Focus on flow: imaging the human microcirculation in perioperative and intensive care medicine
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“We therefore applaud the efforts of Dr. Elbers and his associates who, stimulated by Professor Ince, continue vigorous pursuit of the dichotomy between the macro- and microcirculations and its implication for better management of diverse shock states.”

Max Harry Weil, MD, PhD, ScD, MACP
Resuscitation 2010; 81: 5
Focus On Flow

Imaging the Human Microcirculation
in Perioperative and Intensive Care Medicine
Focus on Flow
Imaging the Human Microcirculation in Perioperative and Intensive Care Medicine

Thesis, University of Amsterdam, The Netherlands, with summary in Dutch

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Focus On Flow
Imaging the Human Microcirculation in Perioperative and Intensive Care Medicine

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Faculteit der Geneeskunde
Bloed moet stromen, niet botsen
Prof. dr. Durk Zandstra

Voor mijn vader en moeder
Voor Josephine
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Introduction
Setting the Scene

The microcirculation is essential for life. This was probably first recognized by Hippocrates. Around 400 BC, the father of modern medicine observed that “in fevers which do not intermit, if the external parts be cold, and the internal burning hot, and fever prevail, it is a mortal sign”. This is likely a description of a septic individual presenting with severely impaired microvascular flow due to multiple pathophysiological processes, although the concept of the microcirculation as an organ that distributes oxygen and nutrients to tissue was far from developed as it is now. Hippocrates was the first physician to reject superstitions, legends and beliefs which credited supernatural or divine forces with causing illness. Therefore he would have been very pleased that today’s technology has enabled physicians to monitor the human microcirculation at the bedside in real time thus allowing objective assessment of possible disturbances and their causative mechanisms both qualitatively and quantitatively.

Indeed, the publication of this thesis only shortly follows the celebration of the 10th anniversary of Orthogonal Polarization Spectral (OPS) imaging [1]. OPS imaging was a revolution as it brought human microvascular imaging to the bedside. Not much later, Sidestream Dark Field imaging was invented, offering superior imaging while continuing to be small, minimally invasive, safe and relatively inexpensive [2]. Both techniques applied known optical methods to study the microcirculation into hand held microscopes, OPS imaging being based on cross polarization [3] and SDF imaging being based on dark field illumination [4]. Both techniques use green light that is absorbed both by oxygenated and deoxygenated hemoglobin present in the red blood cell flowing in the microcirculation. In this way magnified moving images may be recorded representing an area of approximately 1 mm$^2$ of these flowing red blood cells and thus in the functional microcirculation. These techniques can be applied on exposed organ surfaces, nail fold skin and mucous membranes. At the bedside it has been commonly used for microvascular monitoring at the sublingual site because of its proximity to the brain, its phylogenetic relationship to the gut and ease of access.

Before the introduction of OPS- and SDF imaging, monitoring the human microcirculation was hampered by a lack of suitable techniques because only bulky microscopes were available. At that time, only nail fold video microscopy and laser Doppler techniques were apt for use in humans. However, nail fold video microscopy has limited value as it is extremely sensitive to external temperature and vasoconstrictive agents. Laser Doppler can be used to measure gastric or jejunal mucosal blood flow as well as skin and muscle blood flow, but does not take into account microvascular blood flow heterogeneity. Intravital microscopy used to be the gold standard for imaging the microcirculation in that era. However this technique can only be used in animal models and in a limited number of clinical scenarios as it requires large microscopes, fixed tissue for stability and sometimes the infusion of fluorescent dyes.

As early as the beginning of last century, various pioneers started to use intravital microscopy and found that human ungual blood flow was altered in various conditions including heart failure, hemorrhagic shock and sepsis, as reviewed recently [5]. For septic patients,
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This was later confirmed using laser Doppler techniques. These findings were later replicated for various other tissues in many studies using intravital microscopy in animal models of sepsis.

It is interesting that even though microvascular alterations have long been known to exist, clinicians continue to rely on monitoring and optimizing global hemodynamic parameters. This is based on the expectation that optimizing systemic hemodynamic variables will improve oxygen delivery to tissue. Taking sepsis as an example, this idea was strengthened by the so-called goal directive resuscitation strategies that began to emerge during the last decades of last century [6]. This approach has now been embedded in guidelines such as the surviving sepsis campaign that was introduced in 2004 and updated in 2008 [7]. This campaign introduced a treatment bundle based on the concept of early goal directed therapy. Among other interventions, these goals include therapeutic targeting of macrohemodynamic parameters such as arterial and venous blood pressure and central or mixed venous oxygen saturation. Although it is difficult to disentangle the different components of a bundle, its application was repeatedly shown to reduce mortality. This makes quite a strong case for targeting global hemodynamic parameters in sepsis.

However, despite implementation of these goal directed protocols, critically ill septic patients continue to die. In addition, two early trials showed that targeting supra normal values for oxygen delivery did not improve outcome [8, 9]. This may be explained by dissociation between global hemodynamics and microvascular perfusion. Thus, optimizing macrohemodynamic parameters may not necessarily cause all capillaries to be adequately perfused.

This concept made the use of OPS- and SDF imaging highly attractive for characterizing the human microcirculation in sepsis. A landmark study by De Backer et al. included 50 patients with severe sepsis [10]. The density of their microvessels was significantly reduced and these alterations were more severe in nonsurvivors. These findings were later confirmed by Trzeciak et al. [11]. In addition, Sakr et al went on to show that in 49 patients in septic shock persistent lack of small microvessel perfusion was strongly associated with organ failure and death and demonstrated that it was microcirculatory flow which represented the single most specific and sensitive indicator of such outcome [12].

Indeed, a consistent finding from studies using OPS- and SDF imaging in septic patients was that the human microcirculation showed marked heterogeneity in disease. In addition such microcirculatory alterations were found to occur in otherwise normalized global hemodynamic parameters. This indicates that sepsis is a disease of the microcirculation even during treatment. Direct observations of the septic microcirculation have revealed the coexistence of well perfused and non-perfused capillaries which would lead to a decreased oxygen extraction due to increased diffusion distances [13].

This idea of a dissociation between macro- and microhemodynamics was further exemplified by De Backer et al who showed that the beneficial effects of dobutamine are reflected in the microcirculation in patients with septic shock and that these are independent of its systemic effects [14]. Similarly, Spronk et al. reported on a case series of septic patients
who despite a mean arterial blood pressure of more than 60 mm Hg and central venous pressure greater than 12 mm Hg showed stasis of microcirculatory flow [15]. Infusion of 0.5 mg of nitroglycerin intravenously resulted in a marked increase in microvascular flow. They concluded that instead of focusing on macrohemodynamic parameters, recruitment of the microcirculation could be a new resuscitation endpoint in septic shock. In addition, Boerma et al. used OPS imaging in a patient who was given terlipressin for catecholamine-resistant septic shock. Despite correction of macrohemodynamic parameters, this led to microvascular shutdown [16]. Finally De Backer et al. showed that administration of activated drotrecogin alpha rapidly improves sepsis-induced microvascular alterations, relatively independent from global hemodynamic parameters, whereas its cessation was associated with a transient deterioration [17].

Thesis

The key message from these studies is that in sepsis, and during resuscitation in sepsis, microvascular alterations are heterogeneous and are not necessarily reflected by routinely used measures of global hemodynamic parameters. Hemodynamic management of patients in routine clinical practice in other areas than sepsis also focuses on the stabilization of macrohemodynamic variables. In fact, these parameters including heart rate, arterial and venous blood pressure and sometimes cardiac output and oxygen derived parameters are measured in most patients in perioperative and intensive care medicine. In addition, as reviewed recently, many trials using goal directed therapy aiming at aggressive correction of global hemodynamic showed reduced mortality in high risk surgery [18]. Therefore, again, monitoring and treating global hemodynamics makes sense both from a physiological and evidence based point of view. However, if microvascular heterogeneity and disparity between global hemodynamics and microvascular perfusion observed in sepsis, would also hold for non-septic patients in intensive care and perioperative medicine, today’s clinicians may sometimes be directing their attention and therapies to the wrong parameters. It is well known that many non-septic patients in intensive care medicine and perioperative medicine, especially those being critically ill, having been resuscitated from cardiac arrest or having been subjected to major surgery suffer from a systemic inflammatory response syndrome, not unlike sepsis [19]. This might imply that the same microvascular alterations and possibly the absence of a clear relationship with macrohemodynamic parameters may also exist in other patient groups than in septic patients. In fact, De Backer had already shown that microvascular blood flow alterations are frequently observed in patients with severe heart failure and are more severe in patients who do not survive [20]. It was against this background that the studies in this thesis were conceived.

The hypothesis of this thesis is that discrepancy between macrohemodynamic and microvascular parameters also exists in non-septic patients in perioperative and intensive care medicine. To prove this thesis, a large number of studies were executed, several of which form the core of this publication. A large variety of routine clinical settings were studied, all characterized by the fact that clinical management of patients was routinely guided by
measurement of macrohemodynamic variables. The aim of our studies was to characterize microvascular flow in these perioperative settings using SDF imaging and relating these to routinely measured global hemodynamic parameters in order to unveil the possible disparity between these two physiological compartments.

Outline

The vivid images of moving red blood cells and stagnant flow in various disease states as obtained by OPS- and SDF imaging, have served to remind us that the microcirculation is far more complex than just a collection of connecting pipes. In chapter 1, the concept of viewing the microcirculation as an organ is introduced. Just like other organs, the microcirculation is composed of various cell types such as endothelium, smooth muscle cells, red and white blood cells in complex interaction with each other. Further, also similar to other organs, the microcirculation also consists of a large number of other components including platelets, coagulation factors, and a plethora of cytokines and chemokines. These components display an intricate interplay not unlike an orchestra performing a symphony. In this way the microcirculatory organ is highly regulated to perform its main function mainly of transporting oxygen and nutrients to the tissue cells along with the removal of waste products. This chapter will introduce the reader to the various techniques for monitoring microvascular function including OPS- and SDF imaging. In addition it will summarize what is known on microvascular alterations in the critically ill with a focus on septic patients, and outline potential strategies for resuscitating the microcirculation.

Almost 40 years ago, Weil and Shubin proposed their now famous classification of shock states [21]. Based on their understanding they distinguished four types of shock, which they identified as obstructive, hypovolemic, cardiogenic and distributive shock. They recognized the relevance of the microcirculation in distributive shock in which malfunction in the distribution of a normal or even of an elevated cardiac output may hamper microvascular perfusion. Therefore, in chapter 2, it is hypothesized that in addition to classifying shock states based on global hemodynamics, it may be necessary to also classify them according to microvascular hemodynamic patterns as observed by bedside microvascular imaging. Such classification may be helpful to discriminate patterns of microvascular dysfunction and may offer guidance when selecting strategies to improve microvascular flow.

In 2006, an international round table conference was held in Amsterdam, the Netherlands [22]. Here, international experts including delegates from our group, reached consensus on how to best evaluate the microcirculation using OPS- and SDF imaging. Among other recommendations, it was proposed that comprehensive description of the functional state of the microcirculation must include Perfused Vessel Density, Percentage of Perfused Vessels and Microvascular Flow Index as well as an index of flow heterogeneity. This effort should be applauded, as uniform and thorough analysis assures quality and enables direct comparison between studies. However, the proposed approach may be time consuming as despite advances in computer analysis, current practice is still predominantly manual. Therefore, chapter 3 proposes a standard operating procedure for microvascular analysis.
that simplifies and speeds up this often daunting task without any compromise to the proposed recommendations. Although many groups now adhere to the proposed guidelines, some continue to either use their own scoring systems or only partly implement the recommendations. This is addressed in chapter 4, in which the case for uniform microvascular analysis is further reinforced.

The core of this thesis is formed by six studies. Four of these are original papers while two are case reports. All of these use SDF imaging in a large variety of clinical settings in perioperative and intensive care medicine. Their common aim was to relate findings from microvascular imaging to the macrohemodynamic parameters that are routinely used for monitoring and targeting interventions in these settings.

In leukemic patients, the abundance of white blood cells may sometimes cause a syndrome known as leukostasis. It was hypothesized that microvascular flow impairment due to white blood cell cluttering may be the root of the problem. Therefore, SDF imaging was used to characterize microvascular flow in a patient with extreme leukocytosis. The results may be found in chapter 5.

Following cardiac surgery, it is important to treat hypertension to avoid suture line rupture [23]. In the Netherlands, ketanserin is sometimes used to achieve this goal. It reliably decreases blood pressure within minutes. However, it is unknown whether this decrease in blood pressure is accompanied by changes in microvascular perfusion. Therefore SDF imaging was used to study patients before and after ketanserin administration for hypertension after cardiac surgery. The results are given in chapter 6.

During cardiopulmonary resuscitation, clinicians traditionally focus on global hemodynamics. While this is obviously very important, only animal data is available on microvascular flow during cardiopulmonary resuscitation [24]. Human data is lacking and hence the behavior of microvascular flow in this setting is unknown, especially in comparison to that in a resuscitated patient in whom spontaneous circulation has returned. SDF imaging was used to record microvascular images in a victim of submersion trauma both during mechanical cardiopulmonary resuscitation and after return of spontaneous circulation. Chapter 7 contains the results.

Elaborating on the many unknowns in microvascular perfusion in the setting of cardiac arrest, a controlled study was performed to explore the behavior of the microcirculation in the setting of aortic surgery. The response of the microcirculation to intentional deep hypothermic cardiac arrest was assessed by SDF imaging as a model for spontaneous cardiac arrest. Previously it was unknown when microvascular flow would cease after the heart stops, with estimates ranging from 50 seconds up to 5 minutes [25]. In addition, there was no previous information on microvascular kinetics in the different types of microvessels, e.g. capillaries or venules, in this setting. Our results are reported in chapter 8.

During cardiopulmonary bypass for cardiac surgery the heart-lung machine is responsible for maintaining circulation. This can be done in continuous and pulsatile modes. The benefits of pulsatile perfusion are subject of great debate [26]. Advocates claim that pulsatile
perfusion may improve organ function and even survival, however the evidence is conflicting. The possible protective effects of pulsatile perfusion are usually thought to occur because of an improvement in microvascular perfusion. However, there is no evidence for this in humans. Thus a crossover study was designed in which patients were subjected to pulsatile followed by non-pulsatile perfusion during cardiopulmonary bypass or vice versa. SDF imaging was used during both types of perfusion to record microvascular images, the results of which may be found in chapter 9.

In the ICU, intra aortic balloon pump counter pulsation is frequently used to mechanically support the failing heart. There have been conflicting reports on microvascular changes during IABP support [27]. In addition, the best time to cease IABP support in recovered patients is currently unknown. Chapter 10 reports on the effects on microvascular flow when IABP support is terminated in patients deemed ready for its discontinuation.

This thesis concludes with a discussion on how these studies have contributed to a deeper understanding of the behavior of the microcirculation in perioperative and intensive care medicine especially in relation to commonly measured global hemodynamic parameters. In addition the clinical implications and direction of future research will be examined.

References


Chapter 1

The Microcirculation is a Vulnerable Organ in Sepsis

Paul WG Elbers, Can Ince

Update in Intensive Care and Emergency Medicine 2007: 44: 249
The Microcirculation as a Key Organ in Septic Shock

There is now increasing evidence that the microcirculation is one of the key organisms in the pathophysiology of sepsis and septic shock [1, 2]. However, its importance does not seem to be reflected in current clinical practice. In addition, the surviving sepsis campaign, a world wide effort to decrease sepsis related mortality, focuses only minimally on the importance of the microcirculatory organ [3]. By definition, sepsis is initiated by an infectious agent and the ultimate therapeutic strategy will therefore be its removal from the body. However, the systemic hostile inflammatory response that ensues from sepsis is the real culprit of this disease. The microcirculation is severely affected by this inflammatory response. At the same time, it is responsible for maintaining or even fuelling the devastating disease process of sepsis and septic shock. Even in the face of stable systemic hemodynamics, the microcirculation may be at risk giving rise to regional dysoxia, causing multiple organ failure and ultimately death. Monitoring the microcirculation provides sensitive information on the severity of disease and the effect of therapies [4]. In addition, if sepsis is a disease of the microcirculation [5], resuscitating this organ may become as important as antibiotic therapy.

The Microcirculation as a Functional System

The microcirculation is one of the largest organs in the body and by definition comprises vessels with a diameter roughly smaller than 100 µm, i.e. arterioles, capillaries and venules, and the blood flowing in them. The entire length of the organ is lined with endothelial cells, which are surrounded by smooth muscle cells mainly in arterioles. Red blood cells (RBCs) and the various types of white blood cells (WBCs) complete the cellular picture. However, the microcirculation also embraces a large number of other components including platelets, coagulation factors, and a plethora of cytokines and chemokines [6]. Among the many different microcirculatory functions, the delivery of oxygen to tissue is paramount. This is part of the microcirculation's larger function as an exchanger of nutrients and waste products and chemical or cellular signals. Pertaining to sepsis, however, it is also important to realize the pathogenic interplay of WBCs, RBCs, endothelium, and messenger molecules in inflammation and coagulation in the microcirculation [6].

Therefore, it is not surprising that this organ is a highly regulated one. Central to coordinating microcirculatory perfusion, and hence oxygen delivery ($DO_2$), is the endothelium. In order to meet the oxygen requirements of the cells, the endothelium will ultimately control arteriolar smooth muscle cell tone, both directly and via neurohumoral mechanisms, resulting in altered microcirculatory perfusion. This is achieved by mechanisms such as stress and strain sensing as well as detection of oxygen and metabolic waste products [7]. Endothelium produced nitric oxide (NO) deserves special attention in this context. Apart from its role as a mediator of the inflammatory cascade, the vasodilating properties of NO are important in regulating the distribution of perfusion.

The endothelium, helped by WBCs, platelets, and messenger molecules, is also involved in the regulation of inflammation and coagulation [8]. Interestingly, RBCs are nowadays
considered to regulate perfusion by releasing vasodilators, such as NO [9] and ATP [10], when encountering oxygen deprived environments. In addition it has been shown that deoxyhemoglobin can convert nitrite to NO, causing arteriolar dilatation [11]. Thus, apart from transporting oxygen, RBCs effectively redirect flow and oxygen where it is needed.

Scientific Importance of the Microcirculation

Realization of the importance of the microcirculation is growing, although the concept of microcirculatory disturbances in sepsis is not new. For several decades now, microcirculatory alterations have been recognized as important in pathophysiology [12, 13], and given attention as potential therapeutic targets [14]. One reason why the microcirculation has become an organ of increasing interest in critical care medicine is the validation [15] and clinical introduction [16] of orthogonal polarization spectral (OPS) imaging, which has allowed direct visualization of the human microcirculation in solid organs and mucous membranes for the first time. OPS imaging has revealed the important role of microcirculatory abnormalities in patients with sepsis, confirming results from animal models [17-19]. In addition, we recently validated a scoring system for quantification of microcirculatory abnormalities in sepsis [20] and introduced side stream dark field (SDF) imaging [2, 21] as a successor to OPS imaging.

The Septic Microcirculation

In their landmark clinical study of 50 patients with severe sepsis, De Backer and colleagues showed that functional vessel density and the proportion of perfused vessels smaller than 20 μm were significantly lower than in healthy controls, non-septic patients, and post-cardiac surgery patients [17]. In addition, microvascular deterioration was more severe in non-survivors. A later study by the same group showed that septic patients who did not survive their disease showed no improvement in microvascular perfusion whereas survivors did [18].

Our group reported comparable observations of sluggish microcirculatory perfusion in a small group of septic patients. These observations also independently showed sustained flow in larger vessels confirming that shunting of the capillaries of the microcirculation is a key feature of sepsis [2, 19, 22].

These findings are important because they show that there is indeed a microvascular problem in human sepsis, which is associated with organ dysfunction and death. It also shows the importance of looking at the actual vessels. There has been some confusion in the past, where plethysmography [23], xenon dilution [24], and laser Doppler flux [25] have been used as surrogate markers for microcirculatory perfusion. While observations using these techniques have brought useful data, it should be remembered that they cannot account for any degree of microcirculatory heterogeneity, a characteristic property of sepsis. For this reasons, these techniques should be considered as indicators of regional rather than microcirculatory perfusion.
Of particular note is that the clinical picture of a disturbed microcirculation in sepsis is paralleled by the abnormalities found in various animal models using intravital microscopy and carbon injection. Observations in mice, rats, and dogs invariably show a reduction in perfused capillary density, and stopped flow next to areas of hyperdynamic blood flow, resulting in increased heterogeneity in skeletal and intestinal microvascular beds, despite normotensive conditions [26-29]. It has also been shown experimentally that hemorrhagic shock does not affect microvascular perfusion as much as endotoxic shock for the same degree of hypotension [27].

An increased heterogeneity of the microcirculation was shown to provoke areas of hypoxia and generally impaired oxygen extraction, both mathematically and in a porcine model of septic shock [30]. This means that while some parts of the microcirculation may do relatively well after an insult, there may be other more vulnerable areas that are underperfused. We call these areas microcirculatory weak units [22].

**Dysfunction of Individual Microcirculatory Components**

To understand the causes of microcirculatory abnormalities in sepsis, the impact of sepsis on the different components of the microcirculation needs to be considered. A common finding has been the decreased reactivity of smooth muscle cells to vasostimulating drugs in experimental sepsis. This applies to both vasoconstrictors [31, 32] and vasodilators [33]. However, observations in humans show that the response to nitroglycerin and acetylcholine is still preserved, at least partially [17, 19]. Vasoconstrictor activity can be improved by inhibiting the formation of NO [34]. This is in agreement with observations of a severely deregulated state of the endothelium in sepsis, in which there is massive overexpression of inducible NO synthase (iNOS). As this expression is not homogeneous within tissues, the resulting heterogeneous vasodilatation may partly explain the variation in microcirculatory perfusion observed clinically [35-37].

Apart from its central role in sepsis, the endothelium also serves a passive role lining the vessel wall. In sepsis, this barrier becomes swollen and leaky allowing fluids to extravasate passively [38]. This leads to edema formation, which is aggravated by a possible impairment in the glycoalyx [39] and a reduction in the anionic charge on endothelial cells [40, 41], allowing charged proteins to pass.

There are numerous interactions of WBCs and the endothelium during sepsis, representing the crossroads between inflammation and coagulation. Essentially a complex defense system against infectious agents, this interaction is responsible for the inflammatory response. Many mediators are released, including tumor necrosis factor α, interleukin (IL) β, IL-8, E-selectin, P-selectin, and the intercellular adhesion molecules [6,42]. All are responsible for activating neutrophils, while the latter three, produced both in endothelium and monocytes, are also associated with the initiation of a procoagulant state [43]. While leukocytes themselves become less deformable [44], and have a prolonged capillary transit time [45], potentially blocking microcirculatory flow, the procoagulant state can give rise to a coagulopathy of consumption, disseminated intravascular coagulation (DIC). This coagulopathy
gives rise to microthrombi in the smallest of vessels, again disrupting flow, in addition to the induced risk of bleeding as a result of diminished levels of platelets and clotting factors, both in the micro- and macrocirculation [46].

The RBC is an underappreciated cell. By virtue of its hemoglobin content, it is responsible for the bulk transport of oxygen. RBCs have to pass through capillaries smaller than the cell itself, meaning that they have to deform to be able to pass in single file through the smallest vessels, where there is an effective capillary hemodilution, with hematocrits far lower than that of arterial blood [47]. In addition, a consistent finding both clinically and experimentally is that RBC deformability is decreased in sepsis. This decrease may be caused by direct binding of endotoxin to the RBC, complement coating of RBCs, membrane alterations associated with intracellular ATP changes or the formation of schistocytes in DIC [48-50]. Of specific interest is that the reduction in RBC deformability has been shown to be NO dependent [51], suggesting that the excessive NO production in sepsis may contribute to RBC dysfunction.

**Dysoxia and the Oxygen Extraction Paradox**

The factors discussed above lead to a disturbed microcirculation which, if not corrected adequately, is associated with a very poor prognosis [18]. From this perspective, the microcirculation may be considered as the motor of sepsis [2].

The model that fits this viewpoint is that a disturbed microcirculation in sepsis will lead to an uneven distribution of tissue oxygenation leading to regional dysoxia in microcirculatory weak units, loss of cell viability, organ failure and death. It may, therefore, be meaningful to see if there is evidence linking microcirculatory abnormalities and dysoxia.

In terms of clinical practice, it is perhaps surprising that regional monitoring is not more routinely applied. Usually, clinicians rely on global parameters such as oxygen delivery ($\text{DO}_2$), oxygen uptake ($\text{VO}_2$), cardiac output, and arterial and central venous blood pressure. In addition, commonly observed parameters such as urinary output, lactate levels and skin color or temperature are only nonspecific markers of regional perfusion. Circumstantial evidence of abnormal regional perfusion and dysoxia comes from the fact that patients can be dying even in the light of normal or even improving global parameters.

It is a common finding in clinical sepsis that there is a deficit in oxygen extraction rate. This is illustrated by a normal or high mixed venous oxygen saturation ($\text{SvO}_2$). However, trials aimed at maximizing tissue $\text{DO}_2$ did not improve outcome [52, 53]. This means that either the oxygen is not reaching the microcirculation or that cells and their mitochondria are simply not using it. Indeed mitochondrial dysfunction has been found to be associated with the severity and outcome of clinical sepsis [54]. This type of mitochondrial malfunction in the presence of normal to high amounts of tissue oxygenation has been termed cytopathic hypoxia [55]. Postulated mechanisms include reverse cytochrome inhibition by NO and peroxynitrite. One important study supporting the existence of cytopathic hypoxia examined pigs in which oxygen availability, as assessed by Clark electrodes, remained high
while metabolic distress persisted as evidenced by a high intragastric PCO$_2$ [56]. While cytopenic hypoxia may be one of the causes of metabolic dysfunction, evidence is gathering that microcirculatory blood flow is the main determinant of metabolic disturbance. Microcirculatory PO$_2$, assessed by palladium porphyrin phosphorescence, was less than venous PO$_2$ in a pig model of sepsis [22]. This was direct evidence of shunting of oxygen transport from the microcirculation. Further evidence for this theory comes from a recent study by Creteur et al. in which they showed, amongst other findings, that increasing microcirculatory blood flow, as assessed by OPS imaging, with dobutamine, led to an increase in tissue CO$_2$ levels, confirming that capillary blood flow was an important factor in the metabolic challenge in this setting [57].

Microcirculatory and Mitochondrial Distress Syndrome (MMDS)

The pathophysiology of severe sepsis unresponsive to treatment is determined at the level of the microcirculation and probably at the mitochondrial level. The time factor and the nature of treatment being applied are also important elements. We have termed these deleterious changes, the microcirculatory and mitochondrial distress syndrome (MMDS, figure 1), in which time and therapy are considered as important modulating co-factors [2]. It is important to realize that MMDS is caused by the initial septic hit but then acts to maintain the septic process. Keeping in mind the pathophysiological mechanisms described previously, the microcirculation may be considered a motor of sepsis, effectively shutting down oxygen, nutrient, and medication supply to regions of tissue.

In addition, it should be remembered that the intricate process of microcirculatory organ function is very much dependent on the stage of the disease and the therapy given [2]. An intensive care unit (ICU) physician treating many septic patients will only rarely see one in whom at least some form of therapy has not been started, e.g., fluids, vasoactive agents, antibiotics, or steroids. This will also apply to the microcirculation in sepsis, where it would be more correct to take into account time and therapy when defining microcirculatory disorders. Since the microcirculatory organ can now be visualized in humans more readily, it is possible to directly observe the microscopic consequences of sepsis in man.

