THE ADDITIONAL VALUE OF MICROCIRCULATORY IMAGING IN CRITICALLY ILL PATIENTS AND ITS RELATION TO SYSTEMIC HEMODYNAMICS

Eva Klijn

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THE ADDITIONAL VALUE OF MICROCIRCULATORY IMAGING IN CRITICALLY ILL PATIENTS AND ITS RELATION TO SYSTEMIC HEMODYNAMICS

De waarde van monitoren van de microcirculatie bij ernstig zieke patiënten en de relatie met de systemische hemodynamica

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"Hoezo compromis, heb ik het mis dan?"
Loesje

Voor mijn ouders,
omdat ik altijd moest
afmaken waar ik aan
begonnen was.

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PART A
INTRODUCTION

1 GENERAL INTRODUCTION AND OUTLINE OF THIS THESIS

Based on: Dynamische hemodynamische en microcirculatoire monitoring bij sepsis
A&I Jaargang 6,nummer 3, September 2014

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In recent years the understanding and acceptance of the importance of the microcirculation as a vital system in the pathogenesis of a variety of diseases has begun. Most, and probably all physiological or pathological processes are influenced by the microcirculation, the smallest functional unit of the cardiovascular system. The microcirculation is where interaction between blood and tissues provides the necessary environment for adequate cell function. With increasing technological advances clinically relevant methods have been developed to provide real time information about oxygenation and perfusion of the microcirculatory system at bedside. This has largely increased the study and use of microcirculatory (patho)physiology in several fields of medicine, making the microcirculation relevant to the practicing physician.

MICROCIRCULATORY MONITORING

The microcirculation plays a fundamental role in gas and nutrient exchange: it must constantly adapt by controlling vascular tone.[1] The microvasculature consist of arterioles, capillaries and postcapillary venules all smaller than 100 microns. The arterioles are the major determinant of vascular resistance. Blood flow depends on a pressure gradient along the vascular tree, as well as the amount and distribution of resistance across the microvasculature bed. By alternate contraction and relaxation of the smooth muscle cells in arterioles blood flow is regulated. The capillary walls consist of a single layer of endothelium and basement membrane which is the principal site for gas and nutrients exchange between blood and tissue. Changes in systemic blood pressure leads to a corresponding change in microcirculatory flow, which is than compensated for by local readjustment. These changes ensure that the microcirculatory blood flow is adequate to meet the oxygen requirements of the cells, a term called autoregulation. The microvasculature characteristics of each organ system are closely related to the functional role played by a the organ as a whole. The number of capillaries per unit mass of organ or tissue (capillary density) may be related to the organs metabolic requirements (muscle, heart brain) or other functional requirements (skin, intestinal, mucosa, kidney).[2] Although the microvascular beds of most organs and tissues clearly have both metabolic and functional components, one or the other usually predominates. In this way morphology of each microcirculatory bed is designed to fit the function and oxygen requirements of each organ.

TECHNIQUES TO MONITOR THE MICROCIRCULATION

Several techniques are available to monitor the microcirculation. They can be divided in techniques which reflect microcirculatory perfusion, techniques which measure tissue oxygenation and techniques which reflect vasomotor tone. When choosing a method for study of a specific organ and pathological process it is important to consider the different properties of the methods.

Laser Doppler flowmetry

Laser Doppler flowmetry is a non-invasive, continuous measure of microcirculatory blood flow. The principle of this method is to measure the Doppler shift – the frequency change that light undergoes when reflected by moving objects, such as red blood cells. Laser Doppler

flowmetry (LDF) utilizes the Doppler shift to give a value of the mean erythrocyte flux found in the investigated tissue.[3] The main limitation of this technique is that it measures flow in a variable volume of tissue and it is unable to detect it in individual vessels.

Laser Speckle Imaging

Laser Speckle Imaging (LSI) is a non-invasive non-contact method which can be used to evaluate blood flow in the microcirculation. The full field laser technique is used to provide real-time images of blood flow. When an area illuminated by laser light is imaged onto a camera, a granular or speckle pattern is produced. If the scattering particles are in motion, a time varying speckle pattern is generated at each pixel in the image. The temporal and spatial intensity variations of this pattern contain information about the motion of the scattering particles and quantitative flow information can be obtained by measuring the spatial characteristics of the intensity fluctuations.[4-6] Using this approach, two dimensional maps of blood flow can be obtained with very high spatial and temporal resolution by imaging the speckle onto a CCD camera and quantifying the spatial blurring of the speckle pattern that results from blood flow. In areas of increased blood flow, the intensity fluctuations of the speckle pattern are more rapid, and when integrated over the CCD camera exposure time, the speckle pattern becomes blurred in these areas. Therefore low contrast is related to high flow, high contrast to low flow. The contrast image is processed to produce a color-coded image that correlates with blood flow in the tissue.

Thermographic imaging

In thermography, two-dimensional maps of radiation differences are constructed of regional temperature distributions across the skin and are used as a direct measure of blood perfusion.[7-9] The relationship between blood flow and skin surface temperature has been studied, and a strong correlation was found.[10,11] A thermographic (infrared) camera measures the emitted thermal radiation of a surface and translates this measurement into a matrix of temperatures of this surface. One of the major advantages of the thermal infra-red measurement is that the sensor does not make contact with the skin.

Sidestream dark field imaging

SDF imaging illuminates tissue stroboscopic with polarized green light and measures the reflected light from the tissue surface after filtering out the polarized portion of the reflected light.[12] Green light is absorbed by hemoglobin in red blood cells, thereby tracking the movement of the red blood cells (independently of the oxygenation state), which appear as dark moving globules in the microcirculation. The vascular wall cannot be visualized so that vessels can only be detected if they contain red blood cells. These techniques can only be used on organs covered by a thin epithelial layer. The technique provides real time images of vascular perfusion, heterogeneity of perfusion and microvascular perfusion, however the analyses is done in a semi-quantitative manner. The limitations are secretions, movement artefacts and pressure artefacts which comprise image quality.

Peripheral perfusion index

The peripheral perfusion index (PPI), is derived from the photoelectric plethysmographic signal of the pulse oximeter. The PPI is calculated as the ratio between the amplitude of the pulsatile component (arterial compartment) and the non-pulsatile component (venous and capillary blood, and other tissue) of the light reaching the detector of the pulse oximeter.[13,14] This ratio is independent of the hemoglobin oxygen saturation. Because a change in peripheral vasomotor tone primarily causes a corresponding change in the pulsatile component of the signal, the ratio changes accordingly. As a result, the PPI value reflects changes in peripheral vasomotor tone.

Forearm-to-fingertip skin-temperature difference

Forearm-to-fingertip skin-temperature difference ($T_{skin\text{-}diff}$) is measured with two skin probes attached to the index finger and on the radial side of the forearm, midway between the elbow and the wrist.[10,11,13] This temperature gradient can better reflect changes in cutaneous blood flow than the absolute skin temperature itself and increases during vasoconstriction. Basically, when vasoconstriction decreases fingertip blood flow, finger skin temperature decreases, and $T_{skin\text{-}diff}$ increases. In the presence of a constant environmental temperature a change in the skin temperature is the result of a change in skin blood flow.

Reflectance spectroscopy

Reflectance spectrophotometry measures microvascular oxygen hemoglobin saturation (***μ***HbO2) and relative hemoglobin concentration (rHb) in the superficial tissue layers, such as the skin and mucus membranes.[15] Light generated with a rapidly rotating disc at 64 different wavelengths in the range of 502-628 nm is directed to a microlight guide to the tissues. The use of different wavelengths allows oxygen saturation measurements due to light absorption by oxy- and deoxyhemoglobin. The resolution is very sharp, but the depth of the tissue sampled is large, making the sampling volume not so small. Reporting a mean ***μ***HbO2 value gives no information on heterogeneity.

Near infrared spectroscopy

Near-infrared spectroscopy is a technique that uses near infrared light to measure chromophores (oxy- and deoxyhemoglobin, myoglobin and cytochrome AA3) in tissues.[13,16] The fractions of oxy and deoxyhemoglobin are used to calculate tissue oxygen saturation. Total light absorption is used to compute total tissue hemoglobin and the absolute tissue hemoglobin index, which are two indicators of blood volume in the region of microvasculature sensed by the probe. Because most of the blood in this volume is venous it mostly reflects local venous Hb oxygen saturation and therefore does not reflect heterogeneity.

MICROCIRCULATION AND CRITICAL ILLNESS

Optimizing the systemic circulation is the cornerstone of treatment of shock. The aim of hemodynamic resuscitation is to correct and avoid tissue hypoxia.[17] In clinical practice this means optimizing tissue perfusion (microcirculation) because this is ultimately responsible for providing oxygen to the tissues. Because circulatory shock is defined by the inability of the circulatory system to maintain the metabolic demand of the tissue, every form of shock will initially result in tissue hypoperfusion. Until recently, assessing microcirculatory blood flow was difficult, therefore surrogate global markers were used. This makes the microcirculation a black box, whose physiological activities at best could be estimated from more easily accessible upstream and downstream parameters.[18] In patients with severe sepsis and septic shock the traditional used parameters lack sensitivity. This lack of sensitivity is primarily due to the distributive defect associated with septic shock, in which a defect in the distribution of normal cardiac output (or even increased cardiac output) results in regional hypoxia that is not detected by conventional hemodynamic monitoring of the systemic circulation. This occult hypoxia highlights the necessity of finding new endpoints for the treatment of severe sepsis and septic shock.

Although protocolled hemodynamic management has advantages, an alternative approach is more individually titrated hemodynamic therapy. With the use of macrocirculatory as well as microcirculatory parameters, this could be tailored to meet the specific individual needs of the patient.

MICROCIRCULATORY ABNORMALITIES DURING SEPSIS

The microcirculation during sepsis is characterized by a decreased capillary density and an increased heterogeneity. De Backer *et al* demonstrated in their landmark study that patients with sepsis and septic shock had a decreased capillary density, a decreased proportion of perfused vessels and an increase in heterogeneity, compared to healthy volunteers and non-septic critically ill patients.[19] Additionally the severity of microcirculatory perfusion abnormalities were higher in non-survivors compared to survivors. During sepsis, time seems to be an important factor, as several studies demonstrated microcirculatory abnormalities in the early course of sepsis.[20,21] Improving the microcirculatory perfusion in this phase of illness was most associated with survival, compared to other hemodynamic parameters.[22]

RELATION BETWEEN MICROCIRCULATION AND SYSTEMIC CIRCULATION

Although therapy which optimizes systemic hemodynamic parameters has the goal to improve tissue perfusion and oxygenation, the relation between the two is complex. With adequate autoregulatory properties, like during hypodynamic shock, improving systemic hemodynamics will automatically lead to recovery of microcirculatory perfusion. With these types of shock, the microcirculatory disturbances are caused by a cascade of physiological compensatory mechanisms by activation of baroreceptors to guarantee adequate blood flow to vital organs. In contrast to the hyperdynamic

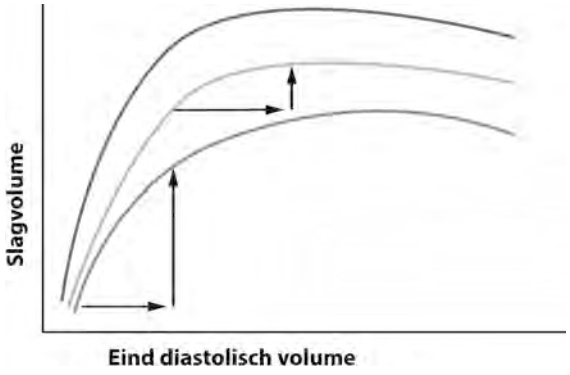
septic shock, where due to loss of autoregulatory properties, there can be persisting microcirculatory abnormalities despite adequate systemic hemodynamic resuscitation. In septic shock microcirculatory abnormalities will not only be the result of compensatory mechanisms but also by inflammatory processes. Multiple factors will be a part of this dysregulation, like endothelial dysfunction, increased leukocyte adhesion, formation of microthrombi and rheological disturbances.[23]

Although it could be expected that there is a critical point were microcirculatory perfusion will be dependent of mean arterial pressure and cardiac output, several studies have demonstrated that in critically ill septic patients microcirculatory perfusion can strongly vary within the generally accepted ranges for cardiac output and mean arterial pressure. This is demonstrated by studies investigating the effect of fluids and vaso-active substances on microcirculatory perfusion.[24-27]

MONITORING OF FLUID RESPONSIVENESS

One of the first therapies to restore cardiac output and tissue perfusion is the administration of fluids. When in the later phases of disease additional fluid is administered, this could result in a positive total fluid balance. However studies show that only half of the patients admitted to the intensive care increase their cardiac output or stroke volume following a bolus of fluids (i.e. are fluid responsive).[28] This is important because there is increasing evidence that a positive total fluid balance, corrected for the severity of disease, is associated with an increased mortality.[29,30] It seems rational that testing fluid responsiveness is useful in critically ill patients. Therefore dynamic hemodynamic monitoring focusses mainly on determining fluid responsiveness.

Ideally the administration of fluid will increase venous return to the heart, subsequently increasing end diastolic volume (EDV) and stroke volume (SV). However the administration of fluid does not necessarily increase stroke volume. This is described in the Frank-Starling curve (figure 1). This curve describes the relationship between the EDV (on the x-axis) and SV (on the y-axis). The shape of the curve is intrinsically determined by the intrinsic properties of the heart, which can vary between patients and in time. Myocardial dysfunction during sepsis, for instance, can change the shape of this curve.

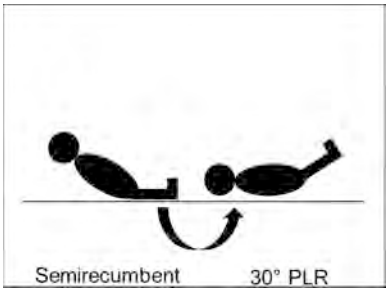


Figuur 1. Frank-Starling curve.

Traditionally central venous pressure (CVP) and pulmonary artery occlusion pressure (PAOP) are used to estimate preload. However several studies have demonstrated that these parameters as well as the change in these parameters following a bolus of fluid have a limited value in estimating fluid responsiveness.[31,32] The predictive value of right and left ventricle volume is also limited. This is probably because fluid responsiveness is not dependent on absolute values but the location of that value on the Frank-Starling curve, for that specific patient on that specific moment.

An alternative for the use of these static parameters is the use of endogenic preload increase by performing a passive leg raising test (PLR). The PLR is a postural change were the legs are lifted passively from the horizontal plane in a lying or semirecumbent subject (figure 2). This induces a gravitational transfer of blood from the lower part of the body to the central volume, increasing the preload. If the patient is fluid responsive, this postural change will increase stroke volume.[33-35]

Fluid responsiveness only indicates that the patient is on the steep part of the Frank-Starling curve, but this does not necessarily imply that the patient needs fluid. Additional parameters (e.g. microcirculatory or tissue perfusion parameters) are needed to determine whether the patient will benefit from fluid administration.



Figuur 2. Passive leg raising test.

AIM AND OUTLINE OF THE THESIS

The aim of this thesis was to investigate whether measuring microcirculatory perfusion is of value in several critical illnesses.

PART A presents two review articles, which comprehensively highlight the importance of the microcirculation in critical diseases. **Chapter 2A** focusses mainly on the anatomy and function of several microvascular beds and describes disturbances found in the microcirculation during sepsis. **Chapter 2B** describes several methods available to monitor the microcirculation and the disturbances found in shock of various etiologies.

In **PART B** we studied a relatively new method – laser speckle imaging (LSI) – to measure microcirculatory blood flow. In **chapter 3** laser speckle imaging is compared to sidestream dark field (SDF) imaging with the aim to validate LSI for assessing microvascular perfusion. For this purpose, LSI and SDF measurements were performed on the human nail fold during gradual occlusion of the

upperarm circulation to modify nail fold perfusion under controlled circumstances. To determine the ability of LSI to detect rapid changes in tissue perfusion during reactive hyperemia a VOT was performed. Additionally, a hyperthermic challenge was applied to measure tissue perfusion using LSI under conditions of maximal functional capillary density. **Chapter 4** describes the results of an experimental study on the effect of perfusion pressure in a pig model for gastric reconstruction. We hypothesized that increasing mean arterial pressure would improve blood flow, measured with LSI and video thermography, at the anastomotic site of the gastric tube. In **chapter 5** we present a study aimed to investigate whether LSI is suitable for mapping functional brain areas during neurosurgical tumor removal procedures. For this purpose, LSI measurements were performed on brain tissue in patients undergoing awake neurosurgery. To determine the ability of LSI to detect local changes in cortical microcirculatory perfusion, patients were asked to perform a motor task, and the results were compared with standard electrocortical stimulation mapping data.

PART C focusses on several microcirculatory parameters in relation to volume and/or preload status. In **chapter 6** we hypothesized that the site and type of microvascular perfusion response to a cardiac preload challenge depends on sympathetic tone alterations.. Therefore we focused on microvascular effects of increasing preload with and without altering sympathetic activity in healthy volunteers. We aimed to investigate which of the microvascular parameters can be used to truly reflect a preload challenge and resultant increase in forward flow, so called fluid responsiveness, by studying effects of two postural changes known to establish a preload challenge but with different effects on the autonomic nervous system. We used the sublingual and cutaneous microvascular beds because these are the most easily accessible and most studied microvascular beds in critically ill patients. **Chapter 7** describes the effect of cardiac output resuscitation on different microvascular perfusion and peripheral perfusion variables during different endotoxemic and obstructive shock states. We hypothesized that only in endotoxemic shock, microvascular and peripheral perfusion abnormalities would persist after the resuscitation of systemic hemodynamics to preshock levels and that these abnormalities would be remedied with additional resuscitation of cardiac output in a different magnitude for the different peripheral tissues and the different regional perfusion variables. In **chapter 8** we studied whether tissue perfusion and oxygenation is able to predict and monitor fluid responsiveness, in critically ill, septic patients considered hypovolaemic on clinical grounds after initial resuscitation. Therefore, critically ill, septic patients underwent infusion of 250 mL of colloids, after initial fluid resuscitation. Prior to and after fluid infusion, systemic hemodynamics, sublingual microcirculatory perfusion and skin perfusion and oxygenation were measured. In **Chapter 9** we investigated whether non-invasive peripheral perfusion indicators prior to and during fluid withdrawal are able to predict and monitor central hypovolemia and subsequent hypotension during progressive fluid withdrawal by CVVH in critically ill patients with AKI. Critically ill AKI patients were subjected to progressive fluid withdrawal. Systemic hemodynamics and peripheral perfusion index by pulse oximetry, forearm-to-fingertip skin temperature gradient and tissue oxygen saturation were measured.

In **part D** (**Chapter 10** and **11**) we discuss the main results of each chapter, and summarize the most important findings of this thesis.

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2a

THE HETEROGENEITY OF THE MICROCIRCULATION IN CRITICAL ILLNESS

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INTRODUCTION

In recent years, interest in the role of microcirculation in critical illness has grown. This seems logical because microcirculation is ultimately responsible for providing oxygen to the tissues. Microcirculation is a complex network, with special morphologic features tailored for the specific function of the tissues it supplies. Recently, new imaging techniques and clinical investigations have identified microcirculation as a pivotal element in the pathogenesis of sepsis.

Although major progress has been made in the treatment of sepsis, mortality and morbidity rates associated with sepsis remain high.[1,2] Hemodynamic treatment is primarily aimed at correcting global hemodynamic and oxygen-derived variables.[2] Despite aggressive correction of global parameters with volume resuscitation and vasoactive agents, some patients progress into multi-organ failure and die.[3,4] The ultimate aim of resuscitation is to correct and avoid tissue hypoxia. However, the end points used to evaluate the achievements of resuscitation therapy (e.g., lactate levels, venous oxygen saturation, and mean arterial pressure) are not sensitive enough to detect regional hypoxia. This lack of sensitivity is primarily due to the distributive defect associated with septic shock, in which a defect in the distribution of normal cardiac output (or even increased cardiac output) results in regional hypoxia that is not detected by conventional hemodynamic monitoring of systemic circulation. This occult hypoxia highlights the necessity of finding new end points for the treatment of severe sepsis and septic shock.

This review first explores the characteristic features of several microvascular beds that can be visualized at the bedside. Second, the distinctive abnormalities occurring during sepsis are discussed in relation to different types of shock. In addition, we review the response of microcirculation to available therapeutic modalities frequently used to treat septic shock patients. Although many methods exist to study microcirculation, either directly or indirectly, this review mainly focuses on the use of either orthogonal polarization spectral (OPS) imaging or sidestream dark field (SDF) imaging for direct clinical visualization of microcirculation with bedside vital microscopy.[5,6] Because the heterogeneity of microvascular alterations among different organs and within individual organs is a key characteristic of the pathophysiology of hemodynamic dysfunction in sepsis, the preferred technique for identifying these alterations is direct visualization.

THE ANATOMY AND FUNCTION OF DIFFERENT MICROCIRCULATORY BEDS

Microcirculation has evolved from being a hypothesized necessary link between the arteries and veins, in Harvey's theory of circulation, to a possible end point of resuscitation in critical illness. In 1661, Malpighi demonstrated, for the first time in vivo, long, thin-walled tubes, which he termed capillaries. Technical advances made in recent years have made it possible to directly or indirectly study microcirculation in different pathologic states. Most of the information concerning the functional morphology of microcirculation has been obtained from extensive studies involving experimental animals.

Microvasculature consists of arterioles, capillaries, and postcapillary venules, of which the arterioles are the major determinant of vascular resistance. Blood flow depends on a pressure

gradient along the vascular tree, as well as the amount and distribution of resistance across the microvasculature bed. Circular smooth muscle cells surround the arteriolar walls. By alternately contracting and relaxing, these muscle cells control microvascular flow and its distribution. In specific microcirculatory beds, such as those of the brain pericytes, surrounding arterioles can also cause constriction and regulate blood flow.[7] This vascular tone controls the diameter of the vessels because, as described by the law of Poiseuille, the resistance to flow is primarily determined by the radius. The proximal arteriole determines the total blood flow to the capillaries, whereas the terminal arterioles and precapillary sphincters control the distribution of blood within the capillaries. Capillary walls, which consist of a single layer of endothelium and basement membranes, represent the principal site of oxygen and nutrient exchange between blood and tissue. Changes in systemic blood pressure lead to a corresponding change in microcirculatory flow, which is then compensated for by local readjustments. These changes ensure that the microcirculatory blood flow is adequate to meet the oxygen requirements of the parenchymal cells, a mechanism called autoregulation.

The microvasculature characteristics of each organ system are closely related to the functional role played by the organ as a whole. The number of capillaries per unit mass of organ or tissue (capillary density) may be related to the organ's metabolic requirements (muscles, heart, brain) or to other functional requirements (skin, intestinal mucosa, kidney).[8] Oxygen transport to the tissues occurs via passive diffusion from the capillaries, as was first described by Krogh early in the 20th century.[9] The diffusion of oxygen in this manner is considered to be the main rate-limiting process in oxygen transport to tissue. Besides diffusion from the capillaries, diffusion of oxygen to the parenchymal cells also occurs from the larger vessels, such as the arterioles and venules. Although the microvascular beds of most organs and tissues clearly have both metabolic and functional components, one or the other usually predominates. In this way, the morphology of each microcirculatory bed is designed to fit the function and oxygen requirements of each organ.

Regarding critical illness, skeletal muscle has been the most extensively studied tissue in experimental studies. This is due to its easy accessibility and to the technical limitations of intravital microscopes, the main instruments used in such studies. Interest later shifted to other microvascular beds, such as those of the intestine and brain. Upon the introduction of OPS and SDF imaging, microcirculation could be observed in humans, and the location most often studied with these techniques has, to date, been the sublingual region. This is because this region is easily accessible and its vascularization is close to the brain and heart. The extent to which studies of this region can be extrapolated to other microvascular beds is uncertain. However, sublingual microcirculatory alterations do have clinical significance. The superior sensitivity of sublingual microcirculation as an indicator linking the severity of disease to systemic hemodynamic and oxygen-derived variables (demonstrated in several clinical studies) makes tissue in the sublingual region clinically relevant for detecting microcirculatory alterations.

ORTHOGONAL POLARIZATION SPECTRAL AND SIDESTREAM DARK FIELD IMAGING FOR CLINICAL MONITORING OF MICROCIRCULATION

The intravital microscope has provided much insight into the physiology and pathophysiology of microcirculation in several models of disease. However, the microscope's technical limitations have limited its impact. The main limitation of this technique is the need for transillumination of the microcirculatory bed from below for visualization. Observing the microcirculation of organ surfaces using epi-illumination causes surface reflection of the incident light, resulting in poor visualization of the underlying microcirculatory bed. Therefore, the intravital microscope has been used mainly for the study of organs that permit transillumination, such as the mesenteric, cremaster, and hind limb muscles. Fluorescence microscopy (illumination from above), in combination with the infusion of fluorescence indicator dyes, has facilitated the study of other organs. Not being able to apply these types of techniques to clinically relevant, large animal models has limited the clinical relevance in studying the microcirculation. In small-animal studies, the relation between macro and microcirculation, an essential link needed to understand the role of microcirculation in functional hemodynamics, had not been sufficiently elucidated. This limited their translational applicability to clinical scenarios. The technical limitations of transillumination were finally overcome with the introduction of the OPS imaging technique.

OPS imaging illuminates tissues with polarized green light and measures the reflected light from the tissue surface after filtering out the polarized portion of the reflected light.[6] OPS imaging thus makes it possible to visualize microcirculation without transillumination. This modality filters out the surface reflection and permits visualization of subsurface structures. Green light is absorbed by hemoglobin in red blood cells, thereby tracking the movement of red blood cells, which appear in microcirculation as moving dark globules. However, due to its inadequate imaging, the OPS technique has only limited sensitivity for studying capillary kinetics and morphology in detail. An improved optical modality to address this shortcoming was introduced with SDF imaging.[5] Covered by a disposable cap, the SDF probe is placed directly on tissue surfaces. The light from the concentrically positioned light-emitting diodes (530-nm wavelength) penetrates 1 mm into the tissue, thus illuminating the microcirculation and its components. Because hemoglobin absorbs this wavelength, erythrocytes can be clearly observed as flowing cells. Also, because there is no direct optical contact with the sensing central core of the probe, surface reflections do not interfere with image collection. Therefore, remarkably clear images of microcirculation can be captured. The resulting improved image quality allows for better automatic analysis of the images, and the low energy requirement of SDF imaging further enhances its utility by allowing battery or portable computer operation. Using these techniques, brain, sublingual, cutaneous, and conjunctival microcirculation have been studied during surgery and intensive care.

CLINICAL EVALUATION OF MICROCIRCULATION DURING SURGERYAND INTENSIVE CARE

Both OPS and SDF imaging can be used to investigate the microcirculation of organ surfaces. OPS imaging has been used to visualize human brain microcirculation during neurosurgery.[6] Given its embryologic association with the gastrointestinal tract and its easy accessibility, sublingual microcirculation has been extensively studied, especially in intensive care medicine. In addition, these techniques have been applied to the study of microcirculation in the nail fold.[10,11] From a historic perspective, however, the study of the human brain during surgery represents a new, uncharted area of clinical research.[6] Figs. 1–4 illustrate several microcirculatory beds described below, as well as their corresponding microcirculatory images, acquired using OPS and SDF imaging.

Brain Microcirculation

The microvascular bed of the cerebral cortex consists of a dense, highly interconnected network that arises from pial arteries. Irregular tortuous capillaries characterize this cerebrocortical capillary bed, and these capillaries are supplied by branches of cortical penetrating arteries, which cannot be visualized in vivo.[12] The arteriolar system is composed of three main vasculature zones: the pial, cortical, and subcortical vascular networks. When the brain surface is examined with intravital microscopy, the capillaries appear to have no preferential orientation or characteristic length. Also, the capillaries cannot usually be traced back to their pial arteries. On the brain surface, the arterial microanastomoses characterize the pial microarterial network.[13] The capillaries drain into postcapillary venules that give rise to the pial veins. It is still unclear whether arteriovenous anastomoses are present in the cerebral microvasculature. Capillary density in the brain is related to the average metabolic rate at steady state.[14] In addition to the classical factors that control microcirculatory blood flow (myogenic, metabolic, and neurohumoral mechanisms), evidence suggests that pericytes located around the capillaries regulate cerebral blood flow in both normal and pathologic states.[7]

The initial observation of normal brain microcirculation using OPS imaging was followed by several other reports on the structure of cerebral cortical microcirculation in different disease states. First, it was shown that, during surgery for subarachnoid hemorrhage, the cortical microvasculature response to hypocapnia was different between patients prone to vasospasm and patients who did not develop vasospasm.[15] In addition, excision of arteriovenous malformations in the brain resulted in increased microcirculatory flow and functional capillary density in the perinidal brain tissue.[16]

Conjunctival Microcirculation

Direct access to brain microvasculature is impossible outside the operating room. However, a recent study suggests that the microcirculation of the conjunctiva might function as an indicator of the cerebral microcirculation.[17] At the bedside, the most accessible part of the eye for evaluating microcirculation is the bulbar conjunctiva, which offers a complete vascular bed with arterioles, capillaries, and small collecting venules. Earlier studies using intravital microscopy have reported on the behavior of the conjunctival microcirculation in sickle cell

patients.[18,19] The anterior segment of the human eye is supplied by the anterior ciliary arteries, which supply the anterior conjunctival, anterior episcleral, and limbal circulation. The episcleral arterial circle broadly resolves into superficial and deep components. [20] With intravital microscopy or OPS and SDF imaging, collecting venules are visualized as heavy, prominent, dark vessels situated parallel to narrower, straighter vessels, which comprise the accompanying terminal arterioles.[21] Capillaries arise at intervals from the end arterioles and form an irregular network of vessels that come together to form the venular system. The arterioles frequently terminate in main arteriovenous channels that communicate directly to the venular tree. This is most notable near the corneoscleral junction.[22]

The bulbar conjunctiva has been most extensively studied in diabetes and hypertensive patients. In these patient groups, several alterations in microcirculatory morphology and flow have been demonstrated. Microcirculatory alterations in the conjunctiva, however, have not yet been investigated in sepsis.

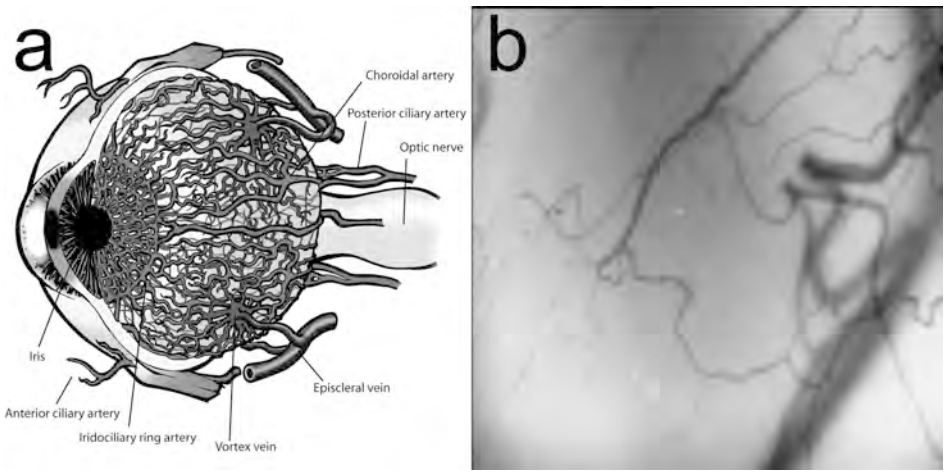

Figure 1. (a) Ocular circulation, including microcirculation. (b) SDF image of conjunctival microcirculation.

