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 focussed 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, focussing 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
of which form the core of this publication.

**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 <20 µm, 2.79 [interquartile range, 1.38-3] to 2.38
[Summary and Conclusions]

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. 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 microcirculation 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 (ScvO$_2$/SvO$_2$) 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 macro-hemodynamic 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. For
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.

References

28. Boerma EC, Koopmans M, Konijn A et al. Effects of nitroglycerin on sublingual microcircula-