Monitoring the Microcirculation

The hallmark of global hemodynamics in septic shock is that of a hyperdynamic circulation. This means an increased cardiac output, low arterial blood pressure, and decreased total peripheral resistance. However, this increased flow does not necessarily result in adequate tissue oxygenation in weak microcirculatory beds in vulnerable organs or their compartments. This paradox can be explained by extreme heterogeneity of the microcirculation or massive arteriovenous shunting of blood flow, effectively bypassing at least some microcirculatory areas.

As has been pointed out above, it is very easy to miss regional perfusion and oxygenation deficits if solely relying on monitoring global parameters. Important studies by LeDou...
et al. [58] and Bourgoin et al. [59] emphasize this idea, showing that resuscitating septic patients to a higher mean arterial pressure (MAP) using norepinephrine actually reduced urinary excretion, increased gastric PCO$_2$, and worsened capillary blood flow.

There is already a myriad of techniques to monitor the microcirculation or at least some form of regional tissue perfusion or oxygenation. Although a detailed overview is not
within the scope of this chapter, some methods should be mentioned. The easiest available today is probably SvO\textsubscript{2} [60]. Although classically considered a global parameter, low SvO\textsubscript{2} values are indicative of tissue at risk of anaerobic metabolism. In the absence of a pulmonary artery catheter the clinician may use the central venous or right atrial oxygen saturation, ScvO\textsubscript{2} or SraO\textsubscript{2}.

Interpretation of these latter values should, however, be performed with caution, as they do not correlate with individual SvO\textsubscript{2} values. However, following their trend may be useful in clinical practice [61]. Also of interest is the arteriovenous PCO\textsubscript{2} difference, essentially monitoring whether cells are actually doing their job and receiving the energy to do so, especially when combined with the arteriovenous O\textsubscript{2} content difference [62].

Monitoring regional oxygenation can be done by gastric pH or gastric, sublingual, buccal, esophageal, or tissue PCO\textsubscript{2} measurement, informing us about the splanchnic vascular bed [36, 58, 64, 65]. For measurement of tissue oxygenation the clinician may use methods based on different forms of spectroscopy to measure microcirculatory hemoglobin saturation [36].

For the moment, the best available monitors of the human microcirculation are SDF and OPS imaging. The SDF imaging technique [21] seems promising as it completely avoids tissue reflectance by illuminating tissue from the side, rendering sharp images of the microcirculation, especially capillaries. An important point to remember, however, is that even though microcirculatory distress, especially measured sublingually, is a serious clinical observation which is associated with a bad prognosis, the microcirculation of other organs may remain unresponsive to therapy and need different recruitment procedures to return to normal function.

It should be noted that images of the septic microcirculation show considerable variation. Again, time and therapy play a very important role here. For example, we observed stagnant capillaries in pressure guided resuscitation in sepsis. In contrast, capillaries with continuous or even hyperdynamic flow may be observed next to capillaries with stopped flow in ongoing fluid resuscitated sepsis. We are currently trying to classify these flow abnormalities in distributive shock based on actual moving pictures. This may be helpful in identifying the causes of these microcirculatory disturbances and perhaps in fine tuning our therapies.

**Resuscitating the Microcirculation**

Knowledge of the pathophysiology of microcirculatory disturbances in sepsis can be used to resuscitate this organ. Loss of barrier function resulting in edema and the heterogeneity of the microcirculation will cause an effective loss of fluids from the global circulation. In addition, there is a flow redistribution at a regional level, predominantly away from vulnerable organs such as those of the splanchnic region [65]. In order to recruit microcirculatory units that are not adequately perfused, it is important to administer fluids and inotropic agents as a first step in microcirculatory resuscitation. Fluids have been shown to increase tissue oxygenation in an animal model [66]. In addition, dobutamine has been shown to
increase microcirculatory perfusion and oxygenation in humans \[57, 67\]. However, this may not hold later on in sepsis underscoring the importance of time in MMDS. In addition, fluids are not effective in consolidating pathological shunting and cause redistribution of blood flow due to both hemorheological effects and altered regulatory properties of the vasculature \[36\].

While normalizing the systemic hemodynamic profile can be considered the first step in rescuing the microcirculation in shock, apparently adequate resuscitation based on systemic variables is not always affective in recruiting the microcirculation. That is why direct monitoring of the microcirculation may be so crucial. Under such conditions other microcirculatory recruitment maneuvers may be considered.

The role of NO in sepsis is complex and incompletely understood \[35\]. However, it is now generally accepted that nonselective inhibition of NOS is not a good thing as it led to increased mortality in human sepsis as shown by the early termination of a phase III trial \[68\]. This is perhaps also the basis of ambiguous results of administering steroids, which non-selectively inhibit NOS in sepsis. However, as mentioned before, from a microcirculatory point of view, selective iNOS inhibition could be favorable in redistributing blood flow away from where it is not needed towards dysoxic regions. In fact, in a porcine model of septic shock, selective iNOS inhibition led to improved intestinal tissue oxygenation and normalization of the gastric PCO$_2$ gap \[36\]. Still, the need for a more robust understanding of iNOS inhibition, including issues such as the best timing and the degree of blockade, calls for cautiousness in clinical use of this strategy.

As far as the microcirculation is concerned, one should probably be careful with vasopressor therapy in sepsis. Although it is obvious from Ohm’s law that at least some perfusion pressure is necessary for blood flow to different organs, resuscitating septic patients to fixed blood pressure endpoints using vasopressor agents may actually jeopardize microcirculatory flow. This was shown by Boerma et al. who administered a relatively high dose of the vasopressin analogue terlipressin to a septic shock patient \[69\]. While urine output and blood pressure improved, sublingual microcirculation came to a halt and the patient died. When using vasopressors it may be advisable to monitor the microcirculation in some way. This has been done by Dubois et al. who showed that vasopressin at lower doses did not affect the sublingual microcirculation \[70\].

Vasodilators could resuscitate the microcirculation by improving flow and by raising capillary hematocrit \[71\]. As previously mentioned, it has been shown that the septic microcirculation is still responsive to acetylcholine \[17\]. Experimentally, we have shown that the NO donor, SIN-1, improved gastric PCO$_2$ in a porcine model of fluid resuscitated shock \[36\]. Commonly used NO donors in intensive care medicine are nitroglycerin and nitroprusside. In septic patients, marked improvement of microcirculatory flow was indeed observed after nitroglycerin infusion \[19\].

It may be counterintuitive that NO donating vasodilators and iNOS inhibiting agents can both be beneficial for the microcirculation, although theoretically, they can be combined.
This problem can be circumvented, however, by using other vasodilators such as ketanserin, a 5-hydroxytryptamine antagonist. Another potentially useful agent in this respect is prostacyclin, which has been shown to improve oxygen consumption and delivery as well as improve gastric intramucosal pH (pHi) in human studies [72, 73].

The vasodilator pentoxifylline is a phosphodiesterase inhibitor and has multiple modes of actions that could resuscitate the microcirculation. Pentoxifylline has experimentally been shown to improve cardiac output, RBC and WBC deformability and to interfere with leukocyte endothelial interaction, causing less WBC stasis [74-78]. In addition, recent research shows that pentoxifylline may act as an iNOS inhibitor thus possibly correcting microcirculatory perfusion maldistribution in sepsis [79]. Indeed, pentoxifylline improved oxygen extraction in an animal model [80], and in septic neonates it was even shown to induce a survival benefit. However, a large clinical trial, in adults or children, has not been conducted so far [81].

Interest in recombinant activated protein C (APC) started because of its anticoagulant activity, inactivating factors Va and VIIIa and increasing fibrinolysis [42]. As such it could counteract DIC and may help resuscitate the microcirculation. APC is currently the only drug that has shown a survival benefit in human sepsis; trials with other anticoagulant drugs have failed to do so [82]. This finding may be explained by the fact that APC also has anti-inflammatory properties. From a microcirculatory perspective, this is beneficial as APC has been shown to reduce endotoxin-induced leukocyte rolling and adhesion as well as improving small vessel blood flow [83]. In addition, APC is also known to block iNOS, which may be another explanation for the observed microcirculatory improvements [84].

Conclusion

The microcirculation is a vulnerable organ in sepsis. At the same time, the diseased microcirculation fuels sepsis, leading to organ failure. Direct monitoring of the microcirculation itself or at least some indicator of regional perfusion may therefore be useful in assessing the course of disease.

However, it should be noted that the effectiveness of many microcirculatory recruitment maneuvers has not yet been confirmed in appropriate clinical trials. Similarly, although there is strong evidence that an improving microcirculation is associated with a better outcome, this is not necessarily a cause and effect relationship and resuscitation of the microcirculation has not been the subject of clinical investigation at the present time. Nevertheless, it is important to remember that normal or improving global hemodynamics or oxygen-derived parameters do not preclude microcirculatory dysfunction, multiple organ failure, and fatal outcome. The microcirculation may be the much-needed end-point of resuscitation of clinical sepsis and septic shock. In addition to accepted therapies, such as fluid resuscitation and inotropic support, promising microcirculatory resuscitating maneuvers including vasodilatation, iNOS inhibition, and multi-action drugs, such as APC, could complement the armamentarium of tomorrow’s ICUs.
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The Microcirculation is a Vulnerable Organ in Sepsis

Chapter 2

Classifying Microcirculatory Flow Abnormalities
Paul WG Elbers, Can Ince

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Abstract

Over 30 years ago Weil and Shubin proposed a re-classification of shock states and identified hypovolemic, cardiogenic, obstructive and distributive shock. The first three categories have in common that they are associated with a fall in cardiac output. Distributive shock, such as occurs during sepsis and septic shock, however, is associated with an abnormal distribution of microvascular blood flow and metabolic distress in the presence of normal or even supernormal levels of cardiac output.

This bench-to-bedside review looks at the recent insights that have been gained into the nature of distributive shock. Its pathophysiology can best be described as a microcirculatory and mitochondrial distress syndrome, where time and therapy form an integral part of the definition. The clinical introduction of new microcirculatory imaging techniques, such as orthogonal polarization spectral and sidestream dark-field imaging, have allowed direct observation of the microcirculation at the bedside.

Images of the sublingual microcirculation during septic shock and resuscitation have revealed that the distributive defect of blood flow occurs at the capillary level. In this paper, we classify the different types of heterogeneous flow patterns of microcirculatory abnormalities found during different types of distributive shock. Analysis of these patterns gave a five class classification system to define the types of microcirculatory abnormalities found in different types of distributive shock and indicated that distributive shock occurs in many other clinical conditions than just sepsis and septic shock.

It is likely that different mechanisms defined by pathology and treatment underlie these abnormalities observed in the different classes. Functionally, however, they all cause a distributive defect resulting in microcirculatory shunting and regional dysoxia. It is hoped that this classification system will help in the identification of mechanisms underlying these abnormalities and indicate optimal therapies for resuscitating septic and other types of distributive shock.

Introduction

Shock is the condition in which there is insufficient transport of oxygen to meet the metabolic demand of the tissue cells. Weil and Shubin [1], in their classic work, classified four states of shock: hypovolemic (loss of intravascular volume), cardiogenic (impaired pump function), obstructive (of the heart, arteries or of the large veins) and distributive shock. They developed a conceptual framework to categorize these states, which gained wide acceptance probably due its clear pathophysiological substrate [2, 3]. The first three categories predictably result in a decrease in cardiac output leading to anaerobic tissue metabolism. However, distributive shock such as septic shock has been more difficult to characterize. This difficulty is primarily due to the fact that this type of shock results from heterogeneous alterations in tissue perfusion caused by microcirculatory dysfunction, resulting in an abnormal distribution of a normal or increased cardiac output [1]. The ensuing disparity between systemic and regional tissue oxygenation makes monitoring difficult and
end-points in the treatment of distributive shock hard to define [2]. Shunting of oxygen transport to the tissues is the main pathogenic feature of distributive shock [4]. It is characterized by hypoxemic shunted microcirculatory weak units, resulting in regional dysxia. Although Weil and Shubin had already identified these concepts, the past decade has provided more insight into the nature of functional shunts and their relationship to impaired oxygen extraction in regional tissue during sepsis [4-8].

The advent of new optical imaging techniques, such as orthogonal polarization spectral (OPS) and sidestream dark-field (SDF) imaging, now allows direct observation of the microcirculation at the bedside. These techniques are applied on organ surfaces and make use of optical modalities to filter out surface reflections of incident light when observations are made. Embodied in a hand-held type of microscope, these techniques allow direct observation of microcirculatory flow at the bedside when placed on organ surfaces. In critically ill patients, these techniques have been applied to the study of sublingual microcirculation and have revealed the central role of microcirculatory function in distributive shock [8-10].

This bench-to-bedside review first briefly describes the different components and functions of the microcirculation in health and disease. The second part of the review discusses how OPS and SDF imaging have exposed microcirculatory abnormalities associated with distributive shock. A five class classification system is introduced for the different types of sublingual capillary flow abnormalities seen during various types of distributive shock.

The microcirculation as an oxygen distributing organ

The microcirculation can be regarded as a vital organ whose function ensures the adequate delivery of oxygen by blood to the various tissue cells [11]. The entire organ is lined with endothelial cells surrounding the plasma and blood cells. A layer of glycocalyx covering the endothelial cells forms an important barrier and transduction system between the lumen of the capillaries and the endothelium and can be disrupted under conditions of inflammation and cardiovascular disease [12]. Smooth muscle cells can be found mainly around arterioles. A large number of cellular components complete the picture: platelets, coagulation factors, cytokines and chemokines. Apart from transporting nutrients and removing waste products, oxygen delivery is the prime function of this organ. The microcirculation is a complex network of resistance and exchange vessels, where perfusion is dependent on numerous factors. These include arterial oxygen saturation, oxygen consumption, blood viscosity, red and white blood cell deformability and flow, shunting of vessels, vasodilatation, vasoconstriction or stasis in arterioles and capillaries, diffusion constants of gasses and nutrients and distances from cells to the nearest blood vessel. The endothelium is an important regulator of oxygen delivery. It responds to changes in blood flow as well as local stimuli. This results in upstream signalling that causes the smooth muscle of the feeding arterioles to dilate [13].

The physical properties of red blood cells, such as deformability and aggregability, play an important role in ensuring optimal perfusion of the microcirculation. Recent findings have shown that red blood cells not only transport oxygen, which is their main function, but can
sense hypoxia and release vasodilator substances such as nitric oxide and ATP [14], indicating that red blood cells have an important role in regulating microcirculatory oxygenation. These mechanisms control highly heterogeneous flow patterns in the microcirculation and ensure homogenous oxygenation of the tissues [15]. Direct diffusion of oxygen from arterioles to other vessels with lower oxygen content, bypassing capillaries, contributes to this process [16]. New recent insights revealing oxygen pressure gradients between flowing red blood cells [17] and oxygen consumption by the vessel wall [18] indicate that oxygen transport kinetics at the capillary level are highly complex.

Marked differences in microcirculatory oxygen pressure ($PO_2$) values can be found in different organs and their subcompartments. For example, epicardial microcirculatory $PO_2$ is high whereas that of the endocardium is lower [19]. In the gut, serosal $PO_2$ is higher [5] than that of the mucosa. Similarly, in the kidney, the cortex $PO_2$ is higher than that of the medulla under normal conditions [20-22].

**The microcirculation in distributive shock**

In sepsis, all the components of the microcirculation listed above are affected, causing a severe dysfunction in its regulatory function and resulting in a regional mismatch of oxygen supply and demand [4]. In summary, endothelial cells are less responsive to vasoactive agents, lose their anionic charge and normal glyocalyx, become leaky and give rise to massive overexpression of nitric oxide. Disturbed gap junctions disrupt intercellular endothelial communication and thus regulation [13]. Both red and white blood cell deformability is reduced, which may cause microvascular plugging. The interaction of white blood cells and endothelium represents the crossroads between inflammation and coagulation. Numerous mediators facilitate intercellular communication and are responsible for white blood cell activation and the induction of a procoagulant state. The latter may give rise to disseminated intravascular coagulation, leading to diminished flow as a result of microthrombus formation. Abnormalities in the nitric oxide system induced by inflammatory activation can be regarded as one of the key mechanisms responsible for the distributive defects associated with severe sepsis and septic shock. Indeed, various studies have shown hemodynamic stabilization after blocking the inflammatory up-regulation of inducible nitric oxide synthase (iNOS) expression (for example, [5]). Inhomogeneous expression of iNOS interferes with regional blood flow and promotes shunting from vulnerable weak microcirculatory units [23]. Inhomogenous expression of endothelial adhesion molecules, such as intercellular adhesion molecules and selectins, can also be expected to contribute to distributive alterations of blood flow through its effect on white blood cell kinetics [24].

Animal experiments have shown a reduction in perfused capillary density, stopped flow next to areas of hyperdynamic blood flow, resulting in increased heterogeneity in skeletal and intestinal microvascular beds, despite frequent normotensive conditions [6, 25]. An increased heterogeneity of the microcirculation was shown to provoke areas of hypoxia and generally impair oxygen extraction, both mathematically and in animal models of septic shock [5, 25, 26]. Microcirculatory $PO_2$ measurements by palladium porphyrin phosphores-
cence revealed that, during various conditions of shock and resuscitation, microcirculatory PO$_2$ levels become lower than venous PO$_2$ levels, providing direct evidence for the action of functional shunting pathways [4, 5, 19, 27, 28]. Acidosis, hypocapnia and hypercapnia occurring during disease and therapy have been reported to have differential effects on the microcirculation, with acidosis (in the presence of nitric oxide inhibition) and hypocapnia causing arteriolar constriction, and hypercapnia resulting in venular dilation [29, 30].

Elevated mixed venous oxygen saturation and metabolic distress, such as occurs during distributive shock, indicates a deficit in oxygen extraction rate. This may be caused by either the oxygen not reaching the microcirculation (e.g. being shunted) [27] and/or that oxygen is not being utilized by the mitochondria of the tissue cells to perform oxidative phosphorylation [31]. The latter has been termed cytopathic hypoxia [32]. This entity, combined with observed microvascular derangements, led us to introduce the term ‘microcirculatory and mitochondrial distress syndrome’ (MMDS) to identify the compartments and pathophysiology of this condition [4]. The nature of MMDS in this definition is not only defined by the condition that led to shock, the comorbidity present and the genetic profile of the patient, but also by the length of time the condition has persisted and the treatment regime that a patient has undergone.

**Classifying microvascular flow abnormalities in shock**

Many of the above insights into the microcirculatory mechanisms underlying distributive defects in sepsis have been obtained from animal experiments. Until recently, observations of microcirculatory hemodynamics in humans were limited to those of skin capillaries in patient nail folds using large microscopes. This changed with the introduction of OPS imaging [33]. This is an optical technique implemented in a hand-held microscope for visualizing the microcirculation in solid organs and mucous membranes using polarized green light and cross-polarized images. We were instrumental in its introduction into the clinic in a surgical setting, which allowed the first observations of the microcirculation in the internal organs of humans [33, 34]. OPS imaging in healthy subjects shows capillaries equally distributed between the tissue cells, ensuring an adequate functional capillary density. One of the most striking findings of OPS imaging in disease is the pathological heterogeneity of microcirculatory flow. Some vascular beds show a preserved functional capillary density whereas others have a sluggish blood flow and some have no flow at all. Capillaries can be recruited and depleted of flow depending on intrinsic and extrinsic factors. When the flow ceases in the capillaries, cells that are close to the capillaries are suddenly far away from their source of oxygen and nutrients, as the diffusion distance of oxygen to the cell increases [6].

An improved optical modality in terms of technology and image quality called SDF imaging has recently been developed for viewing the microcirculation in patients [4, 35]. It uses light-emitting diodes (LEDs) placed around the tip of the light guide with a center core optically isolated from the outer ring (figure 1). When the light guide is placed on tissue surfaces, the light from the outer ring penetrates the tissue, illuminating the microcirculation from the interior. This dark field illumination thus completely avoids reflections from the
tissue surface. This imaging modality yields a clear image of microcirculatory components, with both flowing red and white blood cells. Due to its better image quality, SDF imaging has allowed semi-automated software to be applied in the analysis of the images.

Over the past years, using these new techniques, the human microcirculation has been observed in a large variety of clinical settings both by us and others. Microcirculatory recordings have been made of virtually every type of shock. In hypovolemic, cardiogenic and obstructive shock, microvascular changes are directly related to the limitation in cardiac output. In these conditions, a uniform discontinuity of microcirculatory blood flow in arterioles, capillaries and venules can be observed. All shock states in which the microcirculation was observed were associated with significant metabolic dysfunction (elevated lactate, tissue CO$_2$, strong ion difference). This is in accordance with the findings that metabolic tissue distress, both in hemorrhagic as well as septic shock, is directly dependent on microcirculatory flow [36-38]. In distributive shock, the systemic hemodynamic profile is relatively normal while abnormal disturbed patterns of microcirculatory flow heterogeneity are seen [8, 9]. Over the years we have conducted many clinical microcirculatory observations in a wide range of disease states. These occurred during different types of surgery, infectious and cardiovascular diseases, hematological disorders and critical illness and showed that distributive shock, from a hemodynamic perspective, covers a much wider definition than just sepsis and septic shock. For example, activation of inflammatory pathways and circulatory dysfunction can be caused by cardiopulmonary bypass-pump circuits during cardiac surgery [39], a condition which should also be regarded as distributive shock. Similar conditions can also occur during inflammatory activation during reperfusion injury [40]. Although the main features of normal hemodynamics, inflammation and metabolic distress are common in these different types of distributive shock, the microcirculatory distributive alterations observed by OPS/SDF imaging showed differences in capillary flow patterns under different conditions. To differentiate between the types of flow abnormalities and focusing on sublingual microcirculation due to its clinical accessibility, we clustered similar abnormalities together to establish a classification system that allows a more precise definition of underlying pathologies during different clinical conditions.

At the microcirculatory level, all classes of abnormalities seen during distributive shock show normal to hyperdynamic venular flow [8,9]. It is at the capillary level that the distributive defect is seen, with heterogeneous perfused capillaries resulting in the shunting of areas of the microcirculation. Although the classes of capillary abnormalities we identified may be caused by different mechanisms, they all have in common a distributive defect caused by functional shunting of capillaries in the presence of normal or hyperdynamic venular flow. This is also why we did not make a distinction between stagnant and stopped flow, as both of these result in functional shunting.

Since microcirculatory abnormalities are mainly characterized by a heterogeneous pattern of flow, we summarized the abnormalities per class in two main types of capillary flow patterns. This is shown in cartoon form in figure 2 as two capillaries below each other, each with different flow patterns. Venules are depicted as a single large curved vessel over the
capillaries. In this way, we identified five classes of sublingual capillary flow abnormalities. A Class I abnormality is defined by all capillaries being stagnant in the presence of normal or sluggish venular flow (figure 3). It is a condition that can be found in pressure resuscitated septic patients where vasopressors have been used excessively to normalize blood pressure [8, 9]. Class II microcirculatory flow abnormalities are defined by empty capillaries next to capillaries with flowing red blood cells. This decrease of capillary density makes the diffusion distance between red blood cells in the remaining capillaries and the tissue cells larger, leading to regional hypoxia [6]. The red blood cells in the remaining capillaries show a high microcirculatory hemoglobin saturation, indicating poor oxygen off-loading associated with the reduction in capillary exchange surface area [41]. Class II abnormalities were most frequently found during use of extracorporeal circuits in coronary artery bypass...
grafting (CABG) surgery and extracorporeal membrane oxygenation (ECMO). Class III abnormalities are described by capillaries with stagnant blood cells next to capillaries with normal flow. These abnormalities were most frequently observed in sickle cell patients and critically ill malaria patients, but also in septic patients. In critically ill malaria patients, who are often in a coma, strikingly normal hemodynamics are seen in the presence of high lactate levels. This feature, together with class III microcirculatory abnormalities, also identifies this condition as distributive shock. Class IV abnormalities show hyperdynamic flow patterns in some capillaries next to capillaries with stagnant cells (figure 3). Venules in such cases frequently also show a hyperdynamic flow profile. This condition is seen in resuscitated hyperdynamic septic patients. Class V abnormalities describe the condition where hyperdynamic flow is seen at all levels of the microcirculation.

Blood cells usually travel so fast that individual cells can not be distinguished from each other. Metabolic distress seen under such conditions could be the result of cells moving too fast to off-load their oxygen, or, that they may originate from other organs or compartments being shunted [28]. Interestingly, the class V types of abnormalities are also observed in extreme exercise. The pathogenic nature of class V abnormalities in septic...
patients remains to be determined. In table 1, the diseases observed so far are listed next to the different classes of microcirculatory abnormalities seen in figure 2. They are by no means complete and it is hoped that this list will continue to expand as more insight is obtained into the nature of distributive alterations. Scoring systems developed to quantify such images should greatly aid this process [42]. Examples of OPS/SDF movies of each class of abnormality can be viewed on our web site [43].

The complex interaction of pathology and treatment define the abnormalities seen at the microcirculatory level in distributive shock. From this perspective, it can be expected that the different classes of microcirculatory abnormalities shown in figure 2 are caused by a combination of different regional pathogenic mechanisms while having a similar systemic hemodynamics profile.

Several pathogenic mechanisms associated with disease and therapy could be considered in this context. Normalizing arterial pressure by excessive use of pressor agents, for example, will cause a rise in arterial pressure but at the cost of microcirculatory flow [44]. Such a condition can underlie the class I type of distributive abnormality. Hyperoxia, as applied during the treatment of sepsis with high levels of inspired oxygen, or during cardiopulmonary bypass in CABG surgery, can lead to arteriolar constriction, causing a reduction in functional capillary density and distributive microcirculatory alterations [45]. Hemodilution, applied in various clinical scenarios, causes a decrease in blood viscosity, altered red blood cell rigidity and functional shunting of the microcirculation [28]. The reduced blood viscosity results in a reduction in longitudinal capillary pressure gradient due to reduced resistance of the blood and can result in a fall out of capillary flow. This condition could lead to class II abnormalities. Hemorheological alterations occurring during sepsis and infectious diseases such as malaria [46, 47] are caused by increased red and white blood cell aggregability and rigidity, which can result in the obstruction of capillary blood flow, resulting in class I, III or IV abnormalities. Heterogenous iNOS expression and excessive production of nitric oxide, causing regional vasodilation and an increase in microcirculatory driving pressure, could

<table>
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<th>Class</th>
<th>Capillary Hemodynamics</th>
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<tr>
<td>I</td>
<td>Stagnant</td>
<td>Pressure guided resuscitation from sepsis</td>
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<tr>
<td>II</td>
<td>Continuous/capillary fall-out</td>
<td>On-pump CABG surgery, ECMO</td>
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<td>III</td>
<td>Continuous/stagnant</td>
<td>Resuscitated sepsis, reperfusion injury, sickle cell crises, malaria</td>
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<td>IV</td>
<td>Hyperdynamic/stagnant</td>
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<td>V</td>
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<td>Resuscitated sepsis, exercise</td>
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Table 1. Classifying microcirculatory flow abnormalities in distributive shock. See text for details. CABG, coronary artery bypass grafting; ECMO, extracorporeal membrane oxygenation.
Figure 3. Examples of sidestream dark field images of sublingual microcirculation from septic patients with distributive shock. (Top) Image taken from a resuscitated septic patient with a class I type of microcirculatory abnormality, complete stasis in the capillaries. (Middle) An example of a patient with class IV abnormalities with some capillaries showing stasis and others showing high flow. (Bottom) Image of a healthy volunteer with microcirculatory flow in all vessels.
result in the hyperdynamic images described by class IV and V types of abnormalities. The heterogeneous expression of iNOS in the various organs could explain why, in the presence of similar systemic hemodynamic profiles, regional variation in class V abnormalities might persist [23]. From the above considerations, it can be concluded that a combination of the described pathogenic mechanisms associated with disease and therapy can result in the various microcirculatory abnormalities described in figure 2. Different types of microcirculatory abnormalities could persist in different organ systems, depending on the action of regional pathogenic mechanisms and regional response to applied therapies. Future research using microcirculatory monitoring techniques should identify which disease state combined with which type of therapy underlies these abnormalities. These insights could then identify which microcirculatory recruitment maneuvers are most appropriate for improving organ function in distributive shock.