Sublingual Microcirculation

The sublingual area is one of the most easily accessible human mucosal surfaces, which is why it has been extensively investigated. The external carotid artery, the lingual artery, and the sublingual artery supply blood to the sublingual area. Only a limited number of sublingual arterioles are present, whereas numerous capillaries (diameter <20 mm) and venules (diameter 20–100 mm) are present in the sublingual area. Because perfusion of the sublingual mucosa is related to blood flow in the external carotid artery, sublingual perfusion may, in part, reflect cerebral blood flow. However, only a few studies have taken the cerebral and sublingual perfusion measurements simultaneously.

Perfusion of the sublingual area could represent blood flow in the splanchnic region for two major reasons. First, this is suspected because the tongue shares a common embryogenic

origin with the gut. Second, studies in critically ill patients reported good correlations between sublingual and gastric mucosal carbon dioxide pressures, as measured with sublingual capnometry and gastric tonometry, respectively, further strengthening the hypothesis.[23,24]

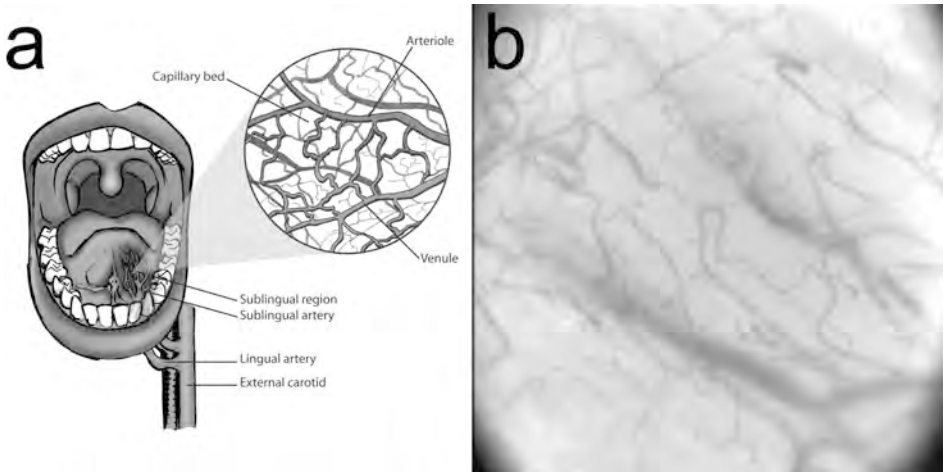

Figure 2. (a) Sublingual circulation, including microcirculation. (b) OPS image of sublingual microcirculation.

Intestinal Microcirculation

Branches from a common submucosal vasculature supply blood to the muscle, submucosal layer, and mucosal layer of the gut. Small arteries that branch extensively in the submucosal layer penetrate the muscularis layer of the intestinal wall. The branches all lead to capillary beds and, though diffusive shunting is thought to occur at the base of the villi, there is no evidence for arteriovenous shunts in the intestinal circulation.[25] Several submucosal arteries that transport blood back to the muscularis layer form a network of arterioles and capillaries around the intestinal smooth muscle cells. Other submucosal arteries supply blood to the mucosa and to each intestinal villus, where dense capillary networks exist. Veins leaving the villi join veins from the mucosal and muscularis layers, and they exit the intestinal wall alongside the mesenteric arteries that supply blood. Because absorption of water and solutes is one of the primary functions of the villi, the capillaries in the mucosal villi are very dense. A critical feature that may facilitate reabsorption of water is that the vessels carrying blood into and out of the villus lie close together, in parallel paths. Because the afferent and efferent blood vessels in the villi are close together, oxygen could diffuse from the arterioles to the venules at the base of the villus.[26] It is probably this organization that makes the tip of the villus the part most vulnerable to shock.

The integrity of the intestinal mucosa is important to preventing the translocation of bacteria and toxins into systemic circulation. Hypoperfusion of the gut mucosa may contribute to mucosal injury.[27,28] In this context, experimental studies have evaluated the effect of sepsis

and also hemorrhagic shock on the intestinal microcirculatory blood flow.[29-31] A recent study by Dubin and colleagues demonstrated that endotoxic shock in a sheep rapidly induced alterations in intestinal and sublingual microcirculation.[31] Fluid resuscitation normalized the systemic and intestinal hemodynamics, while also restoring the microvascular flow at the sublingual and intestinal serosal levels. However, the microvascular flow and percentage of perfused vessels in the intestinal mucosa were not effectively restored after fluid resuscitation.

Human data are scarce, and they are limited to the observations in stomas of patients with abdominal sepsis. Boerma and colleagues, after comparing the microcirculatory alterations between the sublingual region and stomas in patients admitted with abdominal sepsis, reported no correlation between sublingual and intestinal microcirculatory alterations on day 1 of sepsis.[32] After 3 days of sepsis, however, these regional alterations became more systemic and correlations were found between the sublingual and intestinal microcirculatory alterations. The alterations in one microcirculatory bed, therefore, do not necessary reflect alterations in other microvascular beds. However, although microcirculatory beds may differ substantially in different organs, studies have clearly demonstrated the clinical significance of sublingual microcirculatory alterations in sepsis. These alterations indicate the response to treatment and resuscitation and serve as sensitive and specific indicators of bad outcome.

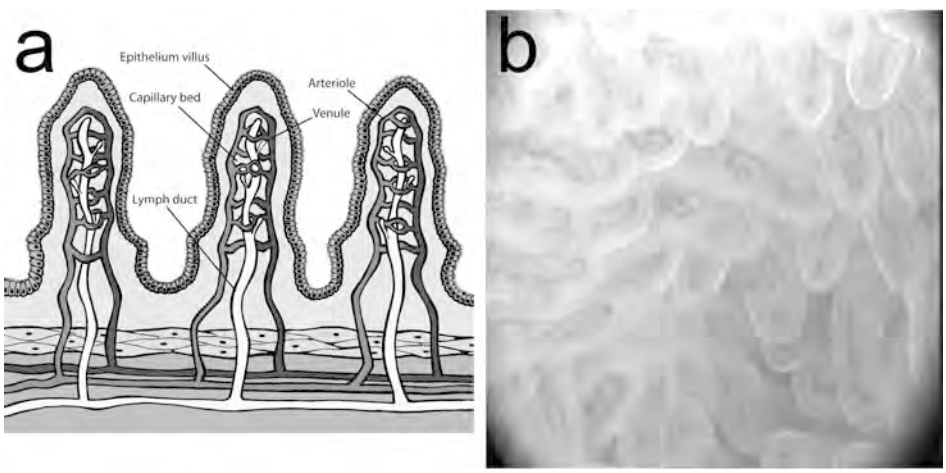

Figure 3. (a) Intestinal microcirculation. (b) OPS image of villus microcirculation.

Cutaneous Microcirculation

The study of cutaneous microvascularization with OPS, SDF, and intravital microscopy has been limited to the nail fold area because of the distinctive construction of its skin, which is a thick epidermal layer. Other techniques have also been used to study skin microcirculatory blood flow in illness. Using laser Doppler flowmetry, Young and Cameron demonstrated an impaired microcirculatory blood flow response of the forearm after transient ischemia.[33] Using

intravital microscopy, the blood flow velocity in the capillary bed of the nail fold was found to be decreased in normotensive febrile patients.[34]

In investigating cutaneous microcirculation, the question remains: To what extent is microcirculatory flow of the skin important in critical illness? This question arises mainly because of the role thermoregulatory properties play in governing and interfering with the skin microvasculature. Because the major role of cutaneous circulation is thermoregulation, blood flow to the skin typically exceeds metabolic requirements. In the deep dermis, branches from subcutaneous arteries that penetrate the dermis form an arterial plexus.[35] The vessels within this plexus generally run parallel to the surface. Arterioles penetrating from the dermal plexus to the subpapillary region form a subpapillary plexus. Capillaries connect to the subpapillary venous plexus, and single capillary loops ascend to each papilla. The descending portion of the capillary joins the subpapillary venous plexus, which drains into the deeper cutaneous venous plexus. Due to the numerous arteriovenous shunts (only in apical regions) and because many cutaneous veins run close to parallel arteries, the resulting countercurrent heat exchange conserves heat. The combination of the richly sympathetic innervations of the cutaneous arterioles with the high blood flow relative to oxygen demand allows a primary control of reflexes.

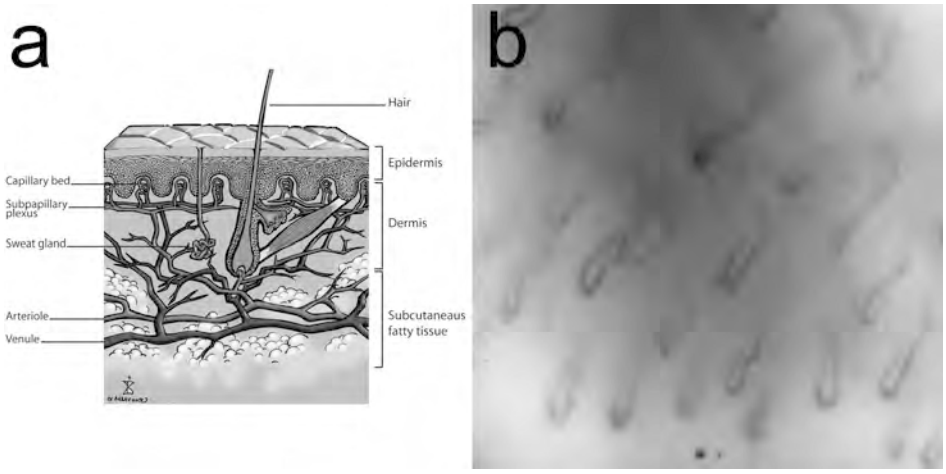

Figure 4. (a) Dermal circulation, including microcirculation. (b) SDF image of nail fold microcirculation.

Other Microcirculatory Beds

In addition to these areas of investigation, other microcirculatory beds have been studied using OPS and SDF imaging. While these applications have been limited to single studies, such studies have demonstrated the applicability of these techniques to other organ surfaces if they can be exposed for investigation. For instance, rectal microcirculation was studied in patients with malaria.[36] During maxillofacial surgery and especially for assessing wound healing, these techniques have also been used to explore additional areas of the oral cavity.[37] Finally,

during surgery in which several organ surfaces are exposed, investigations have examined microcirculation in the liver, pancreas, and kidney.[38-40]

MICROCIRCULATION IN SEPSIS

Observational studies of sublingual microcirculation in sepsis have contributed to the understanding of the pathophysiology of acute circulatory failure and multiple organ dysfunction.[41] Both clinical and experimental studies have previously indicated that sepsis and septic shock are disorders of microcirculation. In the early phases of sepsis, a persistent deficit in microcirculatory perfusion is associated with poor outcome, as was demonstrated by De Backer and colleagues.[42] These investigators compared the sublingual microcirculation in septic patients versus that of healthy volunteers and detected a significant decrease in the proportion and density of small perfused vessels during sepsis. In addition, the alterations were more severe in non-survivors than in survivors. Meanwhile, Sakr and colleagues observed that the recovery of microcirculatory alterations within the first 24 hours indicated survival outcome.[3] In this study, the persistent loss of capillary perfusion was one of the most sensitive and specific hemodynamic predictors of survival from septic shock. Trzeciak and colleagues studied the effects of an early goal-directed protocol on indices of microcirculatory perfusion in early sepsis.[4] They demonstrated that, even in the context of early goal-directed therapy, microcirculatory flow was more markedly impaired and more heterogeneous in septic non-survivors than in survivors. Interestingly, the investigators found a correlation between microcirculatory and macrocirculatory parameters in early sepsis, but this correlation was weaker in later sepsis. This finding emphasizes the importance of early resuscitation, since time is one of the most important factors affecting therapeutic strategies for improving microcirculation.[43]

Many pathogenic mechanisms contribute to the microcirculatory abnormalities that occur during sepsis. In this context, microcirculation can be regarded as the integrative compartment in which all of these factors come together. Left uncorrected, microcirculatory abnormalities can lead to multi-organ failure and death. The most challenging septic patients are those who have received treatment but remain resistant to improvement. Here, the initial hit, in combination with therapy, time, comorbidity, and genetic background, all contribute to the complex pathogenesis of sepsis that leads to organ failure. Together, these are referred to as the microcirculatory and mitochondrial distress syndrome.[41]

Significant increases in leukocyte rigidity are observed during sepsis. This rigidity subsequently decreases with an improvement in the clinical condition.[44] A significant role has also been attributed to the mechanical behavior of white blood cells in microcirculatory alterations.[45] During sepsis, red blood cells are less likely to become deformed and more likely to aggregate.[46-48] The vasodilatory effects of several mediators released during sepsis, in combination with the excessive amounts of fluids given (which reduce viscosity), result in a low systemic resistance and low arterial pressure. The capillary leakage caused by endothelial injury of the capillaries results in hypovolemia and tissue edema, both of which result in larger diffusion distances between oxygen-carrying red blood cells and parenchymal cells. A possible initial contributing factor to the disruption of the barrier

function of the microcirculatory milieu could lie in the disruption of the glycocalyx, which covers the endothelium and forms an important barrier and transduction system.[49] As was previously observed, this layer of the glycocalyx can be disrupted during inflammation and cardiovascular disease. It can also be measured using OPS or SDF imaging.[50,51] Recently, in a volunteer study, administration of endotoxin resulted in a shedding of the glycocalyx detected sublingually using OPS imaging. The shedding could be partially prevented by administration of a tumor necrosis factor scavenger.[52]

In addition to metabolic, myogenic, and neurohumoral regulatory mechanisms that control microcirculatory blood flow, other regulatory factors can alter vascular tone and affect blood flow. Over the last decade, it has been demonstrated that red blood cells play an important role in the regulation of vascular tone through their ability to sense hypoxia and respond by releasing vasodilatory substances, such as nitric oxide (NO) and ATP.[53] It has been suggested that excessive NO production plays a key role in the pathology of microcirculatory abnormalities in sepsis. Excessive NO induced by inducible NO synthase (iNOS) has deleterious effects on red blood cell function because it overrides the naturally occurring regulatory mechanisms of vasodilation and vasoconstriction associated with autoregulation. The iNOS induction caused by cytokine release during bacteremia results in excessive NO formation during inflammation and infection,[54] resulting in the loss of vascular tone due to the reduced responsiveness to vasoconstrictors.[55] The inhomogeneous expression of iNOS between different organ compartments could represent the underlying cause of the shunting of the microcirculation in various organ segments.[56] The heterogeneity caused by shunting results in local areas of hypoxia and impairs oxygen extraction. The shunting theory of sepsis could explain why resuscitation strategies based on the correction of upstream hemodynamic variables do not correct the downstream indicators of hypoxia because they are unable to recruit shunted microcirculatory units.[57] The functionally vulnerable microcirculatory units are bypassed, and oxygen is shunted from the arteriole to the venous compartment.

As described above, various mechanisms in sepsis could contribute to microcirculatory dysfunction and promote shunting. In sepsis, the perfusion in the capillaries is more severely altered, even while the flow in larger microvessels is preserved in septic shock patients, which represents direct evidence of shunting pathways in resuscitated sepsis.[58] Direct evidence of functional shunting pathways is derived from microcirculatory PO2 measurements using palladium porphyrin phosphorescence. During various conditions of shock and resuscitation, microcirculatory PO2 levels become lower than the PO2 of the venous effluent of an organ, demonstrating the action of functional shunting.[57]

Several experimental studies have been performed to elucidate the differences in microcirculatory response to different types of shock. Particular focus has been placed on differentiating between the effects that distributive (e.g., sepsis) and hypovolemic (e.g., hemorrhage) types of shock have on microcirculation.[59] Collectively, these studies have shown that the microcirculatory abnormalities in sepsis can occur in the presence of normal (or even supranormal) systemic hemodynamics. In contrast, with hypovolemic shock, the microcirculation and systemic hemodynamics seem to follow each other more closely. This difference is probably due to the fact that autoregulatory mechanisms still function in hypovolemic shock, whereas these mechanisms are severely impaired in septic shock. In addition, the differing courses of shock could

be the result of differing responses from various microvascular beds. At the arteriolar level, the cremaster muscle and diaphragm respond differently from the small intestine.[60-62] In the intestine, arteriolar constriction occurs at all levels of the arteriolar network. In contrast, in the cremaster muscle and diaphragm, larger arterioles constrict and smaller ones dilate. Therefore, there appears to be heterogeneity not only between organs but also within a single organ. Experimental studies have thus demonstrated that an increase in heterogeneity is associated with a decrease in functional capillary density and diminished red blood cell velocity in models of sepsis.[63]

Several studies have reported that global hemodynamics do not necessarily reflect regional blood flow during sepsis and septic shock.[4,64] Farquhar and colleagues, using a subacute model of sepsis, found that capillary density in the distal small bowel mucosa decreases during normotension.[29] Lam and colleagues used a skeletal muscle preparation in the same subacute sepsis model to observe the distributions of perfused capillaries and red blood cell flow.[63] They demonstrated an increase in capillaries with stopped flow and a decrease in the total number of capillaries. In addition, they demonstrated an increase in the spatial heterogeneity of red blood cell–perfused capillaries, similar to what later was demonstrated in clinical sepsis.[4] To underscore the dissociation of the presence of shock and microcirculatory abnormalities in sepsis, Nakajima and colleagues compared the effects of hemorrhage and endotoxin shock at the microvascular level in a rat model.[30] They found that, in the intestinal villi, the capillary density, red blood cell velocity, and flux all decreased. Yet, at the same level of hypotension, only moderate changes were detected during hemorrhagic shock. Boczkowski and colleagues showed similar results in the microvascular bed of the diaphragm by comparing an acute model of sepsis with hypotensive hypovolemic controls.[60] Fang and colleagues demonstrated similar findings for buccal microcirculation in a subacute model of septic shock compared with hemorrhagic shock.[65] Additionally, they demonstrated that when both models where matched according to the cardiac index, the microcirculatory alterations were similarly altered. After resuscitation, the improved global hemodynamics were not effective in improving the buccal capillary blood flow in septic shock, in contrast to those in hemorrhagic shock, where improved global hemodynamics were found to be effective. Thus, in shock profiles other than distributive shock, microcirculatory alteration seems to be effectively corrected by resuscitating global hemodynamic variables. In septic shock, however, therapeutic modalities aimed at recruiting systemic variables do not always seem to be effective in recruiting microcirculation. Therefore, more specific therapies may be required.[56] These experimental studies have contributed to the view that sepsis is a disease of microcirculation.

RESUSCITATING THE MICROCIRCULATION

Clinical investigations are currently being conducted to determine whether treatment modalities aimed at recruiting microcirculation provide a beneficial therapeutic target. Several agents and resuscitation strategies have been studied in single center intensive care populations and experimental models. Various animal studies have demonstrated that, under conditions in which autoregulatory mechanisms have not been affected, fluid resuscitation successfully improves the microcirculatory abnormalities and oxygen transport to baseline values.[30,65] In septic shock,

however, fluid resuscitation alone is ineffective in improving microcirculatory function. Even when the systemic variables have been normalized, microcirculatory alterations can persist.[3,42] Nevertheless, in a fraction of preload-dependent intensive care patients, a fluid loading of 500 mL within a 15-minute period was found to significantly improve microcirculatory parameters, although it was not clear in that study whether the patients were treated for severe sepsis or septic shock.[66]

In the clinical setting, several therapeutic agents have been demonstrated to effectively improve microcirculatory abnormalities in septic shock patients. One of the important findings from these studies is that the microvasculature, specifically endothelial function, is still responsive in sepsis. This was demonstrated elegantly by De Backer and colleagues, who found that sublingual topical application of acetylcholine was able to recruit microcirculatory units in resuscitated septic patients.[42] The topical sublingual application of acetylcholine reversed the local microcirculation in sepsis shock patients to normal values. Spronk and colleagues demonstrated that intravenous infusion of an NO donor (nitroglycerin) improved sublingual microcirculation in normovolemic, pressure-resuscitated patients.[58] Whether such therapeutic approaches will improve outcome has yet to be determined.

De Backer and colleagues also tested the effect of dobutamine, used at a fixed dosage, on microcirculation of the sublingual region of septic patients.[64] Dobutamine improved the sublingual microcirculatory flow, with acetylcholine-induced reserve being preserved. They also found that lactate levels correlated with the degree of microcirculatory improvement, but not to changes in systemic hemodynamic parameters, such as cardiac output and blood pressure. These studies demonstrate that, in sepsis, microcirculatory alterations can persist independent of resolving macroscopic systemic hemodynamic variables, and that these alterations predict poor outcome.

Since the heterogeneous expression of iNOS is one of the factors contributing to the inflammation-induced autoregulatory dysfunction of microcirculatory flow, iNOS inhibition was hypothesized to improve microcirculatory function in sepsis. It has been demonstrated in several animal studies that a combined therapy of fluid and iNOS inhibitors can resuscitate weak microcirculatory units.[67] Interestingly, in a clinical study, although the mean arterial pressure was improved, the mortality in the group treated with nonselective iNOS inhibitors was significantly greater due to cardiovascular failure, resulting in an early termination of the trial.[68] Clearly, inhibition of the harmful effects of NO requires a more specific approach.

Although several questions remain concerning the mode of action of activated protein C (APC), there is evidence that it improves the microcirculatory flow indices in experimental models.[69,70] In humans, improvement in the microcirculatory flow observed with OPS imaging was reported during treatment with APC, which decreased after cessation of the APC treatment.[71] The latter finding may suggest that the timing and length of treatment with APC may be an essential component in its application.

SUMMARY

One of the key features of both experimental and human sepsis is the distributive alteration of microvascular blood flow. These distributive alterations have been demonstrated by a heterogeneous microvascular blood flow between different vascular beds, as has been clearly

visualized through the use of such imaging techniques as OPS and SDF. Using these techniques, microhemodynamic alterations have been demonstrated to be associated with organ dysfunction and impaired outcome in sepsis. In light of the central role of microcirculatory alterations in the pathogenesis of sepsis, it seems logical to hypothesize that restoring microcirculatory function contributes to the treatment of sepsis. However, several questions remain unanswered. First, what is the clinical impact of sublingual monitoring of microcirculation in terms of clinical benefit and response to conventional therapy? Second, what are the best therapeutic interventions to recruit microcirculation, and how do these differ from conventional therapeutic modalities, in terms of monitoring? Finally, will the outcome of patients with sepsis improve when microcirculation is effectively resuscitated? To answer these questions, more evidence must be gathered. In answering these questions, it is clear that monitoring microcirculation will be an important component in the functional hemodynamic monitoring of critically ill patients.

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2 THE MICROCIRCULATION IN HEALTH AND CRITICAL DISEASE

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ABSTRACT

The microcirculation is a complex system, which regulates the balance between oxygen demand and supply of parenchymal cells. In addition, the peripheral microcirculation has an important role in regulating the hemodynamics of the human body because it warrants arterial blood pressure as well as venous return to the heart. Novel techniques have made it possible that the microcirculation can be observed directly at the bedside in patients. Currently, research using these new techniques is focusing at the central role of the microcirculation in critical diseases. Experimental studies have demonstrated differences in microvascular alterations between models of septic and hypovolemic shock. In human studies, the microcirculation has most extensively been investigated in septic syndromes and has revealed highly heterogeneous alterations with clear evidence of arteriolar-venular shunting. Until now, the microcirculation in acute heart failure syndromes such as cardiogenic shock has scarcely been investigated. This review concerns the physiologic properties of the microcirculation as well as its role in pathophysiologic states such as sepsis, hypovolemic shock, and acute heart failure.

INTRODUCTION

The microcirculation is that part of the circulation where oxygen, nutrients, hormones, and waste products are exchanged between circulating blood and parenchymal cells.[1] The microcirculation consists of a network of blood vessels less than 100 µm in diameter. According to anatomical features and the direction of the blood flow, these microvessels are subdivided into arterioles, capillaries, and venules. Adequate blood flow in capillaries is a prerequisite for normal organ perfusion and function. In critically ill patients, capillary blood flow in tissues may be impaired. In this article, we systematically review morphology and physiologic function of the microcirculation in tissues outside the heart, such as the splanchnic region, skin, muscles, and the sublingual area, because these microcirculatory beds have been studied most extensively. We also comprehensively discuss the current literature on the role of the microcirculation in the pathogenesis of critical illnesses.

NORMAL FUNCTION OF THE MICROCIRCULATION

Capillaries consist of a single layer of epithelium and a basement membrane. The main function of capillaries is to allow exchange of molecules between blood and tissues. Capillary blood flow (a product of driving pressure, arteriolar tone, and hemorheology) and capillary patency are the main determinants of capillary perfusion.[2] Blood flow in one single capillary is not a good indicator of oxygen delivery to the tissue under evaluation due to the temporal and spatial heterogeneity of capillary blood flow.[3,4] Capillary patency is reflected by functional capillary density (FCD), defined as the functional number of capillaries in a given area where functional is defined as capillaries filled with flowing red blood cells. Because oxygen has to diffuse from capillaries to tissue cells, every organ has a rich abundance of microvessels.[5] Density and anatomy of this local microvasculature depend on the metabolic requirement of each specific tissue. Although oxygen transport from the blood in the capillaries to the tissue cells is the key function of the microcirculation, the arteriolar and venular parts of the microcirculation have important functions in maintaining adequate tissue perfusion and hemodynamics. Arterioles are tiny branches of arteries that lead to capillaries and form the major resistive component of the microcirculation. The walls of arterioles are surrounded by circular smooth muscle cells, which are under the control of the sympathetic nervous system. Vascular endothelial cells can respond to alterations in local shear stress and give local stimulatory signals to arterioles via cell-to-cell communication.[6,7] These central and local control mechanisms regulate constriction and dilatation of the arterioles depending on the metabolic requirements of the tissue cells. Under abnormal conditions, such as shock or heart failure, the balance between vascular resistance to preserve arterial pressure (by constriction of the resistance vessels) and peripheral tissue flow may be lost, causing insufficient blood flow to sustain metabolism in specific organs. For instance, the temporary compromise as a response to a sudden decline in blood pressure (e.g., in shock states) is an increase in arterial resistance, which can result in a reduced blood flow to the less vital organs such as the skin and the splanchnic system. The venular part of the microcirculation serves as a large low-pressure reservoir through which blood is returned to the heart. It may contain as much as 75% of total blood volume.[8] Active and passive changes of

venous vascular tone alter the quantity of venous blood, which thereby change cardiac preload and cardiac output and guarantee maintenance of the circulating blood pool.[9]

In addition, intravascular content is important for local regulation of microvascular blood flow. There is increasing evidence that red blood cells (RBCs) not only carry oxygen to the tissues but also able to locally sense and regulate oxygen delivery in the microcirculation.[10] Red blood cells, when exposed to hypoxia, can release nitric oxide and adenosine triphosphate, which are potent vasodilators, thus improving oxygen delivery.[11-14]

METHODS TO ASSESS MICROVASCULAR BLOOD FLOW

Gastric tonometry has been introduced as a technique to assess splanchnic perfusion.[15] Using a special gastric tonometry catheter, CO2 tension (pCO2) can be measured in the stomach, ileum, and sigmoid lumen. The pCO2 values can be used to estimate, for example, the pH of the gastric mucosa (pHi) or can be related to the value of the arterial pCO2 (by calculating the so-called pCO2 gap).[16] Despite substantial initial enthusiasm, this technique has never been widely implemented due to technical problems and difficulties in demonstrating the use of gastric tonometry-derived variables as therapeutic guides.[17] Recently, it became also possible to monitor tissue pCO2 in other sites. Sublingual capnometry is a promising noninvasive example, although the value of this new technique in critically ill patients still has to be determined.

Intravital microscopy (IVM) is considered the gold standard for in vivo investigation of the microcirculation and has been widely used in animal studies to examine the microcirculation in situ. This technique can be used for investigation of thin tissues that allow transillumination, whereas fluorescent dyes have to be used to allow epiillumination of thicker organ surfaces. Unfortunately, the use of dyes in humans is hindered by safety concerns, and IVM studies in humans have therefore been limited to observation of nail fold capillaries that can be observed without using dyes.[18] However, one can question the clinical significance of the nail fold microcirculation due to its high sensitivity to external temperature, which hinders reproducibility of measurements. The large size of an IVM microscope is also a reason why this technique is not extensively used in clinical research and practice.

The recently developed laser-Doppler flowmetry is a technique that is based on frequency shifts in laser light by moving erythrocytes. The technique measures red cell flux in small-volume samples (0.5 mm^3) of organ surfaces, thus providing averages of velocities in all microvessels in the tissue volume that is under investigation. However, this feature of laser-Doppler flowmetry is also an important limitation of the technique because averaged numbers for microvascular blood flow do not provide information about heterogeneity of blood flow, which actually is a major characteristic of the microcirculation. Modifications of this methodology, called laser-Doppler perfusion imaging and laser speckle imaging, allow construction of real-time 2-dimensional perfusion maps of a larger area. Hence, it has become possible to perform successive measurements in exactly the same area of interest. These techniques, however, are still limited by individual blood vessels that cannot be visualized. Moreover, the laser-Doppler-derived signal is expressed in arbitrary units (flux or perfusion units), which implies that results can only be reported in percentage of change rather than in absolute terms.

Near-infrared spectroscopy (NIRS) is a technique that can be used to noninvasively monitor regional and microcirculatory oxygenation at the bedside. In short, this technique uses near infrared light that can easily penetrate biological tissues. Hemoglobin has a well-defined absorption spectrum that is influenced by O2-binding. It is the analysis of the attenuation spectra of such pigments (spectroscopy) that is used to monitor tissue oxygenation (StO2) in vivo. The technique has been validated and has been applied extensively to evaluate cerebral perfusion and to perform peripheral ischemia/reperfusion experiments.[19,20] Near-infrared spectroscopy is a promising method to assess tissue perfusion, although the variety of NIRS devices currently commercially available and the different values generated by these devices hinder comparison of results from different NIRS studies. Careful calibration of each device and standardization of the method are required to completely use the opportunities provided by the NIRS technique.[21]

Modern clinically applicable microscopic approaches to investigate the microcirculation are orthogonal polarization spectral imaging (OPS) and its successor side-stream dark field (SDF) imaging. Orthogonal polarization spectral imaging as well as SDF imaging are both validated techniques to investigate the microcirculation of tissues covered by a thin epithelial layer (Table 1[22-26]). Currently, most studies are performed of the well accessible sublingual microcirculation, and most investigators report the use of OPS imaging to study this particular microvascular bed. In OPS imaging, the tissue embedding the microcirculation is illuminated by polarized green light.[27] Back-scattered, depolarized, light is projected onto a charged coupled device video camera after it passes an analyzer, that is, a second polarizer that is orthogonally oriented with respect to the first polarizer. In this way, surface reflections are filtered out and images can be observed of the microcirculation below the surface under investigation. In OPS imaging, the hemoglobin is actually used as contrast agent, so that red blood cells are imaged as dark moving globules against a white/grayish background. The obtained video clips can be analyzed to quantify various parameters such as flow and morphology via precise semi-quantitative methods or by using dedicated software packages.[28-31] Recently, SDF imaging was introduced as the successor of OPS imaging. The SDF imaging device consists of a central light guide, surrounded by concentrically placed light emitting diodes that emit stroboscopic green light. The center of the probe is optically isolated from the light emitting diodes so that hindering surface reflections are avoided. This results in similar images compared to OPS-derived video clips (Fig 1), although SDF imaging results in better visualization of capillaries due to complete avoidance of surface reflections and the short, stroboscopic illumination intervals that prevent smearing of flowing RBCs.[26]

THE MICROCIRCULATION IN SEPSIS

Sepsis is defined as the clinical syndrome characterized by the presence of both infection and a systemic inflammatory response of the body. The term severe sepsis is often used in the literature and refers to sepsis complicated by organ dysfunction. Septic shock refers to a state of acute circulatory failure characterized by persistent arterial hypotension unexplained by other causes.[32] Although major progress has been made in the treatment of sepsis, a substantial number of patients will not survive despite early, aggressive correction by volume resuscitation and

Table 1. Validation studies for OPS and SDF imaging.

