcirculatie. Opnieuw kon een niet overeenkomen van de doorbloeding tussen de verschillende compartimenten worden waargenomen. Alhoewel in het verleden een relatie tussen de ernst van shock en de temperatuur van de grote teen was beschreven bij andere vormen van shock, lijkt het distributieve karakter zo'n relatie minder duidelijk te maken bij sepsis. Onder deze omstandigheden lijkt een geïntegreerde benadering van de informatie uit alle vaatcompartimenten noodzakelijk om het complete beeld te ontrafelen.

In tegenstelling tot veel parameters van de globale bloedsomloop zijn de gevonden afwijkingen in de microcirculatie gerelateerd aan (orgaan)schade en sterfte. Dit betekent echter niet dat ingrepen ter verbetering van de doorstroming van de microcirculatie ook automatisch tot verbetering van de patiënt leiden. In hoofdstuk 6 worden de resultaten van een prospectieve dubbelblind placebo gecontroleerde klinische studie beschreven. Doel van de studie was het toevoegen van een pro-microcirculatoire strategie, bovenop een behandelingsprotocol, dat reeds als gunstig bekend staande maatregelen bevat (o.a. 'early goal-directed therapie' en het dynamisch vaststellen van een gunstige respons van het hart op vloeistof toediening). Afgaande op eerdere studies viel onze keuze op nitroglycerine als pro-microcirculatoir middel. Zeventig patiënten met orgaan falen als gevolg van sepsis werden in de studie geïnccludeerd. Ondanks het succesvol bereiken van de gestelde einddoelen van het behandelingsprotocol bestonden er nog aanzienlijke afwijkingen van de doorstroming van de microcirculatie onder de tong voor het starten van de studiemedicatie. Binnen 24 uur verbeterde deze doorstroming significant. Er was echter geen verschil waarneembaar tussen patiënten die nitroglycerine toegediend hadden gekregen en patiënten met een placebo (fopmedicijn). Deze onverwachte bevindingen maken verdere studies naar de effecten van nitroglycerine onder verschillende omstandigheden in sepsis noodzakelijk; zelfs het veronderstelde werkingsmechanisme van nitroglycerine als nitricoxide donor komt hiermee in een ander daglicht te staan.

In hoofdstuk 7 komt een ander model van shock ten gevolge van een abnormale verdeling van de bloedstroom aan de orde. Tijdens een operatie met ondersteuning van een hart-longmachine ondergaan patiënten aanzienlijke veranderingen in hun bloedsomloop, die geassocieerd zijn met distributieve verandering van de microcirculatie onder de tong. Bovendien is beschreven dat met name de doorbloeding van de darm kwetsbaar is onder die omstandigheden. Ons doel was na te gaan of er een relatie bestond tussen het voorkomen

van microcirculatoire doorbloedingsstoornissen in de darm en het optreden van zuurstofschuld in darmcellen. Daartoe voerden we een studie uit bij 26 laag-risico hartchirurgie patiënten, die geopereerd waren met ondersteuning van een hart-longmachine; directe observatie van de microcirculatie en ‘tonometrie’ werden gelijktijdig verricht in de endeldarm. In eerdere studies werd er in een vergelijkbare patiëntencategorie een relatie beschreven tussen het optreden van postoperatieve complicaties en tekenen van verminderde darmdoorbloeding, gemeten met behulp van deze tonometrie. Onder de specifieke omstandigheden waarin deze studie werd uitgevoerd bleek het optreden van postoperatieve tekenen van een verminderde darmdoorbloeding en zuurstofschuld niet veel voorkomend. Een uitbreiding van de studie naar de microcirculatie onder de tong bracht geen grote verschillen tussen de 2 vaatbedden aan het licht.

Aan het einde van deze thesis wordt in hoofdstuk 8 getracht een overzicht te geven van de recente literatuur op het gebied van de microcirculatie als een klinisch concept.

Dankwoord

Dankwoord

Promoveren is een bijzondere gebeurtenis in het leven van een eenvoudig intensivist. Meer precies: de weg naar dit proefschrift heeft voor mij nieuwe dimensies geopend. Naast de verdieping in kennis leidde deze zoektocht tot een kritische zelfreflectie en het loslaten van zoveel zekerheden, die van dokter tot dokter worden overgedragen; alle even noodzakelijk als beperkend.

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