**Resuscitating microcirculatory defects underlying distributive shock**

Microcirculation recruitment maneuvers may be able to correct the observed abnormalities [23]. They can be regarded as a two step approach. First, the microcirculation should be opened and kept open. This implies the need for fluids, inotropics, vasodilators and restricted use of vasopressors. Second, pathological flow heterogeneity and microvascular shunting should be corrected. This demands control of inflammation, vascular function and coagulation [4]. In this respect, it is important to realize that MMDS and its distributive alterations are not static entities but evolve in time in interaction with therapy and disease.

The manner in which therapy can improve systemic variables, while leaving the microcirculation unaffected, was shown in an early study by LeDoux and co-workers in septic patients [48]. That therapy can actually impair the microcirculation and affect outcome was reported by Boerma and co-workers in a case study in a septic shock patient receiving the vasopressin analog terlipressin [49]. Here it was found that while this compound was effective in improving hemodynamics and urine output, it resulted in microcirculatory flow stasis and a deterioration of the patient. The finding that a lower dose of vasopressin, in a similar setting of distributive shock, had no such effect on the microcirculation while improving systemic hemodynamics underscores the need to monitor individual cases [50].

Application of microcirculatory recruitment maneuvers has been shown to be effective in promoting microcirculatory blood flow and correct metabolic distress in clinical studies using OPS/SDF imaging (for example [37, 38]). Fluids in combination with nitroglycerine therapy were shown to recruit disturbed microcirculation following pressure guided resuscitation in septic shock patients, suggesting a role for vasodilator therapy in the treatment of sepsis [9, 51]. De Backer and colleagues had also shown that such disturbed microcirculation can be recruited by topical application of acetylcholine [8]. Support of pump function by dobutamine therapy has been shown to improve microcirculatory flow independent of improvement of global hemodynamic parameters [52]. Correction of endothelial function and coagulation abnormalities by activated protein C has been recently shown to recruit microcirculatory function during septic shock [53]. Recently, Spronk and co-workers...
[37] reported a case study where thrombolysis therapy using a recombinant tissue plasminogen activator in fulminant purpura was effective in recruiting sublingual microcirculation and normalizing sublingual capnography. Thus, it is clear that therapies are available that are effective in recruiting the microcirculation. Although persistent microcirculatory abnormalities are associated with a very bad prognosis [54] and may need to be corrected, the efficacy of such microcirculatory recruitment procedures in affecting outcome still has to be determined in controlled trial settings. The availability of microcirculatory imaging technologies and effective scoring methods will greatly aid in answering these questions.

Conclusion

It is now clear that optimizing global hemodynamic and oxygen derived parameters in patients in shock does not necessarily resuscitate the microcirculation. As this is the organ that is ultimately responsible for oxygen delivery to tissue, it seems sensible to monitor this organ and, if necessary, improve its function.

Observing the microcirculation in different shock states shows equally different flow patterns. These depend on the pathophysiology of the disease, its time course and the instituted therapy. The number of affected microcirculatory components and the severity of their disturbance are set by these three factors, which will ultimately determine what we see when recording dynamic images.

The now commonplace classic shock classification based on global hemodynamics is invaluable in optimizing systemic circulation and oxygen delivery. However, microvascular resuscitation could become an adjunct to early goal directed therapy in shock states. Our proposed reclassification system may be a basis for identifying different types of microcirculatory abnormalities and possibly provide a guide for therapeutic interventions.

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Chapter 3

Fast-Track Microcirculation Analysis
Paul WG Elbers

Critical Care 2007; 11: 426
Letter

The recently published report ‘How to evaluate the microcirculation’ [1] should be praised for standardizing analysis of human microcirculation data. This standardization will enable better comparison between studies.

Having worked extensively with both orthogonal polarization spectral (OPS) and side-stream dark field (SDF) imaging and most analysis software, I feel the proposed analysis is extremely useful but also equally time consuming. Despite advances in computer analysis, current practice is still predominantly manual. I therefore wish to make a comment that may greatly simplify the procedure.

The report suggests determining the microvascular flow index (MFI), the perfused vessel density (PVD) and the percentage of perfused vessels (PPV). For the MFI a grid is used dividing the screen into four quadrants, and the vessels are scored according to observed flow: 0 = none, 1 = intermittent, 2 = sluggish, 3 = continuous. For the PVD and the PPV, three equidistant horizontal and vertical lines are drawn and a different score is used: absent, intermittent, present (for details see [1]).

I propose using the same grid for the MFI, the PPV and the PVD. Dividing the MFI quadrants into four sections more effectively creates the PPV and PVD lines (see figure 1). Each vessel is then scored according to the MFI criteria. The PPV and the PVD are calculated as usual. Vessels with MFI scores of 2 or 3 are classified as having flow present. Finally, the MFI is calculated as usual.

I used this method for a recent study [2]. Trzeciak and colleagues used a similar approach but with different scoring definitions [3]. Combining scores and the grid saves time. In addition, the approach potentially allows for distinction between sluggish and continuous flow for PVD and PPV determinations.

References

Figure 1. The same grid is used for MFI, PPV and PVD determinations. Only the bold center lines are used for MFI.
Quality and Consistency in Microvascular Research
Paul WG Elbers, Bektas Atasever

British Journal of Anesthesia, 2009; 102: 886
Letter

We read the recent article by Maier and colleagues [1], in which they assess the microcirculatory effects of phenylephrine during cardiopulmonary bypass. The authors are to be commended for focussing their research on the microcirculation, as that is where actual delivery of oxygen and nutrients to tissue takes place. Their study adds to the body of evidence that systemic haemodynamic measurements do not necessarily reflect microvascular perfusion. However, having extensive experience with the technique used by the authors to visualize the microcirculation, that is, sidestream dark-field (SDF) imaging, we feel the following comments may be of great importance. Many different scoring systems and video acquisition procedures have been developed in microcirculation research. This has the potential for hampered comparison between similar studies, or worse, false conclusions. This is why a round table conference was held in 2006. Using the Delphi methodology, a vast number of recommendations were made in order to improve quality and consistency in microvascular research [2].

Unfortunately, the authors did not fully adhere to these recommendations. Although there are multiple differences from the consensus statement, two may be critical and are discussed below. First, it was recommended to measure at least three, but preferably five sites, per patient per time point, because of the intrinsic variability of the microcirculation. This also allows for better reporting of an index of heterogeneity. The latter is not stated and they only measured two sequences per time-point. It is therefore possible that the measurements they have made do not truly represent the state of the microcirculation. This may be reflected by the unexpectedly low baseline microvascular flow index (MFI) of 2.5 in anaesthetized patients before cardiopulmonary bypass. Secondly, it was recommended to report both MFI, as the authors did, and an index of functional capillary density, which was omitted. The problem with this is that MFI is a semi-quantitative way to describe microvascular flow but does not take into account the number of vessels per area. This is important because with SDF imaging, different imaging sites are used at different time points which may hence have different capillary densities. Obviously from a perspective of oxygen and nutrient delivery to tissue, a large capillary density with some sluggish and some continuous flow would be preferable over very low capillary density albeit with only continuous flow. Because an index of perfused vessel density was not reported, the given MFI values may not truly represent the state of the microcirculation. Of course, trade-offs have to be made in microvascular research between detail of analysis and the time it takes to perform the analysis. However, it was recently suggested that using a fast-track analysis approach, it is quite reasonable to report on both indices of microvascular perfusion [3].

In summary, failure to adhere to the recommendations mentioned above may have led to a biased representation of the microcirculation. This is not to say that the results are false by definition. However, we feel that they should be approached with caution. Their conclusion would be greatly strengthened by reporting on more microvascular sites per time point and by extending the analysis to include an index of functional capillary density. Although the former is not feasible, it is relatively easy to perform the latter using the existing videos of
the microcirculation. Therefore, we would strongly recommend the authors to do so.

References

Chapter

Microvascular Blood Flow in Extreme Leukocytosis
Arend-Jan Meinders, Alaattin Ozdemir, Paul WG Elbers

The New England Journal of Medicine 2009; 360: e9
Case

A 51-year-old woman presented with a 3-month history of fatigue and a 2-week history of a nonproductive cough and night sweats. Chronic myeloid leukemia was diagnosed, with a leukocyte count of 398,000 per cubic millimeter.

On the basis of suspected leukostasis, sidestream dark field (SDF) imaging was performed. SDF imaging uses a handheld microscope which emits green light (530 nm) that is absorbed by hemoglobin to visualize the sublingual human microcirculation in real time.

At the time of diagnosis, SDF imaging (figure 1, panel A and video 1) showed abnormally large gaps between erythrocytes, probably because of leukocytosis (black arrows). We saw areas of hyperdynamic flow (black arrowhead) next to areas with striking microcirculatory stasis (white arrow) and capillary derecruitment (white arrowheads).

After 2 weeks of treatment with hydroxyurea, the leukocyte count normalized, the patient’s symptoms diminished, and the microcirculatory flow pattern returned to normal (figure 2, panel B and video 2). We observed restored flow in previously derecruited capillaries and normalization in the gaps between erythrocytes.

Approximately 2 years later, the patient underwent allogeneic stem-cell transplantation from her HLA-identical sister and since then has been in good health.

Videos may be seen by visiting http://content.nejm.org/cgi/content/full/360/7/e9
Figure 1. Stills of SDF imaging before (A) and after (B) treatment. See text for details.
Chapter 6

Microcirculatory Imaging in Cardiac Anesthesia: Ketanserin Reduces Blood Pressure but not Perfused Capillary Density
Paul WG Elbers, Alaattin Ozdemir, Mat van Iterson, Eric PA van Dongen, Can Ince

Journal of Cardiothoracic and Vascular Anesthesia, 2009; 23: 95
Abstract

Objectives: It has become possible to image the human microcirculation at the bedside using sidestream dark field (SDF) imaging. This may help the clinician when correlation between global and microvascular hemodynamics may not be straightforward. Ketanserin, a serotonin and α-1 adrenoceptor antagonist, is used in some countries to treat elevated blood pressure after extracorporeal circulation. This might hamper microcirculatory perfusion. Conversely, it is also conceivable that microcirculatory flow is maintained or improved as a result of flow redistribution. In order to introduce SDF imaging in cardiac anesthesia, we set out to directly observe the sublingual microcirculation in this setting.

Design: An observational study.

Setting: A large teaching hospital.

Participants: Mechanically ventilated patients with elevated arterial blood pressure immediately after extracorporeal circulation (ECC).

Intervention: An intravenous bolus of ketanserin, 0.15 mg/kg.

Measurements and main results: Five minutes before and 10 minutes after ketanserin administration, global hemodynamic variables were recorded. In addition, we used SDF imaging to record video clips of the microcirculation. Analysis of these allowed for quantification of microvascular hemodynamics including determination of perfused vessel density (PVD) and microcirculatory flow index (MFI). After ketanserin administration, there was a significant reduction in systolic arterial blood pressure (129 (9) to 100 (15) mm Hg, p<0.01). At the level of the microcirculation, the mean MFI did not change significantly for small (diameter <20 µm, 2.79 [interquartile range, 1.38-3] to 2.38 [1.88-2.75], p=0.62) or large (diameter >20 µm, 2.83 [1.4-3] to 2.67 [0.35-2.84] p=1.0) vessels. There was a significant increase in mean PVD for large vessels (1.23 (0.63) to 1.70 (0.79) mm⁻¹, p=0.02) but not for small vessels (5.59 (2.60) to 5.87 (1.22) mm⁻¹, p=0.72) where red blood cell flow was maintained.

Conclusions: SDF imaging clearly showed a discrepancy between global and microvascular hemodynamics after the administration of ketanserin for elevated blood pressure after ECC. Ketanserin effectively lowers arterial blood pressure. However, capillary perfusion is maintained at a steady value. Both effects may be explained by an increase in shunting in the larger vessels of the microcirculation.
Introduction

Clinical research of the microcirculation has long been hampered by a lack of suitable techniques. However, the advent of modern imaging modalities like orthogonal polarized spectral (OPS) imaging [1, 2] and its improved successor, sidestream dark field (SDF) imaging [3], have facilitated observation of the human microcirculation at the bedside in real time. To date, these devices primarily have been used to characterize the microcirculation in sepsis. These studies have consistently shown that there may be large discrepancies between microcirculatory flow and global hemodynamics [4-7].

Interestingly, cardiac anesthesia profoundly relies on monitoring of global hemodynamics, such as arterial and venous pressures. Obviously, these parameters are of great importance. However, if similar discrepancies were to exist in this setting, these routine measurements might not reflect the state of the microcirculation. This would prevent accurate estimation or correction of capillary perfusion and transport of oxygen and nutrients to organs, tissues, and cells. Unfortunately, experience with microcirculatory imaging is limited in this field. To introduce SDF imaging in cardiac anesthesia, we designed an observational pilot study. To this end, we chose to investigate the microcirculation before and after drug treatment for elevated blood pressure after extracorporeal circulation with cardiopulmonary bypass (CPB).

As discussed later, we hypothesized that this clinical setting could show disagreement between microvascular and macrovascular measurements. Thus, it could unveil the maximum potential of the technique.

High blood pressure after CPB may give rise to life-threatening complications such as suture line disruption, aortic dissection, and myocardial ischemia [8]. It may occur in up to 60% of patients undergoing CPB [9] and calls for aggressive treatment to lower blood pressure [8]. At the same time, however, such a reduction in blood pressure must not compromise tissue perfusion to prevent shock and ischemic complications such as stroke.

By augmenting systemic vascular resistance (SVR), both an increase in circulating catecholamines [10] and serotonin (5-HT) [11] are thought to contribute to increased blood pressure in this setting. Thus, a drug blocking adrenergic and 5-HT receptors may be a logical treatment option. Indeed, in Europe, ketanserin, a quinazolinedione derivative with 5-HT-2 receptor and mild 1-α, -β, and -δ adrenoceptor antagonist activity [12], continues to be used successfully in these patients. Intravenous administration reduces systemic blood pressure while moderately increasing heart rate and cardiac output [11].

Ketanserin is generally considered to benefit microcirculatory perfusion based on the observed decrease in SVR. However, this is dependent on the exact site of action. For example, if ketanserin increases precapillary shunting by opening arteriovenous passages, tissue perfusion could actually be hampered. In contrast, if increased capillary flow was the mechanism behind the reduction in SVR, this could possibly increase oxygen and nutrient delivery to tissue. The former could cause a discrepancy between microvascular and global hemodynamic measurements. In addition, it is well known that CPB induces a systemic
inflammatory response [13] which could also contribute to such discrepancy.

For these reasons, we believe that this clinical setting is useful to introduce SDF imaging as a novel technique in cardiac anesthesia. Thus, it was our hypothesis that ketanserin would be able to lower blood pressure but not at the expense of microcirculatory perfusion.

Methods

The local institutional review board approved the study. The need for written informed consent was waived in accordance with the national Law on Experiments with Humans because the study was observational and measurements were considered noninvasive. The study was performed in the intensive care unit (ICU) of our hospital between May and September 2006.

In order to be considered for inclusion, patients had to be over 18 years of age, have undergone cardiac surgery with the use of extracorporeal circulation, and be mechanically ventilated. Patients were considered for eligibility in a nonconsecutive fashion based on the

Figure 1. (A) The handheld SDF imaging device equipped with a 5x magnifying objective. The microcirculation is imaged using green pulsed light-emitting diode illumination. In the tissue, the light is scattered and is absorbed by hemoglobin. The lens system is optically isolated from the outer light-emitting diode ring, thus minimizing surface reflections. Using a charge coupled device (CCD) chip, video can be stored digitally. (B) The CCD chip can be axially translated with respect to the lens system to alter depth of focus. Modified with permission from [3].
availability of microcirculatory recording equipment and its operators. Patients were only included definitively if an attending ICU physician decided that ketanserin administration was needed for blood pressure control within 2 hours of arrival in the ICU. Patients with lacerations of the oral mucosa were excluded because this would interfere with microcirculatory imaging. Additional exclusion criteria were a history of any type of diabetes or hypertension because these are known to affect the microcirculation to various extents [14, 15].

All patients underwent routine and continuous monitoring of global hemodynamics through arterial and central venous catheters as well as rectal temperature and ventilation parameters. Two patients had a pulmonary artery catheter. These data were recorded automatically every minute by a patient data-management system.

As per clinical routine, patients were given a 0.15 mg/kg bolus of ketanserin via a peripheral intravenous catheter. In our experience, this dose predictably lowers arterial blood pressure within minutes, whereas no further decline in blood pressure is seen beyond 6 to 8 minutes after drug administration.

Five minutes before and 10 minutes after drug administration, we recorded video clips of the microcirculation using SDF imaging (figure 1). This technique has been described in detail previously [3]. In brief, it consists of a handheld video microscope that emits stroboscopic green light (530 nm) from an outer ring at the end of a probe. This light is absorbed by hemoglobin. Thus, a negative image of moving red blood cells is transmitted back through the isolated optical core of the probe toward a charge-coupled device (CCD) camera. SDF imaging has been shown to provide a higher image quality with more detail and less motion blur than its predecessor OPS imaging [3]. In a recent roundtable conference, international experts reached consensus on how to best evaluate the microcirculation using OPS and SDF imaging [16]. We implemented all recommendations given in this article. Video clips were directly saved as digital AVI-DV files to a hard drive of a personal computer using an analog-to-digital converter (Canopus, Kobe, Japan) and the freeware program WinDV (http://windv.mourek.cz). We used 5x optical magnification, producing images representing approximately 940x750 µm² of tissue surface. Per time point, clips at 3 sublingual sites yielding at least 20 s of stable video per site were recorded.

Special care was taken to avoid pressure artifacts, adhering to the standard operating procedure previously described by Trzcinski et al. [4] and recommended in the roundtable conference [16]. In brief, secretions were removed with gauze, and, after obtaining good image focus, the probe was pulled back gently until contact was lost and then advanced again slowly to the point at which contact was regained. We paid special attention to the larger vessels at the time of recording because alterations in their flow with probe manipulation may indicate pressure artifacts.

One video file was recorded for each site at each time point. These were stored under a random number. At a later time, these were analyzed by one of the authors (PWGE) using the AVA 3.0 Software program (Microvision Medical, Amsterdam, The Netherlands). According to the recommendations, microvascular flow index (MFI), perfused vessel density
(PVD), proportion of perfused vessels (PPV), and indices of heterogeneity were determined for every patient at both time points. All have been validated previously [4, 17, 18]. As published recently [19], each score was determined for both large and small microvessels with a cut-off diameter of 20 µm. In addition, we defined large-type vessels that split into more large vessels as arterioles. Other large vessels were defined as venules. For PPV and PVD, vessel density was calculated as the number of vessels crossing 3 horizontal and 3 vertical equidistant lines spanning the screen divided by the total length of the lines. Perfusion at each crossing was then scored semi-quantitatively by the eye as follows: 0 - no flow (no flow present for the entire duration of the clip), 1 - intermittent flow (flow present >50% of the duration of the clip), 2 - sluggish flow (flow present >50% but <100% of the duration of the clip or very slow flow for the entire duration of the clip), and 3 - continuous flow (flow present for the entire duration of the clip). PVD was then calculated as the number of crossings with flow scores greater than 1. PPV was calculated as the proportion of crossings with flow scores greater than 1 divided by the total number of crossings. For each time point and each patient, the scores for PPV and PVD were averaged. PPV is expressed as n/mm, whereas PPV is expressed as a percentage. Intraobserver variability ranges between 2.5% and 4.7% for PVD and between 0.9% and 4.5% for PPV. The interobserver variability is slightly higher: between 3.0% and 6.2% and between 4.1% and 10%, respectively [18].

MFI was based on the determination of the predominant type of flow in 4 quadrants

<table>
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<tr>
<td>Nitroglycerin (mg/h)</td>
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Table 1. Patient and procedure characteristics, vital signs and drugs. ECC, extracorporeal circulation; AoX, aortic cross clamp.
adhering to the same scoring system. MFI is the sum of these flow scores divided by the number of quadrants in which the vessel type is visible. The intraobserver agreement of MFI is about 85% (kappa score 0.78) and interobserver agreement about 90% (kappa score 0.85) [17]. For each time point and each patient, the scores for MFI were averaged.

Heterogeneity was assessed in 2 different ways. For PVD, the coefficient of variation was determined. For MFI, we assessed heterogeneity in each patient by subtracting the lowest from the highest quadrant MFI and dividing the result by the mean MFI [4, 18]. We used nonparametric tests for MFI and paired t tests for other data. Results are reported as median and interquartile ranges (IQR) for MFI and as the mean (standard deviation (SD)) for other parameters, unless indicated otherwise. The study was powered to detect a 20% difference in small vessel PVD after ketanserin administration based on the PVD and its SD in patients undergoing thoracic surgery as found by De Backer et al [20]. This showed the need for the inclusion of 6 patients.

Results

Six patients were included. Patient characteristics are shown in table 1, including their analgesic, vasoactive, and sedative drugs. One patient had an intra-arterial balloon pump in place, and one patient had a history of systemic lupus erythematosus and a kidney transplant for which she received immunosuppressants. One patient had a pacemaker functioning in AAI mode. This patient was excluded from heart rate analysis. Intravenous administration of ketanserin, 0.15 mg/kg, had a similar impact on global hemodynamic parameters in all patients. These are shown in table 2. There was no change in temperature or oxygen saturation throughout the experiment.

We were successful in obtaining high-quality images in each patient. Figure 2 shows a typi-
The standard operating procedure for acquisition and analysis of the microcirculation was strictly adhered to in order to avoid artifacts. A total of 36 SDF video clips were recorded. In only 2 of these, an arteriole could be identified. Typically, microvascular architecture consisted of tortuous capillaries and venules. The results for PVD, PPV, MFI, and indices of heterogeneity are shown in table 3 and figure 3.

The administration of ketanserin lowered arterial blood pressure. At the same time, we observed a significant increase in mean perfused vessel density for large vessels. However, capillary perfusion was unaltered by the administration of ketanserin. Other microcirculatory parameters including microvascular heterogeneity remained statistically unaltered.

Figure 4 depicts the relationship between changes in global hemodynamics versus changes in microvascular parameters in individual patients. Individually, there does not seem to be a clear association between the degree of reduction in systemic blood pressure and the response of the microcirculation. Again, on average, PVD in microvessels with a diameter smaller than 20 µm remained fairly stable, whereas that of microvessels with a diameter larger than 20 µm increased.

**Discussion**

We successfully used SDF imaging in cardiac anesthesia to study the microcirculatory effects of ketanserin when administered to correct elevated blood pressure after extracorporeal circulation. Using this novel technique, it was shown that changes in global hemody-
namics were not reflected in the microcirculation. Such discrepancies previously have been described for other clinical settings, most notably in sepsis.

In the present patients, an intravenous bolus of ketanserin significantly increased mean perfused vessel density in vessels larger than 20 µm. Perhaps more importantly, ketanserin did not alter PVD, PPV, or MFI for small vessels. This implies that capillary density remained intact, suggesting that microcirculatory perfusion was not compromised even in the face of

Figure 3. The effect of ketanserin, 0.15 mg/kg, on perfused vessel density for vessels with a diameter (top) smaller and (bottom) larger than 20 µm. Data points represent individual patients. Perfused large vessel density increased significantly (*, table 3), whereas perfused small vessel density remained statistically unaltered.
markedly different global hemodynamics. The latter followed the same pattern as described earlier by Van der Stroom et al. [8] with a slightly elevated heart rate and a reduction in arterial blood pressure.

This is the first study that reports on the state of the microcirculation in normovolemic patients after cardiac surgery using all international consensus recommendations. Bauer et al. [21] reported on functional capillary density after cardiac surgery using OPS imaging. However, they used capillary length per area as a parameter that precludes direct comparison with the present data. Using the same technique, De Backer et al. [20] reported on PVD in patients before cardiac surgery. They found a median of 1.9 (IQR 1.4-2.3) mm\(^{-1}\) for large vessels and 3.8 (IQR 3.2-4.5) mm\(^{-1}\) for small vessels. This is quite different from the present findings: 5.8 (IQR 4.0-7.7) for small vessels and 1.1 (IQR 0.7-1.7) for large vessels.

Focusing on small vessels, the study by De Backer et al. [6] showed median PVD values of 3.1 (IQR 2.8-3.2), 3.1 (IQR 2.9-3.3), and 1.1 (IQR 0.7-1.5) for healthy controls, nonseptic acutely ill ICU patients, and septic patients, respectively. Interestingly, Trzeciak et al. [4] also reported on PVD in septic patients and healthy volunteers but found very different numbers, 8.20 (1.9) and 10.75 (1.2) mm\(^{-1}\).

Only 3 studies have reported on MFI in humans. Boerma et al. [22] used this index in their
study comparing stoma and sublingual microcirculation. In septic patients, they reported a median MFI value for small vessels of 2.08 (IQR 1.25-2.42).

Tzreciak et al. [4] also used MFI, although it is not clear for which vessel size. Their reported median MFI was 1.48 (SD 0.4) for septic patients and 2.82 (SD 0.1) for healthy controls. Den Uil et al. [23] reported the maximum value of 3 as median MFI for all vessel types after cardiac surgery. The present MFI values for small vessels are slightly lower but seem to be in the range of healthy volunteers.

Some of the observed differences in vessel density may be explained by the higher quality of SDF imaging as compared with OPS imaging. It is important to note that none of the previous studies quoted fully adhered to the recommendations of the round table conference. Therefore, differences in methodology between the present study and those by De Backer et al. [6] and Trzeciak et al. [4] may explain part of the large differences in capillary density. For example, Trzeciak et al.’s capillary density was simply a count of vessels seen, whereas De Backer et al., like us, only counted vessels that showed flowing hemoglobin. The importance of standardization in microvascular research cannot be emphasized enough. As discussed in more detail later, the remainder of the difference may be explained by the fact that we studied patients with elevated blood pressure.

Before the present study, only 1 article focused on the human microcirculatory effects of ketanserin. Based on peripheral plethysmography, Konishi et al. [24] concluded that ketanserin beneficially affects microhemodynamics and rheology. As stated earlier, the present study confirmed an increase in large vessel PPV, but capillary density remained unchanged. Animal experiments have shown that 5-HT-2 receptor–mediated constriction of arteries larger than 50 µm is blocked by ketanserin [12]. This is consistent with the observation of an increase in PVD for large vessels, almost exclusively venules.