Reference	Compared techniques	Subjects	Tissue	Investigated conditions	Analysis method	Conclusions
Harris[22]	IVM vs. OPS imaging	10 hamsters	Dorsal skinfold	Ischemia/reperfusion	Cap-Image Software	Good agreement for RBC velocity and vessel diameters
Mathura[23]	IVM vs. OPS imaging	10 healthy volunteers	Nailfold	Baseline and after venous occlusion	Cap-Image Software	Good agreement for RBC velocity and capillary diameter. Better capillary contrast with OPS
Langer[24]	IVM vs. OPS imaging	9 rats	Liver	Ischemia/reperfusion	Cap-Image Software	Good agreement for RBC velocity and vessel diameters
Harris[25]	IVM vs. OPS imaging	9 hamsters	Dorsal skinfold	Standardized hemodilution	Cap-Image Software	Good agreement for vessel diameter and FCD at various levels of hematocrit
Goedhart[26]	OPS vs. SDF imaging	10 healthy volunteers	Nailfold	Baseline, venous and arterial occlusion	Microscan Analysis Software	Good agreement for capillary diameter and RBC velocity
		2 healthy volunteers	Sublingual	-	Image quality analysis	Higher capillary contrast and quality with SDF

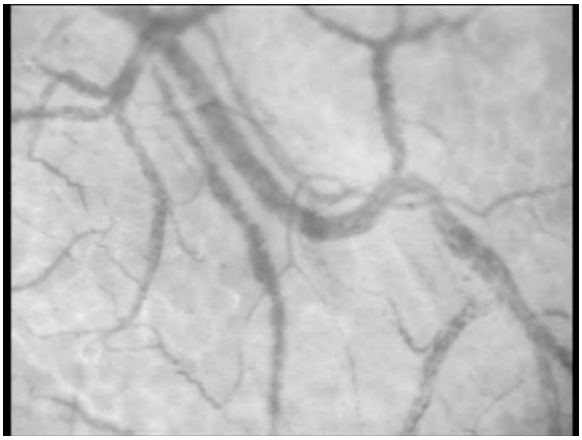

Figure 1. Sidestream Dark Field video frame of the sublingual microcirculation. The image represents a tissue area of 0.98 × 0.73 mm.

administration of vasoactive agents and antibiotics.[33-36] Indeed, severe sepsis is the most common cause of death in general (non-cardiac) critical care units.[37] Current guidelines for resuscitation of patients with severe sepsis and septic shock aim at normalizing global hemodynamic and metabolic parameters (i.e., central venous pressure, mean arterial pressure, urine output, central venous oxygen saturation, and lactate level).[38] Although these parameters are indicative for global tissue perfusion, information about regional perfusion, hypoperfusion, and hypoxia in a septic patient is often lacking. Yet, regional hypoxia and associated organ dysfunction is one of the major features of septic shock. This is the reason why microcirculation research has gained so much interest in the last years. One of the key features of the microcirculation in sepsis is a heterogeneous distribution of blood flow. It has been shown that certain vascular beds, or microcirculatory units, become underperfused, whereas others show a normal or even abnormally high blood flow.[29,39-41] It has been suggested that this phenomenon is explained by autoregulatory dysfunction through heterogeneous expression of inducible nitric oxide synthase, an important system responsible for autoregulatory vasodilation of the microcirculation.[42,43] Several other phenomena play a role in the pathogenesis of sepsis. Smooth muscle cells that line arterioles and regulate perfusion lose their adrenergic sensitivity and tone in sepsis.[44] Erythrocyte deformability decreases, whereas their ability to aggregate increases.[45,46] In addition, leukocytes activated by septic inflammation generate reactive oxygen species that may impair glycocalyx function and coagulatory function.[47-49] These defects may further impede microcirculatory perfusion, which can finally result in an oxygen extraction deficit, severe functional shunting, and (multiple) organ failure.[2,39,50,51] Although the microcirculation seems to play an important role in the pathogenesis of severe septic shock, others have argued that mitochondrial dysfunction is the driving mechanism underlying multi-organ dysfunction in sepsis.[52] However, whether mitochondrial failure is indeed a primary cause of oxygen extraction deficit in sepsis is still a source of debate. De Backer et al used OPS imaging to demonstrate that total vascular density and capillary perfusion

are impaired in patients with sepsis, and these microvascular alterations have been associated with in-hospital mortality.[29,35] Subsequent studies showed that microvascular alterations could be improved with nitroglycerin,[40] dobutamine,[53] and red blood cell transfusion.[54,55] Nevertheless, it remains unknown whether a microcirculation-guided therapy, monitored by OPS or SDF imaging, improves outcome in patients with septic shock.

SEPTIC SHOCK VS HYPOVOLEMIC SHOCK

Kerger et al have demonstrated that functional capillary density can deteriorate in experimental severe hemorrhagic shock with an induced mean arterial pressure (MAP) of 40 mm Hg.[56] These changes were related to mortality. To our knowledge, 3 experimental studies have been performed to investigate whether there is a difference between microcirculatory dysfunction in septic shock relative to mild hypovolemic shock. First, Boczkowski et al used a rat diaphragmatic microcirculation model in which they investigated capillary perfusion using transillumination in experimental sepsis and during controlled hemorrhage.[57] Blood pressure decreased similarly in rats with sepsis vs rats with hemorrhage, but the percentage of non-perfused capillaries was significantly higher in the septic group, whereas it remained at normal values in the group rats with hemorrhage. Second, Nakajima et al used intravital microscopy to investigate the effects of hemorrhagic shock, normotensive sepsis, and septic shock on the microcirculation of mouse small intestine.[58] Significant decreases in red blood cell velocity were observed in the groups with sepsis and septic shock, whereas it remained preserved in mice with hemorrhage-induced hypotension. Although mean FCD also decreased in mice with hemorrhagic shock, this decrease was much worse in mice with septic shock. Finally, Fang et al used OPS imaging to estimate buccal microvascular blood flow in rats with septic shock vs rats with MAP-matched hemorrhagic shock.[59] These investigators demonstrated that microvascular blood flow was worse in the septic group compared to the MAP-matched hemorrhagic shock group. In addition, saline infusion resulted in complete restoration of microvascular blood flow in the MAP-matched hemorrhagic shock group, whereas saline infusion could not improve microvascular blood flow in the septic shock group. Taken together, these studies have demonstrated that the microcirculation in mild hemorrhagic shock is not affected to such an extent as compared to septic shock. In addition, the results presented by Fang et al suggested that microvascular alterations in hypovolemic shock (conversely to septic shock) are at least not completely independent of global hemodynamic parameters.[59] These findings have to be confirmed in human studies, and it should be realized that administration of allogeneic red blood cell transfusions after hypovolemic shock rather than hypovolemic shock itself may have deleterious effects on the microcirculation in humans.[54,55]

CARDIOGENIC SHOCK

Cardiogenic shock is one of the acute heart failure syndromes and is defined as evidence of tissue hypoperfusion induced by heart failure after correction of preload. Cardiogenic shock is characterized by a reduced arterial blood pressure (systolic <90 mm Hg or a drop of MAP >30

mm Hg) and/or low urine output (<0.5 mL/kg per hour), with a heart rate more than 60 beats per minute.[60] In most cases, cardiogenic shock develops after an acute myocardial infarction. In patients admitted with acute myocardial infarction, cardiogenic shock is the leading cause of death.[61] Other causes of cardiogenic shock include cardiomyopathy, valvular heart disease, myocarditis, myocardial contusion, and previous cardiac surgery. Despite advances in treatment, in-hospital mortality of cardiogenic shock patients remains about 50%.[62] For years, it has been accepted that when cardiac output, and thus systemic blood flow, decreases, compensatory redistribution of blood volume (partly by active venoconstriction) results in a decrease in peripheral vascular capacitance with a compensatory increase in cardiac filling pressures and cardiac output. In this classic paradigm of cardiogenic shock, depression of cardiac output also causes arteriolar vasoconstriction and an elevation of systemic vascular resistance. Studies that investigated the peripheral circulation in patients with chronic, severe heart failure (New York Heart Association class III-IV) and cardiogenic shock have supported this hypothesis.[63-65] However, data on the state of the peripheral microcirculation in acute heart failure is scarce and did not consistently confirm the classic notion of systemic vasoconstriction in all patients with cardiogenic shock. For example, Lim et al reported that 40% of patients admitted with cardiogenic shock due to myocardial infarction died with a cardiac index more than 2.2 L/min per square meter.[66] In line with these data, post hoc analysis of data from the SHOCK-trial demonstrated that a considerable number of patients, admitted with cardiogenic shock, developed a systemic inflammation response syndrome (SIRS) during hospital stay. In these patients, systemic vascular resistance (SVR) at shock onset was significantly lower compared to patients who did not develop SIRS, although a wide variation of SVR among patients was observed. A low SVR, suggesting peripheral vasodilation, was a predictor for later development of sepsis.[67] The latter data suggested once again that not all cardiogenic shock patients develop diffuse vasoconstriction as a compensatory response to cardiac pump failure. Fig 2 shows the current theory on the effects of cardiogenic shock on the microcirculation. In fact, inappropriate vasodilation due to SIRS might further impair perfusion at the microvascular level, which might be important in the pathogenesis of multiple organ failure and the persistence of shock.[68] This process may be due to an increased production of nitric oxide by nitric oxide synthase (NOS). Accordingly, strategies were developed to inhibit NOS in patients with cardiogenic shock. Small studies suggested that NOS-inhibition could improve blood pressure.[69-71] The Tilarginine acetate injection in a Randomized International study in Unstable MI Patients with cardiogenic sHock (TRIUMPH)-trial was designed to test whether administration of tilarginine, a NOS-inhibitor, would result in an improved 30-day survival in patients with myocardial infarction complicated by cardiogenic shock. Unfortunately, enrollment of patients was recently terminated at 398 patients because of lack of efficacy. There was no difference in favor of NOS-inhibition in 30-day all-cause mortality between patients who received tilarginine (48% died) relative to placebo (42% died; risk ratio, 1.14; 95% confidence interval, 0.92-1.41).[72]

This negative study underlines that microcirculatory alterations in cardiogenic shock are still not fully understood. The aforementioned sophisticated 2-dimensional imaging techniques may give better insight in the nature of these alterations. De Backer et al used OPS imaging to

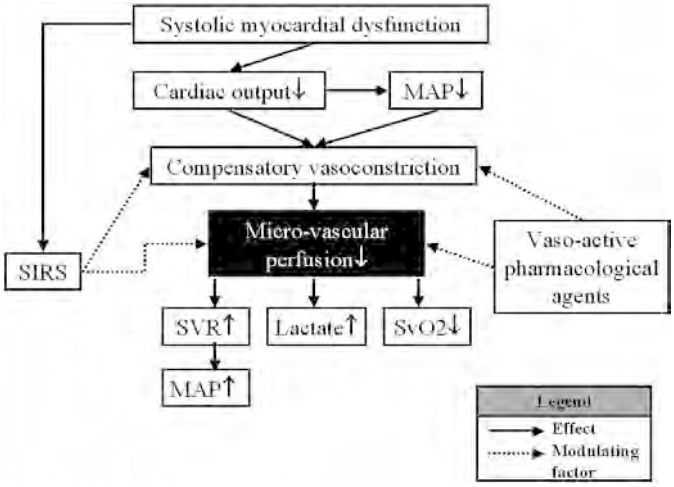

Figure 2. The effects of cardiogenic shock on the microcirculation. A decrease in cardiac output, caused by systolic myocardial dysfunction, results in a decrease in mean arterial blood pressure. These phenomena cause compensatory vasoconstriction of the resistance vessels, which decreases microvascular perfusion. The degree of SIRS and the administration of vasoactive pharmacologic agents can both modulate microcirculatory responses and influence perfusion at the microvascular level. In case of severe hypoperfusion of the microcirculation, lactate levels will increase whereas venous oxygen saturation will decrease. Abbreviations: MAP, mean arterial pressure; SIRS, systemic inflammatory response syndrome; SvO_2, venous oxygen saturation; SVR, systemic vascular resistance

investigate the sublingual microcirculation in 40 patients with acute heart failure, most of them having cardiogenic shock, who were investigated within 48 hours after the onset of acute heart failure.[73] These investigators reported similar alterations to those observed in sepsis, including a lower proportion of perfused capillaries in patients with heart failure compared to control patients. Moreover, these alterations were related to in-hospital mortality. However, these investigators performed only one measurement per patient, and it remained unclear whether the microcirculation could be improved by pharmacologic therapy and whether such interventions could be beneficial in reducing the high mortality rate in cardiogenic shock patients.

MACROCIRCULATION VS MICROCIRCULATION IN SEPTIC AND CARDIOGENIC SHOCK

An important finding in several studies in SIRS and sepsis was that the alterations in microvascular flow occurred independently of systemic hemodynamic variables.[29,53,74] De Backer et al have argued that probably the same principle holds for cardiogenic shock.[73] Conversely, several studies have shown that there is a close correlation between macrocirculation and microcirculation in animals and patients with left ventricular systolic dysfunction. This was recently confirmed with OPS imaging in pigs subjected to ventricular fibrillation followed by advanced life support.[75,76] Measured by OPS imaging, Erol-Yilmaz et al recently reported a better sublingual functional capillary density during biventricular pacing relative to either only right ventricular pacing or no

pacing.[77] Although cardiac output was not measured in this study, these data suggest a relation between cardiac output and sublingual microcirculatory perfusion. Further well-designed studies in patients who had cardiogenic shock, preferably using SDF imaging, are needed to completely explain these results. The exact extent of peripheral vasoconstriction may in fact be dependent of the degree of SIRS present in a patient with cardiogenic shock (Fig 2).

FUTURE PERSPECTIVES IN MICROCIRCULATION RESEARCH

There is overwhelming evidence that sepsis is a disease of the microcirculation.[2,78] However, the exact role in the pathogenesis is still incompletely understood. Ongoing animal studies and 3-dimensional computer models, simulating microvascular blood flow and gradients of hypoxia, will improve our understanding of microvascular alterations and will result in new approaches to monitor and treat patients with sepsis.[79] Furthermore, randomized studies are currently underway to test whether microcirculatory flow-guided therapy might be beneficial for patients with severe sepsis and septic shock. The microcirculation does not seem to play a completely independent role in hypovolemic shock, although this has to be confirmed in human studies. Research focusing on the exact role of the microcirculation in cardiogenic shock is still in its premature stages. We have to learn more on nature, severity, and duration of peripheral microvascular changes in acute heart failure syndromes to comprehensively investigate whether these changes can be improved by pharmacologic and mechanical treatment strategies, it is to be hoped that resulting in improved treatment and survival of heart failure patients.

CONCLUSIONS

An adequate function of the peripheral microcirculation in the different organ systems is necessary to maintain adequate tissue perfusion and to preserve normal hemodynamics. Recent technical developments have enabled investigation of the microcirculation at the bedside and confirmed that the microcirculation is impaired in severe sepsis and septic shock. Heterogeneous microvascular alterations in sepsis occur independently of changes in global hemodynamic parameters. Several recent experimental studies have demonstrated that the microcirculation in hypovolemic shock is not affected to such an extent as in septic shock, although it should be realized that allogeneic RBC transfusions after hypovolemic shock may impair the microcirculation. The role of the peripheral microcirculation in cardiogenic shock is still incompletely understood, although there is growing evidence that systemic inflammation of the body is an important aspect in the pathogenesis. In addition, there is still debate whether microvascular alterations in acute heart failure syndromes occur completely independent of changes in the macrocirculation. Novel bedside technology to image the peripheral microcirculation may be helpful to understand the pathogenesis of cardiogenic shock more comprehensively and to learn whether intervention strategies, aimed at improving peripheral microvascular flow, are beneficial for patient outcome.

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PART B

LASER SPECKLE IMAGING TO MEASURE MICROCIRCULATION

3

VALIDATION OF NEAR-INFRARED LASER SPECKLE IMAGING FOR ASSESSING MICROVASCULAR (RE)PERFUSION

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ABSTRACT

The present study was conducted to compare laser speckle imaging (LSI) with sidestream dark field (SDF) imaging (i.e., capillary microscopy) so as to validate the use of LSI for assessing microvascular (re)perfusion. For this purpose, LSI and SDF measurements were performed on the human nail fold during gradual occlusion of the upperarm circulation to modify nail fold perfusion under controlled circumstances. Additionally, a vascular occlusion test was performed to test the ability of LSI to detect rapid changes in tissue perfusion during reactive hyperemia and a hyperthermic challenge was performed to measure LSI perfusion at maximum functional capillary density. Normalized LSI measurements (i.e., normalized to baseline is 100%) were shown to correlate positively with normalized SDF measurements (Pearson's r=0.92). This was supported by linear regression analysis (slope of 1.01, R2=0.85, pb0.001). During the vascular occlusion test, LSI perfusion decreased from 307±90 AU (baseline) to 42±8 AU (ischemia). Peak perfusion during reperfusion was 651±93 AU (212% of baseline), which had returned to baseline after 2 min. Hyperthermia increased LSI perfusion from 332±90 AU to 1067±256 AU (321% of baseline). The main finding was that changes in perfusion as measured by LSI correlated well with changes in capillary red blood cell velocities as measured by SDF imaging during controlled reduction of the (micro)vascular perfusion. It was further shown that LSI is capable of measuring tissue perfusion at high temporal and spatial resolution. In conclusion, LSI can be employed to accurately quantitate microvascular reactivity following ischemic and hyperthermic challenges.

INTRODUCTION

Microvascular function is considered an important parameter in many clinical scenarios ranging from vascular surgery to critical care medicine.[1-5] A typical test for assessment of microvascular function, defined as the ability to increase microvascular flow after an ischemic challenge, is the vascular occlusion test (VOT), where the arterial and venous flow are transiently stopped and post-ischemic reactive hyperemia is monitored using, for example, laser Doppler velocimetry (LDV)[6], near-infrared spectroscopy (NIRS)[7-9], and thermography.[10] Other tests for assessing microvascular function include thermal challenges, as applied in patients with systemic sclerosis and primary Raynaud's phenomenon[11], and fluid and vasoactive drug challenges, as applied in intensive care patients.[12] However, each of these measurement techniques has specific shortcomings, limiting their clinical applicability.

A technique potentially overcoming the abovementioned shortcomings is laser speckle imaging (LSI).[6,13,14] The main advantage of LSI with respect to clinical applicability is the ability to measure tissue perfusion in large areas (e.g., skin grafts, burnwounds, complete organ surfaces) in a noncontact and thus non-perturbing manner at high spatial and temporal resolution using a simple setup including a laser diode for illumination and a grayscale CCD camera for imaging.[13,14] An additional advantage of using a conventional CCD camera for imaging is that it allows a normal video mode for morphological identification of organ surfaces at high resolution, complementing the quantitative LSI data with anatomical and morphological detail.

To date, LSI has been used mainly in experimental research[15-18] and only scarcely in patients[11,19,20], which is unfortunate inasmuch as LSI may circumvent the shortcomings associated with the currently used techniques.[6,13] However, whether LSI is indeed sensitive to changes in capillary perfusion has not been validated before. Although percentual flow changes determined by LSI have been compared to the changes measured by LDV, LSI has never been juxtaposed to a quantitative technique capable of measuring tissue perfusion at the capillary level.[21,22] In this respect, intravital capillary microscopy constitutes a suitable quantitative technique for the assessment of the cutaneous microcirculation under resting conditions and after different provocation tests.[23-27] Hence, validation of LSI by intravital capillary microscopy would provide strong support for the employment of LSI in settings that require information on (changes in) microcirculatory perfusion.

Sidestream dark field (SDF) imaging is a validated intravital microscopic imaging technique for imaging capillaries on organ surfaces and for measuring red blood cell velocities in individual capillaries.[21,28] In contrast to LDV, SDF therefore constitutes a useful tool for validating LSI responses to alterations in capillary perfusion.

Consequently, the present study was conducted to compare LSI to SDF imaging so as to validate the use of LSI for assessing microvascular (re)perfusion. For this purpose, LSI and SDF measurements were performed on the human nail fold during gradual occlusion of the upperarm circulation to modify nail fold perfusion under controlled circumstances. To determine the ability of LSI to detect rapid changes in tissue perfusion during reactive hyperemia a VOT was performed. Additionally, a hyperthermic challenge was applied to measure tissue perfusion using LSI under conditions of maximal functional capillary density.

METHODS

Subjects

The study was approved by the medical ethics research board and informed consent was obtained for each subject. Ten (n=10) healthy, non-smoking, male subjects receiving no medication voluntarily participated in the validation experiments. The subject population had a mean±SD age of 24±3 years and weight of 73±5 kg. Heart rate was 65±6 beats/min and systolic and diastolic blood pressures were 118±5 and 65±7 mmHg, respectively.

Laser speckle imaging setup

For LSI measurements, a commercially available system was used (Moor Instruments, Devon, UK). A 785-nm class 1 laser diode was employed for illumination of the tissue up to a depth of approximately 1 mm. Directly reflected light by the tissue surface was blocked by a tunable polarization filter placed in front of the lens system since it was not scattered by flowing red blood cells and therefore contained no information on tissue perfusion. Laser speckle images were acquired using a 576×768-pixel grayscale CCD camera at a frame rate of 25 Hz and converted to pseudo-color images, where the level of perfusion was scaled from blue (low perfusion) to red (high perfusion).[13-16] The lens system allowed a variable zoom, ranging from 0.6×0.8 cm (corresponding to 10 ***μm***/pixel) to 9×12 cm at a working distance of 15–45 cm, and focus optimization. Using a 5×5 pixel window to calculate speckle contrast, the maximal image resolution was 50 ***μm***/pixel.

Sidestream dark field imaging setup

For SDF imaging, a MicroScan video microscope (MicroVision Medical, Amsterdam, the Netherlands) was employed, which was fitted with a 5× magnifying objective lens system. The illumination intensity was optimized during imaging to obtain clear microcirculatory images. Focusing was achieved by axial translation of a 576×768-pixel grayscale CCD camera (frame rate of 25 Hz) with respect to the fixed lens system in the tip of the SDF probe. Image resolution was ~1.4 ***μm***/pixel. The device was mounted in a specially engineered holder (Department of Instrumentation, Academic Medical Center, University of Amsterdam, the Netherlands) for accurate positioning and stabilization of the probe. Video output was visualized on a monitor and connected to a computer via a signal converter (ADVC110, Canopus Corporation, Kobe, Japan) for digital recording of the images onto a hard drive. From the SDF images, all in-focus capillaries were selected for further off-line analysis. Image analysis software developed for the SDF video images (Microscan Analysis Software 2.0, MicroVision Medical) was used to generate space–time diagrams of single capillaries for quantitative measurement of red blood cell velocities.[21,29] The use of space time diagrams to determine red blood cell velocities is described extensively elsewhere.[29] In brief, a space time diagram is generated by plotting a selected capillary center line in time. Moving cells and plasma gaps in the selected capillary cause a pattern to appear in the space–time diagram and the orientation of this pattern is indicative for the capillary red blood cell velocity and is converted to an actual velocity value (velocity=distance/***Δtime***).

Validation protocol

The nail fold microcirculation was selected as the site for validation of LSI since it is easily accessible for both techniques and its perfusion can be systematically reduced using a pneumatic cuff placed around the upper arm. The cuff was inflated to 30, 60, 90, 120, 150, and 180 mm Hg. Between each measurement, the cuff was deflated for >5 min to allow stabilization of the nail fold microcirculatory perfusion. Vascular occlusion test and hyperthermic challenge Following the validation protocol, a 3-min VOT was performed with an occlusion pressure of 210 mmHg for 3 min. Subsequently, the occlusion pressure was released and reperfusion was recorded until the LSI perfusion value had restored to baseline. Five minutes after return to baseline, local hyperthermia was created by flowing heated air onto the hand. Skin temperature was measured using a skin temperature sensor (Smiths Medical ASD, Rockland, MA) and kept between 41 and 43 °C by adjusting the distance between the heat source and the hand.

Statistical analysis

All data was analyzed using GraphPad Prism 5.0 (GraphPad Software, La Jolla, CA). All data are presented as mean±SD. Comparison of LSI with SDF imaging was done by linear regression analysis and Pearson's correlation analysis. For the SDF and LSI measurements, the inter-individual coefficient of variability (CV) was calculated as CV=SD/mean. Comparative analysis of tissue perfusion measured with LSI during normothermia and hyperthermia was performed using a paired Student's t-test. Differences were considered statistically significant at $p<0.05$.

RESULTS

LSI perfusion maps for each step of occlusion pressure and the corresponding video image of the nail fold are shown in Fig. 1.

Validation of laser speckle imaging

Perfusion measurements by LSI and SDF imaging for each occlusion pressure are shown in Fig. 2. Baseline LSI perfusion was 321±186 AU (CV=0.58) and baseline capillary red blood cell velocity as measured by SDF imaging was 791±332 ***μm***/s (CV=0.42). The reduction in perfusion following gradual occlusion of the upper arm circulation was similarly measured by LSI and SDF imaging as shown in Fig. 2. Furthermore, Fig. 2 shows that there is considerable spread in the absolute velocity values measured by SDF imaging, which reflects the normal physiological variability between the subjects. Overall, normalized LSI measurements (i.e., normalized to baseline=100%) were shown to correlate positively with normalized SDF measurements (Pearson's r=0.92). This was supported by linear regression analysis as shown in Fig. 3 (slope of 1.01, R2=0.85, $p<0.001$).

Vascular occlusion test and hyperthermic challenge

A typical LSI trace during the VOT is presented in Fig. 4. In Fig. 5 it is shown that baseline (BSLN) LSI perfusion was 307±90 AU (CV=0.29) which was reduced to 42±8 AU (CV=0.19) during ischemia

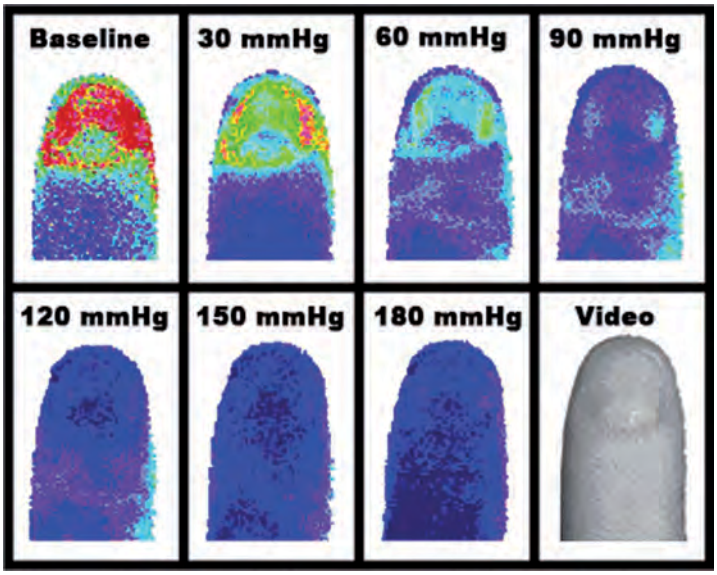

Figure 1. Representative laser speckle imaging perfusion maps for 0 (baseline), 30, 60, 90, 120, 150, and 180 mm Hg occlusion pressure and the corresponding video image. Blue indicates low perfusion and red indicates high perfusion.

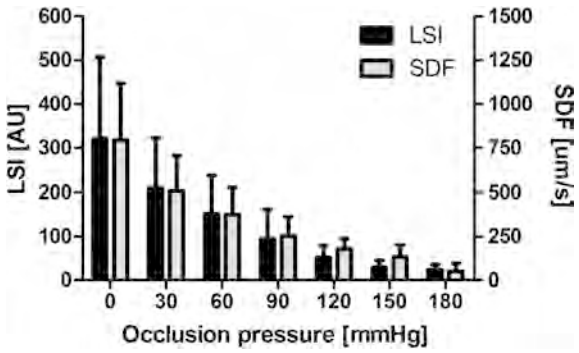

Figure 2. Perfusion data for the laser speckle imaging (LSI) and sidestream dark field (SDF) measurements for each occlusion pressure (0–180mm Hg). LSI data are plotted against the left y-axis and SDF data are plotted against the right y-axis allowing optimal visual comparison of the perfusion data obtained using each technique as a function of occlusion pressure.

(ISCH). Peak perfusion (PEAK) during reperfusion after 3 min of ischemia was 651±93 AU (CV=0.14) (212% of baseline) which decreased to 422±107 AU (CV=0.25) after 1 min of reperfusion (1 min) and was fully restored to baseline level, 309±92 AU (CV=0.30), after 2 min of reperfusion (2 min).

A typical LSI trace during increasing skin temperature is presented in Fig. 6. In Fig. 7 it is shown that tissue perfusion as measured using LSI increased from 332±90 AU (CV=0.27) during normothermia to 1067±256 AU (CV=0.24) (321% of baseline) during hyperthermia. The LSI

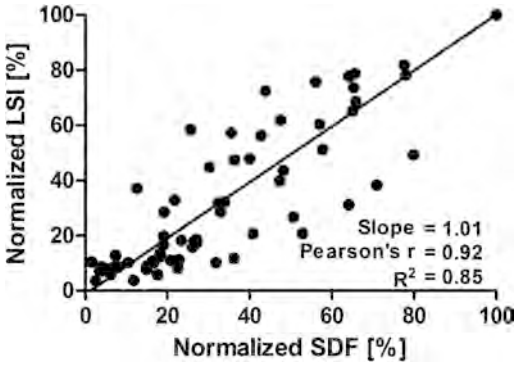

Figure 3. Linear regression analysis on normalized laser speckle imaging (LSI) data versus normalized sidestream dark field (SDF) imaging data. Data were normalized so that baseline = no occlusion pressure = 100%. Pearson's r = 0.92, slope = 1.01, R^2 = 0.85, p < 0.001.

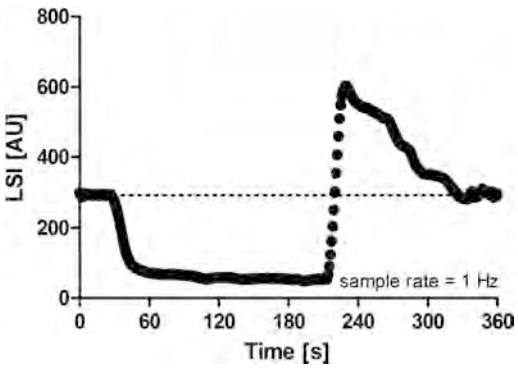

Figure 4. Typical laser speckle imaging (LSI) perfusion trace during the vascular occlusion test.