Regrettably, the number of arterioles in the present recordings was too small to yield meaningful results. In contrast, the α1-blocking effect of ketanserin is known to dilate venous capacitance vessels [25]. Possibly, the increase in PVD for large vessels is the microcirculatory substrate for this observation. However, this is not reflected in the central venous pressure of the present patients, which changed very little after ketanserin administration.

Again, from animal experiments, it has been suggested that ketanserin may open arteriovenular shunts [26]. This is consistent with our finding that small-vessel microcirculatory parameters remained unchanged in the face of lower systemic blood pressure and an increased density of larger microcirculatory vessels. If ketanserin were truly able to open arteriovenous shunts, this means that, in the current patient group, these shunts were closed. This may have been the cause for their elevated blood pressure.

Microcirculatory imaging has been used extensively in sepsis. This led Ince [27] to introduce the concept of microvascular weak units, meaning that in sepsis these weak units feature impaired perfusion even when macrohemodynamics are normalized. The units can exist next to areas in which the microcirculation is normal. In other words, the hallmark of these microcirculatory derangements is heterogeneity. In fact, the distributive shock
seen in sepsis and systemic inflammation may be classified according to microcirculatory observations [28].

This may warrant treating the microcirculation as an organ that may be in need of resuscitation in certain clinical settings [5]. It is feasible that the concept of microcirculatory weak units is also applicable after CPB because this is known to induce a systemic inflammatory response state associated with hypermetabolism and an increase in oxygen consumption. Oudemans-Van Straaten et al. [29] showed that ketanserin decreases endotoxin levels and attenuates the increased VO$_2$ in this setting. However, this could be a reflection of ketanserin compromising the microcirculation by shunting blood away from it, thus preventing cells from using the oxygen. A decreased PPV and PVD and/or an increased heterogeneity of the microcirculation would be consistent with this idea. However, the present study was not able to identify changes in these parameters by ketanserin administration.

The most important finding is that changes in global hemodynamic parameters do not necessarily reflect alterations in the microcirculation and vice versa. This is also a consistent finding in microcirculatory sepsis and systemic inflammatory response state research. It may therefore be prudent to directly visualize this vital organ when trying to optimize perfusion in clinical medicine. Figure 4 is supportive of this statement in the clinical setting we studied.

A limitation of the present study may be that the sample size is small. However, because of its design in which patients function as their own controls, power analysis showed that the sample was adequate to address the specific aims of this study. Theoretically, the problem of sample size would be more severe for the analysis of larger vessels. Since large vessels are less abundant than smaller ones, some may be missed because of the small sampling area of the SDF device. That is why the consensus on microcirculatory measurements recommends a minimum of 3 sites to be evaluated. Still, the low number of subjects may hinder comparison with microcirculatory data from other studies. On the other hand, we found a statistically significant and clinically relevant result for PVD for larger microvessels.

The sublingual area was chosen for microcirculatory assessment for 3 reasons. First, it is not affected by changes in peripheral temperature. Second, it is close to the brain, which is of primary concern from a perfusion standpoint. Third, it is easily accessible. It should be noted that sublingual microvascular perfusion does not always correlate with other microcirculatory beds. Boerma et al. [22] showed this difference comparing sublingual and stoma microcirculation beds.

Further study is needed to assess the microcirculatory effects of other commonly used agents to treat elevated blood pressure such as nitroglycerin, nitroprusside, and nicardipine. Differences in microvascular response may have implication for future drug selection. In addition, our technique now allows for investigation of microvascular effects of other vasoactive agents commonly used in cardiac anesthesia.
Conclusion

We successfully have used SDF imaging in the clinical setting of cardiac anesthesia to assess the effects of ketanserin on the microcirculation. This study adds to the growing body of evidence that changes in global circulatory parameters are poor predictors of microcirculatory changes.

Ketanserin, when given for elevated blood pressure after extracorporeal circulation, reduces systemic blood pressure while increasing microvascular perfused vessel density for vessels with a diameter larger than 20 µm. All other indices of perfusion including those for smaller microvessels remained unaltered.

This suggests that increased arteriovenous shunting is the mechanism of the known reduction in systemic vascular resistance by ketanserin. The fact that indices of capillary perfusion did not change significantly, despite the low range of blood pressures after ketanserin administration, may indicate that ketanserin does not impair oxygen and nutrient delivery in this clinical setting while effectively reducing blood pressure.

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Chapter 7

Imaging the Human Microcirculation during Resuscitation for Cardiac Arrest in a Hypothermic Victim of Submersion Trauma
Paul WG Elbers, Antonius J Craenen, Antoine Driessen, Marco C Stehouwer, Luuk Munsterman, Miranda Prins, Mat van Iterson, Peter Bruins, Can Ince

Resuscitation 2010; 81: 123
Abstract
The microcirculation is essential for delivery of oxygen and nutrients to tissue. However, the human microvascular response to cardiopulmonary resuscitation (CPR) is unknown. We report on the first use of sidestream dark field imaging to assess the human microcirculation during CPR with a mechanical chest compression/decompression device (mCPR). mCPR was able to provide microvascular perfusion. Capillary flow persisted even during brief mCPR interruption. However, indices of microvascular perfusion were low and improved vastly after return of spontaneous circulation. Microvascular perfusion was relatively independent from blood pressure. The microcirculation may be a useful monitor for determining the adequacy of CPR.

Introduction
The microcirculation delivers oxygen and nutrients to tissue. This makes adequate microvascular perfusion a key objective in cardiopulmonary resuscitation (CPR). Animal CPR experiments have shown that global haemodynamic parameters do not always reflect microvascular perfusion [1–6]. It is currently unknown if such discrepancy exists in humans, because human microvascular assessment during CPR has long been a virtual technical impossibility. Such knowledge is essential to determine the efficacy of CPR in establishing adequate blood flow to tissue. As such, microvascular perfusion might serve as an end point for CPR. Orthogonal polarized spectral (OPS) imaging and its improved successor sidestream dark field (SDF) imaging, have enabled human microvascular imaging in real time [7]. For the first time, we applied this technique to directly observe the sublingual microcirculation during CPR in a victim of submersion trauma.

Case Report
A 27-year-old man drove into a canal and was liberated after approximately 45 min of cold submersion. There were no detectable signs of life and protocolized resuscitation was begun using mechanical chest compression/decompression at a rate of 100 min⁻¹ (mCPR, LUCAS®, Jolife, Lund, Sweden). Following intubation, manual interposed ventilations at 15–20 min⁻¹ were started. On arrival at the hospital, mCPR was continued. Pupils were widely dilated and unresponsive to light and the electrocardiogram was isoelectric. Initial tympanic temperature was 22 °C. The patient was transported to the operating room for rewarming using extracorporeal circulation (ECC). During preparation for ECC, a first series of microvascular recordings was made. This was done during a brief period (<20 s) of mCPR switch-off as requested by the surgeon. Up until this time point, there was no spontaneous heart rhythm and mCPR had been ongoing. Full ECC flow (5 L min⁻¹) was reached approximately 2 h after initiation of mCPR, which was used continuously until then. The rectal temperature at this point was 23.9 °C. Slow rewarming was begun according to our local protocol. At 30 °C, successful cardioversion from ventricular fibrillation to sinus rhythm was carried out. Rewarming was continued up to mild hypothermia (33 °C). Pump flow was slowly decreased and subsequently stopped. At this time point circulation
was spontaneous and a second series of microvascular recordings was made. After about 10 minutes, ECC weaning proved unsuccessful because of inadequate oxygenation ($\text{SpO}_2$ 70–80%) at 100% $\text{FiO}_2$. ECC was restarted and the patient was transferred to the ICU. After 72 h, brain stem reflexes were absent and did not improve. Eventually, active treatment was withdrawn and the patient died of pulmonary complications.

**Microcirculatory Imaging**

Sidestream dark field imaging has been described in detail previously [7]. In brief, a hand-held video microscope emits stroboscopic green light (530 nm), which is absorbed by hemoglobin. Thus, a negative image of moving red blood cells is transmitted back towards a camera, representing approximately $940 \times 750 \mu m^2$ of tissue surface. The local ethics committee waived the need for informed consent for studies using SDF imaging as it is considered noninvasive. Following recent guidelines [8], we determined microvascular flow index (MFI), perfused vessel density (PVD) and proportion of perfused vessels (PPV) both for large and small microvessels with a cut-off diameter of 20 µm. Two authors (PWGE, LM) performed the analysis independently and their results were averaged.

**Results**

Figure 1 shows stills of the acquired video clips. Movies are available in the online supplement. mCPR switch-off did not obviously influence microvascular flow. Microvascular perfusion scores, macrohemodynamic parameters and administered drugs may be found in table 1. Microvascular flow was present during mCPR. However, PVD markedly improved after return of spontaneous circulation (ROSC). The change in PVD was much larger than the change in blood pressure.

**Discussion**

This is the first paper to report on the human microvascular response to CPR. We showed that mCPR generates microvascular flow, which persists during short interruptions. However, all microvascular perfusion scores are considerably lower compared to after ROSC. Further, the difference in PVD is much larger than the difference in systemic blood pressure. This adds to the large body of evidence that macrovascular parameters do not necessarily reflect microvascular perfusion [9]. We previously studied hypertensive patients after routine cardiac surgery. These had a PVD of 5.59 for small microvessels (PPV 80%, MFI 2.8), about twice the value for mCPR and two thirds of the value after ROSC [10]. The latter may represent post-hypoperfusion hyperemia. Weil’s group used OPS imaging in experimental cardiac arrest in pigs. They reported a brain and sublingual microvascular MFI of 1.2–1.5 during manual CPR which improved to 3 after ROSC [1, 2, 4, 5]. This is comparable to our results.

There are limitations to our study that need to be addressed. First, it is difficult to draw conclusions from a single case. Second our patient died and showed severe brain damage.
Therefore, we cannot tell whether the observed PVD is associated with adequate microvascular brain perfusion. Further, we cannot be sure that microvascular flow persisted beyond the resuscitation period and can therefore not relate it to outcome. In addition, the sublingual microcirculation, albeit central, may not reflect other microvascular beds [11]. Next, between measurements, vasoactive drugs were given such as adrenalin (epinephrine) and propofol. However, propofol was shown to decrease PVD in humans [12]. The same is true for adrenalin in pigs during CPR [4]. Finally, the difference in PVD between mCPR and ROSC may be caused by rewarming. This is consistent with findings in hamsters where capillary density was reduced twofold at 18 °C versus 37 °C [13]. Conversely, we are currently studying microvascular flow during hypothermic circulatory arrest in humans. Preliminary analysis shows a PVD of approximately 6 mm\(^{-1}\) at 25 °C, similar to our findings.
<table>
<thead>
<tr>
<th></th>
<th>mCPR</th>
<th>Sinus rhythm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Perfused Vessel Density (small vessels, mm(^{-1}))</td>
<td>3.80</td>
<td>9.01</td>
</tr>
<tr>
<td>Percentage of Perfused Vessels (small vessels)</td>
<td>64%</td>
<td>97%</td>
</tr>
<tr>
<td>Microvascular Flow Index (small vessels)</td>
<td>1.8</td>
<td>3.0</td>
</tr>
<tr>
<td>Perfused Vessel Density (large vessels, mm(^{-1}))</td>
<td>1.28</td>
<td>3.31</td>
</tr>
<tr>
<td>Percentage of Perfused Vessels (large vessels)</td>
<td>75%</td>
<td>100%</td>
</tr>
<tr>
<td>Microvascular Flow Index (large vessels)</td>
<td>2.2</td>
<td>3.0</td>
</tr>
<tr>
<td>NIBP (mm Hg)</td>
<td>70/40</td>
<td>n/a</td>
</tr>
<tr>
<td>ABP (mm Hg)</td>
<td>n/a</td>
<td>90/50 (67)</td>
</tr>
<tr>
<td>CVP (mm Hg)</td>
<td>n/a</td>
<td>16</td>
</tr>
<tr>
<td>HR (min(^{-1}))</td>
<td>n/a</td>
<td>108</td>
</tr>
<tr>
<td>PaO(_2) (kPa)</td>
<td>15.3</td>
<td>n/a</td>
</tr>
<tr>
<td>SpO(_2)</td>
<td>96%</td>
<td>80%</td>
</tr>
<tr>
<td>Hb (mM)</td>
<td>5.9</td>
<td>5.9</td>
</tr>
<tr>
<td>Ht</td>
<td>28%</td>
<td>28%</td>
</tr>
<tr>
<td>pH</td>
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<td>7.02</td>
</tr>
<tr>
<td>PaCO(_2) (kPa)</td>
<td>12.9</td>
<td>9.6</td>
</tr>
<tr>
<td>HCO(_3^-) (mM)</td>
<td>17.6</td>
<td>18.1</td>
</tr>
<tr>
<td>Base Excess (mM)</td>
<td>-16</td>
<td>-12.9</td>
</tr>
<tr>
<td>K(^+) (mM)</td>
<td>3.58</td>
<td>3.79</td>
</tr>
<tr>
<td>Temperature (rectal, °C)</td>
<td>24</td>
<td>33</td>
</tr>
<tr>
<td>Midazolam (mg)</td>
<td>5(^a)</td>
<td>5(^b)</td>
</tr>
<tr>
<td>Propofol (mg/h)</td>
<td>200</td>
<td></td>
</tr>
<tr>
<td>Noradrenalin (µg)</td>
<td>500</td>
<td></td>
</tr>
<tr>
<td>Adrenalin (µg)</td>
<td>200</td>
<td></td>
</tr>
<tr>
<td>Dexamethason (mg)</td>
<td>50</td>
<td></td>
</tr>
<tr>
<td>Calcium (mg)</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>Magnesium (mg)</td>
<td>3000</td>
<td></td>
</tr>
</tbody>
</table>

Table 1. Microvascular, macrohemodynamic, laboratory and drug data. NIBP, non-invasive blood pressure; ABP, arterial blood pressure; CVP, central venous pressure; HR, heart rate. \(^a\) Midazolam 5 mg was given at the start of rewarming, >50 min before cardioversion. \(^b\) Adrenalin was given at regular intervals according to the CPR protocol except during surgery for cannulation for ECC.
Conclusion

This is the first report on human microvascular imaging during CPR. mCPR is able to provide microvascular perfusion. However, indices of microvascular perfusion seem low and improve vastly after conversion to sinus rhythm. Microvascular perfusion was relatively independent from global hemodynamic parameters in this setting. The microcirculation may prove a sensitive monitor to determine the adequacy of CPR. Albeit prone to movement artifacts, it may prove possible to use SDF imaging in this context.

Supplementary Data

Supplementary data can be found at doi:10.1016/j.resuscitation.2009.09.032.

References

Chapter 8

Microvascular Hemodynamics in Human Hypothermic Circulatory Arrest and Selective Antegrade Cerebral Perfusion
Paul WG Elbers, Alaattin Ozdemir, Robin H Heijmen, Jos Heeren, Mat van Iterson, Eric PA van Dongen, Can Ince

Critical Care Medicine, 2010; 38: 1548
Abstract

Objective: The behavior of the human microcirculation in the setting of cardiac arrest is largely unknown. Animal experiments have consistently revealed that global hemodynamics do not necessarily reflect microvascular perfusion. In addition, the time it takes for capillary blood flow to stop after the heart arrests is debated. Estimations range from 50 s to 5 minutes, but data in humans are lacking. Aortic arch surgery frequently necessitates deep hypothermic circulatory arrest and subsequent selective antegrade cerebral perfusion. To elucidate microvascular behavior surrounding cessation of human circulation, we used sublingual microvascular imaging in this setting.

Design: Prospective, observational study.

Setting: Operating room of a large tertiary referral center for cardiac surgery.

Patients: Seven patients undergoing elective aortic arch repair.

Interventions: We used sidestream dark field imaging to study the sublingual microcirculation immediately before circulatory arrest, during circulatory arrest, and immediately after selective antegrade cerebral perfusion.

Measurements and Main Results: Results are reported as mean (SD) unless indicated otherwise. Before circulatory arrest, perfused vessel density was 6.41 (1.18) for small (<20 µm) and 1.57 (0.88) mm⁻¹ for large (>20 µm) microvessels. Microvascular flow index was a median of 3.0 (interquartile range 3.0-3.0) for both vessel sizes. After circulatory arrest, there was no equilibration of arterial and venous blood pressure before onset of selective antegrade cerebral perfusion after 59 (17) s (range, 40-80 s). Flow in small microvessels came to a complete stop after 45 (9) s (range, 34-57 s) after transition to circulatory arrest. However, flow in larger microvessels did not completely stop before selective antegrade cerebral perfusion started. Selective antegrade cerebral perfusion restored microvascular flow, reaching pre circulatory arrest levels after 45 (27) s (range, 20-85 s).

Conclusions: In a controlled surgical setting, circulatory arrest in humans induces a complete sublingual small microvessel shutdown within 1 min. However, flow in larger microvessels persists. Selective antegrade cerebral perfusion was able to restore microvascular flow to pre circulatory arrest levels within a similar time frame.
Introduction

The microcirculation is crucial for delivery of oxygen and nutrients to tissue. In the setting of circulatory arrest and resuscitation, upstream hemodynamic parameters such as blood pressure and cardiac output do not necessarily reflect microvascular perfusion. However, this has only been shown in animal models [1–4]. Similarly, there are no conclusive human data on the time it takes for the microcirculation to come to a complete halt after circulatory arrest.

After circulatory arrest, pressures ultimately equalize throughout the circulation causing flow to stop. Guyton et al. [5] calculated that arterial and systemic venous pressure would reach equilibrium 30–50 s after the heart stops beating. However, Schipke et al. [6] concluded that this would take much longer by extrapolating pressure data in the setting of induced ventricular fibrillation for defibrillator implantation in humans. Furthermore, studies in anesthetized pigs have shown that equalization of pressures and microvascular flow does not occur until after 5 minutes of circulatory arrest [1, 7].

Surgery involving the aortic arch necessitates a period of circulatory arrest. This is often combined with deep hypothermia and selective antegrade cerebral perfusion for neuroprotection [8]. As such, this setting may serve as a model for human cardiac arrest and resuscitation. With the advent of side stream dark field (SDF) imaging, it has recently become possible to image the human microcirculation in real time [9]. We used this technique to record videos of the sublingual microcirculation surrounding the phase of circulatory arrest and selective antegrade cerebral perfusion in aortic arch repair.

Our primary objective was to determine the behavior of the human microcirculation after circulatory arrest. In particular, we intended to find out if and when microvascular perfusion comes to a complete halt in this controlled observational setting. Our null hypothesis was that no difference would exist between microvascular perfusion immediately before circulatory arrest and 50 s after circulatory arrest.

A second goal of our study was related to aortic arch surgery itself, which imposes risk of brain injury [8]. Cardiopulmonary bypass has been shown to induce discrepancy between microvascular and global hemodynamic parameters [10, 11]. Selective antegrade cerebral perfusion may have a similar effect. Therefore, we aimed to test whether our selective antegrade cerebral perfusion strategy would impair microvascular flow.

Materials and methods

The local Institutional Review Board approved the study. The need for written informed consent was waived in accordance with the national Law on Experiments With Humans because the study was observational and measurements were considered noninvasive.

We studied adult subjects undergoing surgery involving the aortic arch in which hypothermic circulatory arrest and selective antegrade cerebral perfusion were planned. A schematic overview of the study protocol is given in figure 1. Routine monitoring included arterial
and central venous pressures as well as blood gas parameters. We used bilateral near-infrared spectroscopy sensors for assessment of frontal cortex oxygen saturation and recorded a four-channel electroencephalogram.

Transcranial Doppler was used to measure the velocity of middle cerebral artery blood flow bilaterally. After induction of anesthesia, median sternotomy was performed to expose the heart. After institution of cardiopulmonary bypass, ventilation was stopped. Placement of an aortic cross clamp allowed for initiation of proximal aortic repair while cooling toward a rectal temperature of 25 °C. Cardioplegic solution was administered through a previously placed aortic root needle or selectively through the coronary ostia after opening the aorta. The heart–lung machine kept an automated record of blood flow and temperature. Intermittent blood gas analysis was performed and oxygenator gas flow was adjusted accordingly.

For the second stage of surgery, circulatory arrest was induced by slowing down cardiopulmonary bypass flow to zero within 10 s. This was followed by opening the aortic arch and placement of two cannulas for selective antegrade cerebral perfusion. One of these was placed in the innominate artery and the other in the left common carotid artery. Then, cerebral perfusion was started through the heart–lung machine aiming for a flow of 10 mL/kg body weight. Subsequently, the left subclavian artery was occluded to prevent cerebral steal by back bleeding through the ipsilateral vertebral artery.

We used SDF imaging to visualize the sublingual microcirculation in all patients. This tech-
nique has been described in detail previously [9]. In brief, it consists of a handheld video microscope that emits stroboscopic green light (wavelength 530 nm) from an outer ring at the end of a probe. This light is absorbed by hemoglobin. Thus, a negative image of moving red blood cells is transmitted back through the isolated optical core of the probe toward a charge-coupled device camera.

We implemented all recommendations from a recent round table conference on how to best evaluate the microcirculation [12]. Video clips were digitally stored using 5x optical magnification, producing images representing approximately 940x750 µm² of tissue surface. Special care was taken to avoid external pressure artifacts [12, 13]. Secretions were removed and, after obtaining good image focus, the probe was pulled back gently until contact was lost and then advanced again slowly to the point at which contact was regained. We paid special attention to the larger vessels at the time of recording because alterations in their flow with probe manipulation may indicate pressure artifacts.

We recorded microvascular clips 5–10 minutes before circulatory arrest and 5–10 minutes after the start of selective antegrade cerebral perfusion. At each time point, clips at three different sublingual sites yielding at least 20 s of stable video per site were recorded. In addition, we attempted to record a single video clip from a single sublingual site from the actual onset of circulatory arrest until several minutes after selective antegrade cerebral perfusion.

These were used to determine the exact time points at which microvascular flow and capillary density came to a complete stop after circulatory arrest and at which microvascular flow and vessel density did not improve further after selective antegrade cerebral perfusion. These times were determined visually both for smaller and larger microvessels (cutoff diameter 20 µm) by one of the authors (PWGE). Additionally, from these long video recordings, two smaller clips of stable video of each patient were cut representing 5 s immediately before circulatory arrest and the period surrounding 50 s after circulatory arrest. We chose the time point of 50 s because this is the historical theoretical upper limit for pressure equilibration as proposed by Guyton et al. [5].

All clips were stored under a random number. At a later time, these were analyzed in batch with clips from several other studies by one of the authors (PWGE) using the AVA 3.0 software program (Microvision Medical, Amsterdam, The Netherlands). We determined microvascular flow index (MFI), perfused vessel density (PVD), proportion of perfused vessels (PPV), and indices of heterogeneity for every patient at both time points. All have been validated previously [13–15]. As published recently [16], each score was determined for both large and small microvessels with a cut-off diameter of 20 µm.

For PPV and PVD, vessel density was calculated as the number of vessels crossing three horizontal and three vertical equidistant lines spanning the screen divided by the total length of the lines. Perfusion at each crossing was then scored semi-quantitatively by the eye as follows: 0 - no flow (no flow present for the entire duration of the clip), 1 - intermittent flow (flow present <50% of the duration of the clip), 2 - sluggish flow (flow present >50%
but <100% of the duration of the clip or very slow flow for the entire duration of the clip), and 3 - continuous flow (flow present for the entire duration of the clip). PVD was then calculated as the number of crossings with flow scores greater than 1. PPV was calculated as the PVD divided by the total number of crossings. For each time point and each patient, the scores for PPV and PVD were averaged. PPV is expressed as n/mm, whereas PVD is expressed as a percentage.

MFI was based on the determination of the predominant type of flow in four quadrants adhering to the same scoring system. MFI is the sum of these flow scores divided by the number of quadrants in which the vessel type is visible. For each time point and each patient, the scores for MFI were averaged.

To determine intra- and interrater variability, five percent of video clips from this and other studies were randomly selected. This yielded 17 video clips that were analyzed again by the same (PWGE) and a different observer independently and blinded to the first analysis 10

<table>
<thead>
<tr>
<th>#</th>
<th>Age (y)</th>
<th>Sex</th>
<th>Surgery</th>
<th>HT</th>
<th>Anti-HT</th>
<th>Propofol (mg/h) / Remifentanil (mg/h)/ Nitroglycerin (mg/h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>64</td>
<td>M</td>
<td>Ascending aorta + complete aortic arch + elephant trunk</td>
<td>+</td>
<td></td>
<td>100 / 0.25 / 0</td>
</tr>
<tr>
<td>2</td>
<td>69</td>
<td>M</td>
<td>Aortic valve + supracoronary ascending aorta + partial aortic arch</td>
<td>+</td>
<td>+</td>
<td>100 / 0.25 / 0</td>
</tr>
<tr>
<td>3</td>
<td>62</td>
<td>M</td>
<td>Bentall + partial aortic arch + MAZE</td>
<td>+</td>
<td>+</td>
<td>200 / 0 / 0</td>
</tr>
<tr>
<td>4</td>
<td>71</td>
<td>M</td>
<td>Bentall + complete aortic arch + CABG + mitral valve + tricuspid valve</td>
<td>+</td>
<td></td>
<td>100 / 0.25 / 0</td>
</tr>
<tr>
<td>5</td>
<td>61</td>
<td>M</td>
<td>Aortic valve + supracoronary ascending aorta + partial aortic arch + CABG</td>
<td>+</td>
<td></td>
<td>100 / 0.25 / 1</td>
</tr>
<tr>
<td>6</td>
<td>62</td>
<td>F</td>
<td>Bentall + complete aortic arch + elephant trunk</td>
<td>+</td>
<td>+</td>
<td>300 / 0 / 0</td>
</tr>
<tr>
<td>7</td>
<td>66</td>
<td>M</td>
<td>Bentall + partial aortic arch</td>
<td>+</td>
<td>+</td>
<td>50 / 1 / 1</td>
</tr>
</tbody>
</table>
weeks earlier. Intra- and interrater bias is reported as mean (SD).

For PVD, for all vessel sizes, Bland’s intrarater bias was between 0.03 mm⁻¹ (SD 0.28) and 0.28 mm⁻¹ (SD 0.47) with an absolute mean difference of 0.17–0.41 mm⁻¹, which is 5.6–9.3% of mean PVD [17]. Interrater bias was between 0.03 mm⁻¹ (0.28) and 0.64 mm⁻¹ (0.82) with an absolute mean difference of 0.17–0.78 mm⁻¹, which is 7.3–10.1% of mean PVD. De Backer et al. [14] previously reported intrarater variability ranging from 2.5–4.7% and interrater variability ranging from 3.0–6.2%.

For PPV, for all vessel sizes, intrarater bias was between 0 (0) and 0.66 (2.23) percentage points with an absolute mean difference 0–1.35 percentage points, which is 0–1.41% of mean PPV. Interrater bias was between 0 (0) and 1.34 (2.11) percentage points with an absolute mean difference of 0–1.64 percentage points, which is 0–1.7% of mean PPV. De Backer et al. [14] reported intra- and interobserver variability for PPV of 0.9–4.5% and 4.1–10%, respectively.

For MFI, intraobserver kappa score was between 0.903 and 0.954 [18]. Boerma et al. [15] found a kappa score of 0.78. Interobserver kappa score for MFI was between 0.821 and 1, whereas Boerma et al. [15] and Trzeciak et al. [13] reported values of 0.85 and 0.77.