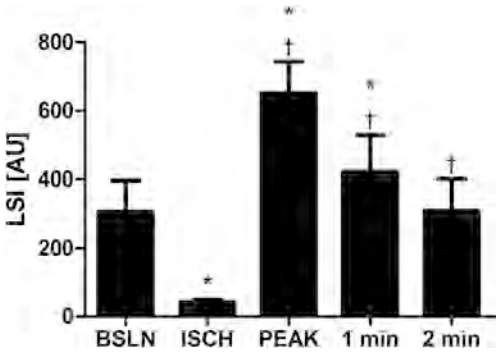

Figure 5. Perfusion changes as measured using laser speckle imaging (LSI) during the vascular occlusion test. BSLN = baseline, ISCH = ischemia, PEAK = peak, 1 min = 1 minute of reperfusion, 2 min = 2 minutes of reperfusion. *p < 0.05 vs. BSLN and †p < 0.05 vs. previous time point.

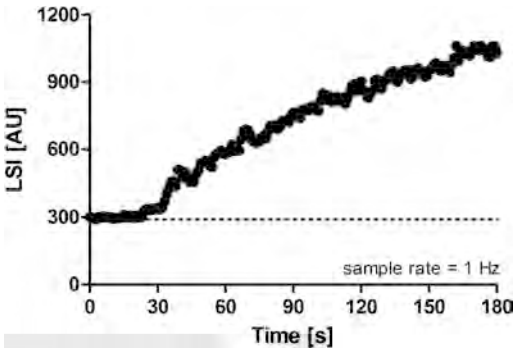

Figure 6. Typical laser speckle imaging (LSI) perfusion trace during heating of the skin, starting at 30 s.

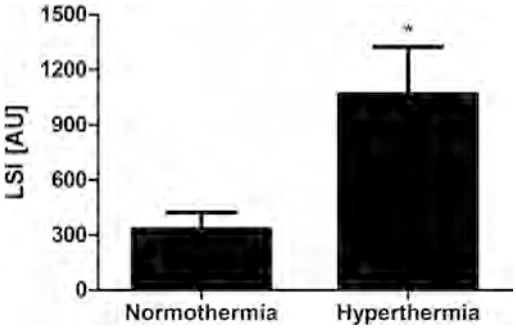

Figure 7. Perfusion changes as measured using laser speckle imaging (LSI) during heating of the skin. *$p < 0.05$ vs. normothermia.

perfusion during hyperthermia was significantly higher than the LSI peak perfusion during reactive hyperemia ($p<0.05$). No statistical differences were identified for the baseline LSI perfusion during the validation experiment, the vascular occlusion test, and the hyperthermic challenge ($p>0.05$) reflecting the reproducibility of LSI measurements of tissue perfusion.

DISCUSSION

The aim of the study was to validate near-infrared LSI for assessing microvascular (re)perfusion by comparison to a technique capable of quantitatively measuring capillary red blood cell velocity, i.e., SDF imaging. The main finding was that changes in perfusion as measured using LSI correlated well with changes in capillary red blood cell velocities as measured using SDF imaging during controlled reduction of the (micro)vascular perfusion. We have furthermore shown that LSI is capable of measuring tissue perfusion in high temporal and spatial resolution and that this technology can be used to assess microvascular reactivity to ischemic and hyperthermic challenges.

This is the first study to validate the use of LSI for the assessment of tissue perfusion at the microcirculatory level by comparison to capillary red blood cell velocity measured quantitatively with SDF imaging. Since SDF imaging allows direct visual observation of red blood cells flowing through individual capillaries, this technique serves as a gold standard for measuring microcirculatory perfusion. Hence, in contrast to LDV, comparison of LSI to SDF measurements constitutes a reliable means to validate LSI responses to alterations in capillary perfusion. During controlled gradual reduction of the nail fold capillary perfusion, LSI and SDF imaging were shown to be in good agreement (correlation and linear regression analysis), indicating that LSI perfusion is a sensitive parameter for the detection of changes in tissue perfusion at the microcirculatory level.

In the validation protocol, the SDF measurements demonstrated a large variability in baseline capillary red blood cell velocity between each volunteer (CV=0.42), which may have been caused by the native perfusion variability between the individuals. Contrastingly, baseline variability in LSI measurements (CV=0.58), although of similar extent as the variability in SDF-derived velocities (Fig. 2), is difficult to interpret due to the fact that LSI measures movement of cells in tissue irrespective of the direction in which they move. The measurement is, among other factors, influenced by the position of the camera with respect to the target tissue. Hence, application of LSI is more appropriate for intra-individual comparison of perfusion (e.g., perfusion at different time points) rather than inter-individual comparison (e.g., perfusion in different patients).

Using LSI in clinical research would overcome some limitations of other commonly used techniques used to measure microcirculatory (re)perfusion, such as SDF imaging, LDV, NIRS, and thermography. Although LSI and SDF imaging were shown to correlate well during controlled reduction of capillary blood flow velocity during the validation protocol, SDF imaging could not be used to quantitatively determine red blood cell velocities during post-occlusive reactive hyperemia. A high red blood cell velocity will result in a vertically rather than diagonally oriented pattern in the space–time diagrams, making it impossible to determine red blood cell velocity (velocity= distance/***Δtime***). On the other hand, LDV as a fiber-based modality is able to measure (micro)vascular perfusion at high speed, but only in a small volume of tissue and is therefore very sensitive to measurement site. As an imaging modality (i.e., scanning LDV), LDV overcomes this shortcoming. However, LDV imaging is a relatively slow technique (typically 10–60 s/image) and therefore not able to detect the rapid perfusion changes during reactive hyperemia.[6] NIRS is a spectroscopic technique for measuring microcirculatory oxygenation and supra-baseline levels of oxygenation following a period of ischemia (i.e., reactive hyperemia).[7] However, since NIRS is measuring oxygenation, this technique provides only limited information on the actual microvascular flow alterations. Thermography, in contrast, is indeed capable of generating images of tissue perfusion at a high frame rate. However, thermography is based on changes in tissue temperature following changes in tissue perfusion and is therefore integrative in both the time- and space-domain. Due to the thermal capacity of the tissue, rapid changes in tissue perfusion do not immediately induce rapid changes in tissue temperature and, furthermore, thermal diffusion limits the spatial resolution of the technique. In this paper we have shown that LSI overcomes these issues and that it is capable of measuring tissue perfusion at both high spatial and temporal resolution at rest and during different provocation tests.

To date, LSI has been applied scarcely in a clinical setting.[11,19,20] In a surgical setting a macroscopic, non-contact technique such as LSI would be preferable over SDF imaging and LDV because the latter techniques are limited by the small measuring surfaces and require physical contact with the tissue surface. Compared to the available macroscopic non-contact imaging techniques such as video thermography and scanning laser Doppler flowmetry, LSI has a better spatial and temporal resolution.[6,11] The high spatial resolution allows for the matching of local perfusion to anatomical characteristics, making it a potentially useful technique for e.g., neuro- , vascular-, and transplantation surgery. Furthermore the high temporal resolution enables the possibility to study responses of the microvasculature to pharmacological interventions during intensive care and surgery.[11,12]

In conclusion, we have shown that LSI responds linearly to changes in red blood cell velocity in the nail fold of healthy volunteers during gradual occlusion. Therefore LSI is a valid modality for measuring microcirculatory perfusion both at rest and during different provocation tests. Additionally, LSI is a macroscopic non-contact imaging technique with a high spatial and temporal resolution, rendering it especially suitable for clinical scenarios where surface contact is undesirable and microcirculatory perfusion in large areas requires online monitoring.

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4

THE EFFECT OF PERFUSION PRESSURE ON GASTRIC TISSUE BLOOD FLOW IN AN EXPERIMENTAL GASTRIC TUBE MODEL

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ABSTRACT

Background

Anastomotic leakage and stricture formation remain an important surgical challenge after esophagectomy with gastric tube reconstruction for cancer of the esophagus. The perfusion of the anastomotic site at the proximal site of the gastric tube depends exclusively on the microcirculation, making it susceptible to hypoperfusion. We hypothesized that increasing the perfusion pressure would improve blood flow at the anastomotic site of the gastric tube.

Methods

A gastric tube was reconstructed in 9 pigs. Laser speckle imaging and thermographic imaging were used to measure blood flow and temperature, respectively, at the base, medial part, future anastomotic site, and top of the gastric tube. Measurements were repeated at every stepwise increase of mean arterial blood pressure (MAP) from 50 to 110 mm Hg.

Results

Besides MAP, global hemodynamics did not change throughout the experiment. The blood flow in the top of the gastric tube was significantly lower than the flow in the base and medial part of the gastric tube at all levels of MAP. Increasing MAP did not have a significant effect on blood flow at any location in the gastric tube. Distribution of temperature was similar to distribution of flow for the different locations. Increases in MAP did not change temperature values at any location of the gastric tube.

Conclusion

Blood flow in the upper part of the gastric tube is decreased compared with more proximal sites. Gastric tissue blood flow does not increase with increased perfusion pressure. Therefore, it is not recommended to increase MAP to supranormal levels to increase anastomotic tissue blood flow and reduce postoperative complications.

INTRODUCTION

Esophagectomy with reconstruction of a gastric tube is associated with significant morbidity, i.e., leakage and stenosis of the gastroesophageal anastomosis.[1] A major cause of impaired anastomotic healing is insufficient local blood flow, which is attributed to poor arterial inflow and venous congestion caused by partial ligation of the vasculature.[2] As a result, the uppermost 20% of the gastric tube is only perfused through the microcirculation.[3]

Several studies have demonstrated that improving venous drainage enhances the blood flow at the anastomotic site.[4-6] However, the effect of arterial perfusion pressure on gastric tube tissue blood flow has never been evaluated. Although increasing arterial blood pressure with the administration of vasoconstrictive drugs does not improve tissue perfusion in tissues of septic patients, it can be hypothesized that in tissue with a severely compromised vascularization, such as the anastomotic site of the gastric tube, increasing perfusion pressure might increase tissue blood flow.[7]

For this purpose, we used laser speckle imaging (LSI), which allows determination of the microvascular blood flow in a large area and with a high sampling rate without any physical contact. In addition, an indirect assessment of tissue blood flow was achieved by temperature measurement of the gastric tube by videothermography. These techniques also allow noncontact measurements in a large tissue area with a high sampling rate, and both techniques are frequently used to reflect tissue perfusion. LSI measures blood flow at a certain depth in the tissue (approximately 0.5–1mm depending on various factors such as hemoglobin content), whereas thermography gives a more global impression of total tissue blood flow, with the blood flow of the most superficial layers (with respect to the position of the camera) contributing most to the surface temperature measurement.[8]

With these techniques, we aimed to investigate the effect of increasing perfusion pressure on blood flow in the gastric tube, with a focus on the future anastomotic site. For this purpose, we used a pig model for gastric tube reconstruction.

METHODS

Animal Preparation

Nine female pigs (Yorkshire, Landrace of Durok) with a mean weight of 30.6 ± 0.6 kg (mean ± sd) were included in this study. The study was approved by the institutional animal investigation committee, and the care and handling of the animals were in accordance with the European Community guidelines. All animals received premedication with azaperone (2.5 mg/kg, IM). Anesthesia was induced with ketamine hydrochloride (30 mg/kg, IM) and midazolam (1 mg/kg, IM). All animals were intubated through a cervical midline tracheostomy and ventilated in a volume-controlled mode (Servo 300, Siemens-Elema, Solna, Sweden). Neuromuscular blockade was induced with pancuronium bromide (0.13 mg/kg, IV), and anesthesia was maintained with a continuous infusion of fentanyl (20 $\mu g \cdot kg^{-1} \cdot h^{-1}$), midazolam (0.3 $mg \cdot kg^{-1} \cdot h^{-1}$), and pancuronium bromide (0.3 $mg \cdot kg^{-1} \cdot h^{-1}$). As maintenance fluid, crystalloid solution was administered at a rate of 10 $mL \cdot kg^{-1} \cdot h^{-1}$. After surgery, hypovolemia was excluded with a fluid challenge consisting of 150 mL of crystalloid solution. When cardiac output (CO) increased by 15%, the animal was considered hypovolemic, and fluid challenges were repeated until CO did not increase further. The left

carotid artery was cannulated for continuous arterial blood pressure monitoring and to obtain blood gas analyses. A pulmonary artery catheter (Edwards, Irvine, CA) was inserted through the right jugular vein for measurements of central venous, pulmonary artery, and pulmonary artery occlusion pressures. In addition, the CO was calculated using a Vigilance CO computer (Edwards). At the end of the experiment, the animals were killed with a bolus of potassium chloride.

Surgical Procedure

After induction of anesthesia, the gastric tube was reconstructed as described by Schröder et al.[9] In brief, after median laparotomy, the stomach was mobilized along the smaller and greater curvature. This included ligation of the 1 or 2 short gastric arteries, the gastric arteries, and the left gastroepiploic artery. The right gastroepiploic artery along the greater curvature was carefully preserved. After opening of the hiatus, the esophagus was dissected and transected at the gastroesophageal junction. Finally, a 3-cm-wide gastric tube was formed by dissection of the lesser curvature and the gastroesophageal junction with a linear stapler (Proximate 55 mm TLC linear cutter, Ethicon, Johnson & Johnson, The Netherlands). In this way, the perfusion of the gastric tube was completely dependent on the right gastroepiploic artery, comparable with the clinical setting.

An important difference with human anatomy is the left dominancy of the gastroepiploic artery.[9] Ligation of the left gastroepiploic artery and short gastric arteries results in profound ischemia of the gastric fundus, limiting the length of the gastric tube. For this reason, it is impossible to perform a transthoracic gastric pull-up to restore continuity in the neck, and measurements were performed with the gastric tube in the abdomen. The gastric tube was carefully positioned on top of the abdominal content and covered to prevent evaporation and cooling.

Experimental Procedures

After construction of the gastric tube, baseline values of blood flow (LSI) and temperature (videothermography) were obtained from 4 positions on the serosal side of the gastric tube: the top (Fig. 1; location A), the virtual anastomotic site (Fig. 1; location B), the medial part (Fig. 1; location C), and the base of the gastric tube (Fig. 1; location D). A small suture was placed centrally in each region of interest to assure that each measurement was taken from exactly the same place. These landmarks correspond with the letters in Figures 1 and 3. Depending on baseline arterial blood pressure, the norepinephrine dose was adjusted to attain a mean arterial blood pressure (MAP) of 50 mmHg. When baseline MAP was higher than the desired values, additional propofol was titrated to decrease MAP. Subsequently, norepinephrine was administered to attain a MAP of 60, 70, 80, 90, 100, and 110 mmHg. After each step of increase in MAP, a 15-min stabilization period was included before both LSI and thermography measurements were performed. The total duration of a single experiment was 6–7 h.

Laser Speckle and Thermographic Imaging and Analysis

LSI was performed with a full-field laser perfusion imager (Moor Instruments, Axminster, UK), which was mounted perpendicular to the gastric tube, at a distance of 30 cm. Scans were

captured with a sampling frequency of 25 Hz. The device uses a Class 1 nearinfrared laser source with a wavelength of 785 ± 10 nm. The charge-coupled device camera incorporates a bandpass filter, which attenuates other wavelengths. The raw speckle images are used to compute the speckle-contrast image. The software calculates the speckle contrast k for any given square of 5 x 5 pixels and assigns this value to the central pixel of the square. This process is then repeated across the image of 576 x 768 pixels to obtain the contrast map. For each pixel in the speckle contrast map, the relative velocity of blood flow is obtained.

The temperature of the gastric tube tissue was registered with a computer-assisted infrared thermograph (ThermaCAM SC2000, Flir Systems, Danderyd, Sweden). The thermal sensitivity is 0.05°C at 30°C, the spectral range 7.5–13 mm, and the built-in digital video 320 x 240 pixels (total 76,800 pixels). Data were obtained through a high-speed (50-Hz) analysis and recording system coupled with a desktop personal computer (ThermaCAM Researcher 2001 HS). Thermograms were stored on a hard disk (14-bit resolution) awaiting further analysis. With an interval of -40°C until 120°C, this results in a resolution of 9.8 x 10^{-3}°C per bit, which fits well in the range of the thermal sensitivity. The thermograph camera produces a matrix of temperature values. These temperature values each represent a pixel in the image measured. The distance between the objective and the gastric tube being measured was adjusted to 68 cm. Thereby, the resolution on the gastric tube was 0.8 x 0.8 mm^2.

Statistics

Values are reported as median and interquartile ranges. Each variable was analyzed using analysis of repeated measurements. When appropriate, *post hoc* analyses were performed using the Dunn multiple comparison test. Comparison of LSI blood flow values and thermographic temperature values was done by linear regression analysis and Pearson correlation analysis. $P<0.05$ was considered statistically significant. All analyses were performed using GraphPad Prism (version 5.0, GraphPad Software, San Diego, CA).

RESULTS

During the stepwise increase of MAP, all other global hemodynamic values remained constant throughout the experiment. Systemic hemodynamic data and norepinephrine dosages after surgery and for each step of MAP are shown in Table 1. The volume of fluids administered was 2170 mL (1830–2177 mL) pulmonary wedge pressures remained constant throughout the experiments, indicating that fluid status remained constant as well.

Laser Speckle Imaging

In Figure 1, the laser speckle images of a typical gastric tube are shown. The gastric tube tissue blood flow is significantly lower in the top than in the base and the medial part of the gastric tube (Fig. 2). MAP levels were categorized as <70 mmHg (hypotension), 70–90 mmHg (normotension), and >90 mmHg (hypertension). Increasing MAP did not have a significant effect on blood flow at any location of the gastric tube.

Table 1. Hemodynamic Data

	BL	50	60	70	80	90	100	110
MAP (mmHg)	80 [69-91]	51 [49-52]	61 [59-63]	71 [70-72]	81 [79-82]	90 [88-90]	100 [100-101]	110 [109-111]
HR (bpm)	120 [99-151]	125 [86-163]	117 [84-156]	126 [98-156]	117 [109-139]	100 [82-117]	106 [77-129]	114 [86-137]
CVP (mmHg)	9 [7-10]	8 [7-9]	10 [9-11]	9 [8-10]	9 [8-10]	10 [9-11]	10 [9-11]	10 [9-10]
PCWP (mmHg)	10 [9-10]	9 [8-10]	10 [9-11]	10 [10-11]	10 [10-11]	11 [9-12]	10 [10-12]	10 [10-11]
CO (L/min)	4.1 [3.7-4.6]	3.8 [3.2-4.8]	3.8 [3.1-4.6]	3.3 [3.3-4.8]	3.9 [3.4-4.4]	4.0 [3.4-4.8]	3.8 [3.1-5.1]	3.9 [3.2-4.9]
SvO_2 (%)	78 [70-91]	78 [63-89]	72 [61-92]	77 [66-91]	77 [67-92]	75 [64-87]	73 [65-91]	78 [59-93]
NE (mcg/kg/min)	-	-	-	-	0.1 [0-0.1]	0.2 [0.1-0.2]	0.4 [0.4-0.5]	0.5 [0.5-0.6]

Values are represented as median and interquartile ranges.

BL = baseline after surgery; MAP = mean arterial blood pressure; HR = heart rate; CVP = central venous pressure; PCWP = pulmonary capillary wedge pressure; CO = cardiac output; SvO2 = venous oxygen saturation; NE = norepinephrine.

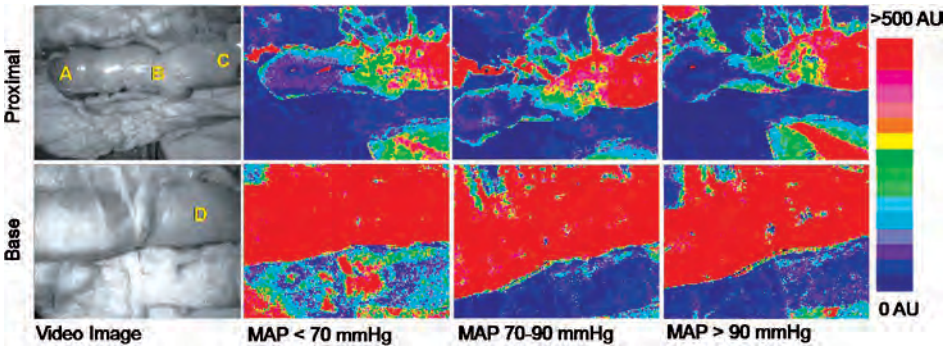

Figure 1. Laser speckle images and video images of the distal part of the gastric tube (upper panel) and base (lower panel) for different values of mean arterial blood pressure (MAP) in a representative pig (#4). Laser speckle images are shown in a color-coded fashion. The colors are related to flow values (in arbitrary units) according to the color bar at the right. A = top; B = anastomosis; C = medial; D = base.

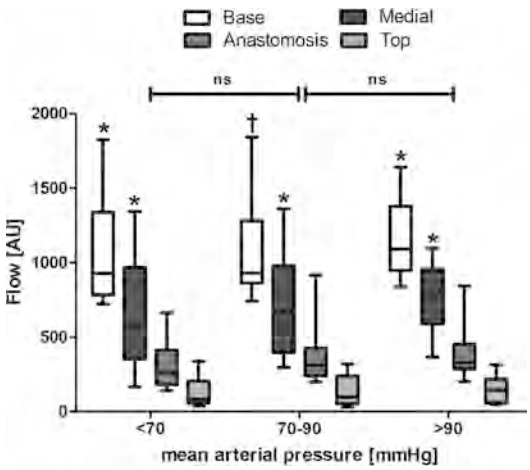

Figure 2. Blood flow for different locations on the gastric tube for different values of mean arterial blood pressure measured with laser speckle imaging. Boxes show median [interquartile range] and bars 5%–95%. †P < 0.01 compared with top; *P < 0.001 compared with top. NS = nonsignificant.

Thermographic Imaging

Figure 3 shows the thermographic images of the gastric tube at the different MAP levels. The size of the black/blue-colored area (lower temperature, less blood flow) even appears to increase at higher MAP levels. Temperature measurements show a similar pattern as LSI, although differences were not significant (Fig. 4). An increase in MAP did not change temperature values at any location.

Flow measured by LSI and temperature measured by thermographic imaging were shown to be positively correlated (Pearson r = 0.84, P<0.001). This was supported by linear regression analysis, as shown in Figure 5 (r^2=0.71).

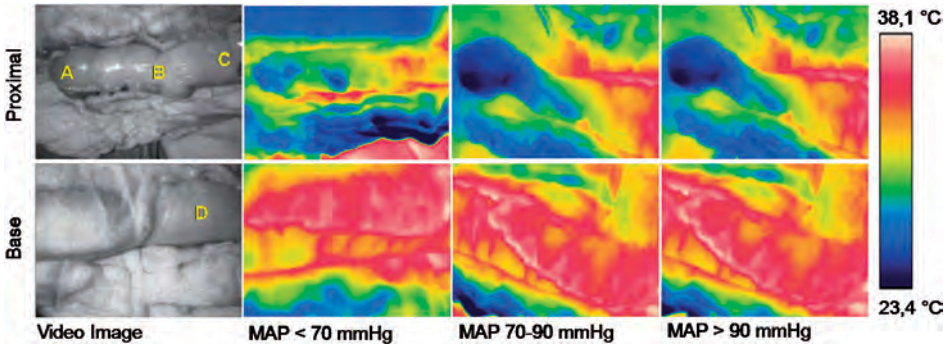

Figure 3. Videothermographic images of the total gastric tube for different values of mean arterial blood pressure (MAP) in a representative pig (#4). The colors are related to temperature values according to the color bar at the right. A = top; B = anastomosis; C = medial; D = base.

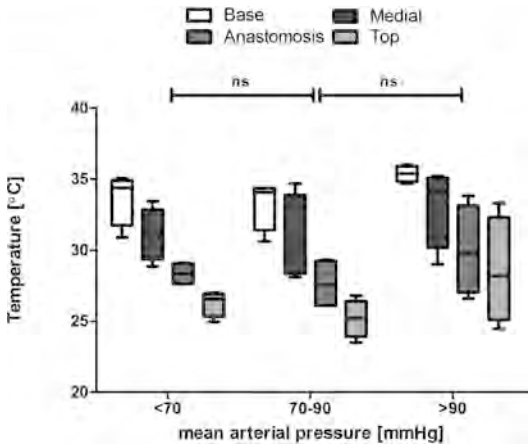

Figure 4. Temperature for different locations on the gastric tube for different values of mean arterial blood pressure measured with thermographic imaging. Boxes show median [interquartile range] and bars 5%–95%. NS = nonsignificant.

DISCUSSION

The results of our study demonstrate that increasing perfusion pressure does not improve the gastric tube tissue blood flow, especially at the site of the future anastomosis, where blood flow is extremely low.

Our results are in accordance with the results in septic patients, in whom no increase in splanchnic perfusion was seen with increasing MAP.[7] However, our model is typical for the local anatomical disruption of the vasculature, which compromises tissue blood flow. It has been shown that improvements can be made either with surgical interventions or by pharmacologically influencing the microvascular bed. Surgical techniques such as "supercharging" the anastomosis of arteries and veins of the gastric tube improve local blood flow, but are technically difficult and

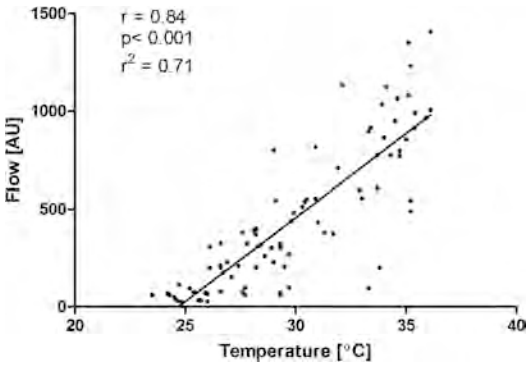

Figure 5. Linear regression and Pearson correlation analysis of flow and temperature.

require an additional operating time of >1 h.[6,10] Studies using pharmacological interventions were able to decrease venous congestion and improve tissue blood flow at the site of the anastomosis.[4,11]

Therefore, the residual function of the vascular bed, although compromised, should be able to supply the top of the gastric tube with sufficient blood. Increasing perfusion pressure, however, does not contribute to an increase in blood flow, as shown by our results. This also implies that venous congestion plays an important role during gastric tube formation, especially when improving tissue perfusion is the aim. This is underlined by the beneficial effect of venous drainage by transient bloodletting on blood flow of the gastric tube.[5] However, Schröder et al. have demonstrated that, regardless of the occurrence of postoperative complications, all gastric tubes had substantially impaired postoperative tissue perfusion.[12] Independent of systemic hemodynamic variables, tissue perfusion recovered to baseline values after 4 days. Considering that this happens in all patients and that anastomotic healing is impaired in only a fraction, it is likely that tissue blood flow is not the only factor to play a role in anastomotic healing.

Our study demonstrates not only that aiming for supranormal arterial blood pressures has no beneficial effects on gastric microvascular blood flow in the anastomotic area but also, maybe even more importantly, that the use of vasoconstrictive substances to induce deliberate short-term hypertension under normovolemic conditions has no acute detrimental effect on already impaired serosal-muscularis gastric tube microvascular blood flow. A previous study has suggested the opposite, but it is very likely that hypovolemic conditions played an important role in those observations.[13] Our results are supported by the study by Banic et al., who demonstrated that increasing arterial blood pressure by 30% with phenylephrine did not have adverse effects on blood flow in free musculocutaneous flaps[14].

Our results emphasize that thermography is a suitable method to evaluate blood flow of the gastric tube during surgery, as is demonstrated by Nishikawa et al.[15] The noninvasiveness of the technique makes it especially suitable in a surgical setting. However, thermography provides a global impression of blood flow, which is attributable to the fact that temperature is a derivate of blood flow and can be influenced by other factors. This could possibly contribute to the variation in the measurements and the fact that there is no significant difference between the

several locations of the gastric tube. Another possible explanation for the increased variation in temperature at supranormal arterial blood pressures could be that thermography measures the result of overall blood flow. It is possible that some heterogeneity occurred, although not significantly, between the different layers of the gastric wall, with LSI measuring exclusively the serosal-muscularis layer and thermography reflecting, in addition, part of the submucosal-mucosal layer. This is underlined by the absence of a linear correlation, which suggests that the 2 techniques do not assess exactly the same depth and perhaps complement each other. Because of different "penetration depths," blood flow changes in the mucosa cannot be excluded. Although LSI and thermography showed a good correlation, we consider LSI to be a more sensitive technique. LSI detects perfusion dynamics in a volume of tissue (approximately 0.5- to 1-mm depth) in a fashion similar to laser Doppler flowmetry (LDF). As a matter of fact, our LSI data are similar to those of previous studies using LDF in the gastric tube.[4,16,17] Although LDF provides sensitive tissue perfusion variables, it measures only small areas, whereas blood flow alterations, especially in the case of gastric tube formation, occur more heterogeneously. Moreover, LDF requires tissue contact for optimal performance, which could compromise reliability by the introduction of a potential pressure artifact and reduce its usefulness during surgery.

Potential limitations of our study, in addition to those concerning the techniques, include differences in the vascular anatomy of the stomachs of humans and pigs, the study's nonrandomized design without washout periods, and the relatively long duration of a single experiment.

In conclusion, we demonstrate in an experimental model of gastric tube formation that the impaired microvascular perfusion in the anastomotic area of the gastric tube after surgery cannot be improved with higher perfusion pressures. Other pharmacological or surgical interventions should be used to improve microcirculatory blood flow of the gastric tube to improve postoperative outcome.

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5

LASER SPECKLE IMAGING IDENTIFICATION OF INCREASES IN CORTICAL MICROCIRCULATORY BLOOD FLOW INDUCED BY MOTOR ACTIVITY DURING AWAKE CRANIOTOMY

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ABSTRACT

Object

The goal of awake neurosurgery is to maximize resection of brain lesions with minimal injury to functional brain areas. Laser speckle imaging (LSI) is a non-invasive macroscopic technique with high spatial and temporal resolution used to monitor changes in capillary perfusion. In this study, the authors hypothesized that LSI can be useful as a noncontact method of functional brain mapping during awake craniotomy for tumor removal. Such a modality would be an advance in this type of neurosurgery since current practice involves the application of invasive intraoperative single-point electrocortical (electrode) stimulation and measurements.

Methods

After opening the dura mater, patients were woken up, and LSI was set up to image the exposed brain area. Patients were instructed to follow a rest-activation-rest protocol in which activation consisted of the handclenching motor task. Subsequently, exposed brain areas were mapped for functional motor areas by using standard electrocortical stimulation (ECS). Changes in the LSI signal were analyzed offline and compared with the results of ECS.

Results

In functional motor areas of the hand mapped with ECS, cortical blood flow measured using LSI significantly increased from 2052 ± 818 AU to 2471 ± 675 AU during hand clenching, whereas capillary blood flow did not change in the control regions (areas mapped using ECS with no functional activity).

Conclusions

The main finding of this study was that changes in laser speckle perfusion as a measure of cortical microvascular blood flow when performing a motor task with the hand relate well to the ECS map. The authors have shown the feasibility of using LSI for direct visualization of cortical microcirculatory blood flow changes during neurosurgery.

INTRODUCTION

In patients suffering from low-grade glioma, the extent of tumor resection has been shown to be an independent prognostic factor for survival.[1,2] However, the preservation of neurological function after surgery is essential for a good quality of life in these patients. Therefore, the goal during these neurosurgical procedures is to maximize tumor resection while minimizing permanent injury to functionally important brain tissue.[3] This strategy emphasizes the critical importance of adequate identification and mapping of eloquent areas of the brain during neurosurgery.