Heterogeneity was assessed in two different ways. For PVD, the coefficient of variation was determined [14]. For MFI, we assessed heterogeneity in each patient by subtracting the lowest from the highest quadrant MFI and dividing the result by the mean MFI [16].

We used paired Student’s t tests for all data except for MFI for which we used Wilcoxon matched pairs tests because MFI is considered a discrete variable. Results are reported as median and interquartile ranges for MFI and as the mean (SD) for other parameters.

We intended to be able to detect an 80% difference between small-vessel PVD immediately before and 50 s after circulatory arrest. In addition, we wanted to be able to show a 25% difference between small-vessel PVD before circulatory arrest and after selective antegrade cerebral perfusion. Power analysis showed the need for inclusion of three and seven patients, respectively, based on previous studies by us and others [14, 19, 20].

Results

Seven subjects were studied. Patient and procedure characteristics can be found in table 1. Mean time between circulatory arrest and selective antegrade cerebral perfusion was 115 s (25 s). There were no surgical complications and after 48 hours, there was no evidence of brain injury in any patient.

Electro-encephalographic (EEG) data analysis was possible in all patients, whereas near-infrared spectroscopy data were available for analysis in six patients. Three patients underwent transcranial Doppler monitoring. Global hemodynamics, blood gas data, and neuromonitor parameters can be found in table 2. In all patients, a silent EEG was observed approximately 60 s after circulatory arrest.
Arterial blood pressure decreased markedly and central venous pressure rose, but there was no equilibration of pressures at the onset of selective antegrade cerebral perfusion in any patient. In the three patients with transcranial Doppler monitoring, bilateral cerebral artery blood flow velocity decreased below noise level within s.

As intended, cardiopulmonary bypass flow was significantly lower during selective antegrade cerebral perfusion compared with systemic perfusion before circulatory arrest. Base excess was also significantly lower during selective antegrade cerebral perfusion. EEG, transcranial Doppler, and near-infrared spectroscopy parameters as well as mean arterial pressure returned to similar values as before circulatory arrest.

In all patients, we managed to complete a full series of high-quality microvascular recordings before circulatory arrest and selective antegrade cerebral perfusion. Drug infusion

<table>
<thead>
<tr>
<th></th>
<th>Before Circulatory Arrest</th>
<th>During Selective Antegrade Cerebral Perfusion</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Q, full body (L/min)</td>
<td>4.06 (0.42)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Q, brain (L/min)</td>
<td>-</td>
<td>0.96 (0.14)</td>
<td>-</td>
</tr>
<tr>
<td>Mean arterial pressure (mm Hg)</td>
<td>43 (7)</td>
<td>42 (11)</td>
<td>0.88</td>
</tr>
<tr>
<td>Central venous pressure (mm Hg)</td>
<td>1 (1)</td>
<td>3 (4)</td>
<td>0.10</td>
</tr>
<tr>
<td>pH</td>
<td>7.33 (0.06)</td>
<td>7.34 (0.06)</td>
<td>0.89</td>
</tr>
<tr>
<td>PCO₂ (kPa)</td>
<td>6.1 (1.1)</td>
<td>5.3 (0.8)</td>
<td>0.15</td>
</tr>
<tr>
<td>PO₂ (kPa)</td>
<td>27.5 (10.4)</td>
<td>24.9 (3.5)</td>
<td>0.47</td>
</tr>
<tr>
<td>Base excess (mM)</td>
<td>-2.0 (1.4)</td>
<td>-4.7 (2.5)</td>
<td>0.03</td>
</tr>
<tr>
<td>Hemoglobin (mM)</td>
<td>5.6 (1.3)</td>
<td>5.7 (1.2)</td>
<td>0.40</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>26.0 (5.1)</td>
<td>26.4 (4.7)</td>
<td>0.67</td>
</tr>
<tr>
<td>T, nasa (°C)</td>
<td>25.6 (1.0)</td>
<td>23.8 (2.0)</td>
<td>0.08</td>
</tr>
<tr>
<td>SpO₂ (%)</td>
<td>99.4 (0.5)</td>
<td>99.4 (0.4)</td>
<td>0.76</td>
</tr>
<tr>
<td>SO₂, NIRS (%)</td>
<td>70 (8)</td>
<td>68 (8)</td>
<td>0.58</td>
</tr>
<tr>
<td>V-MCA (cm/s)</td>
<td>24.0 (11.5)</td>
<td>25.0 (9.5)</td>
<td>0.57</td>
</tr>
</tbody>
</table>

Table 2. Macrohemodynamic and blood gas parameters. Q, flow; T, nasa, nasal temperature; SO₂ NIRS, average brain tissue oxygen saturation (near-infrared spectroscopy); V-MCA, mean blood flow velocity in middle cerebral arteries. Values are reported as mean (SD). Blood gas parameters were determined at 37°C.
Figure 2. Stills from sublingual microvascular video recordings immediately before the transition to circulatory arrest (top), just before onset of selective antegrade cerebral perfusion (middle), and after selective antegrade cerebral perfusion (bottom). Capillary fallout can be seen in the middle screen shot. The actual video shows markedly reduced microvascular flow after circulatory arrest.
rates did not change throughout the measurement period. However, in one patient, 100 µg of noradrenalin was given 8 minutes before SDF measurements. In five patients, stable recordings at one site covering the period from the start of circulatory arrest until several minutes after selective antegrade cerebral perfusion could be obtained. However, in one of these, saliva artifacts precluded formal analysis of the 5-sec period immediately before circulatory arrest. A typical microvascular video recording during transition to circulatory arrest and selective antegrade cerebral perfusion is available as supplemental digital content (see Supplemental Digital Content 1, http://links.lww.com/CCM/A144). Stills of microvascular recordings before and 50 s after circulatory arrest and after selective antegrade cerebral perfusion are depicted in figure 2.

Results of microvascular analysis during transition to circulatory arrest and selective antegrade cerebral perfusion can be found in table 3. In all five patients, flow in small microvessels came to a complete stop after transition to circulatory arrest. This occurred after 45 (9) s (range, 34–57 s). In one patient, the complete halt was only reached 5 s after the start of selective antegrade cerebral perfusion. Flow in one or more larger microvessels did not completely stop in any of the patients before selective antegrade cerebral perfusion started after 59 (17) s (range, 40–80 s).

Selective antegrade cerebral perfusion markedly improved microvascular flow in all patients, sometimes abruptly. Microvascular flow stopped to further ameliorate after 45 (27) s (range, 20–85 s). This is not significantly different from the time it took the small micro-

<table>
<thead>
<tr>
<th></th>
<th>At 0 s during circulatory arrest</th>
<th>At 50 s during circulatory arrest</th>
<th>p</th>
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</thead>
<tbody>
<tr>
<td>ø &lt; 20 µm</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PVD (mm⁻¹)</td>
<td>8.83 (1.46)</td>
<td>0.0 (0.0)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>PPV (%)</td>
<td>99.2 (1.6)</td>
<td>0.0 (0.0)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>MFI</td>
<td>3.0 [3.0-3.0]</td>
<td>0.0 [0.0-0.0]</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>ø &gt; 20 µm</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PVD (mm⁻¹)</td>
<td>2.91 (0.65)</td>
<td>0.57 (0.36)</td>
<td>0.01</td>
</tr>
<tr>
<td>PPV (%)</td>
<td>100.0 (0.0)</td>
<td>33.1 (10.5)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>MFI</td>
<td>3.0 [3.0-3.0]</td>
<td>0.50 (0.25-0.63)</td>
<td>0.01</td>
</tr>
</tbody>
</table>

Table 3. Microvascular flow scores immediately before circulatory arrest and after 50 s of circulatory arrest. MFI, microvascular flow index; PVD, perfused vessel density; PPV, proportion of perfused vessels. For MFI, values are reported as median interquartile range. Other values are reported as mean (SD). See text for details.
vessels to come to a complete hold after circulatory arrest (p=.39). Table 4 shows microvascular parameters 5–10 minutes before circulatory arrest and 5–10 minutes after selective antegrade cerebral perfusion. Indices of microvascular flow and heterogeneity did not differ significantly between these time points.

**Discussion**

This is the first report on human microvascular imaging in the setting of hypothermic circulatory arrest and selective antegrade cerebral perfusion. We found that perfusion of smaller microvascular vessels stops after 45 s (SD 9), whereas flow in larger microvessels persists.

Almost 90 years ago, Krogh [21] introduced the classic model of oxygen transport by diffusion from capillaries. This makes small microvessel PVD a crucial factor for oxygen delivery, because this determines the distance to tissue that oxygen needs to bridge. This would

<table>
<thead>
<tr>
<th></th>
<th>Before circulatory arrest</th>
<th>During selective antegrade cerebral perfusion</th>
<th>p</th>
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<tbody>
<tr>
<td><strong>ø &lt; 20 µm</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PVD (mm(^{-1}))</td>
<td>6.41 (1.18)</td>
<td>6.61 (0.69)</td>
<td>0.75</td>
</tr>
<tr>
<td>PPV (%)</td>
<td>92.2 (7.0)</td>
<td>96.0 (3.7)</td>
<td>0.30</td>
</tr>
<tr>
<td>HI-PVD</td>
<td>0.28 (0.29)</td>
<td>0.21 (0.13)</td>
<td>0.54</td>
</tr>
<tr>
<td>MFI</td>
<td>3.0 [2.8-3.0]</td>
<td>3.0 [2.8-3.0]</td>
<td>0.89</td>
</tr>
<tr>
<td>HI-MFI</td>
<td>0.31 (0.55)</td>
<td>0.26 (0.36)</td>
<td>0.80</td>
</tr>
</tbody>
</table>

| **ø > 20 µm**        |                           |                                             |        |
| PVD (mm\(^{-1}\))   | 1.57 (0.88)               | 1.88 (0.52)                                 | 0.44   |
| PPV (%)              | 94.1 (14.0)               | 100.0 (0)                                   | 0.31   |
| HI-PVD               | 0.58 (0.42)               | 0.39 (0.20)                                 | 0.33   |
| MFI                  | 3.0 [3.0-3.0]             | 3.0 [3.0-3.0]                               | 1.00   |
| HI-MFI               | 0.32 (0.55)               | 0.11 (0.19)                                 | 0.25   |
imply that oxygen delivery stops within 1 min after circulatory arrest despite a difference between arterial and venous blood pressure and persistent larger microvascular flow. However, in hamsters, Ellsworth and Pittman [22] found that larger microvessels may transfer some of their oxygen to neighboring capillaries. It is unknown if this type of oxygen transport is important in the setting of human circulatory arrest. The complete shutdown of smaller microvessels adds to the large body of evidence that microvascular perfusion is relatively independent of global hemodynamic parameters. Interestingly, EEG signals only showed silence after 1 min, which is later than PVD reaches zero.

Starr [23] first described the concept of pressure equilibration after circulatory arrest. Guyton et al. [5] proposed that this would occur after 30–50 s. This is consistent with our findings for capillaries but not for larger microvessels. However, recently, it was argued that the time for pressure equilibration may take much longer [6, 24, 25] based on data from patients in which ventricular fibrillation was induced to test their defibrillators.

It is known that the arterial system tends to behave like a waterfall or Starling resistor. The flow in front of a waterfall is not affected by its height. This may explain why global and regional blood flow may stop while arterial is still above venous pressure [26, 27]. This can be explained by the closure of certain microvascular beds causing blood to use the few circuits that are still patent. This causes macrohemodynamic blood flow cessation because net vascular resistance is increased, whereas some microvascular flow remains [6]. This is consistent with our current observations, although we only observed this in larger microvessels.

Weil’s group [2] was the first to use microvascular imaging in experimental cardiac arrest. They reported a dramatic fall in dural and sublingual MFI of both small and large microvessels within 30 s after cardiac arrest. However, in the majority of their studies, at least some microvascular flow persisted for over 120 s [1, 4, 28, 29]. In one study, 44% of the animals studied even showed signs of microcirculatory movement 5 minutes after cardiac arrest [1]. In our study, human microvascular shutdown occurred much earlier. In addition, the animal studies reported similar behavior of small and large microvessels, whereas we now show persistent flow in human larger microvessels when smaller microvessel flow has already come to a complete stop.

Another important finding is that the microcirculation is restored after 45 (27) s of selective antegrade cerebral perfusion. This implies that resuscitation after circulatory arrest is feasible at the level of the microcirculation and not held back by possible no-reflow phenomena. Indices of microvascular perfusion were not different 5–10 minutes before circulatory arrest and during selective antegrade cerebral perfusion. All patients remained without brain injury, which may indicate that our regimen provides adequate flow. However, our study may have been underpowered to exclude a clinically relevant difference in microvascular perfusion.

Several shortcomings of our study need to be kept in mind when interpreting our results. First, the procedure under which circulatory arrest occurred was carried out under hypothermic conditions. Temperature is known to influence vascular waterfalls [30] and possibly
microvascular flow. In hamsters, hypothermia (18 °C) reduces functional capillary density almost twofold compared with normothermia [31]. In contrast, the values for PVD 5–10 minutes before circulatory arrest are similar to those we reported earlier in normothermic patients in our intensive care unit after cardiac surgery [19].

Second, it may be tempting to extrapolate our results to the clinical setting of naturally occurring cardiac arrest. However, in addition to other major differences such as absence of hypothermia and anesthesia, mean arterial pressure is usually much higher immediately before the event than it was before circulatory arrest in our study. Although speculative, this may imply that small microvessel shutdown occurs at a later time point in cardiac arrest.

Finally, we chose to monitor the sublingual microcirculation because it is close to the brain, above the level of the carotid arteries and in the core temperature zone. However, this site may not represent cerebral microcirculation [32].

Conclusion

In a controlled surgical setting of extended aortic repair using deep hypothermia with selective antegrade cerebral perfusion, we have shown that circulatory arrest in humans induces a complete sublingual small microvessel shutdown within 1 min. In contrast, flow in larger microvessels persisted. Selective antegrade cerebral perfusion was able to restore microvascular flow to pre circulatory arrest levels within a similar time frame.

References

10. den Uil CA, Lagrand WK, Sproonk PE et al. Impaired sublingual microvascular perfusion during


Chapter 9

Direct Observation of the Human Microcirculation during Cardiopulmonary Bypass: Effects of Pulsatile Perfusion

Paul WG Elbers, Jeroen Wijbenga, Frank Solinger, Aladdin Yilmaz, Mat van Iterson, Eric PA van Dongen, Can Ince

Journal of Cardiothoracic and Vascular Anesthesia, 2010; epub ahead of print
Abstract

Objectives: Possible benefits of pulsatile perfusion during cardiopulmonary bypass are often attributed to enhanced microvascular flow. However, there is no evidence to support this in humans. Therefore, we assessed whether pulsatile perfusion alters human microvascular flow.

Design: A prospective, randomized observational crossover study.

Setting: A tertiary cardiothoracic surgery referral center.

Participants: Sixteen patients undergoing routine cardiopulmonary bypass for cardiac surgery.

Interventions: All patients underwent both pulsatile and nonpulsatile perfusion in random order.

Measurements and Main Results: We used sidestream dark field imaging to record video clips of the sublingual human microcirculation. Perfusion was started either in the pulsatile (n=8) or the nonpulsatile mode. After 10 minutes, microvascular recordings were made. The perfusion mode was then switched, and after 10 minutes, new microvascular recordings were taken. We quantified pulsatile perfusion-generated surplus hemodynamic energy by calculating pulse pressure and energy-equivalent pressure. Microvascular analysis included determination of the perfused vessel density (mean (standard deviation)). This did not differ between nonpulsatile and pulsatile perfusion (6.65 (1.39) v 6.83 (1.23) mm⁻¹, p=0.58, and 2.16 (0.64) v 1.96 (0.48) mm⁻¹, p=0.20 for small and large microvessels, respectively, cutoff diameter 20 µm). Pulse pressure and energy-equivalent pressure was higher during pulsatile perfusion. However, there was no correlation between the difference in energy-equivalent pressure or pulse pressure and perfused vessel density (r =-0.43, p =0.13, and r=-0.09, p=0.76, respectively).

Conclusions: Pulsatile perfusion does not alter human microvascular perfusion using standard equipment in routine cardiac surgery. Changes in pulse pressure or energy-equivalent pressure bear no obvious relationship with microcirculatory parameters.
Introduction

The possible benefits of pulsatile perfusion (PP) over non-pulsatile perfusion (NP) during cardiopulmonary bypass (CPB) for cardiac surgery remain heavily debated. Some studies have suggested that PP induces hemodynamic and metabolic benefit with improved organ function, but conflicting findings have also been reported [1, 2]. Indeed a recent review states that no recommendation for PP over NP or vice versa can be made when focussing on mortality and incidence of myocardial infarction, stroke, or renal failure [2].

There may be at least two reasons why some studies fail to show benefits of PP. First, the goal of PP is to increase hemodynamic energy. It has been proposed that a certain minimum PP induced surplus hemodynamic energy (SHE) is necessary to unveil its benefits [2]. Negative PP trials may have been conducted using too little SHE. However, the magnitude of a SHE minimum is not known and there is considerable discussion on how it should be measured [2].

Second, the benefits of PP may remain invisible amidst many other clinical factors influencing these commonly reported outcomes. In addition, the incidence of these adverse events in routine cardiac surgery is already very low, necessitating very large trials for a beneficial effect to become apparent. This implies that routinely applying PP using standard CPB equipment may lack rationale because SHE may be insufficient or its effects are too marginal.

Mechanistically, PP is thought to improve microvascular flow. This is consistent with the concept that the microcirculation is crucial for oxygen and nutrient delivery to tissue and ultimately for organ function [3]. As reviewed by Ji and Undar, evidence for microvascular improvement is available but scarce and mainly based on surrogate markers such as laser Doppler flux and ultrasonic flow probes [4]. While valuable, these techniques cannot distinguish capillary from larger vessel flow.

Until recently, the lack of suitable techniques has limited direct microscopic observation of the microcirculation to animal studies. This has provided evidence of improved capillary flow in rats and goats [4]. However, with the recent advent of side stream dark field (SDF) imaging it has now become possible to observe the human microcirculation in real time [5]. An intriguing and consistent finding using this technique in a variety of clinical settings has been that microvascular flow is relatively independent from global hemodynamics.

Against this background, it is relevant to assess whether PP generated using standard CPB equipment does actually alter microvascular perfusion. Therefore we used SDF imaging to record video clips of the sublingual microcirculation in patients undergoing cardiac surgery using commonly available CPB equipment. Using a crossover design, this enabled us to assess their capillary perfusion during both PP and NP. It was our hypothesis that indices of microvascular perfusion would improve using PP as compared to NP.
Methods

This study was approved by our local institutional review board. The need for written informed consent was waived in accordance with the national Law on Experiments with Humans because routine procedures and equipment were used and microvascular measurements were considered non-invasive.

We studied adult patients undergoing coronary artery bypass grafting or aortic valve replacement necessitating CPB. Lacerations of the oral mucosa were a criterion for exclusion because of interference with microcirculatory imaging. Another exclusion criterion was bolus injection of vasopressors. Routine monitoring of global hemodynamics included invasive arterial and central venous blood pressures.

General procedure

Patients received fentanyl, pancuronium, propofol and remifentanil for anesthesia induction and maintenance. No volatile anesthetics were given. After midline sternotomy and systemic heparinization, a 24 F cannula and a 36/51 F two-stage cannula (Medtronic, Heerlen, The Netherlands) was used for aortic and right atrial cannulation, respectively. Cardiopulmonary bypass was initiated using a Jostra-HL30 (Maquet, Hilversum, The Netherlands) heart-lung machine with a RP150 arterial roller pump, Quadrox membrane oxygenator, and Maquet custom pack tubing. A Veri-Q Doppler flow meter (Medistim, Deisenhofen, Germany) and pressure transducer were connected to the arterial side of the CPB circuit, distal to the oxygenator. For cardioplegia, 800-1000 mL cold low sodium crystalloid cardioplegic solution was administered. Flow rate during CPB was aimed at 2.4 L/min/m² at body temperature of 32 °C using either PP or NP (see below).

Experimental procedure

Patients were randomly assigned to start on either PP or NP by opening sealed envelopes after patients were included. For PP we used pulsatility settings with a baseline flow of 35% of the average flow and a pulse starting at 55% and stopping at 80% of the 65 min⁻¹ cycle. This is our routine setting producing a maximum pulse while preventing negative oxygenator pressures.

Ten minutes after administration of cardioplegic solution, we obtained microvascular video recordings of the sublingual microcirculation (see below). We chose the sublingual area for its phylogenetic relation to the gut and ease of access. Fifteen minutes after administration of cardioplegic solution, CPB mode was switched, i.e. patients that started on PP were put on NP and vice versa. Ten minutes later, we obtained a second series of sublingual microvascular video recordings.

Microvascular recordings

We used SDF imaging to obtain microvascular recordings. This technique has been de-
scribed in detail previously [5]. In brief, it consists of a handheld video microscope that emits stroboscopic green light (wavelength 530 nm) from a probe that is absorbed by hemoglobin. Thus, a negative image of moving red blood cells is transmitted back through the isolated optical core of the probe towards a charge-coupled device camera. SDF imaging has been shown to provide a higher image quality with more detail and less motion blur than its predecessor OPS imaging [5].

Recordings were performed in accordance with recommendations from a recent round table conference [6]. Video recordings yielding at least 20 second of stable images were digitally stored and represent approximately $940\times750\ \mu m^2$ of tissue surface. For each time point, SDF recordings at 3 different sublingual sites were recorded within five minutes. Special care was taken to avoid pressure artifacts [6, 7].

**Microvascular analysis**

Evaluation of microvascular recordings was in accordance with recommendations from a recent round table conference [6]. All images were given a random number and analyzed offline by one of the authors (PWGE). We determined perfused vessel density (PVD), proportion of perfused vessels (PPV), microvascular flow index (MFI) and indices of heterogeneity [8-11]. For PPV and PVD, vessel density was calculated as the number of vessels crossing 3 horizontal and 3 vertical equidistant lines spanning the screen divided by the total length of the lines. Perfusion at each crossing was then scored semi-quantitatively as follows: $0 = $ no flow (no flow present for the entire duration of the recording), $1 = $ intermittent flow (flow present <50% of the duration of the recording), $2 = $ sluggish flow (flow present >50% but <100% of the duration of the recording or very slow flow for the entire duration of the clip), and $3 = $ continuous flow (flow present for the entire duration of the recording). PVD was then calculated as the number of crossings with flow scores greater than 1. PPV was calculated as the proportion of crossings with flow scores greater than 1 divided by the total number of crossings. For each time point and each patient, the scores for PPV and PVD were averaged.

PPV is expressed as n/mm, whereas PVD is expressed as a percentage. MFI was based on the determination of the predominant type of flow in 4 quadrants adhering to the same
scoring system. MFI is the sum of these flow scores divided by the number of quadrants in which the vessel type is visible. Heterogeneity was assessed in 2 different ways. For PVD, the coefficient of variation was determined [7]. For MFI, we assessed heterogeneity in each patient by subtracting the lowest from the highest quadrant MFI and dividing the result by the mean MFI [11].

To determine intra- and interrater variability, 17 randomly chosen video recordings were analyzed again after 10 weeks independently by the same and a different observer. For PVD, for all vessel sizes, Bland’s intrarater bias was between -0.03 (0.28) and 0.28 (0.47) mm\(^{-1}\) with an absolute mean difference of 0.17-0.41, which is 5.6%-9.3% of mean PVD [12]. Interrater bias was between -0.03 (0.28) and 0.64 (0.82) mm\(^{-1}\) with an absolute mean difference of 0.17-0.78 mm\(^{-1}\) which is 7.3–10.1% of mean PVD. De Backer et al., studying microvascular blood flow in septic patients, previously reported intrarater variability ranging from 2.5%-4.7% and interrater variability ranging from 3.0-6.2% (8). For PPV, for all vessel sizes, intrarater bias was between 0 (0) and 0.66 (2.23) % with an absolute mean difference 0-1.35%, which is 0–1.41% of mean PPV. Inter-rater bias was between 0 and -1.34 (2.11) % with an absolute mean difference of 0 to 1.64% which is 0 to 1.7% of mean

<table>
<thead>
<tr>
<th>Mean (SD) or n (%)</th>
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<tbody>
<tr>
<td>Male</td>
<td>8 (50%)</td>
</tr>
<tr>
<td>Age (y)</td>
<td>69.6 (11.7)</td>
</tr>
<tr>
<td>CABG</td>
<td>7 (44%)</td>
</tr>
<tr>
<td>AVR</td>
<td>8 (50%)</td>
</tr>
<tr>
<td>Morrow</td>
<td>1 (6%)</td>
</tr>
<tr>
<td>Re-operation</td>
<td>2 (13%)</td>
</tr>
<tr>
<td>Chronic hypertension</td>
<td>3 (19%)</td>
</tr>
<tr>
<td>Diabetes</td>
<td>3 (19%)</td>
</tr>
<tr>
<td>Beta blocker use</td>
<td>4 (25%)</td>
</tr>
<tr>
<td>ACEI/ARA use</td>
<td>4 (25%)</td>
</tr>
<tr>
<td>Calcium antagonist use</td>
<td>3 (19%)</td>
</tr>
<tr>
<td>Propofol (mg/h)</td>
<td>241 (58)</td>
</tr>
<tr>
<td>Remifentanil (mg/h)</td>
<td>0.60 (0.55)</td>
</tr>
<tr>
<td>Nitroglycerin (mg/h)</td>
<td>0.56 (0.51)</td>
</tr>
</tbody>
</table>

Table 1. Patient characteristics. ACEI/ARA, angiotensin converting enzyme inhibitor/angiotensin receptor antagonist; AVR, aortic valve replacement; CABG, coronary artery bypass grafting; Morrow – morrow procedure, i.e. ventricular septal myotomy/myectomy.
PPV. De Backer reported intra- and interobserver variability for PPV to be between 0.9-4.5% and 4.1-10%, respectively [8]. For MFI, intraobserver kappa score was between 0.903 and 0.954 [13]. Boerma et al. and Trzeciak et al. previously performed intra- and interrater variability in septic patients. Boerma et al. found an intraobserver kappa score of 0.78 [11]. Interobserver kappa score for MFI was between 0.821 and 1 whereas Boerma and Trzeciak reported values of 0.85 and 0.77 [7, 11].

Quantification of hemodynamic energy

Pulse pressure was calculated as the difference between systolic and diastolic radial artery pressure. We continuously and simultaneously monitored pressure waveforms just proximal to the aortic cannula and flow waveforms just distal to the oxygenator to quantify CPB circuit Energy Equivalent Pressure (EEP). This was calculated using Shepard’s formula to quantify the pressure and flow waveforms in terms of hemodynamic energy. It is based on the ratio between the area beneath the hemodynamic power curve (integral $fpdt$) and the area beneath the pump flow curve (integral $fdt$) during each pulse cycle (14): $\text{EEP} = \frac{\text{integral } fpdt}{\text{integral } fdt}$. Where $f$ is the pump flow rate, $p$ is the arterial pressure (mm Hg), and $dt$ indicates that the integration is performed over time ($t$). The unit of the EEP is mm Hg. The difference between the EEP and MAP represents the surplus hemodynamic energy (SHE) generated by each pulsatile or non-pulsatile device.