Currently, mapping of functional areas of the brain mainly consists of preoperative fMRI or intraoperative ECS. Electrocortical stimulation mapping is the surgical gold standard for establishing a real-time functional map of the brain surface.[4-6] However, it is a laborious and time-consuming technique that yields occasional false-positives and exposes the patient to the risk of seizures. In fMRI, blood oxygen level changes that influence the MR signal are used to visualize functional brain areas when performing specific tasks, such as finger tapping. This technique relies on the concept of flow metabolism coupling.[7,8] The results off MRI are difficult to interpret during surgery because of brain shift consequent to opening the skull and the large-vessel effect extending the signal toward the draining vessels.[9]

A relatively new technique that may overcome the aforementioned shortcomings is LSI, a macroscopic noncontact imaging technique with high spatial and temporal resolution that directly measures microcirculatory blood flow.[10-12] It is an optical modality that uses a low-power laser diode for illumination of organ surfaces and a grayscale CCD camera for imaging the area of interest at a normal video acquisition rate (for example, 25 Hz). Laser speckle contrast analysis exploits the fact that a random speckle pattern is generated when tissue is illuminated by laser light. The pattern is dependent linearly on the movement of red blood cell flow. The contrast imaging is processed to produce a color-coded live image that correlates with perfusion in the tissue. It is specifically sensitive to microcirculatory flow, and we validated this fact in humans by comparison with microcirculatory flow measurements obtained by direct observation of the nailfold microcirculation.[12] Laser speckle imaging has been extensively tested in animals and humans to measure blood flow in different organs and on surfaces, such as skin, retina, and mucosal surfaces.[13-16] In these settings, LSI has proven to be a simple, safe, and reliable method for continuous online measurement of microcirculatory blood perfusion. To our knowledge, only 2 studies have utilized this imaging technique during neurosurgery, but the technique has yet to be explored for use during awake craniotomy for functional brain mapping.[17,18] The technique is especially suitable for clinical scenarios in which surface contact is undesirable and microcirculatory perfusion in large areas requires online monitoring.

Our aim in the present study was to investigate whether LSI is suitable for mapping functional brain areas during neurosurgical procedures. For this purpose, LSI measurements were performed on brain tissue in patients undergoing awake neurosurgery. To determine the ability of LSI to detect local changes in cortical microcirculatory perfusion, patients were asked to perform a motor task, and the results were compared with ECS mapping data.

METHODS

Patient Population

This study was a prospective, single-center observational study. The medical ethics committee of the Erasmus Medical Center, Rotterdam, approved the protocol. All procedures were performed in accordance with the Helsinki declaration. Written informed consent was obtained from all patients.

All patients with an indication for an awake craniotomy procedure were eligible for inclusion in the study. The indication for an awake procedure at our center is suspicion of a low-grade glioma in or near eloquent brain areas, such as motor or language areas.

Anesthesia Procedure

Patients received 1.5 mg of lorazepam on the evening before surgery. All patients were on a regimen of 8 mg of dexamethasone twice per day, while regular personal drug regimens were continued. Thirty minutes before inducing anesthesia, patients were given 7.5 mg of piritramide and 25 mg of promethazine. Piritramide was used to reduce pain perception during skull infiltration with 40 ml of bupivacaine 0.375% and epinephrine 1:300,000. Additionally, low doses of propofol were used for sedation and remifentanil for analgesia.

We also performed continuous monitoring of electrocardiographic activity, peripheral oxygen saturation, and invasive arterial pressure through the insertion of an indwelling catheter into the radial artery ipsilateral to the lesion.

Surgical and Stimulation Procedure

Craniotomy was performed with the patient's head immobilized in a Mayfield headrest system (OMI, Inc.). A standard protocol was followed for all patients, which included preoperative fMRI for intraoperative neuronavigation, bipolar cortical and subcortical motor mapping, and continuous clinical evaluation of motor responses. A cortical stimulator (Grass Technologies, Astro-Med, Inc.) was used to deliver square-wave pulses to induce depolarization of motor cortices. The intensity of the working current was increased from 6 to 12 mA (60 Hz frequency, 1-msec duration) until a motor response was evoked. Cortical stimulation was considered to be positive when the anesthesiologist and the patient observed a motor response. Areas with a positive response after cortical stimulation were labeled with numbers. When the exposed brain area was stimulated, a photograph of the exposed area was taken, with labels representing the total functional ECS map. This photograph was used for the offline laser speckle analysis.

Experimental Procedure

After the dura was opened, LSI and subsequent electrocortical mapping were performed while the patient was awake. After setting up the LSI system, patients were instructed to perform the following rest-activation-rest protocol for mapping the motor cortex. During baseline measurement, the patient and operating room staff were instructed to be completely silent for 1 minute. Subsequently, the patient was asked to start fiercely contracting his or her fist for a period of 1 minute, followed by a 1-minute period of complete rest and silence.

To correlate patient motor movement with cortical activity registered by the LSI device, we acquired simultaneous images using a synchronized security video camera focused on the patient's hand.

Laser Speckle Imaging and Analysis

Laser speckle imaging was performed with a fullfield laser perfusion imager (Moor Instruments, Ltd.), which was mounted in the craniotomy opening perpendicular to the brain at a distance of 30 cm. The LSI was set for low-resolution high-speed images, with a display rate of 25 Hz, time constant of 1 second, and camera exposure of 20 msec. The device used a Class 1 near-infrared laser source with a wavelength of 785 ± 10 nm. The CCD camera incorporated a band pass filter, which attenuates other wavelengths. The raw speckle images were used to compute the speckle contrast image. Software was used to calculate the speckle contrast k for any given square of 5 × 5 pixels and assigned this value to the central pixel of the square. This process was then repeated across the image of 576 × 768 pixels to obtain the contrast map. For each pixel in the speckle contrast map, the relative velocity of blood flow was obtained.

Although the LSI software provided a real-time flux time curve in specific ROIs, a customized program was written for postacquisition analyses for research purposes. The software was written in MATLAB (The MathWorks, Inc.) by our department (Erasmus Medical Center, Rotterdam, the Netherlands). The software was customized to analyze the changes in microcirculatory blood flow (flux) over time for any selected location of the laser speckle image. The raw images were loaded in the program, which enabled 4 sampling windows to be superimposed on the laser speckle images. The sampling windows could be varied in length and width. Sampling windows were placed on areas depicted as functional brain areas of the hand, as indicated on the photograph of the functional ECS map, which was made with ECS during surgery. For those 4 sampling windows, the program generated flux curves, depicted as line scans of the mean flux in the area of the sampling window over the time course of the total measurement. It also displayed a bar chart reflecting the mean LSI flux in a specified sampling window over a selected interval. Figure 1 features an example of the analysis procedure for the LSI signal.

Statistical Analysis

Values are reported as the means ± standard deviations. Each variable was assessed using analysis of repeated measurements (2-way ANOVA). When appropriate, post hoc analyses were performed using Dunn multiple comparison tests. A $p < 0.05$ was considered statistically significant. All analyses were performed using Prism (version 5.0, GraphPad Software, Inc.).

RESULTS

Eight patients were included in our study. Table 1 shows the baseline demographics and surgical covariates of the study group. In all of the patients, functional motor areas were mapped with ECS. Figure 2 shows an example of the results of ECS, with the white numbered labels representing the locations of mapped functional areas (speech and motor areas). Figure 1 shows the setup for

Table 1: Baseline demographics of the study population.

Patient	Age	Sex	Location	Pathology	[Hb]	ECS confirmed	LSI confirmed
1	29	M	Parietal-occipital right	Low grade astrocytoma	8.9	+	+
2	42	F	Temporal right	Low grade oligodendroglioma	8.6	+	+
3	43	M	Frontal right	Low grade mixed oligo-astrocytoma	7.8	+	-
4	34	F	Frontal-parietal left	Low grade oligodendroglioma	7.2	+	-
5	42	F	Frontal-parietal right	Low grade astrocytoma	7.7	+	+
6	38	F	Temporal left	Glioblastoma multiforme	8.2	+	+
7	41	M	Frontal left	Low grade astrocytoma	9.3	+	+
8	25	F	Frontal-parietal left	Low grade astrocytoma	7.7	+	+

* Hb = hemoglobin.

offline analysis of the LSI signal. We used the ECS topography overview to choose the location of the ROIs and the control regions (with no functional activity during ECS mapping). Changes in cortical blood flow for the entire study population are depicted in the left panel of Fig. 3. In the cortical area involved in manual motor skills mapped by ECS, cortical blood flow increased from 2052 ± 818 AU to 2471 ± 675 AU ($p < 0.001$) during the stimulation protocol. In regions in which no functional motor areas were mapped during ECS (control regions), no changes in blood flow occurred. In the right panel of Fig. 3, changes in blood flow in the functional motor areas and

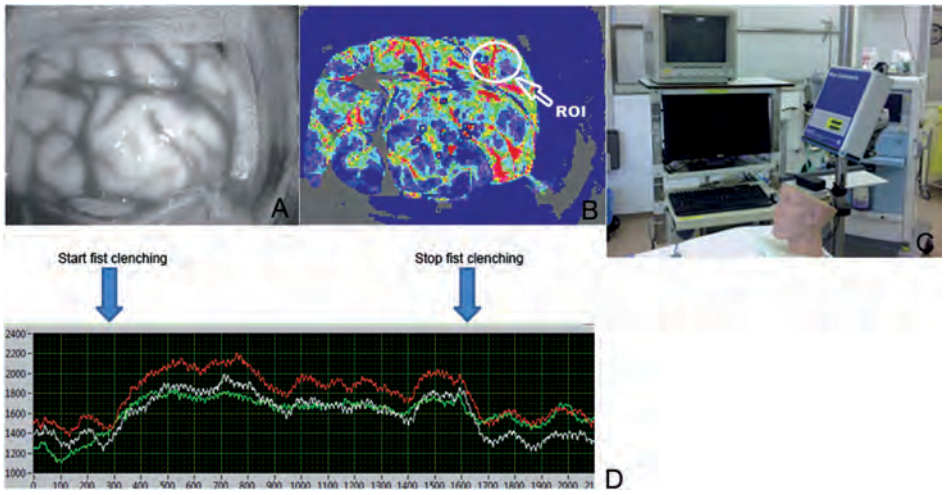

Figure 1. Laser speckle imaging identifies cortical microcirculatory blood flow increase following fist clenching during awake neurosurgery. A: Online black-and-white image seen using LSI. This image allows direct identification of the morphological location of flow changes. **B:** Processed online image in pseudo color showing areas of high flow *(red)*. **C:** Photograph of operative setting. **D:** Offline analysis of sequences of images identified the ROI in panel B as being that part of the cortex responsive to motor activity. Analysis of the changes in contrast in this area allows quantification of the response to fist clenching to be measured (each color is 1 subarea of the area of interest shown).

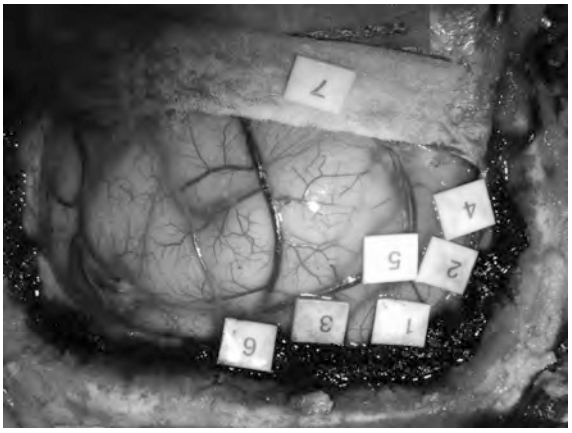

Figure 2. Photograph of the total ECS map of the brain. White labels with numbers represent the functional brain areas mapped with ECS (including motor areas).

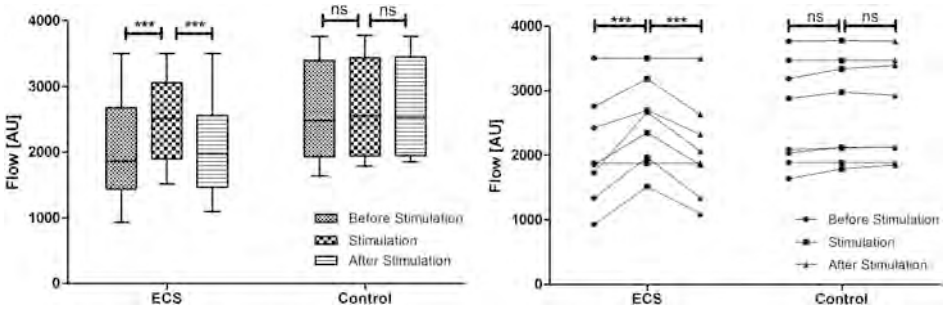

Figure 3. Graphs showing perfusion data for LSI measurements before, during, and after stimulation for the ECS-confirmed and control ROIs. Data for the entire group are represented (left), as are the LSI measurement data for individual patients (right). ***p < 0.001 versus the previous time point. ns = nonsignificant.

control areas are represented for individual patients. In 6 of the 8 patients, a significant increase in cortical blood flow was seen during the stimulation protocol. In addition to the offline analyses, local changes in cortical blood flow in the functional motor areas of the hand could be visualized intra-operatively in the color-coded laser speckle images (Fig. 4). We did not observe any changes in mean arterial pressure, heart rate, or peripheral oxygen saturation during the experiments.

DISCUSSION

In this study, for the first time, LSI was applied for functional mapping of cortical perfusion in awake patients during resections of low-grade gliomas. The changes in laser speckle perfusion, as a measure of cortical microvascular blood flow when performing a motor task, correlated with the ECS mapping data. This correlation was demonstrated objectively by

Figure 4. Representative laser speckle perfusion imaging maps before (A) and during (B) the hand motor task with the corresponding video image (C). Blue indicates lower perfusion, and red represents higher perfusion. Circled regions represent areas of interest (ECS-confirmed areas).

significant changes in laser perfusion blood flow in areas determined to be functional motor areas by ECS during hand clenching as compared with control regions (offline analyses). Furthermore, we saw visible changes in the color-coded laser speckle images when the motor task was performed during surgery. A specific advantage of LSI is the ability to superimpose morphological images of the cortex surface on functional perfusion images, allowing direct and detailed neurosurgical identification of functional locations on the brain during surgery. In addition, it is a rapid-response imaging modality, which allows direct and instant assessment of cortical perfusion changes to motor or speech activity. Other functional imaging modalities that might be used during awake craniotomy for functional neurosurgery, such as infrared thermography and Doppler imaging, do not have such advantages.

To date, LSI has been used mainly in preclinical experimental research settings and rarely has been translated to human research. Most human studies utilizing LSI have assessed changes in skin perfusion in different pathological states.[14,15,19] Only one of these studies has used LSI to study cerebral blood flow in humans. Hecht et al. studied 3 patients who underwent a cerebral revascularization procedure for aneurysm treatment.[17] These authors concluded that LSI was a suitable technique for assessing bypass graft patency with high spatial resolution. Several experimental studies have already been focused on neurovascular coupling in the rat somatosensory cortex.[20,21] Royl et al. performed LSI of the rat somatosensory cortex during a functional challenge via electrical forepaw stimulation.[22] They found a correlation between LSI-measured cerebral blood flow and the underlying neuronal activity measured using the somatosensory evoked potentials recorded with epidural electroencephalography. These authors concluded that LSI is a reliable method of visualizing local changes in cerebral blood flow and suggested that it may be more accurate than oxygen-related methods, such as fMRI, in localizing neuronal activity. In several other experimental studies on cerebral blood flow, LSI has been shown to provide high-resolution images of the spatiotemporal dynamics of cerebral blood flow.[11,23] However, none of these studies has addressed the question of whether LSI can be used to identify functional brain areas.

Because our study was designed as a feasibility study, it does not allow exact calculation of the spatial and temporal resolution in our setting; however, the spatial and temporal resolutions of LSI have been extensively studied elsewhere. Durduran et al. demonstrated that LSI can effectively measure, map, and characterize cerebral blood flow's response to electric somatosensory forepaw and hindpaw stimulation with high temporal and spatial resolution.[21] Their stimulus protocol was adapted to exactly calculate temporal resolution. They placed subdermal electrodes and used a mode of triggering that allowed an exact coregistration at the time of data acquisition and stimulation, permitting exact calculation of temporal resolution. In our setting this coregistration was much more difficult given the nature of the stimulation, that is, asking the patient to start fist clenching. Therefore, exact timing of the start of the motor task was not possible.

Using LSI in clinical research would overcome some limitations of other commonly used techniques for measuring cerebral blood flow and brain function. Fiber-based laser Doppler flowmetry enables reliable measurement of vascular perfusion but only in a small volume of tissue, requiring physical contact with the tissue surface.[24] As a scanning modality, laser Doppler

flowmetry does overcome this shortcoming but is a relatively slow technique and therefore cannot detect rapid perfusion changes.[25] Thermography can generate tissue perfusion at a high frame rate but is based on changes in tissue temperature, and rapid changes in tissue perfusion do not immediately induce changes in temperature; thus, thermography is limited in its temporal resolution.[26] Near-infrared spectroscopy measures microvascular oxygenation; therefore, it offers only limited information on actual flow alterations and can only provide measurements in a small volume of tissue.[27] The newly developed full-field laser Doppler imaging is a promising technique but is still limited by small imaging areas, making it more laborious to visualize the total exposed brain surface, and it cannot yet provide real-time data.[28] We have shown that LSI is capable of measuring microvascular tissue perfusion at rest and during different provocation tests and is related to capillary blood flow, as assessed by direct microscopic observation.[12]

In clinical practice, intraoperative ECS mapping is routinely combined with preoperative fMRI to aid maximal resection of tumors located near functional brain areas. Although it is the gold standard, ECS is a time-consuming method of mapping different functional brain areas and carries the risk of afterdischarge activity, such as seizures.[29] Alternatively, preoperative fMRI is noninvasive, but the potential loss of spatial validity from intraoperative displacement and deformation of the brain during surgery and the large-vessel effect extending the signal toward the draining vessels give it limited value in the precise intraoperative detection of functional cortex areas.[30] Although LSI cannot be used for preoperative mapping, it does overcome most of the intraoperative drawbacks of ECS and fMRI. The technique has the advantage of providing visualization of video images combined with functional blood flow images, making it possible to accurately couple functional with anatomical images in a noncontact manner during surgery. In our setting we performed an offline analysis to determine the exact magnitude and location of increases in blood flow and to confirm our intraoperative observations. However, changes in local blood flow can be directly observed during surgery because the contrast imaging is processed to produce color-coded live images that correlate with perfusion in the tissue. This processing is done and can be visualized directly on screen using the software supplied by the manufacturer.

Despite these promising results, some limitations must be addressed. First, only 6 of our 8 patients showed changes in blood flow with LSI. The non-responders can be explained by multiple factors, which must be ruled out in control experiments. One factor could be inter-subject variability in task execution. A second factor is probably that electrophysiological activation to motor stimulus is more sensitive and has a higher resolution than blood flow changes to motor stimulation. Currently, LSI is not a substitute for the already applied techniques of mapping but could instead be used for rapid and initial characterization, which may reduce the duration of the operation by its instant mapping. If LSI does not respond, conventional time-consuming electrophysiological examination can be performed. A third factor could be blunting of the cerebral blood flow response via the effects of drugs used during the procedure. A second limitation of our study was that we only tested the ability of LSI to visualize functional motor cortex corresponding to the hand. Future studies must be conducted to assess whether LSI is useful in mapping other functional areas in the cortex, such as somatosensory and language areas in humans. Given the limited penetration depth of LSI (< 1 mm), it is only possible to study

exposed areas of the brain; therefore, LSI is a technique that can only be used during surgery. Because of its limited penetration depth, LSI yields information about the cortical surface alone. Additionally, given that the measurement is influenced by, among other things, the position of the camera with respect to the imaged tissue, the application of LSI is more appropriate for intra-individual than inter-individual comparisons of perfusion maps.

CONCLUSIONS

In this study, we demonstrated that LSI can be used to identify increases in cortical microcirculatory blood flow induced by motor activity during awake neurosurgical procedures. Using LSI as a macroscopic noncontact method to visualize cortical microcirculatory blood flow intra-operatively would overcome some of the limitations of ECS. Further research should be conducted to determine whether LSI could eventually replace ECS to map functional brain areas during surgery. It is anticipated that LSI will enter the realm of neurosurgery as a noninvasive online assessment of cerebral blood flow during neurosurgical procedures.

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PART C
MICROCIRCULATION AND ITS RELATION TO VOLUME STATUS

6

POSTURAL CHANGE IN VOLUNTEERS: SYMPATHETIC TONE DETERMINES MICROVASCULAR RESPONSE TO CARDIAC PRELOAD AND OUTPUT INCREASES

Clinical Autonomic Research (in press)

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ABSTRACT

Purpose

Microvascular perfusion may be a non-invasive indicator of fluid responsiveness. We aimed to investigate which of the microvascular perfusion parameters truly reflects fluid responsiveness independent of sympathetic reflexes.

Methods

Fifteen healthy volunteers underwent a postural change from head up tilt (HUT) to supine position, diminishing sympathetic tone, followed by a 30° passive leg raising (PLR), with unaltered tone. Prior to and after the postural changes, stroke volume (SV) and cardiac output (CO) were measured as well as sublingual microcirculatory perfusion (sidestream dark field imaging), skin perfusion and oxygenation (laser Doppler flowmetry and reflectance spectroscopy).

Results

HUT to supine posture change increased CO, SV and pulse pressure, while heart rate, systemic vascular resistance and mean arterial decreased, in the responders (≥ 10% increase in CO). In the latter, microvascular flow index, laser Doppler flow, microvascular hemoglobin oxygen saturation and concentration increased. During PLR, only CO, SV and sublingual functional capillary density increased in responders (≥ 5% increase in CO), whereas systemic vascular resistance decreased less than from HUT to supine.

Conclusion

When preload and forward flow increase in addition to diminishing sympathetic activity, microvascular blood flow in the skin as well as the sublingual area increase. When preload and forward flow increase with little altering of sympathetic activity, only sublingual functional capillary density increases. The latter is thus the best parameter to evaluate fluid responsiveness independent from changes in sympathetic tone in future studies.

INTRODUCTION

In the critically ill, non-invasively obtained microcirculatory perfusion abnormalities measured sublingually or cutaneously are associated with a poor outcome, relatively independent of global hemodynamics.[1] They can be ameliorated by fluid infusion and, in fact, perfusion abnormalities may be surrogate indicators of fluid responsiveness, i.e. an increase in cardiac output (CO)/ stroke volume (SV) with cardiac preloading, suggesting that systemic blood flow still feeds the microcirculation in the critically ill.[2,3] Sympathetic activation that may result in peripheral vasoconstriction may partly explain the sometimes observed discrepancy between global and microvascular hemodynamics in the critically ill.[4,5] Changes in sympathetic tone concomitantly with changes in volemic status (eg decreasing sympathetic tone by fluid infusion) may thus confound microvascular perfusion abnormalities to reflect global hemodynamics and resultant tissue oxygenation remote from the sublingual or cutaneous microvasculature.

Because of the potential detrimental effects of fluids in critically ill patients, the concept of fluid responsiveness is an important guide for fluid administration. Fluid does not necessarily increase SV and CO, because of the curve-linearity of the Frank-Starling curve. When the heart is operating on the steep part of the curve an increase in preload results in an increase in stroke volume (i.e. responders). If the heart is operating on the distal part of the curve an increase in preload will not result in an increase in SV (i.e. non-responders).

Head up tilting (HUT) is used in healthy volunteers and patients with vagal syncope to diminish preload and activate the sympathetic nervous system, so that the reverse positioning is expected to lower sympathetic activity.[6] The head up tilt to supine position induces a large preload challenge as during the postural change approximately 500 ml of pooled blood redistributes to the central circulation.[7,8] The passive leg raising (PLR) test is a frequently used test in clinical practice which also induces a preload challenge due to redistribution of blood, however in a smaller amount (150 - 300 ml).[9,10] This test may not alter, or in a much lesser degree than HUT, sympathetic activity, at least in sedated patients, with little alterations of carotid and baroreceptor positioning, and is more likely to increase than decrease sympathetic activity. Comparing the two maneuvers in microvascular perfusion parameters may thus give insight into the true parameters associated with fluid responsiveness independent of alterations in sympathetic tone.

We hypothesized that the site and type of microvascular perfusion response to a cardiac preload challenge depends on sympathetic tone alterations, e.g. peripheral vasodilation with diminished activity. Therefore, in the current study, we focused on microvascular effects of increasing preload with and without altering sympathetic activity in healthy volunteers. We aimed to investigate which of the microvascular parameters can be used to truly reflect a preload challenge and resultant increase in forward flow, so called fluid responsiveness, by studying effects of two postural changes known to establish a preload challenge but with different effects on the autonomic nervous system. We used the sublingual and cutaneous microvascular beds because these are the most easily accessible and most studied microvascular beds in critically ill patients.

PATIENTS AND METHODS

Subject population

This study was performed in the Erasmus MC University Medical Center (Rotterdam, The Netherlands) and was approved by the local ethics committee. Written informed consent was obtained in each case. We studied 15 healthy volunteers (9 men, 6 woman; median age 27 yrs [23-28.5]) with no history of cardiovascular disease or the use of any vaso-active medication. The phase of menstrual cycle of the female subjects at the time of the study was not recorded. Subjects were recruited from the Erasmus University of Rotterdam.

Protocol

Study measurements took place in a quiet, temperature-controlled room and were performed in four stages. Subjects were comfortably restrained on an electric tilt table with footplate support. Following the positioning of the non-invasive recorders, subjects underwent a passive head up tilt to an angle of 70°, and remained tilted for 5 minutes after which baseline measurements were made. Subjects were returned to the supine position (0°) and measurements were repeated after 5 minutes. Subjects were placed in a regular hospital bed, were positioned in a semirecumbent position of 30°, all measurement equipment was recalibrated and measurements were made. Secondly, a PLR test was performed, resulting in a 0° supine position of the thorax and the legs elevated 30°, for five minutes, as described previously and measurements were repeated.[11] The different positions are demonstrated in Figure 1. During these postural changes both hands (one used to measure peripheral perfusion, other used to measure global hemodynamics) were passively immobilized at heart level using a sling.

Measurements of systemic hemodynamics

Global hemodynamic parameters included heart rate (HR), CO, SV, mean arterial pressure (MAP) and pulse pressure (PP) which were continuously measured non-invasively with a Finometer® (Finapres Medical System, Amsterdam, the Netherlands). The Finometer gives waveform measurements similar to intra-arterial recordings and computes beat-to-beat hemodynamic

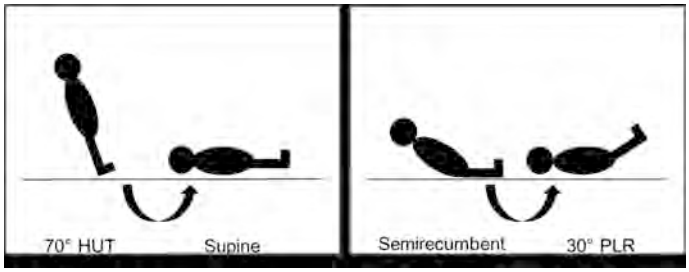

Figure 1. Postural changes. 70° head up tilt (HUT) to supine posture change induces a large preload challenge which redistributes approximately 500 mL of pooled blood to the central circulation. The semirecumbent position to 30° passive leg raising (PLR) induces a smaller preload challenge which redistributes approximately 150-300 mL of pooled blood to the central circulation.

parameters including CO and SV. The Finometer measures brachial pressure and corrects for finger pressure accordingly. Finger arterial pressure was measured using a finger cuff (on the left hand index finger) in combination with an infrared plethysmograph consisting of a light source (infrared light-emitting diode) and a light detector (infrared photodiode).[12] Systemic vascular resistance (SVR) was calculated using the following formula: SVR = MAP/CO * 80 (dyn.sec.cm-5).

Sidestream dark field imaging (SDF)

Sublingual microvascular blood flow was evaluated using SDF (MicroVision Medical, Amsterdam, The Netherlands). Image acquisition and subsequent analyses were performed according to published consensus criteria.[13] In brief, after removal of saliva with gauze the device was gently applied to the sublingual area by investigators well trained in SDF imaging. For each stage five sequences of 20 seconds from different adjacent areas were recorded. The sequences were stored under a random number and later analyzed according to the recent consensus with dedicated software (Microcirculatory Analysis Software (MAS 3.0) Academic Medical Center, Amsterdam). Microvascular flow index (MFI) was calculated after dividing each image into four equal quadrants. Quantification of flow was determined using an ordinal scale (0, no flow; 1, intermittent flow; 2, sluggish flow; 3, normal flow; 4, hyperdynamic flow;.[14] MFI is the average score of all quadrants for a given time point. Vessel density was calculated, according to the consensus, in two manners. First, functional capillary density (FCD) was calculated by measuring total length of perfused capillaries divided by image area. Second, vessel density (VD) was calculated by inserting a grid of three equidistant horizontal and three equidistant vertical lines over the image. VD is equal to the number of vessels crossing these lines divided by their total length. Flow was then categorized as present, intermittent or absent, allowing calculation of the proportion of perfused vessels (PPV). To determine the intrarater variability of the sublingual microvascular parameters, the complete image analysis on 90 SDF sequences was repeated at a later time point. The intrarater variability of the SDF analysis was determined by calculating a intraclass correlation coefficient on consistency, considered good when ≥0.6. The intraclass correlation for MFI was 0.75, for FCD 0.72, for VD 0.73 and for PPV 0.93.

Laser Doppler flowmetry (LDF) and reflection spectrophotometry (RS)

LDF and RS were performed using an O2C device (Oxygen to See, LEA Medizintechnik GmbH, Giessen, Germany). The tissue was illuminated with a pulsed 830 nm class 1 laser diode and the backscattered light was spectrally analyzed to assess the velocity-dependent frequency shifts caused by flowing red blood cells. The microvascular hemoglobin oxygen saturation (μHbSO2) and relative hemoglobin concentration (rHb) were measured by illuminating tissue with visible white light (500-630 nm), which is backscattered and changed in color according to its O2 saturation. The mean flow and μHbSO2 was recorded and averaged over a steady-state period of 1 minute.

Statistical analysis

Statistical analyses were performed in SPSS (version 19.0, SPSS, Chicago, IL, USA). Non-parametric test were used because of relatively small numbers, even though variables were

mostly normally distributed. Intragroup comparisons were done with help of the Wilcoxon matched pairs test. Comparison between groups was done with a Mann-Whitney U test. To correct for the different magnitudes in change of CO during the HUT to supine and PLR the data was normalised for the change in CO. Therefore the changes in parameters of microvascular perfusion during the HUT to supine or PLR were divided by the changes in CO during the corresponding postural change. To test if the changes in parameters of microvascular perfusion corrected for CO differed between the two postural changes we compared the for CO normalized changes in parameters of microvascular perfusion during HUT to supine with these changes during PLR using a Wilcoxon matched pairs test. We defined fluid responsiveness by an increase in CO ≥10 and 5% for HUT to supine and PLR, respectively, because of their difference in preload augmentation. In critically ill patient fluid responsiveness is defined as an increase in CO of > 10% following a fluid bolus of 500 ml, therefore we used this cut off value for HUT to supine, as approximately 500 ml of blood is redistributed to the central volume.[15] Data are summarized by mean ± standard deviation. A P value <0.05 was considered statistically significant. Exact P values are given.