Statistics

We used paired t tests to analyse all data except for MFI for which we used Wilcoxon signed rank tests. Results are reported as median and interquartile ranges (IQR) for MFI and as the mean (SD) for other parameters. Spearman tests were used to detect possible correla-
tion between small vessel PVD and both EEP and pulse pressure. Power calculation was performed before the start of the study. We calculated that 14 patients would be required to detect a 15% difference in PVD between NP and PP based on previous studies by us and others [8, 15, 16]. In order to account for possible data loss, we planned to include 16 patients.

Results

We included 16 patients. Half of these started CPB in PP mode. Figure 1 shows a typical flow recording of both PP and NP modes. Patient characteristics including details on surgery are listed in table 1. One patient received noradrenaline during imaging and was excluded. In all other patients, no dose adjustments of anesthetic and/or vasoactive drugs were made between measurements. One patient was excluded from hemodynamic energy analysis because of technical failure in pressure and flow recordings.

Table 2 lists the hemodynamic results. There was no difference in CPB flow rate or mean arterial pressure (MAP) between groups. As intended, pulse pressure and EEP was signifi-

<table>
<thead>
<tr>
<th></th>
<th>NP</th>
<th>PP</th>
<th>Difference and 95%-CID</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Q (L/min)</td>
<td>3.71 (0.39)</td>
<td>3.62 (0.46)</td>
<td>-0.09 (-0.7 to 0.08)</td>
<td>0.28</td>
</tr>
<tr>
<td>MAP (mm Hg)</td>
<td>54 (13)</td>
<td>50 (10)</td>
<td>-4 (-8 to 1)</td>
<td>0.15</td>
</tr>
<tr>
<td>CVP (mm Hg)</td>
<td>3.3 (3.4)</td>
<td>3.3 (3.5)</td>
<td>0.0 (-1.3 to 1.3)</td>
<td>1.00</td>
</tr>
<tr>
<td>pH</td>
<td>7.41 (0.06)</td>
<td>7.42 (0.06)</td>
<td>0.01 (-0.01 to 0.02)</td>
<td>0.75</td>
</tr>
<tr>
<td>PCO₂ (kPa)</td>
<td>5.2 (0.7)</td>
<td>5.2 (0.8)</td>
<td>0.0 (-0.3 to 0.1)</td>
<td>0.54</td>
</tr>
<tr>
<td>Hb (mM)</td>
<td>4.3 (0.6)</td>
<td>4.4 (0.6)</td>
<td>0.1 (-0.1 to 0.2)</td>
<td>0.68</td>
</tr>
<tr>
<td>Ht (%)</td>
<td>20.6 (3.0)</td>
<td>20.8 (2.9)</td>
<td>0.2 (-4 to 0.8)</td>
<td>0.51</td>
</tr>
<tr>
<td>SaO₂ (%)</td>
<td>99.6 (0.5)</td>
<td>99.8 (0.4)</td>
<td>0.2 (-0.0 to 0.4)</td>
<td>0.08</td>
</tr>
<tr>
<td>ScvO₂ (%)</td>
<td>83.9 (8.2)</td>
<td>84.1 (5.1)</td>
<td>0.3 (-2.7 to 3.2)</td>
<td>0.85</td>
</tr>
<tr>
<td>T, nasal (°C)</td>
<td>30.7 (2.7)</td>
<td>30.8 (2.6)</td>
<td>0.1 (-0.1 to 0.3)</td>
<td>0.37</td>
</tr>
<tr>
<td>EEP (mm Hg)</td>
<td>150 (27)</td>
<td>184 (33)</td>
<td>34 (22 to 46)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Pulse Pressure (mm Hg)</td>
<td>7 (2)</td>
<td>27 (6)</td>
<td>19 (16 to 22)</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

Table 2. Hemodynamic parameters. NP, non-pulsatile perfusion; PP, pulsatile perfusion; 95%-CID, 95% confidence interval of the difference; Q, flow; CVP, central venous pressure; EEP, energy equivalent pressure; MAP, mean arterial pressure; NP, non-pulsatile perfusion; PP, pulsatile perfusion; SaO₂, arterial oxygen saturation; ScvO₂, central venous oxygen saturation ; T, temperature.
A still of a typical microvascular video recording is shown in figure 2. Results of microvascular analysis may be found in Figure 3 and Table 3. There was no difference in any microvascular parameter between NP and PP. This is true both for smaller and larger microvessels. Similarly, indices of microvascular heterogeneity did not differ between groups for either vessel size. Figures 4 and 5 plot the difference in small vessel PVD against the difference in pulse pressure and EEP. No significant correlation could be found for either (Spearman $r=-0.43$, $p=0.13$ for EEP and Spearman $r=-0.09$, $p=0.76$ for pulse pressure).

### Discussion

This is the first study to report on the microvascular response to PP during routine cardiac surgery using standard CPB equipment. The most important result is that PP does not alter microvascular perfusion as compared to NP in this setting in the face of a significant rise.
in both EEP (and thus SHE) and pulse pressure. This adds to the large body of evidence that macrovascular hemodynamic parameters do not necessarily reflect microvascular perfusion. This implies that global hemodynamics and microvascular flow are dissociated in this setting. However it must be noted that our results pertain only to the sublingual microvascular bed at a single level of pulsatility.

Multiple mechanisms have been postulated for a possible PP induced beneficial microvascular effect. Among these are recruitment and prevention of derecruitment of capillaries, prevention of capillary sludging, and promotion of lymph movement [17, 18]. While the latter cannot be visualized using SDF imaging, capillary recruitment and sludging can readily be seen and quantified. This would be consistent with a higher PVD and PPV and lower indices of heterogeneity when using PP. However, as stated above, no differences in these parameters were found.

With SDF imaging, arterioles are usually not visible and the images usually consist of capillaries and venules only. No pulsatility in any vessel type in any patient in any perfusion mode could be confirmed during direct visualization. We did measure heterogeneity, but found no change during PP compared to NP. This could mean that even in patients with large EEP differences, the surplus energy is still insufficient to propel pulsatility beyond the arterioles.
An alternative explanation would be that pulsatility cannot be achieved in these downstream microvascular vessels, regardless of the amount of surplus energy. Instead, this energy might ultimately be used to open up previously closed capillaries. Indeed, this was the case in the goat studies showing an almost a twofold increase in PVD following PP [17, 19-21]. However, these changes could not be reproduced in a goat having only a left ventricular assist device [19]. Also using intravital microscopy in a goat model, a Canadian study by Lee et al could not show a difference in perfused capillary density in the flexor carpi ulnaris muscle using PP [22].

There may be several possible explanations for the fact that we failed to observe microvascular improvement using PP whereas several animal studied did. First, aortic cannulas are known to induce energy loss both in general and for pulsatility added energy specifically [23]. Similarly, Undar et al. have clearly shown that different pumps producing the same pulse pressure may have very different hemodynamic energy levels [24]. Unfortunately we only measured EEP in the CPB circuit. This implies that the absence of microvascular alterations in our study may have been caused by a failure to transmit SHE to the arterial
system. However, as clearly shown in figures 4 and 5, there is no correlation between the change in pulse pressure or EEP and microvascular perfusion. This may support the view that even if SHE is properly transmitted microvascular alterations may still not emerge. Voss et al recently submitted pigs to near physiological PP by a custom made device and found no difference in renal and intestinal blood flow [25]. This is a finding that probably points in the same direction. Second, our patients underwent PP and NP for a relatively short time. Perhaps a difference in microcirculatory indices would have emerged if PP would have been applied longer. Finally, possible PP benefits may only become apparent in fragile patients, especially those with disseminated atherosclerotic pathology [2, 4]. Perhaps our routine patients were too healthy to show any PP induced microvascular improvement, although we did not quantify their chronic health. In fact, all PVD values are similar to those in routine patients after cardiac surgery in our ICU [16].

It was our specific goals to assess the microvascular effects of PP using a readily available heart lung machine. Interestingly our type of heart lung machine and oxygenator is known to provide more hemodynamic energy than others [24, 26]. Therefore, although speculative, it seems likely that other commercially available setups will similarly fail to improve

Figure 5. Plotted is the difference in energy equivalent pressure at the level of the extracorporeal circuit (EEPpump) between pulsatile and non-pulsatile perfusion versus the corresponding difference in perfused vessel density for small microvessels (Ø <20 µm). There does not seem to be an obvious relation between these parameters.
The Human Microcirculation during Cardiopulmonary Bypass: Effects of Pulsatile Perfusion

microvascular flow using PP.

We recognize that our study has some important limitations. First, we chose the sublingual area for microvascular imaging for its ease of access, its location in the core temperature shell, its phylogenetic relation to the gut and its proximity to the brain. However the sublingual microcirculation does not necessarily reflect other microvascular beds [27, 28]. Further, the technique of SDF imaging carries a risk of operator induced movement bias. However, by strictly applying every recommendation given in the round table conference, we believe this risk was minimal. Finally, it is known that initiating CPB per se may compromise microvascular flow though this is mild and restored towards normal during the course of CPB [29, 30]. However, we circumvented this potential source of bias by starting with NP in half of our patients while starting with PP in the other half.

In summary, we studied microvascular effects of PP in a clinically relevant setting of cardiac surgery. Despite a significant PP induced increase in pump generated hemodynamic energy and pulse pressure, we found no PP induced improvement in microvascular flow. However it should be remembered that we only imaged the sublingual microcirculation at one level of pulsatility in routine cardiac surgery. Further studies may be needed to characterize the microvascular effects of PP in a more comorbid population.

References

Withdrawing Intra-Aortic Balloon Pump Support Paradoxically Improves Microvascular Flow
Luuk DH Munsterman, Paul WG Elbers, Alaattin Ozdemir, Mat van Iterson, Eric PA van Dongen, Can Ince

Critical Care, 2010; 14: R161, epub ahead of print
Abstract

Introduction

The Intra-Aortic Balloon Pump (IABP) is frequently used to mechanically support the heart. There is evidence that IABP improves microvascular flow during cardiogenic shock but its influence on the human microcirculation in patients deemed ready for discontinuing IABP-support has not yet been studied. Therefore we used sidestream dark field imaging (SDF) to test our hypothesis that human microcirculation remains unaltered with or without IABP-support in patients clinically ready for discontinuation of mechanical support.

Methods

We studied 15 ICU patients on IABP therapy. Measurements were performed after the clinical decision was made to remove the balloon catheter. We recorded global hemodynamic parameters and performed venous oximetry during maximal IABP-support (1:1) and ten minutes after temporarily stopping the IABP therapy. At both time points, we also recorded video clips of the sublingual microcirculation. From these we determined indices of microvascular perfusion including perfused vessel density (PVD) and microvascular flow index (MFI).

Results

Ceasing IABP-support lowered mean arterial pressure (74 (8) mm Hg to 71 (10); p=0.048) and increased diastolic pressure (43 (10) to 53 (9) mm Hg; p<0.01). However, at the level of the microcirculation we found an increase of PVD of small vessels <20 µm (5.47 (1.76) to 6.63 (1.90); p<0.01). PVD for vessels >20 µm and MFI for both small and large vessels were unaltered. During the procedure global oxygenation parameters (ScvO₂/SvO₂) remained unchanged.

Conclusions

In patients deemed ready for discontinuing IABP-support according to current practice, SDF-imaging showed an increase of microcirculatory flow of small vessels after ceasing IABP-therapy. This observation may indicate that IABP impairs microvascular perfusion in recovered patients, although this warrants confirmation.
Introduction

In cardiogenic shock, intra-aortic balloon counterpulsation is frequently used to mechanically support the failing heart [1,2]. IABP-support improves coronary blood flow by augmenting systemic and coronary diastolic blood pressure and increases cardiac index by reducing left ventricular work [1,3]. As a bridge to recovery, its goal is to facilitate the heart and continuously provide adequate systemic perfusion.

However, the microcirculation is ultimately responsible for delivering oxygen and substrates to tissue [4]. The recent emergence of Orthogonal Polarization Spectral Imaging and its successor Sidestream Dark Field Imaging has enabled imaging of the human microcirculation in real time [5-7]. These techniques have been used to characterize the microcirculation in various clinical situations including cardiogenic shock.

For example, De Backer et al. [8] demonstrated that microvascular blood flow worsens in severe cardiac failure and cardiogenic shock and is associated with in-hospital mortality. Importantly, there is now a large body of evidence that microvascular flow may be relatively independent from global hemodynamics [4]. For example, arterial and venous blood pressure, cardiac output as well as central or mixed venous oxygen saturation may not necessarily reflect microvascular perfusion [9-14]. However, in current clinical practice, these very global hemodynamic parameters frequently guide the decision when to start and withdraw IABP-therapy.

Three recent trials examined the microcirculatory effects of counterpulsation during cardiogenic shock and high risk percutaneous coronary intervention (PCI) [15-17]. Two of these reported IABP-induced improvement in microvascular flow whereas the other did not. Therefore our understanding of microvascular perfusion during IABP-support remains based on limited and conflicting data. This paucity of data is even more apparent in deciding when to best withdraw IABP-support. No previous study has addressed this issue.

To examine the influence of IABP-support on microcirculation of recovered patients, we studied patients deemed ready for discontinuation of IABP-support as judged by their treating physicians. We used SDF-imaging to test our hypothesis that microvascular flow is unaltered with or without IABP-support in this clinical setting.

Materials and Methods

The local institutional review board approved the study protocol. Since the study was observational and given the non-invasive nature of SDF-imaging, the need for a written informed consent was waived in accordance with the national Law on Experiments with Humans. The study was performed at the intensive care unit (ICU) of a large teaching hospital between April 2007 and October 2008.

We included adult patients that had an IABP in place. Patients were only included if and when the responsible ICU physician had made the decision that the subjects were clinically ready for discontinuation of IABP-support. We excluded patients that showed signs of sepsis (suspected or proven systemic infection with ≥2 SIRS criteria: tachycardia > 90
beats/minute and/or tachypnoea >20 breaths/minute or arterial PCO₂ < 32 mm Hg/4.2 kPa and/or body temperature >38 °C or < 36 °C and/or WBC count > 12,000 cells/mm³ or < 4,000 cells/mm³ or > 10% immature cells). Disruption or laceration of the oral floor mucosa was an exclusion criterion because this would interfere with microcirculatory imaging. As per clinical routine, all patients underwent continuous invasive monitoring of arterial and central venous blood pressure and some patients had a surgically placed pulmonary artery catheter.

A Datascope® CS300 intra aortic balloon pump system (Datascope Corporation, Mahwah, NJ, USA) was used in all studied patients. The IABP system was set automatically, using the electrocardiogram for optimal timing so that inflation and deflation occurred at the diastolic notch and immediately before systolic upstroke, respectively. Optimal balloon size was chosen depending on patient height before insertion. Routine chest X-rays were examined to define correct intra aortic placement of the IABP balloon, 2-3 cm distal to the origin of the left subclavian artery. All hemodynamic parameters were recorded continuously by our patient data management system.

The decision to discontinue IABP-support was a clinical one and left completely at the discretion of the ICU-team. In most cases, this included a weaning trial in which the IABP-assist ratio was lowered step by step over several hours. Possible changes in routinely measured macrocirculatory and laboratory parameters were observed during this process.

Microcirculatory measurements were performed using SDF imaging, which has been described in detail elsewhere [7]. In brief, it consists of a handheld video microscope that emits stroboscopic green light (wavelength 530 nm) from an outer ring at the tip of the probe. This light is absorbed by haemoglobin. A negative image of moving red blood cells is sent back through the isolated optical core of the probe toward a charge-coupled device (CCD) camera. SDF imaging has been shown to provide a higher imaging quality with more detail and less motion blur than its predecessor OPS imaging [7]. A typical example of a SDF-image is shown in figure 1.

Within two hours after the decision to discontinue IABP-support had been made, we performed SDF imaging at two points in time. First, the IABP device was set to a 1:1 assist ratio, if this was not already the selected mode. After 10 minutes, to allow for a new steady state to occur, the first series of microvascular recordings was made. Next, the IABP device was temporarily stopped. After another 10 minutes, we recorded a second series of SDF video clips. At both measuring points, venous and arterial blood gas analyses were collected. In patients with a pulmonary artery catheter mixed venous saturation (SvO₂) was determined, otherwise central venous saturation (ScvO₂) was measured. After the procedure the IABP device was switched back on at the pre-measurement settings. During the procedure dosage of continuous intravenous drugs were recorded and no dosing adjustments were made.

In a recent round table conference, international experts reached consensus on how to best evaluate the microcirculation using OPS and SDF imaging [18]. We implemented all
recommendations given in this conference. Video clips were immediately saved as digital AVI-DV files to a hard drive of a personal computer using an analogue-to-digital converter (Canopus, Kobe, Japan) and the freeware program WinDV (http://windv.mourek.cz). We used 5x optical magnification, producing images representing approximately 940 x 750 µm² of tissue surface. Per measuring point, clips at 3 sublingual sites yielding at least 20 s of stable video per site were recorded. Special care was taken to avoid pressure artefacts, adhering to the standard operating procedure previously described by Trzeciak et al. [19] and recommended in the round table conference [18]. In brief, secretions were removed with gauze, and, after obtaining good imaging focus, the probe was pulled back gently until contact was lost and then advanced again slowly to the point at which contact was regained. The authors paid special attention to the larger vessels at the time of recording because alterations in their flow with probe manipulation may indicate pressure artifacts.

One video file was recorded for each location at each measuring point. These were stored under a random number. At a later time, these were analyzed by one of the authors (PWGE) using the AVA 3.0 software program (Microvision Medical, Amsterdam, The Netherlands). According to the recommendations, microvascular flow index (MFI), perfused vessel density (PVD), proportion of perfused vessels (PPV), and indices of heterogeneity were determined for every patient at both time points. All have been validated previously [19-21]. As published recently, each score was determined for both large and small microvessels, with a cut-off diameter of 20 µm [22]. In addition, the authors defined large-type vessels that split into other vessels as arterioles. Other large vessels were defined as venules.

For PPV and PVD, vessel density was calculated as the number of vessels crossing 3 horizontal and 3 vertical equidistant lines spanning the screen divided by the total length of the lines. Perfusion at each crossing was then scored semi quantitatively by the eye as follows:

Figure 1. A screenshot showing a typical sublingual microvascular recording using SDF imaging.
0 = no flow (no flow present for the entire duration of the clip), 1 = intermittent flow (flow present <50% of the duration of the clip), 2 = sluggish flow (flow present >50% but <100% of the duration of the clip or very slow flow for the entire duration of the clip), and 3 = continuous flow (flow present for the entire duration of the clip). PVD was then calculated as the number of crossings with flow scores greater than 1. PPV was calculated as the proportion of crossings with flow scores greater than 1 divided by the total number of crossings. For each measuring point and each patient, the scores for PPV and PVD were averaged. PVD is expressed in n/mm, whereas PPV is expressed as a percentage. Intra-observer variability ranges between 2.5% and 4.7% for PPV and between 0.9% and 4.5% for PVD. The inter-observer variability is slightly higher: between 3.0% and 6.2% and between 4.1% and 10%, respectively [20].

MFI was based on the determination of the predominant type of flow in 4 quadrants.

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**Table 1. Patient characteristics and drugs. Catecholamine and vasopressor doses represent those used during SDF imaging.**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Mean (SD) or n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td>15</td>
</tr>
<tr>
<td>Age (y)</td>
<td>65.7 (11.8)</td>
</tr>
<tr>
<td>Male</td>
<td>12 (80%)</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>175 (11)</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>81 (14)</td>
</tr>
<tr>
<td>Hb (mM)</td>
<td>6.2 (0.7)</td>
</tr>
<tr>
<td>Ht (%)</td>
<td>30.0 (3.5)</td>
</tr>
<tr>
<td>Lactate (mM; 11 patients)</td>
<td>1.2 (0.4)</td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td>36.9 (1.0)</td>
</tr>
<tr>
<td>Apache II score</td>
<td>14.5 (5.3)</td>
</tr>
<tr>
<td>History of vascular disease</td>
<td>5 (33%)</td>
</tr>
<tr>
<td>Hypertension</td>
<td>6 (40%)</td>
</tr>
<tr>
<td>Diabetes</td>
<td>3 (20%)</td>
</tr>
<tr>
<td>Chronic renal insufficiency</td>
<td>1 (7%)</td>
</tr>
<tr>
<td>Dopamine (mg/h; 9 patients)</td>
<td>17.7 (8.7)</td>
</tr>
<tr>
<td>Norepinephrine (mg/h; 5 patients)</td>
<td>0.26 (0.2)</td>
</tr>
<tr>
<td>Dobutamine (mg/h; 2 patients)</td>
<td>15.0 (7.1)</td>
</tr>
<tr>
<td>Nitroglycerin (mg/h; 6 patients)</td>
<td>1.0 (0.0)</td>
</tr>
<tr>
<td>Mechanical Ventilation</td>
<td>11 (7.3)</td>
</tr>
</tbody>
</table>
adhering to the same scoring system. MFI is the sum of these flow scores divided by the number of quadrants in which the vessel type is visible. The intra-observer agreement of MFI is about 85% (kappa score = 0.78) and inter-observer agreement about 90% (kappa score = 0.85) [21]. For each measuring point and each patient, the scores for MFI were averaged.

Heterogeneity was assessed in two different ways. For PVD, the coefficient of variation was determined. For MFI, the authors assessed heterogeneity in each patient by subtracting the lowest from the highest quadrant MFI and dividing the result by the mean MFI [19, 20].

We used Wilcoxon matched pairs tests for MFI and paired t tests for other data. We used spearman tests to detect correlation between global and microvascular parameters. Results are reported as median and interquartile ranges (IQR) for MFI and as the mean (standard deviation) for other parameters, unless indicated otherwise. The study was powered to detect a minimum of 15% difference in small-vessel PVD after switching off the IABP with $\alpha =0.05$ and $1-\beta = 0.80$. Based on previous studies by others and us [8, 10, 20] this showed the need for inclusion of 14 patients.

Results

 Patients

We included 15 patients. Baseline characteristics of the study population, including risk factors for cardiovascular disease and continuous intravenous dosage of vasoactive drugs are shown in table 1. All participants were admitted at the intensive care unit of St. Antonius Hospital, Nieuwegein, The Netherlands.

Mean APACHE II score 24 hours after admission at the ICU was 14.5 (5.3). Indications for IABP placement were urgent coronary artery bypass grafting due to unstable angina pectoris with severe coronary disease (n=6, 40 %), cardiogenic shock due to myocardial infarction without heart surgery (n=2, 13%), cardiogenic shock after heart surgery (n=2, 13 %), due to acute prosthetic aortic valve displacement after surgery (n=1, 7 %) or after cardiac arrest with or without percutaneous coronary intervention (n=4, 27 %). Mean duration of IABP-therapy at the time of measurement was 3 days (range 1-5 days). During the experiment, six patients had a pulmonary artery catheter in place and 11 patients were mechanically ventilated.

 Systemic hemodynamic data

Table 2 depicts the differences in global hemodynamic parameters between the two points of interest. The institution of IABP-support significantly increased mean arterial pressure (MAP). However, recorded diastolic blood pressure was significantly lower. It is important to point out that this represents the lowest pressure recorded during the cardiac cycle, which is the purpose of balloon counterpulsation. After switching off IABP-support no differences in venous oxygen saturation occurred ($\text{SvO}_2$/$\text{SvO}_2$; 70.9 (7.2) % vs. 71.4 (7.5) %; p=0.90).
The authors successfully obtained high-quality images in each patient. In total 90 video clips were recorded. Results are shown in table 3. PVD of small vessels (<20 µm) was significantly lower during IABP-support; 5.47 (1.76) vs. 6.63 (1.90); p<0.01 (figure 2). Other microcirculatory parameters were not significantly altered.

Spearman tests did not show a statistically significant correlation between changes in mean arterial pressure, diastolic arterial pressure and S(c)vO₂ versus changes in PVD in individual patients comparing maximal support vs. no support (r=-0.1; p=0.71, r=0.1; p=0.72, r=0.1; p=0.69 respectively). In addition, no correlation was found between small vessel PVD and APACHE II score (r=0.04; p=0.88).

Discussion

To the best of our knowledge, this is the first study to report on the microvascular effects of IABP switch-off in recovered patients deemed ready for IABP removal. Our most prominent finding is that cessation of IABP-support resulted in a significant and paradoxical increase in small-vessel PVD, independent from initial disease severity. This is in contrast with the loss of IABP-induced raise of mean arterial pressure in these patients [1-

<table>
<thead>
<tr>
<th></th>
<th>Assist ratio 1:1</th>
<th>No assist</th>
<th>Difference (95% CI)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABPs (mm Hg)</td>
<td>115 (16)</td>
<td>110 (15)</td>
<td>-5 (-12 to 2)</td>
<td>0.16</td>
</tr>
<tr>
<td>ABPm (mm Hg)</td>
<td>74 (8)</td>
<td>71 (10)</td>
<td>-3 (-6 to 0)</td>
<td>0.05</td>
</tr>
<tr>
<td>ABPd (mm Hg)</td>
<td>43 (10)</td>
<td>54 (9)</td>
<td>11 (6 to 15)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>HR (min⁻¹)</td>
<td>83 (12)</td>
<td>85 (13)</td>
<td>2 (-1 to 4)</td>
<td>0.19</td>
</tr>
<tr>
<td>CVP (mm Hg)</td>
<td>13 (6)</td>
<td>13 (7)</td>
<td>0 (-1 to 2)</td>
<td>0.44</td>
</tr>
<tr>
<td>ScvO₂ / SvO₂ (%)</td>
<td>71 (7)</td>
<td>71 (8)</td>
<td>0 (-1 to 1)</td>
<td>0.90</td>
</tr>
<tr>
<td>SpO₂ (%)</td>
<td>96 (2)</td>
<td>972 (2)</td>
<td>1 (-1 to 1)</td>
<td>0.72</td>
</tr>
<tr>
<td>PAPs (mm Hg)</td>
<td>46 (8)</td>
<td>46 (7)</td>
<td>0 (-2 to 3)</td>
<td>0.74</td>
</tr>
<tr>
<td>PAPm (mm Hg)</td>
<td>29 (4)</td>
<td>29 (4)</td>
<td>0 (-2 to 1)</td>
<td>0.47</td>
</tr>
<tr>
<td>PAPd (mmHg)</td>
<td>23 (8)</td>
<td>23 (8)</td>
<td>0 (-1 to 1)</td>
<td>0.61</td>
</tr>
</tbody>
</table>

Table 2. Global hemodynamic data. ABPs/m/d, systolic, mean and diastolic arterial blood pressure; HR, heart rate; CVP, central venous pressure; ScvO₂, central venous oxygen saturation; SvO₂, mixed venous oxygen saturation; SpO₂, peripheral oxygen saturation; PAPs/m/d, systolic, mean and diastolic pulmonary artery pressure; CI, confidence interval.
3] and, perhaps more importantly, in contrast with SvO\textsubscript{2} or ScvO\textsubscript{2} values, which remained unchanged after IABP switch-off. Therefore, this study adds to the large body of evidence that global hemodynamic parameters and venous oximetry do not necessarily reflect microvascular perfusion [4, 9, 10, 20, 21]. It is the first time that such a discrepancy is demonstrated after withdrawal of IABP-support.