RESULTS

Effects of HUT to supine position

Table 1 shows global hemodynamics and microvascular perfusion values during the HUT to supine position in responders (n = 12) defined by a ≥10% increase in CO and non-responders (n = 3). There were no differences in hemodynamic and microvascular perfusion parameters between responders and non-responders in the HUT position. The change from HUT to supine increased

Table 1: Hemodynamics and microvascular perfusion values during HUT and supine position, in responders (R) and non-responders (NR) based on a ≥10% increase in cardiac output to the posture change.

	Responders (n=12)			Non-responders (n=3)		
	HUT	**Supine**	**P_1**	**HUT**	**Supine**	**P_2**
Heart rate (bpm)	78 ± 7	67 ± 8	0.002	77 ± 4	61 ± 3	0.11
Stroke volume (ml)	59 ± 16	85 ± 20	0.002	73 ± 14	91 ± 22	0.11
Mean arterial pressure (mmHg)	107 ± 15	96 ± 8	0.028	104 ± 15	103 ± 15	0.66
Pulse pressure (mmHg)	44 ± 4	48 ± 5	0.023	46 ± 8	50 ± 10	0.11
Systemic vascular resistance (dyn.s.cm^{-5})	2049 ± 772	1438 ± 384	0.002	1523 ± 268	1555 ± 348	1.00
Microvascular flow index (AU)	2.8 ± 0.4	3.0 ± 0	0.034	3.0 ± 0	3.0 ± 0	0.32
Vessel density (1/mm)	9.2 ± 1.1	9.9 ± 0.6	0.099	8.9 ± 2.1	9.7 ± 1.8	0.11
Proportion of perfused vessels (%)	95 ± 9	100 ± 0.5	0.063	100 ± 0	100 ± 0	1.0
$\mu HbSO_2$ (%)	65 ± 10	70 ± 9	0.009	70 ± 16	77 ± 5	0.11
rHb (AU)	37 ± 8	46 ± 7	0.005	48 ± 11	57 ± 13	0.29

P1; HUT vs supine position in R, P2; HUT vs supine position in NR. μHbSO2: microvascular hemoglobin oxygen saturation, rHb: relative hemoglobin concentration, AU: arbitrary units. Mean ± standard deviation.

CO, SV and PP, while HR, SVR and MAP decreased, in the responders. Additionally, MFI, LDF, μHbSO2 and rHb increased. In the non-responders, systemic hemodynamics as well as regional tissue perfusion parameters did not change. The change in SVR following HUT to supine position differed between responders and non-responders (P=0.021). Figure 2 shows the changes in CO, LDF and FCD during the HUT to supine position in responders and non-responders.

Effects of PLR

Table 2 shows the global hemodynamic and microvascular perfusion values during the PLR, in responders (n = 6) defined by a ≥5% increase in CO and non-responders (n = 9). There were no differences in hemodynamic an microvascular perfusion parameters between responders and non-responders in the semirecumbent position. During the PLR, CO, SV and FCD increased in responders, while SVR decreased. In the non-responders, systemic hemodynamic and microvascular perfusion parameters remained unaltered. The change in SV (P=0.001), SVR (P=0.001) and FCD (P=0.018) during PLR differed between responders and non-responders. Figure 2 shows the changes in CO, LDF and FCD during the PLR in responders and non-responders. An increase in CO of ≥5% to a PLR did not predict responsiveness of HUT to supine position postural change (sensitivity 42%, specificity 66%), suggesting that the preload effect of the two maneuvers on CO was modulated by different effects on afterload and/or contractility.

Global hemodynamics with HUT to supine vs PLR

The increase in CO and SV were greater with HUT to supine than with PLR (P=0.008); the decrease in HR (P=0.001) and SVR (P=0.006) were greater too. In responders to PLR, the decrease in HR was greater with HUT to supine than with PLR (P=0.028), at similar increase in CO.

Table 2: Hemodynamics and microvascular perfusion values during passive leg raising (PLR), in responders (R) and non-responders (NR) based on a ≥5% increase in cardiac output to PLR.

	Responders (n=6)			Non-responders (n=9)		
	Baseline	**PLR**	**P_1**	**Baseline**	**PLR**	**P_2**
Heart rate (bpm)	64 ± 6	66 ± 10	0.75	65 ± 9	65 ± 11	0.17
Stroke volume (ml)	76 ± 15	85 ± 15	0.028	85 ± 13	81 ± 17	0.21
Mean arterial pressure (mmHg)	99 ± 11	96 ± 9	0.25	96 ± 8	91 ± 9	0.051
Pulse pressure (mmHg)	45 ± 6	45 ± 6	0.35	45 ± 8	42 ± 8	0.09
Systemic vascular resistance (dyn.s.cm^{-5})	1716 ± 494	1430 ± 348	0.030	1429 ± 170	1443 ± 189	0.37
Microvascular flow index (AU)	3.0 ± 0.1	3.0 ± 0.1	1.0	3.0 ± 0	3.1 ± 0.1	0.08
Vessel density (1/mm)	9.5 ± 1.0	10.1 ± 1.4	0.12	10.0 ± 1.3	9.9 ± 1.2	0.68
Proportion of perfused vessels (%)	100 ± 0	100 ± 0	1.0	99.7 ± 0.9	99.6 ± 1.3	0.66
$\mu HbSO_2$ (%)	65 ± 9	68 ± 11	0.46	68 ± 12	67 ± 14	0.52
rHb (AU)	39 ± 8	44 ± 5	0.12	40 ± 7	39 ± 9	0.77

P1; Baseline vs PLR in R, P2; Baseline vs PLR in NR. μHbSO2: microvascular hemoglobin oxygen saturation, rHb: relative hemoglobin concentration, AU: arbitrary units. Mean ± standard deviation.

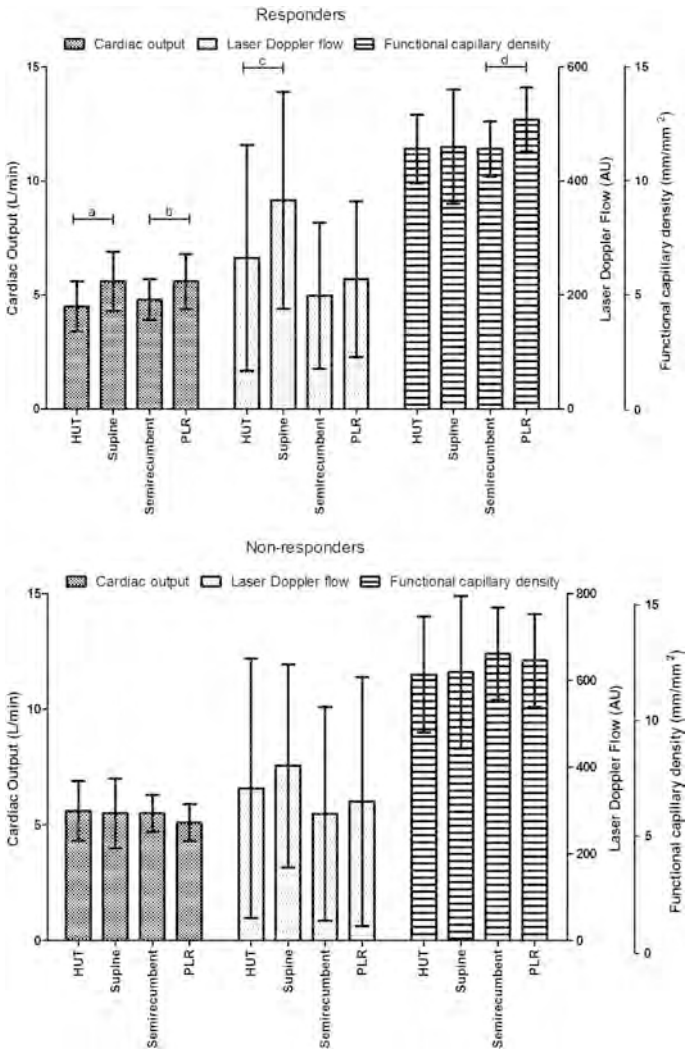

Figure 2. Changes in cardiac output (CO), laser Doppler flow (LDF) and functional capillary density (FCD) during HUT to supine and PLR posture change. The upper panel represents the results for the responders and the lower panel for the non-responders. (a; P=0.002, b; P=0.027, c; P=0.003, d; P=0.046). Mean ± standard deviation.

Microvascular effects normalized for CO

Table 3 shows the changes in microvascular perfusion values adjusted for the change in CO during HUT to supine and PLR in responders (n = 12) and non-responders (n = 3) based on a ≥10% increase in CO to the posture change from HUT to supine. In responders, the for CO- adjusted change in microvascular flow index increased during HUT to supine as compared to PLR. In responders, there is a trend to an increased functional capillary density during PLR as compared to the HUT to supine maneuver.

Table 3: Changes (Δ) in microvascular perfusion values adjusted for change of cardiac output (CO) during head up tilt to supine (HUT) and passive leg raising (PLR) (e.g. change in microvascular flow index during HUT divided by change in CO during HUT) in responders (R) and non-responders (NR) based on a ≥ 10% increase in CO to the posture change from HUT to supine.

	Responders HUT (n=12)			Non responders HUT (n=3)		
	HUT	**PLR**	**P_1**	**HUT**	**PLR**	**P_2**
Microvascular flow index (AU)	0.24 ± 0.42	-0.08 ± 0.24	0.037	-0.1 ± 0.23	0.9 ± 1.6	0.18
Δ Functional capillary density (mm/mm^2)	-0.09 ± 3.0	6.4 ± 22	0.060	-7.5 ± 13.5	3.0 ± 3.3	0.29
Δ Vessel density (1/mm)	0.61 ± 1.4	1.2 ± 3.1	0.43	2.3 ± 5.1	-7.5 ± 5	0.11
Δ Proportion of perfused vessels (%)	5.7 ± 11.1	-1.1 ± 5.6	0.18	0 ± 0	0 ± 0	1.0
Δ Laser Doppler flow (AU)	96 ± 94	241 ± 616	0.75	341 ± 323	-196 ± 277	0.11
Δ $\mu HbSO_2$ (%)	6 ± 8	36 ± 115	0.81	36 ± 55	-4 ± 20	0.11
Δ rHb (AU)	10 ± 8	3 ± 40	0.75	109 ± 96	-7 ± 29	0.11

P1; changes HUT vs changes PLR in R, P2; changes HUT vs changes PLR in NR. μHbSO2: microvascular hemoglobin oxygen saturation, rHb: relative hemoglobin concentration, AU: arbitrary units. Mean ± standard deviation.

DISCUSSION

Our proof-of-principle study suggests that in healthy subjects the two postural maneuvers (HUT to supine and PLR) are not comparable in their effects on systemic and microvascular perfusion variables. When cardiac preload and forward flow increase in addition to diminishing sympathetic activity (i.e. postural change from HUT to supine) as evidenced by a greater decrease in heart rate and vascular resistance than evoked by PLR, microvascular perfusion, i.e. blood flow to the skin as well as blood flow to the sublingual area, increase. When cardiac preload and forward flow increase without or only mildly altering sympathetic activity (i.e. PLR), as evidenced by an unchanged heart rate and a small decrease in systemic vascular resistance, only sublingual functional capillary density increases. Therefore our results indicate that sublingual functional capillary density is the best parameter to use when evaluating fluid responsiveness independent from alterations in sympathetic tone.

The HUT maneuver has been extensively used to study orthostatic hypotension and autonomic failure.[6,16] There is only scarce data on microcirculatory perfusion during HUT, however. A recent study in children using near infra-red spectroscopy demonstrated a decrease in regional tissue oxygenation in the splanchnic region when the subject was tilted.[17] A study measuring femoral as well as brachial blood flow using ultrasound Doppler demonstrated a decrease in blood flow in the upper and lower limbs during a 60° HUT.[8]

The differences in systemic hemodynamic and microvascular perfusion responses following the HUT to supine postural change and PLR test could be explained by the fact that HUT initially leads to a decreased blood volume in the central circulation and baroreflex mediated increases in peripheral vascular resistance to maintain arterial blood pressure, that reverses during subsequent repositioning.[8,18,19] The difference in HR and SVR response between the HUT to supine posture change and the PLR underlines the fact that sympathetic activity decreases following the HUT to

supine posture change and exhibits little change during the PLR. This differential effect may also explain the imperfect overlap in cardiac output responses to HUT to supine and PLR maneuvers.

The HUT serves as a model for central hypovolemia, while in semirecumbent position the subject is supposed to be normovolemic. The preload challenge from HUT to supine was of larger magnitude than the PLR test explaining the differences in effect on hemodynamic as well as regional tissue perfusion parameters. We therefore, studied microvascular responses for equal changes in CO responses. This data shows that when the microvascular perfusion parameters are normalized for the changes in CO there is still a trend in increase in sublingual functional capillary density during the PLR compared with the HUT to supine.

Although this was a proof-of-principle study several limitations need to be addressed. First, we have used a low number of study subjects, making it difficult to study potential gender differences and subgroup comparisons, especially in the HUT to supine posture change were the number of non-responders was low. Additionally, our study population consisted of young subjects, it is not clear if our results can be extrapolated to an older population. Third, we have not accounted for the possibility of relative hypovolemia, we have assumed our study population was normovolemic due to normal fluid intake of every subject before start of the study.

CONCLUSION

When cardiac preload and forward flow increase with diminishing sympathetic activity (i.e. postural change from HUT to supine) microvascular perfusion indices in skin as well as the sublingual area increase. When only preload and flow increase without, or in a much lesser degree than HUT to supine, altering sympathetic activity (i.e. PLR test) sublingual functional capillary density is increased. Therefore our results indicate that sublingual functional capillary density is the best parameter to use when evaluating fluid responsiveness independent of reflex activity of the autonomic nervous system.

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7

MICROVASCULAR PERFUSION AS A TARGET FOR FLUID RESUSCITATION IN EXPERIMENTAL CIRCULATORY SHOCK

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ABSTRACT

Objective

To study regional perfusion during experimental endotoxemic and obstructive shock and compare the effect of initial cardiac output-targeted fluid resuscitation with optimal cardiac output-targeted resuscitation on different peripheral tissues.

Design

Controlled experimental study.

Setting

University-affiliated research laboratory.

Subjects

Fourteen fasted anesthetized mechanically ventilated domestic pigs.

Interventions

Domestic pigs were randomly assigned to the endotoxemic (n = 7) or obstructive shock (n = 7) model. Central and regional perfusion parameters were obtained at baseline, during greater than or equal to 50% reduction of cardiac output (T1), after initial resuscitation to baseline (T2), and after optimization of cardiac output (T3).

Measurements and Main Results

Regional perfusion was assessed in the sublingual, intestinal, and muscle vascular beds at the different time points and included visualization of the microcirculation, measurement of tissue oxygenation, and indirect assessments of peripheral skin perfusion. Hypodynamic shock (T1) simultaneously decreased all regional perfusion variables in both models. In the obstructive model, these variables returned to baseline levels at T2 and remained in this range after T3, similar to cardiac output. In the endotoxemic model, however, the different regional perfusion variables were only normalized at T3 associated with the hyperdynamic state at this point. The magnitude of changes over time between the different vascular beds was similar in both models, but the endotoxemic model displayed greater heterogeneity between tissues.

Conclusions

This study demonstrates that the relationship between the systemic and regional perfusion is dependent on the underlying cause of circulatory shock. Further research will have to demonstrate whether different microvascular perfusion variables can be used as additional resuscitation endpoints.

INTRODUCTION

Systemic hemodynamic variables, such as blood pressure, heart rate, and cardiac output, are routinely used as indicators of shock severity and target for intervention. Accordingly, it is well known that inadequate systemic circulation in hypodynamic and hyperdynamic shock states may also be accompanied by impaired tissue perfusion.[1] Especially in septic shock, impaired vasomotor tone and the disturbed regulation of regional tissue perfusion lead to microcirculatory dysfunction and a regional mismatch of tissue oxygenation.[2,3] Even when systemic hemodynamics are normalized, microcirculatory alterations can be present and may impair tissue perfusion and oxygenation.[4] Monitoring of regional tissue perfusion is therefore of prime importance to assess the adequacy of resuscitation. The development of new techniques that directly visualize the microcirculation,[5] measure tissue oxygenation (Sto_2),[6] or determine peripheral perfusion[7] has improved the evaluation of the effect of various hemodynamic interventions in different peripheral tissues. The current Surviving Sepsis Campaign guidelines recommend early structured resuscitation that targets systemic hemodynamic variables, which underscores the importance of the early identification and resuscitation of tissue perfusion alterations.[8] Although early fluid resuscitation protocols have been demonstrated to improve outcomes in patients with severe sepsis,[9-11] still little is known about the use of regional tissue perfusion variables as a direct target for resuscitation. This is remarkable because microcirculatory abnormalities are more prevalent in nonsurvivors after circulatory shock due to cardiac arrest,[12] following cardiogenic shock,[13] and after septic shock.[14] Because these abnormalities recover over time only in survivors,[15] the question rises if early optimization of circulatory conditions (i.e., cardiac output) could exert a beneficial effect on microvascular perfusion. This is supported by the positive effects of early goal-directed therapy on outcome in acute sepsis patients.[16] However, it is as yet unknown whether microvascular perfusion abnormalities in the early phase of circulatory shock are caused by relatively insufficient systemic hemodynamic resuscitation or by local microvascular pathology. For this purpose, we examined the effect of cardiac output resuscitation on different microvascular perfusion and peripheral perfusion variables during different endotoxemic and obstructive shock states. We hypothesized that only in endotoxemic shock, microvascular and peripheral perfusion abnormalities would persist after the resuscitation of systemic hemodynamics to preshock levels and that these abnormalities would be remedied with additional resuscitation of cardiac output in a different magnitude for the different peripheral tissues and the different regional perfusion variables.

MATERIAL AND METHODS

The local animal experimental committee approved these studies in accordance with the National Guidelines for Animal Care and Handling. Fourteen female pigs (Yorkshire, Landrace of Durok) with a mean body weight ± SD of 30.2 ± 2.1 kg were included in our study.

Animal preparation

Animals were fasted overnight with free access to water and premedicated with intramuscular ketamine (30 mg/kg) and midazolam (1 mg/kg). IV access was obtained by cannulation of an ear vein. Anesthesia was maintained with a combination of IV midazolam (0.2 mg/kg bolus followed by 0.3 mg/kg/hr) and propofol (2 mg/kg/hr). Analgesia was provided with continuous administration of fentanyl (20 μg/kg/hr) and supplemented with bolus doses (20–50 μg) when needed. Muscle relaxation was obtained with pancuronium bromide (0.1 mg/ kg bolus followed by 0.2 mg/kg/hr).

Through a midline cervical tracheostomy, an endotracheal tube (9.0F) was placed in the trachea. Ventilation was performed in a volume-controlled mode (Servo 300; Siemens-Elema, Solna, Sweden) with a minimal oxygen-in-air fraction of 0.40, frequency adjusted to achieve normocapnia, and a positive end-expiratory pressure of 5 cm H2O to prevent atelectasis. Core temperature was monitored by the thermistor at the tip of the pulmonary artery catheter and was maintained at approximately 37°C with a heating pad or external cooling if necessary. Normoglycemia (arterial blood glucose level 4.5–7 mmol/L) was maintained throughout the whole experiment using 20% glucose infusion as needed.

Catheters were placed in the right carotid artery to measure arterial blood pressure and collect arterial blood samples and in the right jugular vein to administer fluid. A pulmonary artery thermodilution catheter (Edwards Lifesciences, Irvine, CA) was positioned in the pulmonary artery. With the pig in supine position, a urinary catheter was placed into the bladder for drainage of urine. In the animals randomized to the obstructive shock group, the pericardial sac was accessed through the midline incision, after removal of the xiphoid. After making a small opening in the pericardium, a purse-string suture was placed with polydioxanone 4.0 and a 8.0F tube. Urinary catheter was inserted in the pericardium. The purse-string suture was knotted watertight, fixing the catheter to the pericardium. When all surgical procedures were completed, the abdominal wall was closed and the animal was covered in blankets to minimize heat loss.

Different regional perfusion measurements

Regional perfusion was assessed using different microvascular perfusion and peripheral perfusion variables. Accordingly we evaluated microvascular perfusion in the sublingual, intestinal, and muscle vascular beds using a sidestream dark field (SDF) imager (Microscan; Microvision Medical, Amsterdam, the Netherlands) as described previously. The SDF imager contains a × 5 stroboscopic light-emitting diode whose light is absorbed by hemoglobin of the RBCs, making the capillaries of the microcirculation visible in different tissues. To obtain access to the intestinal tissue, a loop of the ileum was exteriorized through a 5-cm lateral abdominal incision, and a small ileum segment was opened along its antimesenteric border using electrocautery. With the mucosal surface facing upward, the edges of the opened intestinal segment were fixed at the skin, creating a double-lumen ileostomy. In the proximal part of this ileostomy, the microcirculation was visualized with the SDF imager at 5–7 cm from the margin of the loop. In between measurements, the ileostomy was protected with moisturized wet gauze to prevent desiccation. To obtain a view on muscle microcirculation, a skin incision was made in

the lateral side of the upper hind leg, and the tip of the SDF imager was cautiously positioned on the exposed muscle tissue. Muscle tissue was protected with moisturized wet gauze to prevent desiccation. The sublingual microcirculation was assessed by placing the tip of the SDF imager gently on the sublingual mucosa, avoiding the midline region and the salivary glands.

An investigator, blinded to the study model and the order of sequences, analyzed the video images semiquantitatively.[17] Vascular flow (i.e., microcirculatory flow index, MFI) and density (i.e., perfused capillary density, PCD and; proportion of perfused vessels, PPV) were calculated by the following flow classification: no flow, sluggish, intermittent, or continuous using dedicated software (Automated Vascular Analysis, AVA 3.0, Microvision Medical, Amsterdam, the Netherlands).[18] This approach has been validated previously with low interobserver and intraobserver variability. Capillaries were defined as microvessels with a diameter less than or equal to 20 µm.

Using near-infrared spectroscopy (NIRS), Sto_2 was continuously monitored using an InSpectra Tissue Spectrometer Model 650 (Hutchinson Technology, Hutchinson, MN) in the different peripheral vascular beds. Sublingual and intestinal Sto_2 were assessed with a 15-mm probe. Simultaneously, sublingual Sto_2 was determined by placing the NIRS probe under the base of the tongue and the intestinal Sto_2 by inserting the probe into the distal part of the ileostomy, which was closed and reinserted into the abdominal cavity afterward. Muscle Sto_2 was assessed with a multidepth NIRS probe attached over the biceps femoris muscle eminence.

Peripheral perfusion was assessed using the proximal-to-distal leg (peripheral Tskin-diff) and central-to-distal leg (central Tskin-diff) skin temperature gradients, which were obtained from two skin probes that were attached to the proximal and distal leg as estimates of peripheral skin perfusion. Central temperature was observed by inserting the tip of the thermistor into the rectum. Temperature gradients can better reflect changes in cutaneous blood flow than the absolute skin temperature itself, and they increase during vasoconstriction.[19]

Hemodynamic measurements

Heart rate, mean arterial pressure (MAP), central venous pressure (CVP), pulmonary artery occlusion pressure, mean pulmonary artery pressure, and cardiac output were measured. Cardiac output was continuously monitored using a thermodilution method (Vigilance; Edwards Lifesciences), indexed to body surface area, and presented as a cardiac index.[20] Mixed venous hemoglobin oxygen saturation (SvO2) from the pulmonary catheter and arterial blood gases were measured using a blood gas analyzer (ABL 825FLEX, Radiometer, Copenhagen, Denmark). Systemic oxygen delivery, systemic oxygen consumption, and the oxygen extraction ratio were calculated using standard equations.[21]

Experimental procedure

The pigs were randomly assigned to the endotoxemic or obstructive shock model after baseline measurements were obtained (Fig. 1). Shock initiation (T1) was defined as a decrease in cardiac output of approximately 50% compared with baseline in both models. Endotoxemic shock was induced by an IV infusion of a bolus of 5 µg/kg lipopolysaccharide (LPS—*Escherichia coli* O127:B8, L-3880; Sigma, St. Louis, MO) in 1 minute, followed by a continuous infusion of 2 µg/

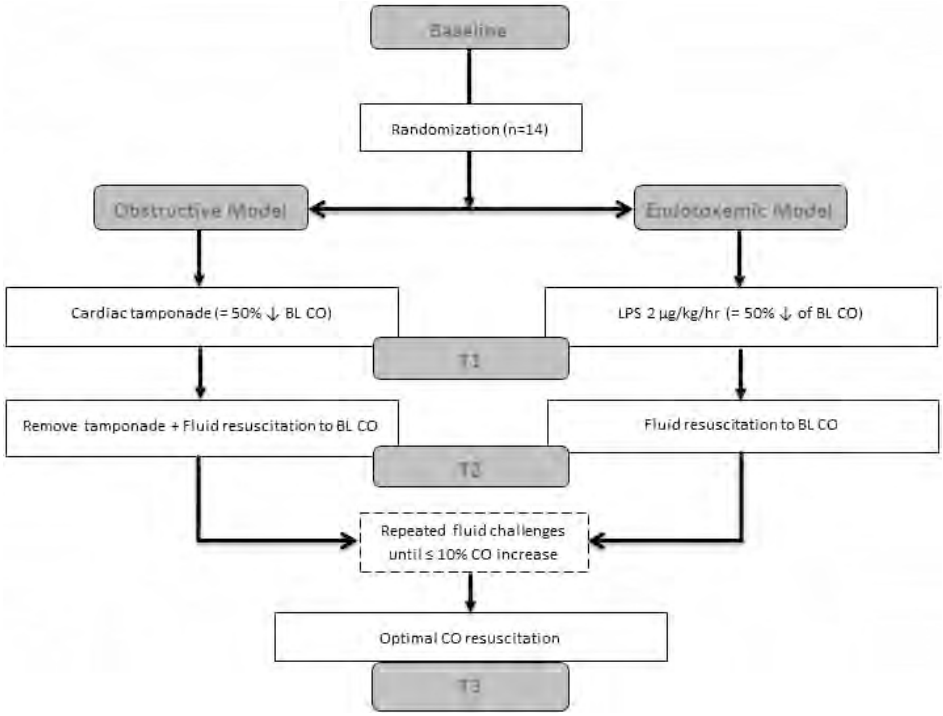

Figure 1. Overview of model classification and hemodynamic support. Obstructive shock is induced with cardiac tamponade, and endotoxemic shock is induced with lipopolysaccharide (LPS). BL = baseline, CO = cardiac output, T1 = 60 minutes after shock state, T2 = 60 minutes after successful resuscitation back to BL, T3 = 60 minutes after CO was optimized.

kg/hr during the rest of the experiment. Obstructive shock was induced by infusing 0.9% saline solution of 37°C (via knotted catheter in the pericardium) into the pericardial space.[22]

Initial resuscitation (T2) was performed by the withdrawal of pericardial fluid content and additional fluid administration in the obstructive model, if necessary, and with continuous fluid administration to achieve baseline cardiac output values in the endotoxemic model. Finally, in both models, optimal fluid resuscitation was continued with repeated fluid challenges until cardiac output increased no more than 10% (T3). Fluid resuscitation was performed with an infusion of colloidal fluids (Voluven, Fresenius-Kabi, Belgium). All systemic hemodynamic variables, metabolic state, and peripheral microcirculatory perfusion variables were collected after a stable 60-minute period once each physiological endpoint was reached in both models.

Statistical analysis

Data are presented as mean ± SD unless otherwise specified. After logarithmic conversion of not normally distributed data (Kolmogorov-Smirnov test $p > 0.05$) to normalize distributions where appropriate, data were suitable for mixed-model analysis. Accordingly, we used a linear mixed-model analysis to assess differences in the different systemic hemodynamic variables and

between the different microvascular perfusion and peripheral perfusion variables, over time (BL, T1, T2, and T3), and between models (endotoxemic shock vs obstructive shock). Changes from baseline were computed dividing the variable value at specific time points into the baseline value and expressed as percentile changes (% of baseline × 100). We then compared the magnitude of changes between models (endotoxemic shock vs obstructive shock) for cardiac output and the different microvascular perfusion variables (i.e., MFI, PCD, and PPV) for the different vascular beds at specific time points to evaluate the effect of resuscitation on these variables. Differences between model means were compared using nonparametrical Mann-Whitney U test or Student t test, if normally distributed. A p value of less than 0.05 was considered statistically significant. Note that because the distribution of the percentile changes often showed differences in the variances between the groups and considerable skewness, these results have to be interpreted cautiously. Statistical analysis was performed using SPSS (version 20.0; IBM, SPSS, Chicago, IL).

RESULTS

Systemic hemodynamics

Systemic hemodynamic and oxygenation variables at each of the three equivalent time points in both models are presented in Table 1. The baseline characteristics were similar in both models, and shock initiation (T1) produced a similar decrease in cardiac output in both models (Fig. 2). Accordingly, MAP and Svo2 decreased at T1 ($p < 0.05$ vs all time points). This was paralleled by a marked reduction in systemic oxygen delivery and an increase in oxygen extraction rate.

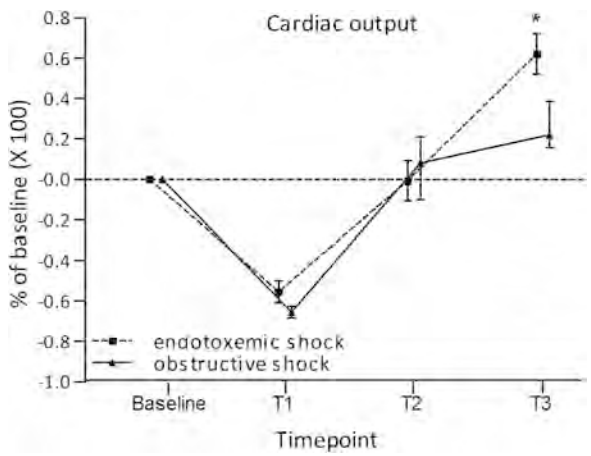

Figure 2. Behavior of cardiac output during the experimental procedure. Cardiac output plotted as proportional changes from baseline for each time point for both models. Following T2, IV fluid resuscitation was maintained to maximize cardiac output, until no more than 10% increase in cardiac output was observed (T3). At this stage, cardiac output reached significative higher values in the endotoxemic shock model (dashed line) compared with the obstructive shock model (continuous line) (*p<0.05 vs obstructive shock by Mann-Whitney test). *Initial volume resuscitation (T2) normalized cardiac output in both models. Following additional fluid resuscitation (T3) however, cardiac output reached significantly higher values in the endotoxemic model compared with the obstructive model.*

Table 1. Evolution of systemic hemodynamic variables over time in the endotoxemic shock model (n = 7) and the obstructive shock model (n = 7)

Variables	Shock model	Baseline	T1	T2	T3
Cardiac index (L/min·m²)	Endotoxemic	2.6 (0.5)	1.2 (0.3) [a]	2.6 (0.5)	4.4 (0.6)
	Obstructive	2.3 (0.3)	0.9 (0.2) [a]	2.4 (0.3)	2.8 (0.5) [a, b]
Heart rate (beats/min)	Endotoxemic	106 (29)	200 (12) [a]	150 (29) [a]	137 (23) [a]
	Obstructive	98 (17)	215 (31) [a]	137 (28) [a]	90 (48) [b]
Mean arterial pressure (mm Hg)	Endotoxemic	110 (16)	71 (27) [a]	95 (22)	118 (30)
	Obstructive	112 (18)	61 (15) [a]	95 (17)	108 (18)
Central venous pressure (mm Hg)	Endotoxemic	8 (2)	8 (4)	10 (2) [a]	14 (3) [a]
	Obstructive	8 (3)	15 (6) [a, b]	10 (6)	13 (6) [a]
Pulmonary artery occlusion pressure (mm Hg)	Endotoxemic	9 (3)	16 (6) [a]	13 (8)	13 (9)
	Obstructive	10 (3)	11 (7)	11 (6)	11 (8)
Mean pulmonary artery pressure (mm Hg)	Endotoxemic	24 (9)	39 (7) [a]	41 (6) [a]	43 (3) [a]
	Obstructive	23 (7)	25 (5) [b]	23 (3) [b]	26 (7) [b]
Fluid volume (mL)	Endotoxemic	688 (74)	223 (127) [a]	778 (112)	1425 (117) [a]
	Obstructive	1022 (171)	190 (74) [a]	398 (114) [a, b]	492 (78) [a, b]
Urine output (mL)	Endotoxemic	121 (51)	57 (45) [a]	20 (31) [a]	35 (23) [a]
	Obstructive	262 (108) [b]	55 (19) [a]	90 (54) [a, b]	88 (74) [a]

T1 = during ≥ 50% reduction of cardiac output, T2 = after initial resuscitation to baseline, T3 = after the optimization of cardiac output.
[a] $p < 0.05$ versus baseline.
[b] $p < 0.05$ between models (endotoxemic and obstructive) in the specific time point, by linear mixed-model analysis.
Data are presented as mean ± SD.