Three recent studies examined the effects of IABP on human microcirculation [15-17]. Jung et al. included 13 patients with cardiogenic shock after acute myocardial infarction. The authors recorded SDF video images before and shortly after the IABP-support was temporarily stopped. MFI of small and medium vessels (10-50 µm) was significantly higher in patients with IABP-support [15]. In contrast, Den Uil et al. studied a heterogeneous group of 13 patients suffering from cardiogenic shock of variable severity and found no differences in perfused capillary density and red blood cell velocity [16]. This was despite the fact that mean arterial pressure and cardiac index were significantly lower after the IABP-assist ratio was switched from 1:1 to 1:8. Finally, more recently, Jung et al. studied 6 patients immediately following high-risk PCI. MFI of both small and large vessels decreased significantly immediately after a short period of IABP-support discontinuation.

<table>
<thead>
<tr>
<th></th>
<th>Assist ratio 1:1</th>
<th>No assist</th>
<th>Difference (95% CI)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>PVD (µm)</td>
<td>5.47 (1.76)</td>
<td>6.63 (1.90)</td>
<td>1.16 (0.44 to 1.89)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>PPV</td>
<td>88.4 (14.1)</td>
<td>88.8 (16.4)</td>
<td>0.4 (-5.6 to 6.5)</td>
<td>0.88</td>
</tr>
<tr>
<td>HI-PVD</td>
<td>0.38 (0.23)</td>
<td>0.40 (0.37)</td>
<td>0.02 (-0.15 to 0.19)</td>
<td>0.83</td>
</tr>
<tr>
<td>MFI</td>
<td>2.75 (2.00-3.00)</td>
<td>3.00 (2.58-3.00)</td>
<td>0.25</td>
<td>0.42</td>
</tr>
<tr>
<td>HI-MFI</td>
<td>0.68 (0.68)</td>
<td>0.57 (0.87)</td>
<td>-0.11 (-0.49 to 0.27)</td>
<td>0.55</td>
</tr>
<tr>
<td>PVD (µm)</td>
<td>1.34 (0.88)</td>
<td>1.64 (0.63)</td>
<td>0.31 (-0.21 to 0.83)</td>
<td>0.22</td>
</tr>
<tr>
<td>PPV</td>
<td>95.5 (9.6)</td>
<td>96.9 (7.7)</td>
<td>1.38 (-2.18 to 4.94)</td>
<td>0.42</td>
</tr>
<tr>
<td>HI-PVD</td>
<td>0.59 (0.25)</td>
<td>0.50 (0.25)</td>
<td>-0.08 (-0.25 to 0.08)</td>
<td>0.30</td>
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<tr>
<td>MFI</td>
<td>3.0 (2.5 to 3.0)</td>
<td>3.0 (2.78 to 3.0)</td>
<td>0</td>
<td>0.11</td>
</tr>
<tr>
<td>HI-MFI</td>
<td>0.44 (0.58)</td>
<td>0.18 (0.29)</td>
<td>-0.26 (-0.57 to 0.05)</td>
<td>0.10</td>
</tr>
</tbody>
</table>

Table 3. Microcirculation. MFI is reported as median with interquartile ranges in square brackets. Other microcirculatory parameters are mean values (standard deviation). PVD, perfused vessel density; PPV, percentage of perfused vessels; MFI, microvascular flow index; HI, heterogeneity index; CI, confidence interval.
and returned to baseline after restarting therapy. Again, no correlation with global hemodynamic parameters was found [17].

Both groups chose not to fully incorporate recent recommendations regarding image acquisition or reporting standard microvascular flow parameters [18]. This hampers direct comparison with our study. More importantly, our population of recovered patients is markedly different. However, this does not dismiss our sharply contrasting finding of improved PVD after IABP switch-off. It is plausible that IABP has different microvascular effects at different stages of disease and recovery.

Although speculative, one possible explanation for the observed difference in microvascular perfusion could be a mechanical effect of IABP. The pressure decrease induced by balloon deflation during diastole could create a collapse of certain parts of capillary microcirculation. This effect is consistent with the concept of vascular waterfalls: the flow in front of a waterfall is not affected by its height. This analogy explains why sometimes global and regional blood flow ceases at positive arterial pressures well above venous pressure [23, 24]. Interestingly, an IABP-induced transient blood flow reversal in basal cerebral arteries has been reported previously [25, 26], which is consistent with this theory. However, correlation between the difference in diastolic pressure and the difference in PVD

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Figure 2. The effect of IABP on perfused vessel density (PVD) for vessels < 20 µm. The perfused vessel density increased significantly, 5.47 (1.76) to 6.63 (1.90), p<0.01, 10 minutes after IABP switch-off.
between the study time points did not reach statistical significance. Conversely, this may not invalidate our hypothesis, as microvascular derecruitment is probably an all or nothing stochastic process.

There are several important limitations to our study. First, we did not routinely measure cardiac output. However, this is highly representative of current practice. In addition, it may be reasonable to assume that cardiac output remained constant as no changes in venous oximetry and heart rate were observed and no drug dosing adjustments were made during the study period. Interestingly, if IABP would have increased cardiac output in our patients, as it has been reported to do so in other clinical situations, this would perhaps even strengthen our findings as this would mean that despite increased cardiac output during IABP support microvascular flow would be relatively impaired as compared to no IABP support. Second, we chose the sublingual site for its proximity to the brain, ease of access and its phylogenetic relationship to the gut. There is conflicting data on whether sublingual microvascular perfusion represents other vascular beds [27,28]. Interestingly a recent animal study shows that in the setting of resuscitation, cerebral microcirculation is relatively protected as compared to sublingual microvascular perfusion [29]. If applicable to the setting studied by us, this would imply that cerebral microvascular perfusion improves to a greater extent after IABP switch-off. Third, the fact that our findings could possibly be explained by temporal changes in microcirculation instead of IABP cannot be ruled out because we did not perform a final measurement after reinitiating IABP-support. Finally, but perhaps most important, a heterogeneous group was studied in which a minority initially had cardiogenic shock. However, no correlation of initial disease severity (APACHE II score) and microcirculatory perfusion at the time of measurement could be found. Further, there were no differences in microcirculatory perfusion between patients with initial shock and those without shock. The absence of correlation between microvascular flow and APACHE II and shock state may not be surprising as patients were only studied during a phase in which they were deemed ready for discontinuation of IABP support.

Given these limitations, our findings and their clinical consequences should be interpreted with caution. In addition, while we have shown that IABP may impair tissue perfusion in hemodynamically recovered patients, we did not observe signs of cell ischemia (e.g. lactate acidosis) nor signs of progressive organ dysfunction during IABP-therapy on the day measurements were performed. In addition PVD values during IABP-support are similar to those found in healthy controls by De Backer [8] and to those found after routine cardiac surgery by us [10].

However, given the current controversy on the evidence of IABP-support and therefore its indication [30], coupled with complication risks [31], it may be prudent not to ignore our results. Perhaps the best strategy is to optimize the duration of support and our findings could possibly be the consequence of the fact that our patients may have been too long IABP-treated. The role of microvascular imaging to achieve this goal merits further study.
Conclusions

The results of this study show that cessation of IABP-support resulted in a paradoxical increase of microvascular flow in small vessels when clinicians deemed IABP-support was no longer necessary. These changes are independent from global hemodynamic parameters and oxygen derived variables.

Clinicians that routinely rely on these parameters for their decision-making should again be reminded that these do not necessarily reflect microvascular perfusion in a wide range of clinical setting including that of cessation of IABP support.

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Summary and Conclusions
Introduction

The microcirculation is essential for life as it delivers oxygen and nutrients to tissue. Therefore, monitoring this vital organ in perioperative and intensive care medicine may provide essential information for clinical decision making. However, in clinical practice, microvascular monitoring was not routinely performed. Instead, anesthesiologists and intensivists continued to rely on global hemodynamic parameters to guide patient management. These include blood pressure, heart rate and sometimes cardiac output and oxygen derived parameters such as systemic oxygen delivery (DO$_2$) and -consumption (VO$_2$) and mixed or central venous oxygen saturation (SvO$_2$ and ScvO$_2$).

One reason why monitoring the microcirculation had not been considered part of routine clinical monitoring was the lack of availability of suitable techniques. However, the last decade has witnessed the advent of Orthogonal Polarization Spectral (OPS) imaging [1] and its improved successor Sidestream Dark Field (SDF) imaging [2]. These techniques have enabled clinical monitoring of the human microcirculation at the bedside. Both provide the clinician with moving magnified images of the microcirculation and are usually applied sublingually.

Human application of these techniques first focused on critically ill septic patients. The density of their microvessels was shown to be significantly reduced and these alterations were more severe in nonsurvivors [3]. In addition, persistent lack of small microvessel perfusion was strongly associated with organ failure and death [4-7]. A consistent finding from these and other studies using OPS- and SDF imaging in septic patients was that the human microcirculation showed marked heterogeneity in disease and that global hemodynamic parameters do not necessarily reflect microvascular perfusion.

Thesis

As discussed above, focusing on normalization of macrohemodynamic variables in routine clinical practice was not limited to septic patients. In fact, clinical decisions based on these parameters, including heart rate and arterial and venous blood pressure were made on a daily basis throughout the world in virtually all patients in perioperative and intensive care medicine.

However, if microvascular heterogeneity and disagreement between global hemodynamics and microvascular perfusion observed in sepsis, would also hold for non-septic patients in intensive care and perioperative medicine, today’s clinicians may sometimes be watching and correcting the wrong parameters. This might not have been unlikely as critically ill non-septic patients, for example those undergoing major surgery or those in shock suffer from a systemic inflammatory response syndrome that might have similar microvascular implications as sepsis [8].

Therefore the hypothesis of this thesis was that discrepancy between macrohemodynamic and microvascular parameters also exists in non-septic patients in perioperative and intensive care medicine. To prove this thesis, a large number of studies were conducted, several
Proof of thesis

Chapter 1 introduced the concept of the microcirculation as a vulnerable organ in sepsis. Just like other organs, the microcirculation is composed of various cell types such as endothelium, smooth muscle cells, red and white blood cells. Further, also similar to other organs, the microcirculation consists of a large number of other components including platelets, coagulation factors, and a plethora of cytokines and chemokines. The microcirculatory organ is a highly regulated one in order to perform its main function: oxygen and nutrient delivery to tissue along with the removal of waste products. It is easy to imagine that disease and especially sepsis may lead to dysfunction of this microvascular organ by interfering with its individual components. Examples are decreased red and white cell deformability which may cause microvascular plugging; and the pathological release of nitric oxide (NO) causing harmful vasodilation. These processes may cause microvascular heterogeneity. In other words, areas of normal or increased microvascular flow may exist right beside areas in which flow is stagnant or absent. This may give rise to an oxygen extraction paradox in which oxygen is effectively shunted away from some tissues whereas other areas receive oxygen in superfluous amounts. The result is tissue ischemia while venous oxygen saturation may be normal or even increased. The various microvascular alterations in sepsis are greatly influenced by the natural course of the disease and therapeutic interventions. In addition, the diseased microcirculation may also fuel the clinical syndrome giving rise to multiple organ failure.

This chapter further discussed the various techniques available for monitoring the microcirculation. It also introduced the reader to OPS- and SDF imaging which have enabled bedside imaging of the human microcirculation in real time. Finally it outlined potential strategies for resuscitating the microcirculation including vasodilatation, fluids and transfusion, inducible nitric oxide synthetase inhibition, and multi-action drugs, such as activated drotrecogin alfa. It is important to remember that normal or improving global hemodynamics or oxygen-derived parameters do not preclude microcirculatory dysfunction, multiple organ failure, and fatal outcome. It was argued that the microcirculation may be the much needed end point of resuscitation of clinical sepsis and septic shock.

Almost 40 years ago Weil and Shubin proposed a re-classification of shock states and identified hypovolemic, cardiogenic, obstructive and distributive shock [9]. The first three categories have in common that they are associated with a fall in cardiac output. Distributive shock, such as occurs during sepsis and septic shock, however, is associated with an abnormal distribution of an otherwise normal or supra normal cardiac output to the microcirculation and metabolic distress. Chapter 2 explored the insights that have been gained into the nature of distributive shock. Its pathophysiology can best be described as a microcirculatory and mitochondrial distress syndrome (MMDS), where time and therapy form an integral part of the definition.

The clinical introduction of new microcirculatory imaging techniques, such as OPS- and
SDF imaging, have allowed direct observation of the microcirculation at the bedside. Images of the sublingual microcirculation during septic shock and resuscitation have revealed that the distributive defect of blood flow occurs at the capillary level. In this chapter, the different types of heterogeneous flow patterns of microcirculatory abnormalities found during different types of distributive shock were examined. Analysis of these patterns gave a five class classification system to define the types of microcirculatory abnormalities found in different types of distributive shock and indicated that distributive shock occurs in many other clinical conditions than just sepsis and septic shock. It is likely that different mechanisms defined by pathology and treatment underlie the different abnormalities observed in the different classes. Functionally, however, they all cause a distributive defect resulting in microcirculatory shunting and regional dysoxia. It is hoped that this classification system will help in the identification of mechanisms underlying these abnormalities and indicate optimal therapies for resuscitating septic and other types of distributive shock.

It is important to standardize analysis of microvascular images using OPS- and SDF imaging as this ensures quality and facilitates comparison between studies. Therefore the recommendations from a round table conference in 2007 on how to perform such analysis should be praised [10]. However, while rigorous, the proposed methods were time consuming. Therefore chapter 3 proposed a modification that greatly speeds up the analysis process while maintaining the robustness of the recommendations. In chapter 4, the case for uniform microvascular analysis that was introduced in chapter 3 was further reinforced.

**Chapters 5 to 10** contain two case reports and four original papers. These formed the core of this thesis. Results were reported as mean (SD) unless indicated otherwise.

SDF imaging was performed in a patient suffering from chronic myeloid leukemia in whom leukostasis was suspected based on a leukocyte count of 398,000/mL. As shown in chapter 5, there were abnormally large gaps between erythrocytes and areas of hyperdynamic flow next to areas with striking microcirculatory stasis and capillary derecruitment. Following treatment, the microcirculatory flow pattern returned to normal.

Ketanserin, a serotonin and α-1 adrenoceptor antagonist, is used in some countries to treat elevated blood pressure after extracorporeal circulation [11]. This might have hampered microcirculatory perfusion. Conversely, it was also conceivable that microcirculatory flow is maintained or improved as a result of flow redistribution. In chapter 6, SDF imaging was used to directly observe the sublingual microcirculation in this setting. Mechanically ventilated patients with elevated arterial blood pressure immediately after extracorporeal circulation were given an intravenous bolus of ketanserin, 0.15 mg/kg. Five minutes before and 10 minutes after ketanserin administration, global hemodynamic variables were recorded. In addition, video clips of the microcirculation were recorded. Analysis of these allowed for quantification of microvascular hemodynamics including determination of perfused vessel density (PVD) and microcirculatory flow index (MFI). After ketanserin administration, there was a significant reduction in systolic arterial blood pressure (129 (9) to 100 (15) mm Hg, p<0.01). At the level of the microcirculation, the mean MFI did not change significantly for small (diameter <20µm, 2.79 [interquartile range, 1.38-3] to 2.38
[Summary and Conclusions]

[1.88-2.75], p=0.62) or large (diameter ≥20 µm, 2.83 [1.4-3] to 2.67 [0.35-2.84] p=1.0) vessels. There was a significant increase in mean PVD for large vessels (1.23 (0.63) to 1.70 (79) mm⁻¹), p = 0.02) but not for small vessels (5.59 (2.60) to 5.87 (1.22) mm⁻¹, p=0.72) where red blood cell flow was maintained. SDF imaging clearly showed a discrepancy between global and microvascular hemodynamics after the administration of ketanserin for elevated blood pressure after ECC. Ketanserin effectively lowered arterial blood pressure. However, capillary perfusion was maintained at a steady value. Both effects may be explained by an increase in shunting in the larger vessels of the microcirculation.

The microvascular response to cardiopulmonary resuscitation in humans was unknown although it had been extensively investigated in animal models [12]. Chapter 7 reported on the first use of sidestream dark field imaging to assess the human microcirculation during CPR with a mechanical chest compression/decompression device (mCPR). mCPR was able to provide microvascular perfusion. Capillary flow persisted even during brief mCPR interruption. However, indices of microvascular perfusion were low and improved vastly after return of spontaneous circulation. Microvascular perfusion was relatively independent from blood pressure. The micrcirculation may therefore be a useful monitor for determining the adequacy of CPR.

The behavior of the human microcirculation in the setting of cardiac arrest was further explored in chapter 8. Animal experiments had consistently revealed that global hemodynamics do not necessarily reflect microvascular perfusion. In addition, the time it takes for capillary blood flow to stop after the heart arrests was debated. Estimations ranged from 50 s to 5 minutes, but data in humans was lacking [13]. Aortic arch surgery frequently necessitates deep hypothermic circulatory arrest and subsequent selective antegrade cerebral perfusion. To elucidate microvascular behavior surrounding cessation of human circulation, SDF imaging was used in this setting. Seven patients undergoing elective aortic arch repair were included. Sublingual microvascular recordings were made immediately before circulatory arrest, during circulatory arrest, and immediately after selective antegrade cerebral perfusion. Before circulatory arrest, perfused vessel density was 6.41 (1.18) for small (<20 µm) and 1.57 (0.88) mm⁻¹ for large (>20 µm) microvessels. Microvascular flow index was a median of 3.0 for both vessel sizes. After circulatory arrest, there was no equilibration of arterial and venous blood pressure before onset of selective antegrade cerebral perfusion after 59 (17) s; range, 40-80 s. Flow in small microvessels came to a complete stop after 45 s (9) s; range, 34-57 s. after transition to circulatory arrest. However, flow in larger microvessels did not completely stop before selective antegrade cerebral perfusion started. Selective antegrade cerebral perfusion restored microvascular flow, reaching pre circulatory arrest levels after 45 s (median, 27 s; range, 20-85 s). Thus, in a controlled surgical setting, circulatory arrest in humans induced a complete sublingual small microvessel shutdown within 1 minute, while flow in larger microvessels persisted. Selective antegrade cerebral perfusion was able to restore microvascular flow to pre circulatory arrest levels within a similar time frame.

Possible benefits of pulsatile perfusion during cardiopulmonary bypass are often attributed
to enhanced microvascular flow. However, there was no evidence to support this in humans [14]. Chapter 9 assessed whether pulsatile perfusion alters human microvascular flow. In a prospective randomized observational cross-over study, 16 patients undergoing routine cardiopulmonary bypass for cardiac surgery were included. All patients underwent both pulsatile and non-pulsatile perfusion in random order. Sidestream Dark Field Imaging was used to record video clips of the sublingual human microcirculation. Perfusion was started either in pulsatile (n=8) or non-pulsatile mode. After 10 minutes, microvascular recordings were made. Perfusion mode was then switched and after ten minutes, new microvascular recordings were taken. Pulsatile perfusion generates surplus hemodynamic energy. This was quantified by calculating pulse pressure and energy equivalent pressure. Microvascular analysis included determination of perfused vessel density. This did not differ between non-pulsatile and pulsatile perfusion (6.65 (1.39) versus 6.83 (1.23) mm⁻¹, p=0.58 and 2.16 (0.64) versus 1.96 (0.48) mm⁻¹, p=0.20 for small and large microvessels respectively, cut-off 20 µm. Pulse pressure and energy equivalent pressure were higher during pulsatile perfusion. However, there was no correlation between the difference in energy equivalent pressure or pulse pressure and perfused vessel density (r=-0.43, p=0.13 and r=-0.09, p=0.76 respectively). Pulsatile perfusion did not alter human microvascular perfusion using standard equipment in routine cardiac surgery. Changes in pulse pressure or energy equivalent pressure did not bear any obvious relationship with microcirculatory parameters.

The Intra-Aortic Balloon Pump (IABP) is frequently used to mechanically support the heart. There was evidence that IABP improves microvascular flow circulation during cardiogenic shock but its influence on the human microcirculation in patients that have recovered from cardiogenic shock had not yet been studied [15]. Therefore in chapter 10, Sidestream dark field imaging (SDF) was used to test the hypothesis that human microcirculation remains unaltered with or without IABP support in patients deemed clinically ready for discontinuation of mechanical support. Fifteen ICU patients on IABP therapy were studied. Measurements were performed after the clinical decision was made to remove the balloon catheter. We recorded global hemodynamic parameters and performed venous oximetry during maximal IABP-support (1:1) and ten minutes after temporarily stopping the IABP therapy. At both time points, video clips of the sublingual microcirculation were recorded. From these recordings indices of microvascular perfusion were determined including perfused vessel density (PVD) and microvascular flow index (MFI). Ceasing IABP-support lowered mean arterial pressure (74 (8) mm Hg to 71 (10); p=0.048) and increased diastolic pressure (43 (10) to 53 (9) mm Hg, p<0.01). However, at the level of the microcirculation an increase in PVD of small vessels was found (diameter <20 µm (5.47 (1.76) to 6.63 (1.90); p<0.01). PVD for vessel diameter >20 µm and MFI for both small and large vessels were unaltered. During the procedure, global oxygenation parameters (ScvO₂/SvO₂) remained unchanged. Thus, in patients deemed ready for discontinuing IABP-support according to current clinical practice, SDF-imaging showed an increase of microcirculatory flow of small vessels after ceasing IABP-therapy. This observation may indicate that IABP impairs microvascular perfusion in recovered patients, although this warrants confirmation.
Conclusions

The hypothesis of this thesis was that discrepancy between microvascular flow and macrohemodynamic parameters as seen in sepsis also exists in non-septic patients in perioperative and intensive care medicine. Indeed the studies presented here clearly prove this hypothesis. Microvascular flow was studied in a wide range of clinical settings as diverse as cardiopulmonary resuscitation, extreme leukocytosis and mechanical cardiopulmonary support devices. Significant changes in microcirculatory hemodynamics were observed whereas no such large changes were found in systemic hemodynamic variables and vice versa.

The character of this dichotomy in non-septic clinical scenarios also seems to differ from that in sepsis. In the latter, heterogeneity of the microcirculation is a prominent feature. Consistent observations included those of areas with severely impaired microvascular flow immediately adjacent to areas with normal or hyperdynamic microvascular perfusion. The dichotomy in the clinical settings studied in this thesis is generally not characterized by a heterogeneous microcirculation. However, even while relatively homogenous, the microvascular compartment was consistently shown not to correlate well with global hemodynamic parameters.

Naturally, as the various studies have been performed in a variety of clinical settings, their results have different impacts. These are highlighted in the discussion sections of the various chapters. The key message, however, is the same every time: microvascular flow is not necessarily reflected by global hemodynamic parameters such as heart rate, arterial or venous blood pressure, cardiac output or oxygen derived parameters such as venous oximetry.

Clinical implications

Even though microvascular perfusion may be dissociated from systemic hemodynamics, it cannot be currently recommended to solely focus on microvascular imaging to guide clinical interventions. For this, there are two very important reasons.

First, there have been no clinical trials examining if using microvascular flow to guide clinical interventions leads to improved outcome. As discussed below, many therapeutic strategies have been found to improve microvascular flow. However, none have been used to assess whether improvement of the microcirculation is actually therapeutic for patients. Therefore it is as yet uncertain if goal directed recruitment of the microcirculation would lead to reduced mortality, morbidity or improvement in organ function.

Second, there is substantial evidence that optimizing global hemodynamic parameters results in reduced mortality and morbidity, although results are conflicting. These so called goal directed therapy protocols have been extensively studied both in perioperative and intensive care medicine.

Relying on global hemodynamics and in particular improving oxygen delivery has produced mixed results as was recently reviewed by Rampal et al. [16]. Its effects seem to be dependent on which patient group is studied and also during which stage of their disease.
example, goal directed therapy does not confer benefits in patients with established critical illness. However, in the resuscitation of sepsis, clear benefits were shown. Generally this is also true for pre-, intra- and postoperative optimization of systemic hemodynamics in high risk surgery, although conflicting results have been reported.

The findings from goal directed therapy are consistent with the theory that a certain minimum of cardiac output and arterial blood pressure is required for a functioning microcirculation. Below that minimum, microvascular flow may be compromised. However, this minimum level remains ill defined and is probably subject to large interindividual variations. Further, even above that minimum, there may be dissociation between systemic hemodynamics and microvascular flow. These concepts are supported by the results of the studies in this thesis.

In addition, the effects of time and therapy may increase the dichotomy between systemic hemodynamics and microvascular flow. Again, this dichotomy may show large interindividual variations. Trials by Jhanji et al. and Dubin et al. may serve as examples using vasopressors as circulatory therapy [17, 18]. Both applied noradrenalin in septic patients targeting different levels of mean arterial pressure. Both showed that in general this did not affect microvascular perfusion. However Dubin showed that the response to increasing doses of noradrenalin was different between individual patients. Those who had reduced microvascular flow at baseline, showed marked improvement upon administration of noradrenalin, whereas patients with normal microvascular flow showed a deterioration in their indices of microvascular perfusion after noradrenalin.

Therefore it can be expected that monitoring the microcirculation using these techniques in various clinical settings in perioperative and intensive care medicine may become an important component in the monitoring armamentarium of the clinician

**Future directions**

This thesis has underscored that the discrepancy between systemic and microvascular hemodynamics seen in sepsis may also be found in a wide variety of clinical settings in intensive care and perioperative medicine. With the advent of imaging techniques such as OPS- and SDF imaging, the microcirculation has earned a firm place in the minds of today’s anesthesiologists and intensivists. Monitoring systemic hemodynamic parameters remains important. But it is hoped that this thesis has contributed to the introduction of the microcirculation as a clinical concept.

The great strength of imaging modalities such as OPS- and SDF imaging is that these can differentiate between different mechanisms of microvascular failure. This is partly reflected in the commonly used scoring systems, where a decreased microvascular flow index may indicate flow limiting pathophysiology whereas a decreased perfused vessel density may indicate diffusion limiting disease, although there is considerable overlap between the scores. In addition, possible heterogeneity of the microcirculation can be clearly visualized. These insights may ultimately lead to tailor made therapy based on microvascular imaging.
Multiple interventions have been shown to improve microvascular flow in various clinical settings. These include fluid therapy [19], blood transfusion [20], inotropic [21] and vasodilator therapy [22-24], corticosteroids [25], activated drotrecogin alpha [26] vasopressors [18] and extracorporeal membrane oxygenation [27]. However, except for vasodilation, these were only studied in septic patients and only used microvascular flow as an end point. A recent study by Boerma et al., also in septic patients, showed that after protocolized optimization of systemic hemodynamic flow, administration of nitroglycerin therapy did not improve microvascular flow or outcome [28]. However, the nitroglycerin dose was low and initial microvascular perfusion was already quite good.

Latest developments include two original studies, one review and one abstract that have now directly linked optimizing systemic hemodynamics to improvements in microvascular flow, both in the setting of surgery and sepsis [6, 29-31]. This may represent a stepping stone implying that guiding therapy based on microvascular flow may be shifting from a physiology based to an evidence based concept.

However, the true challenge that remains is to prove that therapeutic interventions based on improving or normalising microvascular flow improve patient outcome in perioperative and intensive care medicine. The good news is that some barriers preventing such trials have now been lifted. For example, microvascular scoring systems have now been extensively validated and recently good correlation between sublingual and splanchnic microvascular flow was shown [32]. Further, there are promising developments in the automated analysis of microvascular images, which would greatly facilitate microvascular research. Even though trials using mortality as an endpoint require inclusion of a large number of patients and are therefore both challenging and daunting, these do represent the only way forward.