Concurrently, lactate concentrations increased almost five-fold, and urine output decreased significantly in both models (Tables 1 and 2).

Initial volume resuscitation (T2) normalized cardiac output, MAP, and Svo2 in both models. Systemic oxygen delivery and oxygen extraction rate returned to pre-shock levels. Following additional fluid resuscitation (T3) however, cardiac output reached significantly higher values in the endotoxemic model compared with the obstructive model (+65% vs +22% of baseline, respectively, p= 0.001) (Fig. 2). As a result, systemic oxygen delivery increased (p= 0.007) and the oxygen extraction rate decreased further compared with T2. Notably, systemic oxygen consumption, which had remained unchanged as yet, increased as well at this point. Lactate clearance was comparable between both models after initial resuscitation (obstructive shock, -18% [0.01] vs endotoxemic shock, -20% [0.14]). After additional resuscitation, lactate persisted to elevate in the endotoxemic model, whereas in the obstructive model, lactate decreased further with 50% (0.06); (p= 0.007 vs endotoxemic shock). Additional fluid had been administered to achieve optimal cardiac output, although this fluid did not produce a significantly higher cumulative fluid balance compared with the obstructive model (3,110 [1,035 mL] vs 2,100 [528 mL]; p= 0.062, respectively). Still, CVP increased in both groups.

Table 2. Oxygenation and metabolic variables in endotoxemic and obstructive shock

Variables	Shock model	Baseline	T1	T2	T3
Hemoglobin concentration (mmol/mL)	Endotoxemic	6.8 (0.3)	8.5 (0.6) [a]	6.3 (0.7)	5.3 (0.9) [a]
	Obstructive	7.2 (0.4)	8.8 (0.5) [a]	7.2 (1.0)	5.6 (1.2) [a]
Lactate (mmol/L)	Endotoxemic	1.1 (0.6)	5.5 (1.6) [a]	4.0 (2.1) [a]	3.6 (1.6) [a]
	Obstructive	0.7 (0.1)	5.1 (1.5) [a]	4.2 (1.2) [a]	2.1 (0.8) [a, b]
Oxygen delivery (ml/min/m^2)	Endotoxemic	501.5 (144.6)	331.3 (100.5) [a]	513.3 (125.3)	731.9 (183.6) [a]
	Obstructive	521.6 (48.1)	211.9 (24.7) [a, b]	546.9 (75.6)	505.5 (86.7) [b]
Oxygen consumption (ml/min/m^2)	Endotoxemic	173.6 (77.7)	177.7 (20.2)	231.2 (128.3)	267.0 (102.2) [a]
	Obstructive	195.4 (47.6)	180.1 (27.5)	218.4 (33.9)	183.1 (54.1)
Oxygen extraction rate (ml/min/m^2)	Endotoxemic	0.33 (0.15)	0.56 (0.11) [a]	0.44 (0.14)	0.36 (0.07)
	Obstructive	0.37 (0.08)	0.85 (0.06) [a, b]	0.40 (0.03)	0.38 (0.09)
Arterial pH	Endotoxemic	7.52 (0.05)	7.30 (0.05) [a]	7.32 (0.06) [a]	7.36 (0.02) [a]
	Obstructive	7.50 (0.03)	7.36 (0.09) [a]	7.37(0.05) [a]	7.42 (0.05) [a, b]
PaO_2 (mm Hg)	Endotoxemic	229 (102)	103 (29) [a]	104 (32) [a]	121 (43) [a]
	Obstructive	214 (41)	149 (53)	183 (20) [b]	184 (24) [b]
$PaCO_2$ (mm Hg)	Endotoxemic	34 (5)	44 (9) [a]	43 (5) [a]	42 (6) [a]
	Obstructive	34 (4)	34 (9)	36 (6)	36 (6) [b]
Bicarbonate concentration (mmol/L)	Endotoxemic	27.9 (0.9)	20.7 (1.3) [a]	20.8 (2.0) [a]	22.0 (2.5) [a]
	Obstructive	27.7 (0.7)	21.0 (2.9) [a]	21.8 (1.2) [a]	23.2 (1.1) [a]
Mixed venous oxygen saturation (%)	Endotoxemic	61 (7)	42 (12) [a]	53 (12)	60 (10)
	Obstructive	63 (9)	14 (4) [a, b]	60 (3)	62 (7)
Central temperature(°C)	Endotoxemic	38.8 (0.3)	38.8 (0.3)	39.0 (0.3)	38.7 (0.5)
	Obstructive	38.9 (0.4)	38.6 (0.2)	38.8 (0.3)	38.5 (0.3)

T1 = during ≥ 50% reduction of cardiac output, T2 = after initial resuscitation to baseline, T3 = after the optimization of cardiac output.
ap < 0.05 versus baseline.
bp < 0.05 between models (endotoxemic and obstructive) in the specific time point, by linear mixed-model analysis.
Data are presented as mean ± SD.

Regional perfusion

The time course of the different microvascular perfusion variables, observed with the SDF imager, is presented in Figure 3. At baseline, there was no statistical difference between the different microvascular perfusion variables and between both models. All these variables decreased simultaneously in a similar magnitude during shock in both models and increased toward baseline levels in the obstructive model at T2 and remained in this range after T3. However, in the endotoxemic model, microvascular variables increased after initial resuscitation, and they did not reach baseline values. These variables improved toward baseline levels only after additional resuscitation of cardiac output at T3. Hence, although the PCD (*n*/mm2) increased significantly during this stage compared with T2, this index was lower than the baseline values in the intestinal tissue (T3, 21.8 [1.22] vs baseline, 23.2 [0.64]; p= 0.040) (Fig. 3B) and for PPV (%)

in the sublingual (T3, 98.5 [0.47] vs baseline, 99.8 [0.13]; p= 0.034) and intestinal tissue (T3, 93.2 [1.72] vs baseline, 99.2 [0.32]; p= 0.014) (Fig. 3C).

Changes in the peripheral Tskin-diff and central Tskin-diff are presented in Table 3. Similar to the different microvascular perfusion variables (i.e., MFI, PCD, and PPV), these different peripheral perfusion variables exhibited the same magnitude of change at T1 in both models (Table 3). Peripheral Tskin-diff returned to baseline values after T2 and remained in this range after T3 in the obstructive model but not in the endotoxemic model. In this model, peripheral Tskin-diff only increased toward baseline levels after additional fluid resuscitation of cardiac output. Central Tskin-diff followed the same pattern of changes as peripheral Tskin-diff during resuscitation, but these changes were not significant. Additionally, Sto_2 followed an identical pattern of change as the other microvascular perfusion and peripheral perfusion variables in the obstructive shock model (Table 3). In the endotoxemic model however, StO_2 was similarly decreased during shock but persisted following initial resuscitation and additional resuscitation of cardiac output.

DISCUSSION

This experimental study demonstrated that the correction of cardiac output to normal levels is adequate for resuscitation of the peripheral circulation in different tissues after obstructive shock but not after endotoxemic shock. In the endotoxemic model, only additional cardiac output-targeted fluid resuscitation could further improve microvascular and peripheral perfusion abnormalities. These

Table 3. Tissue perfusion variables in three different vascular beds.

Variables	Shock model	Baseline	T1	T2	T3
Tissue oxygen saturation					
Sublingual Sto_2	Endotoxemic	66 (9)	33 (16) [a]	44 (9) [a]	52 (9) [a]
	Obstructive	74 (7)	29 (9) [a]	64 (12) [b]	66 (6) [b]
Intestinal Sto_2	Endotoxemic	78 (12)	53 (14) [a]	64 (16) [a]	66 (15) [a]
	Obstructive	76 (12)	37 (6) [a, b]	72 (13)	77 (9)
Muscle Sto_2	Endotoxemic	69 (2)	37 (6) [a]	53 (4) [a]	56 (7) [a]
	Obstructive	68 (6)	27 (9) [a, b]	61 (8) [a, b]	63 (9)
Tskin-diff					
Tskin-diff peripheral	Endotoxemic	1.3 (1.63)	3.3 (1.48) [a]	3.3 (1.04) [a]	0.7 (2.42)
	Obstructive	2.2 (1.71)	4.7 (2.25) [a]	3.7 (1.09)	3.2 (2.00)
Tskin-diff central	Endotoxemic	9.2 (2.42)	11.2 (3.52) [a]	10.3 (3.25)	7.1 (2.78)
	Obstructive	9.5 (1.43)	11.0 (1.57) [a]	11.6 (2.40)	9.8 (2.69)

T1 = during ≥ 50% reduction of cardiac output, T2 = after initial resuscitation to baseline, T3 = after the optimization of cardiac output, Tskin-diff = body temperature gradient (Materials and Methods section).
ap < 0.05 versus baseline.
bp < 0.05 between models (endotoxemic and obstructive) in the specific time point, by linear mixed-model analysis.
Data are presented as mean ± SD.

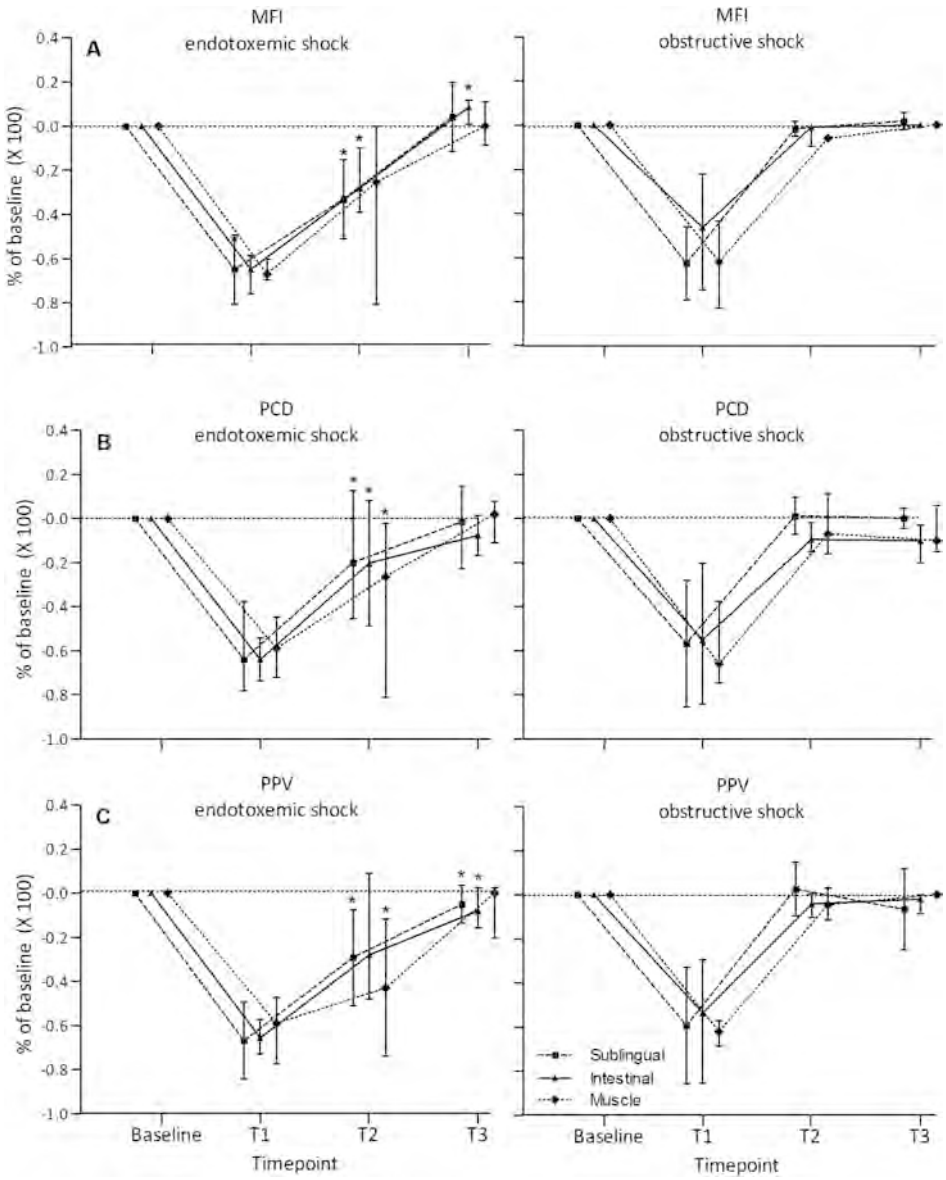

Figure 3. Relative changes (plotted as proportional changes from baseline) of different microvascular perfusion variables visualized with the sidestream dark field imager in different vascular beds. This graph demonstrates the relative changes of the microvascular flow index (MFI) **(A)**, perfused capillary density (PCD, *n*/mm2) **(B)**, and the proportion of perfused vessels (PPV, %) **(C)** for the three vascular beds at each time point for the endotoxemic shock and obstructive model. Bars represent median ± interquartile range. *p value of less than 0.05 versus obstructive shock. All microvascular perfusion variables decreased simultaneously in a similar magnitude during shock in both models and only increased toward baseline levels in the obstructive model after initial resuscitation (T2) and remained in this range at T3. In the endotoxemic model however, microvascular perfusion variables only improved toward baseline levels after additional resuscitation of cardiac output. Dashed line = sublingual, continuous line = intestinal, dotted line = muscle.

data confirm our hypothesis and highlight that additional resuscitation at the microcirculatory level might be a complementary target if the conventional global resuscitation goals have been achieved.

Despite success associated with the implementation of early resuscitation during sepsis, the value of microcirculatory abnormalities as resuscitation target remains uncertain.[23,24] Obstructive shock, unlike septic shock, maintains intact autoregulatory mechanisms that preserve vital organ perfusion and ensure that the depressed microcirculation can be adequately resuscitated with normalized systemic hemodynamics.[25,26] However, compensatory autoregulatory mechanisms are severely disrupted during sepsis. This disruption, rather than systemic hemodynamic depression, results in impaired microvascular perfusion, characterized by endothelial dysfunction and heterogeneous flow patterns in otherwise homogeneously perfused microvascular beds.[1,27,28] Preservation or loss of autoregulation explains why the different microvascular perfusion variables returned to normal in the obstructive model even when slightly resuscitated and why they could be restored only after supranormal resuscitation during endotoxemic shock.

Several experimental and clinical studies have demonstrated that early goal-directed cardiac output fluid resuscitation is associated with improved outcomes prior to high-risk surgery,[29] directly after surgery,[30] after trauma,[31] and in sepsis.[32] However, these studies did not take the microcirculation into account. This is important, because persistent microvascular perfusion abnormalities during sepsis are associated with increased organ failure and mortality independent of systemic hemodynamic variables.[7,14,33] Retrospective[15] and prospective[34] studies suggest that these abnormalities can be additionally resuscitated independent of cardiac output or blood pressure. Furthermore, a previous study showed that a surrogate of gut mucosal perfusion, the intramucosal-arterial Pco2 gradient, is only corrected by supranormal increases of intestinal blood flow and not by its normalization.[35] Our study complements these investigations by demonstrating that persistent microvascular perfusion abnormalities in different peripheral tissues during endotoxemic shock were only improved after additional systemic resuscitation compared with obstructive shock.

Clearly, our data show that the early phase of sepsis is characterized by a generalized perfusion deficit: the impairment of the global circulation (i.e., cardiac output) has a major impact on microvascular perfusion. This is in agreement with observations in patients with acute sepsis[15] and with the beneficial effect of early goal-directed therapy in patients.[16] In the later phase of sepsis, the development of mitochondrial dysfunction and impaired cellular energy metabolism are most likely the predominant causes of organ dysfunction[36,37] This is supported by our own observation that the lactate levels in the endotoxemic model remained elevated despite restoration of microvascular perfusion. This can very well explain the poor effect of "late" supranormal cardiac output therapy.[38]

As such, the relation between microvascular perfusion and organ dysfunction at this stage remains unclear. Although the landmark study by Sakr et al[14] has associated the persistence of microvascular hypoperfusion in late-phase sepsis with poor outcome, the early intervention study by Boerma[39] suggested that outcome was not related to differences in microvascular perfusion. Additionally, in an observational study in our own department we found no differences between survivors and non-survivors when comparing microvascular perfusion during the first week of admission.[40]

Of special interest is the comparison of the microvascular perfusion (SDF) and Sto_2 (NIRS) data. In obstructive shock, both microvascular perfusion and Sto_2 were restored with the resuscitation of cardiac output, whereas during endotoxemic shock, a disparity occurred. At hyperdynamic cardiac output levels, microvascular perfusion was restored, but tissue hemoglobin saturation remained below baseline levels in all tissues. Because low Sto_2 has been associated with decreased local blood flow,[4] it must be concluded that recovery of local microvascular perfusion was not sufficient to completely restore oxygen delivery during sepsis, resulting in increased local oxygen extraction. This is supported by the elevated lactate levels. Most likely this is due to a difference between SDF and NIRS in terms of catchment area: SDF provides an image of only the superficial microvascular bed, whereas the NIRS signal reaches a depth of 15–25 mm. It can be hypothesized that especially during sepsis, where increased heterogeneity of regional blood flow occurs, this difference in technical features becomes apparent.

Based on our observations, it is difficult to identify the microvascular bed or monitoring technique that is most suitable as target during resuscitation. Especially in the obstructive shock model, all microvascular perfusion variables displayed a rather similar pattern, whereas in the endotoxemic group, patterns were a little more heterogeneous. As a result, no microvascular bed stood out as more sensitive to the changes during shock or resuscitation. This is in accordance with a previous study, showing that sublingual and gut microvascular perfusion were significantly related during experimental sepsis.[41] As such, the sublingual microvascular bed might be the most useful for monitoring under clinical conditions.

On the other hand, from a practical point of view, the lack of bedside, real-time information, and the dependency on experienced observer analysis do not favor the use of SDF imaging. Because changes in peripheral skin temperature difference and SDF were comparable in our study, it is easily hypothesized that real-time assessment of peripheral skin perfusion at the bedside could very well be a more valuable monitor of the effect of resuscitation in the clinical setting. If so, the underlying cause of shock might have to be taken into account: a correlation between microvascular perfusion and skin perfusion was observed in cardiogenic shock[10] but not in septic shock patients.[42]

Although we found no significant difference in the cumulative fluid balance between the models after optimal resuscitation, the administered volume of fluids was almost 50% higher in the endotoxemic model compared with the obstructive model. This can be explained by the underlying pathophysiological mechanism of endotoxemic shock, that is, the decreased vasomotor tone and endothelial leakage, contributing to a hyperdynamic circulation. The beneficial effect of fluid resuscitation on the microvascular perfusion was recently demonstrated in patients with sepsis.[43,44] In addition, because excessive fluid administration may be harmful and can potentially cause pulmonary fluid overloading, we administered repeated fluid challenges based on cardiac fluid responsiveness so that fluid overloading was most likely avoided.

Following the restoration of cardiac output to baseline levels in the endotoxemic animals, microcirculatory perfusion did not completely restore to baseline levels. The sharp increase in cardiac output from T2 to T3 (almost 70%), reaching the state of fluid unresponsiveness, is probably related to the normalization of the microcirculatory variables. The multiple insult of the endotoxemic/septic shock not only induces ischemia/ reperfusion injury like in the obstructive

shocked animals but also induces tissue and endothelial injury, which might result in much higher global blood flow levels when the microcirculation is restored. So, the increased cardiac output could therefore very well present the severity of the damage and is not at much directly the consequence of fluid resuscitation. A causal relationship between the increase in cardiac output and the normalization of the microcirculation can however not be made from these experiments.

This study has some limitations that should be acknowledged. First, the endotoxemic shock and resuscitation model consisted of a continuous but relatively short-term endotoxin infusion. A more clinically oriented model of sepsis might have more adequately represented the inflammatory and metabolic derangements that occur during septic shock.[45,46] However, profound hyperlactatemia (as a sign of anaerobic tissue metabolism or catecholamine-stimulated aerobic glycolysis) was present during shock in both models, and the lactate clearance was clearly altered in the endotoxemic model compared with the obstructive model. Therefore, we concluded that our models had sufficient analogy with clinical conditions.

Second, because we did not perform a time control, an adaptive effect of the animals to LPS infusion cannot be ruled out. However, we do not think this is an explanation for our observations. The severity of the shock state was not likely to resolve spontaneously within the time frame of the experiment. Especially because we continued the LPS infusion, to mimic clinical conditions and prevent spontaneous recovery of a short lasting insult, we believe the increase in regional perfusion can be attributed to the increase in cardiac output. Nevertheless, the time frame of our model might have been too short for the development of profound tissue edema, which is a very common problem in patients and as such might hamper the translation of our results to clinical practice.

Third, SDF imaging is a valuable technique, but it is based on semi-quantitative analyses. Therefore, data reliability may be affected by technical expertise and interobserver bias despite the use of standardized analysis software. Finally, we recognize that the sample size was limited for a multivariate analysis between the two groups. However, the sample size was adequate to address the specific aims of this study. Definitive determinations of the relationship between microcirculatory and systemic variables in sepsis require a more robust sample of longitudinal data.

CONCLUSIONS

Resuscitation of cardiac output to preshock levels produced a full recovery of the peripheral circulation in obstructive but not in endotoxemic shock. Apparently, the relationship between the systemic circulation and different microvascular and different peripheral perfusion variables is dependent on the underlying cause of circulatory shock. Our data show that different microvascular and peripheral perfusion variables can be used to assess the adequacy of hemodynamic resuscitation during different types of shock. Supranormal optimization of cardiac output is needed to restore these different variables in sepsis but might still not lead to full recovery of tissue oxygenation. Further research is required to assess the reproducibility of our findings in a clinical setting and further elucidate the relationship between systemic and different peripheral circulation and oxygenation variables as targets for systemic therapeutic interventions during the early phase of septic shock.

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TISSUE PERFUSION AND OXYGENATION TO MONITOR FLUID RESPONSIVENESS IN CRTICALLY ILL, SEPTIC PATIENTS AFTER INITIAL RESUSCITATION: A PROSPECTIVE OBSERVATIONAL STUDY

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ABSTRACT

Fluid therapy after initial resuscitation in critically ill, septic patients may lead to harmful overloading and should therefore be guided by indicators of an increase in stroke volume (SV), i.e. fluid responsiveness. Our objective was to investigate whether tissue perfusion and oxygenation are able to monitor fluid responsiveness, even after initial resuscitation. Thirty-five critically ill, septic patients underwent infusion of 250 mL of colloids, after initial fluid resuscitation. Prior to and after fluid infusion, SV, cardiac output sublingual microcirculatory perfusion (SDF: sidestream dark field imaging) and skin perfusion and oxygenation (laser Doppler flowmetry and reflectance spectroscopy) were measured. Fluid responsiveness was defined by a ≥5 or 10% increase in SV upon fluids. In responders to fluids, SDF-derived microcirculatory and skin perfusion and oxygenation increased, but only the increase in cardiac output, mean arterial and pulse pressure, microvascular flow index and relative Hb concentration and oxygen saturation were able to monitor a SV increase. Our proof of principle study demonstrates that non-invasively assessed tissue perfusion and oxygenation is not inferior to invasive hemodynamic measurements in monitoring fluid responsiveness. However skin reflectance spectroscopy may be more helpful than sublingual SDF.

INTRODUCTION

In critically ill, septic patients, fluid administration to improve tissue perfusion and oxygenation is generally guided by systemic haemodynamic parameters; fluid responsiveness is defined by a cardiac preload challenge by fluid infusion resulting in augmented stroke volume (SV) and cardiac output (CO).[1,2] Currently, several techniques are available to assess tissue perfusion, such as sidestream dark field imaging (SDF) for sublingual microcirculatory perfusion, and laser Doppler flowmetry (LDF) and reflectance spectroscopy (RS) for perfusion and oxygenation of the skin, respectively.[3,4] Since the parameters obtained with these techniques may be sensitive predictors of outcome in critically ill and septic patients[5,6], the effect of resuscitation measures has been studied.[7,8] However, these studies did not focus on fluid infusion and thus do not clarify if and how tissue perfusion and oxygenation are affected by fluid responsiveness. Indeed, the effect of fluid infusion on (SDF) tissue perfusion in septic patients is controversial, regarding dependency on systemic haemodynamics, time and prior resuscitation, among others.[9-12] Indeed, in the late phase after resuscitation, the clinician may have to decide on additional fluids when the risk of harmful fluid overloading is increased. Non-invasively assessed tissue perfusion and oxygenation helping to predict and monitor fluid infusion could contribute to proper fluid management particularly at this stage.

Therefore, we studied whether tissue perfusion and oxygenation is able to predict and monitor fluid responsiveness, in critically ill, septic patients considered hypovolaemic on clinical grounds after initial resuscitation.

PATIENTS AND METHODS

Patients

This single center study was performed in the general intensive care unit (ICU) of the Erasmus MC University Medical Centre. Ethical approval for this study was provided by the ethical committee of the Erasmus MC University Medical Centre (MEC-2009-112). Written informed consent was obtained from each patient or his or her legal representative. Consecutive patients admitted in our general ICU with sepsis and on haemodynamic monitoring with a central venous catheter and a femoral artery catheter connected to a PiCCOplus™ device (Pulsion Medical Systems, Munich, Germany) were eligible. These monitoring tools are standard in our institution when sepsis is accompanied by hypotension and extensive fluid administration and vasopressor support is considered or performed. Sepsis was defined as the presence of two or more systemic inflammatory response criteria with a suspected or confirmed infection.[13] The second inclusion criterion was the presence of clinical signs of residual hypovolaemia after initial fluid resuscitation, prompting the clinical to consider additional fluid administration. These included, but were not limited to, hypotension, i.e. systolic blood pressure ≤90 mmHg, tachycardia, i.e. heart rate ≥100 bpm, central venous O2 saturation (ScvO2)<65 %, increasing vasopressor requirements, decreasing urine output and mottled skin. Patients were included[8 h up to 10 days after ICU admission to allow for initial resuscitation by fluid administration and vasopressor infusion and to obtain informed consent. Exclusion criteria were admission for intracranial catastrophes, known intra-abdominal

hypertension, known extensive peripheral vascular disease and known congestive heart failure. Patients were treated by intensive care staff, with, among others, appropriate broad-spectrum antibiotics, source control and, if needed, intubation and mechanical ventilation according to guidelines for standard practice in our institution. None of the patients received drotrecogin alpha activated or hydrocortisone. One patient received intravenous nitroglycerin. Settings of the ventilator and of vasoactive agent infusions were unaltered during the study.

Protocol

Patients were studied in the supine position; 250 mL of colloid solution (Voluven®, Fresenius Kabi, Bad Homburg, Germany) were infused in 15 min, after which measurements were repeated.

At baseline, patients were placed in supine position and after calibration and zeroing to atmospheric pressure at midchest level, mean arterial pressure (MAP) was taken from the femoral artery catheter and heart rate (HR) from the recorded electrocardiogram. They were measured continuously throughout the experiment. The pulse contour-derived SV and CO (PiCCOplus® device, Pulsion Medical Systems, Munich, Germany) were also continuously measured. Calibration of the pulse contour-derived CO was performed by transpulmonary thermodilution involving three separate central venous injections of 20 mL of ice cold NaCl 0.9 %, at baseline. To calculate cardiac index CO was divided by body surface area. Sublingual microvascular blood flow was evaluated using SDF (MicroVision Medical, Amsterdam, The Netherlands). Image acquisition and subsequent analyses were performed according to published consensus criteria.[4,14] In brief, after removal of saliva with gauze the device was gently applied to the sublingual area by investigators well trained in SDF imaging. For each stage five sequences of 20 s from different adjacent areas were recorded. The sequences were stored under a random number and later analysed according to the recent consensus with dedicated software (Microcirculatory Analysis Software (MAS 3.0) Academic Medical Centre,Amsterdam). Microvascular flow index was calculated after dividing each image into four equal quadrants. Quantification of flow was determined using an ordinal scale (0, no flow; 1, intermittent flow; 2, sluggish flow; 3, normal flow; 4, hyperdynamic flow).[14] Microvascular flow index is the average score of all quadrants for a given time point. Vessel density was calculated, according to the consensus, in two manners. First, functional capillary density was calculated by measuring total length of perfused capillaries divided by image area. Second, vessel density was calculated by inserting a grid of three equidistant horizontal and three equidistant vertical lines over the image. Vessel density is equal to the number of vessels crossing these lines divided by their total length. Flow was then categorized as present, intermittent or absent, allowing calculation of the proportion of perfused vessels. In our healthy volunteers averages (median and interquartile ranges) for microvascular flow index is 3.0 [3.0–3.0] AU, for functional capillary density is 11.97 [10.5–13.1] mm/mm2, for vessel density is 9.9 [9.1–10.3]/mm and for proportion of perfused vessels 100 [100–100] % (unpublished data). To determine the intrarater reproducibility of the sublingual microvascular parameters, the complete image analysis on 75 randomly selected SDF sequences was repeated at a later time point, in the absence of knowledge of interventions and the intraclass correlation coefficient on consistency was calculated, considered good when ≥0.6. The intraclass correlation

was 0.79 for microvascular flow index, 0.76 for functional capillary density, 0.73 for vessel density and 0.74 for proportion of perfused vessels. LDF and RS were performed using an O2C device (Oxygen to See, LEA Medizintechnik GmbH, Giessen, Germany) applied to a finger. The tissue was illuminated with a pulsed 830 nm class 1 laser diode and the backscattered light was spectrally analysed to assess the velocity dependent frequency shifts caused by flowing red blood cells. The microvascular haemoglobin oxygen saturation and relative haemoglobin concentration were measured by illuminating tissue with visible white light (500–630 nm), which is backscattered and changed in colour according to its O2 saturation. The mean laser Doppler flow and microvascular haemoglobin oxygen saturation was recorded and averaged over a stable period of 1 min. In our healthy volunteers average values (median and interquartile ranges) for laser Doppler flow is 352 [185–488] AU, for microvascular haemoglobin oxygen saturation 72 [68–79] % and for relative haemoglobin concentration 47 [41–52] AU (unpublished data).