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Samenvatting en Conclusies
Introductie

De microcirculatie is letterlijk van levensbelang omdat zij zuurstof en voedingsstoffen aflevert aan de weefsels. Het bewaken van dit vitale orgaan kan daarom informatie verschaffen die essentieel is voor de klinische besluitvorming. In de klinische praktijk werd de microcirculatie echter niet routinematig in de gaten gehouden. In plaats daarvan vertrouwden anesthesiologen en intensivisten op de zogenaamde systemische hemodynamische parameters om hun patiënten te behandelen. Hiertoe behoren bloeddruk, hartfrequentie en soms ook het hartminuutvolume oftewel cardiac output en de zogenaamde oxygen derived parameters zoals het systemisch zuurstofaanbod ($\text{DO}_2$) en -verbruik ($\text{VO}_2$) en gemengd- of centraal veneuze zuurstofsaturatie ($\text{SvO}_2$ en $\text{ScvO}_2$).

Eén van de redenen waarom de microcirculatie niet routinematig werd bewaakt was de beperkte beschikbaarheid van geschikte technieken daarvoor. In de laatste tien jaar is hier echter verandering in gekomen door de introductie van Orthogonal Polarization Spectral (OPS) imaging en haar verbeterde opvolger Sidestream Dark Field (SDF) imaging. Deze technieken hebben het mogelijk gemaakt om aan het bed van de patiënt de menselijke microcirculatie klinisch te vervolgen. Beide methodes genereren bewegende vergrote beelden van de microcirculatie en worden meestal toegepast onder de tong.

Bij mensen werden zij eerst vooral ingezet bij patiënten met bloedvergiftiging oftewel sepsis. Bij deze patiënten bleek dat de dichtheid van de kleinste bloedvaten significant verminderd was ten opzichte van gezonde mensen en dat deze veranderingen meer uitgesproken waren bij patiënten die hun sepsis niet overleefden. Ook werd gevonden dat blijvende slechte doorbloeding van de kleinste bloedvaten geassocieerd is met orgaanfalen en dood. Een terugkerende bevinding bij het gebruik van OPS en SDF imaging in deze -maar ook andere- studies was een opvallende heterogeniteit van de microcirculatie bij sepsis. Dit houdt in dat er gebieden gevonden werden met een erg slechte microcirculatie in de onmiddellijke nabijheid van gebieden met een normale of zelfs hyperdynamische microcirculatie. Ook werd consequent gevonden dat systemische hemodynamische parameters niet noodzakelijkerwijs de microcirculatie weerspiegelden.

These

Zoals hierboven vermeld beperkte de focus op systemische hemodynamische parameters zich in de dagelijkse klinische praktijk lang niet alleen tot sepsis. Sterker nog, deze parameters vormden de basis waarop vele dagelijkse klinische beslissingen bij bijna alle patiënten in de hele wereld gemaakt werden in de perioperatieve en intensive care geneeskunde.

Maar als de discrepantie tussen systemische hemodynamische parameters en de microcirculatie alsook haar heterogeniteit zoals die gevonden is bij sepsis ook van toepassing zou zijn voor niet-septische patiënten in de perioperatieve en intensive care geneeskunde, zou het kunnen dat de clinici van vandaag soms de verkeerde parameters in de gaten houden en proberen te optimaliseren. Dit leek zelfs niet zo onwaarschijnlijk, omdat kritisch zieke niet-septische patiënten, zoals na een grote operatie of in shock, vaak lijden aan het zogenaamde
systemic inflammatory response syndrome, dat mogelijk dezelfde consequenties heeft voor de microcirculatie als sepsis.

Daarom was de hypothese van dit proefschrift dat de discrepantie tussen systemische en microcirculatoire parameters ook bestaat in niet-septische patiënten binnen de perioperatieve en intensive care geneeskunde. Om dit te bewijzen, werd een groot aantal studies uitgevoerd. Sommige daarvan vormen het hart van dit proefschrift.

**Bewijs van de these**


Dit hoofdstuk besprak daarnaast de verschillende beschikbare methodes voor het bewaken van de microcirculatie. Het introduceerde OPS en SDF imaging, technieken die het mogelijk hebben gemaakt om de microcirculatie aan het bed in beeld te brengen. Tenslotte gaf dit hoofdstuk een overzicht van mogelijkheden om de microcirculatie te behandelen. Hiertoe behoren vasodilatatie, volumetherapie en bloedtransfusie, onderdrukking van inducible stikstofmonoxide synthetase en geactiveerd proteïne C. Het is belangrijk om te onthouden dat normale of verbeterende systemische hemodynamische parameters niet betekenen dat er geen microvasculaire dysfunctie, multi-orgaanfalen en mortaliteit optreedt. Daarom is de microcirculatie wellicht het langzogehete eindpunt van de behandeling van sepsis en septische shock.

Bijna 40 jaar geleden stelden Weil en Shubin een nieuwe indeling van shock voor. Ze onderscheidden hypovolemische, cardiogene, obstructieve en distributieve vormen. De eerste drie hebben gemeen dat ze geassocieerd zijn met een vermindering van het hartminuutvo-
lume. Distributieve shock, zoals die bijvoorbeeld bij sepsis voorkomt, is echter geassocieerd met een abnormale verdeling van een op zichzelf normaal of verhoogd hartminuutvolume richting de microcirculatie waarbij de stofwisseling in het gedrang komt. In hoofdstuk 2 werd uitgelegd wat de laatste inzichten zijn over distributieve shock. Haar pathofysiologie laat zich het beste beschrijven als het zogenaamde microcirculatory and mitochondrial distress syndrome (MMDS). Hierbij vormen tijd en behandeling een integraal onderdeel van de definitie.

De klinische introductie van nieuwe technieken om de microcirculatie in beeld te brengen, zoals OPS en SDF imaging hebben het bewaken van de microcirculatie naar het bed van de patiënt gebracht. Beelden van de microcirculatie onder de tong tijdens sepsis en septische shock en haar behandeling hebben inzichtelijk gemaakt dat distributiedefecten op capillair niveau optreden. In dit hoofdstuk werden de verschillende soorten heterogene bloedstroom patronen die zichtbaar zijn in verschillende types distributieve shock onderzocht. Dit leverde een classificatiesysteem op dat bestaat uit vijf categorieën om deze verschillende types te definiëren. Daarnaast werd duidelijk dat distributieve shock niet alleen bij sepsis en septische shock voorkomt, maar ook bij vele andere ziektes. Waarschijnlijk zijn verschillende mechanismen verantwoordelijk voor de verschillende geobserveerde vormen van abnormale microcirculatie. Hierin hebben zowel de ziekte zelf als de toegepaste therapie een rol. Functioneel leiden ze echter allen tot een distributief defect dat leidt tot shunting in de microcirculatie en regionale dysoxie. Het is te hopen dat het voorgestelde classificatiesysteem zal helpen om de mechanismes die deze afwijkingen veroorzaken te ontrafelen en zal bijdragen aan het ontwikkelen van geschikte vormen van therapie om de verschillende soorten distributieve shock te behandelen.

Het is erg belangrijk om de analyse van beelden van de microcirculatie zoals die worden gemaakt met OPS en SDF imaging te standaardiseren. Dit maakt vergelijkingen tussen verschillende studies gemakkelijker. Het is daarom te prijzen dat er in 2007 een rondetafel bijeenkomst is gehouden. Deze voorgestelde methode is erg rigoureus en kost daardoor veel tijd. In hoofdstuk 3 werd daarom een kleine wijziging voorgesteld die de procedure aanzienlijk kan versnellen zonder af te doen aan de robuustheid van de aanbevolen methode. De voordelen van een gestandaardiseerde analyse werden nog eens benadrukt in hoofdstuk 4.

De hoofdstukken 5 tot en met 10 bevatten twee casuïstische mededelingen en vier originele artikelen. Deze vormden het hart van dit proefschrift.

In een patiënt met chronische myeloïde leukemie werd SDF imaging toegepast. Deze patiënt had een leukocytengetal van 398,000/mL en werd verdacht van leukostase. Hoofdstuk 5 liet zien dat er sprake was van abnormaal grote lege plekken tussen de rode bloedcellen. Daarnaast waren er gebieden met een hyperdynamische microcirculatie te zien in de onmiddellijke nabijheid van gebieden met opvallend gestagneerde bloedstroom en uitval van capillairen. Na behandeling was de microcirculatie weer normaal.

Ketanserin is een serotonine en α-1 adrenoceptor antagonist die in sommige landen wordt
gebruikt om hoge bloeddruk na extracorporele circulatie te behandelen. Dit zou microvasculaire perfusie kunnen belemmeren maar het is ook mogelijk dat deze juist behouden blijft of zelfs verbetert door een redistributie van de bloedstroom. In hoofdstuk 6 werd SDF imaging gebruikt om de microcirculatie onder de tong in beeld te brengen in deze setting. Beademde patiënten die onmiddellijk na het ondergaan van extracorporele circulatie een hoge bloeddruk hadden, kregen een intraveneuze bolus van 0.15 mg/kg ketanserin toegediend. Vijf minuten vóór en 10 minuten na de toediening werden de systemische hemodynamische parameters gemeten. Tevens werden videoclips opgenomen van de microcirculatie. Deze werden geanalyseerd om onder andere de perfused vessel density (PVD) en microcirculatory flow index (MFI) te bepalen. Na de toediening van ketanserin was er een significante daling van de systolische bloeddruk. Op het niveau van de microcirculatie veranderde de MFI niet significant voor kleinere en grotere vaten van de microcirculatie, waarbij als afkappunt een diameter van 20 µm werd aangehouden. Er was wel een significante toename van de PVD in de grotere vaten, maar niet in de kleinere. SDF imaging toonde dus een duidelijke discrepantie tussen systemische en microvasculaire hemodynamische parameters. Ketanserin verlaagde effectief de arteriële bloeddruk, maar de capillaire perfusie bleef op hetzelfde niveau. Beide effecten zouden kunnen worden verklaard door een toename in shunting richting de grotere vaten van de microcirculatie.

Het gedrag van de microcirculatie rondom reanimatie bij mensen was onbekend hoewel het in diermodellen al uitgebreid was onderzocht. Hoofdstuk 7 deed verslag van het allereerste gebruik van SDF imaging om de microcirculatie tijdens reanimatie in kaart te brengen. Het ging om een reanimatie waarbij gebruik werd gemaakt van een mechanisch borstkas compressie/decompressie systeem. Dit bleek in staat microvasculaire perfusie te bewerkstelligen. De bloedstroom in de capillairen bleef in stand, zelfs tijdens korte onderbreking van de compressies. De waardes voor de parameters die de microcirculatie beschrijven waren echter laag en ze verbeterden enorm nadat er weer spontane circulatie was verkregen. De microvasculaire perfusie bleek relatief onafhankelijk van de bloeddruk. Daarom zou het zo kunnen zijn dat het bewaken van de microcirculatie nuttig is om de effectiviteit van reanimaties te bewaken.

Het gedrag van de microcirculatie rondom circulatiestilstand werd verder onderzocht in hoofdstuk 8. Experimenten in diermodellen hadden bij herhaling duidelijk gemaakt dat systemische hemodynamische parameters niet noodzakelijkerwijs een afspiegeling zijn van microvasculaire perfusie. Verder was het onbekend hoe lang het duurde voordat de microcirculatie volledig tot stilstand komt nadat het hart stopt met pompen. Schattingen varieerden van 50 seconden tot 5 minuten, maar er was geen data voorhanden in de mens. Chirurgie aan de aortaboog maakt een diep hypotherme circulatiestilstand in combinatie met selectieve antegrade cerebrale perfusie noodzakelijk. Om licht te werpen op het gedrag van de microcirculatie rondom het stoppen van de bloedomloop in de mens, werd SDF imaging toegepast in deze setting. Zeven patiënten die electieve chirurgie ondernamen aan de aortaboog werden geïncludeerd. Er werden opnames van de microcirculatie onder de tong gemaakt onmiddellijk vóór en tijdens circulatiestilstand en onmiddellijk na het starten van selectieve antegrade cerebrale perfusie. Na circulatiestilstand was er geen equilibratie.
tussen de arteriële en veneuze bloeddruk op het moment dat selectieve antegrade cerebrale perfusie werd gestart na gemiddeld 59 seconden. De bloedstroom in de kleinste vaten van de microcirculatie kwam echter tot stilstand na gemiddeld 45 seconden na circulatiestilstand. De bloedstroom in de grotere vaten van de microcirculatie ging echter nog door. Selectieve antegrade cerebrale perfusie bracht de microcirculatie weer op het niveau van vóór circulatiestilstand.

Vaak worden de mogelijke voordelen van pulsatiele perfusie tijdens het gebruik van de hartlongmachine toegeschreven aan een verbetering in de microvasculaire bloeddroom. Maar hiervoor was bij mensen geen bewijs. In hoofdstuk 9 werd gekeken of pulsatiele perfusie de menselijke microvasculaire bloeddroom daadwerkelijk beïnvloedt. Hiertoe werden in een prospectief, gerandomiseerd cross-over onderzoek 16 patiënten geïncludeerd die een hartoperatie ondergingen waarbij gebruik werd gemaakt van een standaard hartlongmachine. Alle patiënten werden zowel pulsatiel als niet pulsatiel geperfundeerd in willekeurige volgorde. SDF imaging werd gebruikt om videoclips van de microcirculatie onder de tong te nemen. De perfusie werd opgestart in de pulsatiele (n=8) of de non-pulsatiele variant. Na 10 minuten werd de microcirculatie in beeld gebracht. Daarna werd de perfusiemodus veranderd en werden opnieuw opnames van de microcirculatie gemaakt. Pulsatiele perfusie genereert zogenaamde surplus hemodynamic energy. Deze werd gekwantificeerd door de polsdruk en de zogenaamde energy equivalent pressure te berekenen. De PVD voor kleiner en grotere vaten van de microcirculatie verschilde niet tussen beide perfusievormen. Polsdruk en energy equivalent pressure waren beide hoger tijdens pulsatiele perfusie. Maar er was geen correlatie tussen deze variabelen en PVD. Pulsatiele perfusie beïnvloedt de microcirculatie dus niet in mensen die routine hartchirurgie ondergaan met gebruik van een standaard hartlongmachine.

De intra-aortale ballonpomp wordt vaak gebruikt om het falende hart mechanisch te ondersteunen. Er was bewijs dat een dergelijke ballonpomp de microcirculatie verbetert, maar haar invloed op de microcirculatie in patiënten die hersteld zijn van cardiogene shock was nog niet onderzocht. In hoofdstuk 10 werd daarom SDF imaging gebruikt om de hypothese te testen dat de microcirculatie onveranderd blijft met of zonder ballonpomp in patiënten die klinisch klaar lijken te zijn voor het verwijderen van een dergelijke pomp. Vijftien patiënten die werden behandeld met een ballonpomp werden geïncludeerd. Alle metingen werden uitgevoerd nadat de klinische beslissing was genomen dat de ballonpomp verwijderd zou kunnen worden. Gedurende maximale ondersteuning van de ballonpomp werden de systemische hemodynamische parameters gemeten inclusief veneuze oximetry. Dit werd 10 minuten na het tijdelijk stoppen van de ballonpomp herhaald. Op beide punten werden tevens videoclips van de microcirculatie onder de tong opgenomen. Het stoppen van de ballonpomp verlaagde de gemiddelde arteriële bloeddruk en verhoogde de diastolische bloeddruk. In de microcirculatie werd een significante verhoging van de PVD voor kleiner bloedvaten van de microcirculatie gevonden na het stoppen van de ballonpomp. Andere microcirculatoire parameters veranderden niet, net als de gemengd of centraal veneuze saturatie. Dit betekent mogelijk dat in deze patiënten de ballonpomp de microcirculatie hinderde.
Conclusies

De hypothese van dit proefschrift was dat de discrepantie tussen de microcirculatie en systemische hemodynamische parameters zoals die gezien wordt bij sepsis tevens bestaat in niet-septische patiënten binnen de perioperatieve en intensive care geneeskunde. De studies in dit proefschrift bewijzen deze hypothese overduidelijk. De microcirculatie werd bestudeerd in uiteenlopende klinische settings die varieerden van reanimaties tot leukemie en tijdens ondersteuning van de bloedsomloop door verschillende mechanische appara- ten. Er werden significante veranderingen gevonden in microcirculatoire hemodynamische parameters terwijl dergelijke veranderingen niet of niet in dezelfde mate optraden in de systemische hemodynamische variabelen en vice versa.

Het karakter van de discrepantie in niet septische klinische settings lijkt ook anders dan dat bij sepsis. Bij de laatste is heterogeniteit een in het oog springende eigenschap. Dit blijkt uit terugkerende observaties waarbij gebieden met een ernstig verstoorde microcirculatie in de onmiddellijke nabijheid van gebieden met een normale of hyperdynamische microcirculatie werden gezien. De discrepantie in de klinische settings die bestudeerd werden in dit proefschrift werden niet gekarakteriseerd door heterogeniteit van de microcirculatie. Maar hoewel de microcirculatie hierbij dus homoge was, bleek bij herhaling dat zij niet goed correleert met systemische hemodynamische parameters.

Uiteraard hebben de bevindingen uit de individuele studies, omdat die in verschillende settings zijn uitgevoerd, verschillende implicaties. Deze werden aan de orde gesteld in de discussie gedeeltes van de verschillende hoofdstukken. De belangrijkste boodschap is echter telkens dezelfde: de microcirculatie wordt niet noodzakelijkerwijs weerspiegeld door systemische hemodynamische parameters zoals hartfrequentie, arteriële en veneuze bloeddruk, hartminuutvolume of de zogenaamde oxygen derived parameters waaronder veneuze oximetrie.

Gevolgen voor de kliniek

Ondanks de dissociatie tussen de microcirculatie en de systemische hemodynamiek kan het nog niet aanbevolen worden om alleen de microcirculatie als basis te gebruiken voor klinische interventies. Hiervoor zijn twee belangrijke redenen.

In de eerste plaats zijn er geen klinische trials uitgevoerd die hebben onderzocht of het gebruik van de microcirculatie als basis voor klinische interventies leiden tot een verbeterde uitkomst. Zoals hieronder uiteengezet wordt, is van vele interventies bekend dat ze de microcirculatie verbeteren. Maar er is geen studie gedaan waarbij gekeken is of een verbeterde microcirculatie de patiënt beter maakt. Daarom is het nog steeds onzeker of recrutering van de microcirculatie leidt tot een verminderde mortaliteit, morbiditeit of een verbetering van orgaanfuncties.

In de tweede plaats is er overtuigend bewijs dat het optimaliseren van systemische hemodynamische variabelen wel degelijk resulteert in een verminderde morbiditeit en mortaliteit. Dit is de conclusie van de studies naar de zogenaamde goal directed therapy protocollen,
die uitgebreid zijn bestudeerd in de perioperatieve en intensive care geneeskunde.


De resultaten van de studies naar goal directed therapie zijn in overeenstemming met de theorie dat een zeker minimum aan hartminuutvolume en arteriële bloeddruk nodig zijn voor een functionerende microcirculatie. Beneden zo’n minimum kan de microcirculatie in het gedrang komen. Maar dit minimum is niet goed gedefinieerd en vertoont waarschijnlijk grote interindividuele variaties. Daarnaast kan het zo zijn dat zelfs boven zo’n minimum er dissociatie optreedt tussen systemische hemodynamische parameters en de microcirculatie. Deze concepten worden ondersteund door de resultaten van de studies in dit proefschrift.

Ook kunnen de effecten van tijd en therapie de discrepantie tussen systemische hemodynamische parameters en de microcirculatie vergroten. Ook hierbij zijn grote interindividuele variaties mogelijk. Als voorbeeld kunnen twee recente studies worden genomen waarin vasopressoren werden gebruikt als circulatietherapie. Bij beide werd noradrenaline gebruikt om bij septische patiënten een bepaald artereel bloeddrukniveau te bereiken. Beide toonden aan dat een verschillend bloeddrukniveau in het algemeen geen invloed had op de microcirculatie. Maar één van de studies toonde aan dat de reactie op steeds hogere doseringen noradrenaline sterk verschilde tussen individuen. Patiënten die vóór de studie begon een slechte microcirculatie hadden, toonden behoorlijke verbeteringen na noradrenaline. Maar patiënten die reeds een normale microcirculatie hadden, toonden juist een verslechtering.

Daarom ligt het voor de hand dat het vervolgen van de microcirculatie in verschillende klinische settings binnen de perioperatieve en intensive care geneeskunde een belangrijk gereedschap wordt in het hemodynamisch bewakingsarsenaal van de clinicus.

**Toekomstverkenning**

Dit proefschrift heeft onderstreep dat de discrepantie tussen systemische hemodynamische parameters en de microcirculatie zoals die wordt gezien in sepsis ook optreedt in veel verschillende klinische settings in de perioperatieve en intensive care geneeskunde. De opkomst van beeldvormende technieken zoals OPS- en SDF imaging heeft ervoor gezorgd dat de microcirculatie een stevige plek heeft gekregen in het gedachtoegend van de anesthesioloog en intensivist van vandaag. Het bewaken van systemische hemodynamische parameters blijft belangrijk, maar het is te hopen dat dit proefschrift heeft bijgedragen aan de microcirculatie als klinisch concept.

De grote kracht van technieken zoals OPS- en SDF imaging ligt in het feit dat zij kunnen
differentiëren tussen de verschillende mechanismen van het falen van de microcirculatie. Dit komt gedeeltelijk tot uitdrukking in het gebruik van de verschillende scoringsystemen. Zo kan een vermindere microvascular flow index wijzen op pathologie waarbij de bloedstroom in het gedrang is terwijl een vermindere perfused capillary density kan wijzen op pathologie waarbij zuurstofdiffusie in het nauw komt. Overigens is er een grote overlap tussen beide scoringsystemen. Verder kan eventuele heterogeniteit in de microcirculatie duidelijk in beeld worden gebracht. Deze inzichten kunnen uiteindelijk leiden tot op maat gemaakte therapie gebaseerd op beeldvorming van de microcirculatie.

Van verschillende interventies is aangetoond dat zij de microcirculatie verbeteren in een verscheidenheid aan klinische settings. Onder ander zijn dit vloeistoftherapie, bloedtransfusie, inotropica, vasodilatantia, corticosteroïden, geactiveerd drotrecogine alfa, vasopressoren en extracorporele membraanoxygenatie. Behoudens vasodilatatie zijn deze strategieën echter alleen onderzocht in septische patiënten. Daarnaast hadden ze alleen de verbetering van de microcirculatie als eindpunt. Een nieuwe studie van Boerma et al., ook in septische patiënten, toonde aan dat het toedienen van nitroglycerine geen effect had op de microcirculatie en ook niet op de overleving. De bestudeerde dosis was echter laag en de microcirculatie bij de bestudeerde patiënten was al vrij goed.

Bij de laatste ontwikkelingen horen twee recente studies, een overzichtsartikel en een abstract die een direct verband aantonen tussen het optimaliseren van systemische hemodynamische parameters en de microcirculatie. Misschien betekent dit een stap voorwaarts omdat hiermee het sturen van therapie op de microcirculatie verschuift van een concept dat gebaseerd is op fysiologie naar een concept dat gebaseerd is op bewijs.

De ware uitdaging blijft echter nog altijd om te bewijzen dat therapeutische interventies gebaseerd op het verbeteren of normaliseren van de microcirculatie zorgen voor een betere overleving in de perioperatieve en intensive care geneeskunde. Het goede nieuws is dat sommige obstakels die onderzoek hiernaar belemmerden, nu verdwenen zijn. Zo zijn de methodes om de microcirculatie te kunnen scoren nu uitgebreid gevalideerd en recent nog werd er een goede correlatie tussen de microcirculatie onder de tong en die van de darm gevonden. Verder zijn er veelbelovende ontwikkelingen in de automatisering van de analyse van de microcirculatie, hetgeen studies aanzienlijk zou kunnen versnellen. Een trial met mortaliteit als eindpunt vergt de inclusie van een groot aantal patiënten. Dat is een intimiderende uitdaging, maar wel de enige weg vooruit.
Dankwoord
De perioperatieve en intensive care geneeskunde is erg breed. Daarom past nederigheid ten aanzien van de studies in dit proefschrift. Het zijn slechts kleine, maar wellicht waardevolle, bijdrages aan het voortdurend uitdijende medische kennisgebied. Nederigheid past echter niet ten aanzien van de vele personen die op verschillende manieren hebben bijgedragen aan de totstandkoming van dit proefschrift. Dat komt vooral doordat de onderzoekslijn die daartoe heeft geleid van de grond af aan is opgebouwd tijdens de drukke dagelijkse klinische bezigheden in een topklinisch perifeer opleidingsziekenhuis. Zonder deze mensen was dit onmogelijk geweest en daarom wil ik hen hieronder heel hartelijk danken!


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List of Publications and Curriculum Vitae
Publications related to this thesis


Other publications


Curriculum Vitae

Paul Willem Gerhard Elbers was born on March 18, 1975 in IJsselstein, The Netherlands. He attended pre-university education at Gymnasium Juvenaat H Hart in Bergen op Zoom. Following graduation, he briefly studied Technical Business Administration at Universiteit Twente, Enschede. After this he spent a year working in Paris, France.

In 1994, he started his medical education at Universiteit van Amsterdam. It was here where he first met Prof. dr. ir. Can Ince. This led to a long lasting collaboration. Its results include pioneering research on microvascular imaging, various symposia on Microcirculation and Mitochondrial Dysfunction in Intensive Care Medicine and of course the studies on the human microcirculation that have resulted in this thesis.

During his medical studies, he developed a career in computer science. For this, he spent one year in Dublin, Ireland working at Gateway Computers. Another year was spent in The Hague. There he was appointed team leader to work on the millennium problem at the Ministry of Health of The Netherlands.

He obtained his M.Sc. in Medicine in 2001. During his clinical rotations, he was employed as a research associate at the department of Anesthesiology (head: Prof. dr. Misa Dzoljic) to investigate ischemic spinal cord ischemia. After obtaining his medical degree in 2003 he briefly worked as an intensive care physician at Meander Medisch Centrum, Amersfoort.

In 2004, he started training in Anesthesiology at St. Antonius Hospital, Nieuwegein (head: Dr. Eric PA van Dongen). Besides clinical work he participated in implementing a Patient Data Management System in this hospital. In addition, the studies on the human microcirculation described in this thesis were conducted during this residency. In 2009 he was registered as anesthesiologist with special focus on cardiac anesthesiology.

During his medical career, he also developed a keen interest in acid-base physiology, especially using the quantitative approach developed by the late Peter A Stewart. This led to the development of the web site www.acidbase.org, which hosts Stewart’s classic text. In 2009, together with Prof. John A Kellum (University of Pittsburgh, PA, USA) he published Stewart’s Textbook of Acid-Base, which helped revive clinical quantitative acid-base medicine.

Large parts of this thesis were written on the island of Curacao, Netherlands Antilles where he resided intermittently after his residency in Anesthesiology. This was alternated with a position as Anesthesiologist at Onze Lieve Vrouwe Gasthuis, Amsterdam (head: Dr. Jasper A Kal).

At the end of 2009, he started training in Intensive Care Medicine at Onze Lieve Vrouwe Gasthuis, Amsterdam (heads: Prof. dr. Durk Zandstra and Dr. Peter van der Voort). In 2010, he was registered as anesthesiologist-intensivist. In 2011, he will take up a position as cardiac anesthesiologist and intensivist at Medisch Centrum Leeuwarden.