Statistical analysis

In line with other studies and 50 % fluid responses, we estimated that 35 patients would be sufficient to reach the proof of principle study goals. Patients in whom the 250 mL fluid infusion induced an increase in SV by ≥5 % were defined as fluid responders, since the 10 % cut-off usually applies to 500 mL infusion. We nevertheless also evaluated fluid responses of ≥10 %. Non-parametric test were used because of relatively small numbers, even though most variables were distributed normally (Kolmogorov– Smirnov P >0.05). Groups were compared using Mann–Whitney or Fisher exact tests, where appropriate. Intragroup comparisons were done with help of the Wilcoxon matched pairs test. Receiver operating characteristics (ROC) were calculated and compared to assess predicting and monitoring values of parameters for fluid responsiveness. Baseline values were used for prediction and the changes in parameters following the fluid challenge were used for monitoring. The areas under curves (AUC) ± standard error and sensitivity, specificity, positive and negative predictive values (PPV and NPV, respectively) are given. An AUC >0.70 was considered clinically useful. The cut-offs were determined as the cut-off values with the highest sensitivity and specificity combined. The Spearman correlation coefficient was used to express relations. The intraclass correlation coefficient was used to evaluate reproducibility of microcirculation measurements. Data are expressed as median and interquartile ranges. A two-sided P<0.05 was considered statistically significant. Exact P values are given unless <0.001.

RESULTS

Baseline characteristics

The baseline characteristics of the 35 consecutive patients included in the study are shown in Table 1. The median APACHE II score and vasopressor requirements were high and almost all patients were on mechanical ventilation. Following fluid infusion, SV increased by ≥5 % in 19 (54 %) responders and by <5 % in 16 (46 %) non-responders. There were no baseline differences between the fluid response groups (Table 2).

Table 1: Baseline characteristics of responders and non-responders based on a ≥5 % increase in stroke volume to a colloid fluid infusion of 250 mL

	Responders (n=19)	Non-responders (n=16)	p
Age, year	69 [55-78]	66 [57-73]	0.46
Sex, male	11 (58)	10 (63)	0.78
Female	8 (42)	6 (37)	
BMI, kg/m^2	24.7 [22.5-29]	24.2 [20-27.3]	0.22
Premorbidity			
Cardiovascular	11 (58)	11 (69)	0.51
Liver disease	1 (5)	3 (19)	0.21
Malignancy	6 (32)	5 (31)	0.98
Source of sepsis			
Abdominal	7 (37)	8 (50)	0.43
Respiratory	8 (42)	5 (31)	0.51
Other	4 (21)	3 (19)	0.86
Bacteraemia	12 (63)	10 (63)	0.97
Time since admission (h)	24 [5-60]	48 [16-144]	0.16
APACHE II	28 [20-32]	24 [18-27]	0.08
SOFA	11 [7-14]	11 [8-12]	0.81
Mechanical ventilation	19 (100)	13 (81)	0.05
Vasopressor support	13 (68)	12 (75)	0.67
NE, μg.kg^{-1}.min^{-1}	0.06 [0-0.3]	0.18 [0-0.37]	0.55
Prior fluid balance, mL	4838 [2921-10216]	7281 [2725-9537]	0.74
Survival	9 (47)	10 (63)	0.37

Median [interquartile ranges] or number and percentage, where appropriate. BMI body mass index, APACHE II acute physiology and chronic health evaluation score, SOFA sequential organ failure assessment, NE norepinephrine

Effects of fluid infusion

Tables 2 and 3 show the haemodynamic and microcirculatory values for fluid responders and non-responders, when defined on the basis of ≥5 and 10 % increase in SV, respectively. The SV, CO, MAP and pulse pressure (PP) increased in responders but not in non-responders. In responders (and not in non-responders), microvascular flow index, vessel density, functional capillary density, laser Doppler flow and microvascular haemoglobin concentration and oxygen saturation increased.

Prediction and monitoring of fluid responsiveness

Except for baseline SV and CO there were no baseline predictors among global haemodynamic and tissue perfusion variables. Table 4 shows the ROC curves for monitoring of fluid responsiveness ≥5 and 10 %, by changes in variables (except for SV and CO). The AUC values indicative of global haemodynamics and tissue perfusion did not differ from each other.

Table 2 Haemodynamics and tissue perfusion before and after a fluid infusion, in responders and non-responders based on a ≥5 % increase in stroke volume to fluid infusion of 250 mL

	Responders (n=19)			Non-responders (n=16)		
	Before	**After**	**P**	**Before**	**After**	**P**
Heart rate, bpm	89 [78-100]	92 [71-105]	0.18	96 [82-106]	95 [80-105]	0.046
Stroke volume, mL	70 [45-87]	78 [56-97]	na	82 [65-116]	78 [64-110]	na
Cardiac output, L/min	5.8 [5.2-7.6]*	7.0 [6.0-8.1]	<0.001	7.3 [6.6-9.9]	7.1 [6.0-8.6]	0.009
Cardiac index, L/min/m^2	3.3 [2.4-3.7]†	3.6 [3.1-4.4]	<0.001	4.0 [3.4-5.1]	3.9 [3.3-4.4]	0.009
Mean arterial pressure, mmHg	72 [64-75]	75 [70-82]	0.001	74 [68-81]	76 [69-84]	0.16
Pulse pressure, mmHg	64[51-75]	72 [63-85]	0.009	61 [52-82]	63 [52-84]	0.68
Microvascular flow index, AU	3.3 [2.9-3.8]	3.9 [3.0-4.0]	0.007	3.2 [3.0-3.7]	3.6 [3.0-3.8]	0.20
Functional capillary density, mm/mm^2	15.0 [13.2-17.4]	16.2 [15.0-17.8]	0.030	16.0 [14.3-16.8]	15.7 [15.5-18.0]	0.24
Vessel density, /mm	10.9 [10.3-12.1]	11.9 [11.4-13.0]	0.006	11.0[10.6-13.1]	11.6 [11.2-12.7]	0.09
Proportion of perfused vessels, %	96 [94-100]	97 [96-100]	0.06	99 [95-100]	99 [96-100]	0.14
Laser Doppler flow, AU	115 [31-331]	169 [36-347]	0.030	270 [41-311]	287 [123-358]	0.50
$\mu HbSO_2$, %	49 [26-55]	51 [43-59]	0.020	55 [38-65]	52 [40-65]	0.12
rHb, AU	27 [23-33]	31 [26-38]	0.10	32 [25-37]	3 [25-37]	0.96

Median [Interquartile ranges]. μHbSO2 microvascular haemoglobin oxygen saturation, rHb relative haemoglobin concentration, AU arbitrary units, na not applicable * $P = 0.049$, † $P = 0.02$ before fluid challenge in responders versus non-responders; For changes in cardiac output $P < 0.001$; mean arterial pressure $P = 0.015$; pulse pressure $P = 0.009$; rHb $P = 0.030$

Correlations

The increase in SV related to the increase in rHb (rs = 0.41, P = 0.014) and μHbSO2 (rs = 0.38,P = 0.086).

DISCUSSION

Our results suggest that in critically ill, septic patients with clinical hypovolemia after initial resuscitation and persistent fluid responsiveness, fluid infusion augments several indicators of tissue perfusion, so that these non-invasively derived indicators can be used to monitor fluid infusion.

In fact, our results suggest vascular recruitment and flow increments (SDF) by fluid infusion in responders. However, the effect of fluid infusion and the subsequent increase in SV on the parameters of tissue perfusion was relatively small in our study, and changes in SDF variables in responders did not differ from that in non-responders. We included patients beyond the initial phase of fluid resuscitation and SDF measurements suggest that the sublingual microcirculation was sometimes hyperdynamic at this stage. De Backer et al. noted amelioration of microcirculatory alterations in the course of sepsis.[12] This was also seen in the study by Boerma et al. who observed similar values for sublingual microvascular perfusion as in our study, at 24 h after admission and administration of approximately 6 L of fluids.[8] The study by Pottecher et al. demonstrated a much larger effect of fluids on SDF measurements.[11] A possible explanation for the discrepancy with our study could be that their patients were likely to be more severely

Table 3 Haemodynamics and tissue perfusion before and after a fluid infusion, in responders and non-responders based on an ≥10 % increase in stroke volume to fluid infusion of 250 mL

	Responders (n=15)			Non-responders (n=20)		
	Before	**After**	**P**	**Before**	**After**	**P**
Heart rate, bpm	89 [78-120]	89 [71-118]	0.12	98 [82-101]	97 [80-104]	0.09
Stroke volume, mL	59 [43-75]*	70 [55-97]	Na	82 [68-116]	82 [66-116]	na
Cardiac output, L/min	5.5 [4.7-6.6]†	6.7 [5.9-8.0]	0.001	7.5 [7.0-9.9]	7.3 [6.5-8.6]	0.35
Cardiac index, L/min/m^2	2.9 [2.2-3.6]‡	3.4 [3.0-4.1]	0.001	4.0 [3.4-5.2]	3.9 [3.4-4.8]	0.082
Mean arterial pressure,mmHg	72 [64-75]	77 [71-82]	0.002	73 [68-81]	75 [69-84]	0.040
Pulse pressure, mmHg	64 [46-74]	71 [63-79]	0.021	63 [56-82]	64 [55-86]	0.36
Microvascular flow index, AU	3.2 [2.9-3.8]	3.9 [3.0-4.0]	0.006	3.2 [3.0-3.8]	3.5 [3.0-3.8]	0.24
Functional capillary density, mm/mm^2	15.2 [13.2-17.4]	16.3 [15.0-17.8]	0.046	15.5 [14.2-17.2]	15.9 [15.3-18.0]	0.14
Vessel density, /mm	11.1 [10.3-12.1]	12.1 [11.4-13.0]	0.019	10.9 [10.5-12.8]	11.7 [10.9-12.8]	0.032
Proportion of perfused vessels, %	97 [94-100]	98 [97-100]	0.13	98 [93-100]	99 [95-100]	0.041
Laser Doppler flow, AU	216 [31-347]	278 [36-365]	0.035	203 [41-303]	221 [51-313]	0.49
$\mu HbSO_2$, %	51 [34-59]	54 [47-61]	0.006	52 [20-60]	51 [22-64]	0.27
rHb, AU	27 [21-29]	31 [26-38]	0.030	32 [25-37]	33 [25-37]	0.60

Median [interquartile ranges]. µHbSO2 microvascular haemoglobin oxygen saturation, rHb relative haemoglobin concentration, AU arbitrary unit, na not applicable * $P = 0.005$, † $P = 0.002$, ‡ $P = 0.001$ responders versus non-responders before fluid challenge; for changes in cardiac output $P < 0.001$; mean arterial pressure $P = 0.011$; pulse pressure $P = 0.006$; µHbSO2 $P = 0.024$; rHb $P = 0.042$

hypovolaemic than ours. Indeed, their patients were included early during resuscitation, i.e. within 24 h after admission, although the fluid balance before inclusion is unclear. Additionally, baseline microcirculatory perfusion was lower than in our study. Conversely, 'late' inclusion may mitigate an effect of fluid administration on tissue perfusion (SDF), but in our study effects seemed independent of time from admission.[10]

Tissue perfusion parameters seemed, at least in part, dependent on systemic haemodynamics. This is in agreement with some studies [11] but in contrast to other observations, early (<24 h) and late (>48 h) in the disease course of critically ill, septic patients.[9,10,12] However, prior resuscitation had not similarly affected perfusion and oxygenation of sublingual and cutaneous tissue. In contrast to SDF, LDF and RS parameters were still in the low range (compared to healthy volunteers) at baseline in our study.[5] A poor skin and sublingual perfusion is often observed in septic patients, and has been identified as a sensitive predictor of outcome, independent from systemic haemodynamic parameters.[5,6] The redistribution of CO with regional over- and underperfusion relative to demand is a central hemodynamic abnormality of septic shock.[14] The fact that both systemic and sublingual perfusion were sometimes judged hyperdynamic could point to a close relationship between the two. However, relatively low skin perfusion and oxygenation was associated with a relatively low SV and CO, in patients responding to fluid infusion. This suggests that peripheral (rather than sublingual) tissue blood flow is partly dependent on total forward flow. Conversely, fluid administration had a greater effect on LDF/RS- than on SDF-

Table 4 Monitoring values of haemodynamic parameters for fluid responsiveness

	AUC±Std. error	P	Optimal cutoff	Sens	Spec	PPV	NPV
SV ≥5 %							
Delta PP, mm Hg	0.76±0.09	0.003	4	68	88	87	70
Delta MAP, mm Hg	0.74±0.09	0.006	6	58	88	85	64
Delta rHb, AU	0.72±0.09	0.015	-0.7	44	93	68	73
SV ≥10%							
Delta PP, mm Hg	0.77±0.09	0.004	6	73	90	85	82
Delta MAP, mm Hg	0.750.09	0.006	6	73	90	85	82
Delta μHbSO$_2$,%	0.73±0.09	0.015	2	64	79	69	75
Delta rHb, AU	0.71±0.09	0.025	1.5	50	89	78	71
Delta MFI, AU	0.69±0.10	0.048	0	71	69	67	73

AUC area under the curve, Std. standard, Sens sensitivity, Spec specificity, PPV positive predictive value, NPV negative predictive value, PP pulse pressure, SV stroke volume, MAP mean arterial pressure, μHbSO2 microvascular haemoglobin oxygen saturation, rHb relative haemoglobin concentration, MFI microvascular flow index

derived parameters. Of the tissue perfusion parameters studied, changes in skin haemoglobin concentration and saturation were most helpful in monitoring fluid responsiveness.

The limitations of our study include the relatively small number of patients. Future research on guiding fluid resuscitation by tissue perfusion parameters is also needed to study benefits of this approach of increasing tissue oxygenation by fluid administration in critically ill, septic patients, since our study suggests reasonable intrarater and intrapatient reproducibility of some of these non-invasive measurements. Additionally, we cannot exclude that prediction and monitoring values of microcirculatory parameters are different from ours in patients with less prior resuscitation. Finally, real time analysis would be needed to utilize SDF images for guiding fluid administration, the necessary off-line analysis makes it currently unsuitable for clinical use.

In conclusion, the value of non-invasively assessed skin perfusion and oxygenation for monitoring fluid responsiveness in critically ill, septic patients after initial resuscitation is not inferior to that of invasive hemodynamic measurements. This may help fluid management in septic patients, even though an outcome benefit has yet to be demonstrated.

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PERIPHERAL PERFUSION INDEX PREDICTS HYPOTENSION DURING FLUID WITHDRAWAL BY CONTINUOUS VENO-VENOUS HEMOFILTRATION IN CRITICALLY ILL PATIENTS

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ABSTRACT

Aim

Peripheral perfusion may predict harmful hypovolemic hypotension during fluid withdrawal by continuous veno-venous hemofiltration (CVVH) in critically ill patients with acute kidney injury.

Methods

Twenty-three critically ill AKI patients were subjected to progressive fluid withdrawal. Systemic hemodynamics and peripheral perfusion index (PPI) by pulse oximetry, forearm-to-fingertip skin temperature gradient ($T_{skin-diff}$) and tissue oxygen saturation (StO_2, near infra-red spectroscopy) were measured.

Results

Most hemodynamic values decreased with fluid withdrawal, particularly in the hypotensive group, except for stroke volume (SV) and cardiac output which decreased more in the non-hypotensive patients. Increases in systemic vascular resistance were less in hypotension. Baseline pulse pressure and PPI were lower in hypotensive (n=10) than non-hypotensive patients and subsequent PPI values paralleled SV decreases. A baseline PPI ≤0.82 AU predicted hypotension with a sensitivity of 70%, and a specificity of 92% (AUC 0.80 ± 0.11, P=0.004).

Conclusion

Progressive fluid withdrawal during CVVH is poorly tolerated in patients with less increases in systemic vascular resistance. The occurrence of hypotension can be predicted by low baseline PPI.

INTRODUCTION

Critically ill patients admitted to the intensive care unit (ICU) often receive large amounts of fluid to maintain hemodynamic stability. In this setting fluid overload is frequent especially in patients with concomitant acute kidney injury (AKI).[1] In fact, there is increasing evidence that overzealous fluid administration resulting in fluid overload is harmful for the patient. Several studies have demonstrated a relation between a positive fluid balance, increased risk of AKI, non-recovery of renal function and mortality.[2-4] A positive fluid balance is difficult to resolve, even when renal replacement therapy by continuous veno-venous hemofiltration (CVVH) has been instituted. On the one hand, fluid withdrawal could benefit the patient but may also lead to hypovolemic hypotension if insufficiently compensated for by sympathetic stimulation and refilling from the interstitial compartment.[5,6] Therefore, it is advised to closely monitor the cardiovascular response during hemofiltration. Development of hypotension, believed to result from hypovolemia, often prompts to discontinue or decrease fluid withdrawal during CVVH.

The purpose of this study was to investigate whether non-invasive peripheral perfusion indicators prior to and during fluid withdrawal are able to predict and monitor central hypovolemia and subsequent hypotension during progressive fluid withdrawal by CVVH in critically ill patients with AKI.

PATIENTS AND METHODS

Subject population

This study was performed in the Erasmus MC University Medical Centre and was approved by the local ethics committee. Written informed consent was obtained from each patient or his or her legal representative. Consecutive patients (n=23) admitted in our general intensive care unit (ICU) receiving CVVH and on haemodynamic monitoring with a central venous catheter and a femoral artery catheter connected to a PiCCOplus™ device (Pulsion Medical Systems, Munich, Germany) were eligible. Patients were excluded when they had pre-existing end stage renal disease or severe pre-existing vascular disease compromising measurements of peripheral perfusion. The inclusion number was considered sufficient for predicting CVVH-induced hypotension that occurs almost uniformly after start of fluid withdrawal, dependent on its volume. A formal power analysis was not done for this exploratory study. The monitoring tools are standard in our institution when the patient has hypotension and extensive fluid therapy and vasopressor support is considered or performed. The indication to start CVVH was done by the treating physician based on the presence of oliguria/anuria, acute azotaemia, marked hyperkalaemia and/or fluid overload. The treating physician decided if fluid withdrawal was warranted, which was then executed following the study protocol. Patients were treated by intensive care staff, with, among others, and if needed, appropriate broad-spectrum antibiotics, source control, sedation, intubation and mechanical ventilation according to guidelines for standard practice in our institution. None of the patients received drotrecogin alpha activated, hydrocortisone or intravenous nitroglycerin. Settings of the ventilator and of vasoactive agent infusions were unaltered during the study.

CVVH

In our institution patients are hemofiltered with 2 L post-dilution mode (Aquarius CRRT, Dirinco). Blood flow was set at 200 mL/min and regional citrate anticoagulant was used. The filters used were ethylene oxide (ETO)- sterilized Aquamax HF 19, with an in vitro cut-off point of 55 kDa. The replacement solution was warmed to a temperature between 36 and 38°C. During the experiment patients received other fluids as little as possible. The only fluids administrated were the fluids used for sedation, analgesia, vaso-active substances and antibiotics.

Protocol

Patient characteristics and disease severity measures were collected. Cumulative fluid balance from admission on was calculated as well as the percentage fluid overload (balance divided by pre hospital body weight). Study measurements were performed continuously, starting at baseline and after every 500 mL of fluid withdrawn. The net fluid withdrawal was gradually increased, starting at 50 mL/h. Every fifteen minutes net fluid removal was doubled to a maximum rate of 500 mL/h. Therefore, the first 500 mL of fluid was withdrawn in approximately 100 min. Fluid withdrawal could be stopped at every time point by the treating physician, on the basis of perceived intolerance by the patient.

Measurements of systemic haemodynamics

At baseline, patients were placed in supine position and after calibration and zeroing to atmospheric pressure at mid-chest level, systolic blood pressure (SBP), diastolic blood pressure (DBP) and mean arterial pressure (MAP, mmHg) were taken from the femoral artery catheter and heart rate (HR) from the recorded electrocardiogram. At baseline central venous pressure (CVP) was measured via a central venous catheter. Pressures were measured continuously throughout the experiment, after calibration, zeroing to atmospheric pressure and referenced at the mid chest level with patients in supine position. The pulse contour-derived cardiac output (CO) and stroke volume (SV) were measured continuously also (PiCCO plus, Pulsion, Munich, Germany). Calibration was performed by transpulmonary thermodilution involving three separate central venous injections of 20 mL of ice cold NaCl 0.9%, at baseline. Systemic vascular resistance (SVR) was calculated using the following formula: SVR = MAP/CO * 80 (dyn.sec.cm-5). Additionally, lactate and haemoglobin concentrations were measured in heparinised arterial blood samples on ice at baseline, using a blood gas analyser (ABL 700, Radiometer, Copenhagen, Denmark).

Measurements of peripheral perfusion

Peripheral skin perfusion was determined with the peripheral perfusion index (PPI), which was derived from the photoelectric plethysmographic signal of the pulse oximeter Masimo SET Radical-7 (Masimo Corp., Irvine, CA, USA). The adhesive sensor was attached onto the index finger (Masimo SET®LNCS Adtx, adult sensor). The PPI is calculated as the ratio between the amplitude of the pulsatile component (arterial compartment) and the non-pulsatile component (venous and capillary blood, and other tissue) of the light reaching the detector of

the pulse oximeter. This ratio is independent of the hemoglobin oxygen saturation. Because a change in peripheral vasomotor tone primarily causes a corresponding change in the pulsatile component of the signal, the ratio changes accordingly. As a result, the PPI value reflects changes in peripheral vasomotor tone, with a median value of 1.4 AU (arbitrary units) for normality in healthy volunteers.[6] For the same purpose, forearm-to-fingertip skin-temperature difference ($T_{skin-diff}$) was measured with two skin probes (Hewlett Packard 21078A, Palo Alto, CA, USA) attached to the index finger and on the radial side of the forearm, midway between the elbow and the wrist. This temperature gradient can better reflect changes in cutaneous blood flow than the absolute skin temperature itself and increases during vasoconstriction.[5] The ICU has single-person closed rooms and the ambient bedside temperature was controlled at 22°C. Tissue oxygen saturation (StO_2) was continuously obtained from the thenar using an InSpectra Tissue Spectrometer Model 650 (Hutchinson Technology, Hutchinson, MN, USA) with a 15-mm near infrared spectroscopy probe placed over the thenar eminence, as described previously.[7,8]

Statistical analysis

Unless otherwise specified, data are presented as median and interquartile ranges [IQR]. The amount of fluid withdrawn (0, 500, 1000, 1500, 2000, 2500, and 3000 mL) was plotted against systemic hemodynamic and peripheral perfusion indicator results. For data analysis purposes, the continuous measurements for systemic hemodynamic data, which were sampled every 12 seconds, and for peripheral perfusion data, which were sampled every 2 seconds, were averaged over a stable period of 5 minutes, at baseline and at the time when 500, 1000, 1500, 2000, 2500 and 3000 ml of fluid was withdrawn. Hypotension was defined according to the guidelines for intradialytic hypotension as a decrease in SBP by ≥20 mmHg as compared to the previous time point.[9] Because of relatively small numbers we used non-parametric test throughout. Otherwise, generalized estimated equations (GEE) were used to test the evolution over time and which parameter predicts (baseline values) or monitors (changes) the occurrence of hypotension, with a first order interaction between hypotension and time. A logarithmic transformation was performed on non-normally distributed parameters in order to perform the GEE. Receiver operating characteristic (ROC) curves were constructed to assess predicting values of parameters for occurrence of hypotension. The areas under the curves ± standard error, sensitivity, specificity and positive and negative predictive values (PPV and NPV, respectively) are given for the cut-off values at the highest sensitivity and specificity combined. We used SPSS software (version 20.0; SPSS Inc., Chicago, IL) for statistical analysis. A P value <0.05 was considered statistically significant. Exact P values are given, unless P<0.001.

RESULTS

Baseline characteristics

The characteristics of the 23 patients are shown in Table 1, according to the occurrence of hypotension (n=10) or not (n=13) during fluid withdrawal. Of the 10 patients, 7 had hypotension at 500 mL ultrafiltration, 1 at 1000 mL, 2 at 1500 mL (2 of these patients had a second episode

of hypotension), 1 at 2000 mL and 1 at 2500 mL (second episode). Most patients were admitted because of septic shock. Almost all patients required mechanical ventilation and the majority required vasopressor support. The patients with hypotension had a lower cumulative fluid balance and percentage of fluid overload than patients without hypotension. Other baseline characteristics did not differ between the groups.

Effects of fluid withdrawal

Fluid withdrawal until hypotension was less (P=0.03) in patients developing hypotension vs those without. The baseline values and values per volume fluid withdrawn for systemic and regional hemodynamic parameters, according to hypotension, are shown in Table 2. It shows that only

Table 1: Baseline patient characteristics at study inclusion, according to the occurrence of hypotension (a decrease in systolic blood pressure of ≥ 20 mmHg) during fluid withdrawal.

	Patient with hypotension (n=10)	Patients without hypotension (n=13)	P
Age, year	60 [57-66]	70 [38-73]	0.58
Sex, n (%), male	6 (60)	8 (62)	0.94
female	4 (40)	5 (38)	
Initial diagnosis, n (%)			0.22
Septic shock	6 (60)	10 (78)	
Severe sepsis	1 (10)	0	
Intoxication	0	2 (15)	
Respiratory failure	2 (20)	0	
Hypovolemic shock	1 (10)	1 (7)	
APACHE II	28 [20-35]	21 [19-32]	0.34
SOFA	12 [11-13]	13 [11-14]	0.57
Haemoglobin, mmol/L	5.2 [4.7-5.7]	5.1 [5.0-5.3]	0.88
Hematocrit, L/L	0.24 [0.23-0.28]	0.25 [0.23-0.26]	0.80
Albumin, g/L	22 [16-25]	21 [19-23]	0.85
C-reactive protein, mg/L	99 [50-287]	103 [61-211]	0.92
Lactate, mmol/L	1.6 [1.1-1.8]	1.8 [1.7-2.3]	0.12
Vasopressor support, n (%)	4 (40)	10 (78)	0.07
Norepinephrine dose, mcg/kg/min	0 [0-0.07]	0.04 [0.02-0.4]	0.13
Mechanical ventilation, n (%)	9 (90)	12 (92)	0.85
Central venous pressure, mmHg	10 [8-15]	15 [10-16]	0.25
Cumulative fluid balance, L	10.5 [3.0-15.2]	17.5 [10.8-25.6]	0.04
Fluid overload, %	14 [4-19]	27 [15-32]	0.04
Time on CVVH before fluid withdrawal, hr	27 [14-48]	46 [28-52]	0.21

Median [IQR] or number (%), where appropriate. Cumulative fluid overload was calculated using the following formula (fluid balance/bodyweight) x 100.

absolute values for PP and PPI were lower, from the start, in hypotensive than non-hypotensive patients. Most values decreased in time, particularly in the hypotensive group (as evidence by a first order interaction), except for SV and CO which decreased most in the non-hypotensive patients. Decreases in SBP, MAP and PP and increases in $T_{skin-diff}$ were greater in hypotensive patients, irrespective of time. The SVR increased more in the non-hypotensive than the hypotensive group and the StO_2 somewhat increased regardless of hypotension. The change in PPI did not differ among hypotension groups. No patient developed atrial fibrillation during fluid withdrawal.

Relation of systemic hemodynamic parameters and peripheral perfusion parameters during fluid withdrawal and predictive value of the latter

The PPI (and not $T_{skin-diff}$ and StO_2) was associated with the SV and CO (P<0.001) and changes in SV were reflected by changes in PPI (P<0.001). There was a direct association between PPI and StO_2 and an inverse association with $T_{skin-diff}$ (P<0.009 or lower), but $T_{skin-diff}$ and StO_2 did not have predictive value. Figure 1 shows the ROC curve for prediction of hypotension by a baseline PPI ≤0.82 AU, with a sensitivity of 70%, and a specificity of 92% (AUC 0.80 ± 0.11, P=0.004, PPV 88%, NPV 79%). The change in PPI (0-500 mL) >-0.2 AU (AUC 0.74 ± 0.11, P=0.03, sensitivity 60%, specificity 83%), change in CO (0-500 mL) < -0.8 L/min (AUC 0.75 ± 0.13, p=0.048, sensitivity 70%, specificity 92%) and change in SV < -9 ml (AUC 0.74 ± 0.11, p=0.025, sensitivity 50%, specificity 92%) predicted CVVH-induced hypotension. A fluid balance of ≤ 20 L (AUC 0.76 ± 0.10, p=0.013, sensitivity 100%, specificity 46%) and a percentage fluid overload of ≤ 25% (AUC 0.75 ± 0.11, p=0.019, sensitivity 90%, specificity 62%) predicted CVVH-induced hypotension.

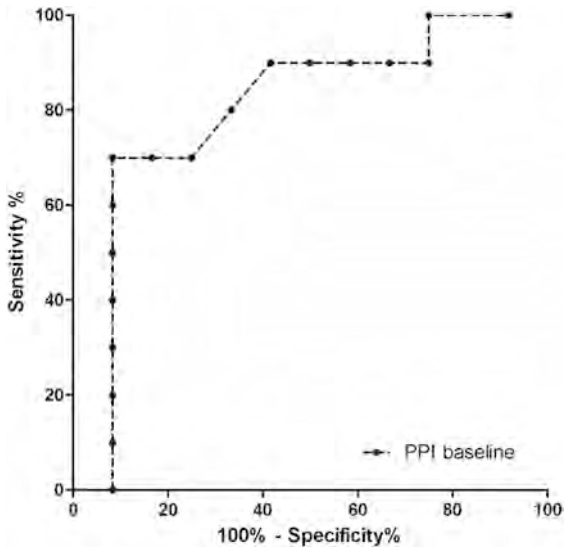

Figure 1. Receiver operating characteristic curve to predict hypotension during fluid withdrawal. The curves show the predictive values of baseline peripheral perfusion index (PPI; area under the curve 0.80 ± 0.11, P= 0.004). Optimal cutoff 0.82 AU.

Table 2: Systemic hemodynamics and peripheral perfusion parameters at the different fluid removal volumes for patients with and without hypotension during fluid withdrawal.

Fluid volume removed, mL	**0**	**500**	**1000**	**1500**	**2000**	**2500**	**3000**	**P**
Hypotension +	(n=10)	(n=10)	(n=6)	(n=5)	(n=3)	(n=1)	(n=1)	Hypotension,
Hypotension -	(n=13)	(n=13)	(n=11)	(n=6)	(n=6)	(n=4)	(n=2)	time, interaction
Heart rate, bpm								
Hypotension +	105 [96-117]	100 [95-117]	102 [96-121]	95 [94-103]	67 [56-92]	94	95	0.93, <0.001, 0.001
Hypotension -	105 [87-109]	105 [88-112]	104 [84-113]	99 [90-106]	74 [46-88]	103 [93-110]	95 [90-99]	
For changes								0.08, <0.001, 0.006
Stroke volume, mL								
Hypotension +	69 [49-85]	60 [41-80]	69 [43-80]	69 [37-78]	74 [40-91]	61	62	0.15, <0.001, <0.001
Hypotension -	81 [67-92]	76 [57-90]	65 [51-96]	64 [49-82]	60 [46-80]	56 [48-73]	66 [57-74]	
For changes								0.31, <0.001, <0.001
Cardiac output, L/min								
Hypotension +	7.9 [4.8-10.4]	7.3 [4.0-8.7]	7.5 [4.5-7.8]	6.3 [3.5-7.3]	7.0 [4.0-9.5]	5.7	5.9	0.23, <0.001, <0.001
Hypotension -	7.7 [7.0-9.1]	7.7 [6.2-8.8]	6.4 [5.8-8.1]	6.3 [5.2-7.6]	5.8 [4.9-7.6]	5.5 [5.1-7.0]	6.3 [5.2-7.3]	
For changes								0.006, <0.001, <0.001
Systolic blood pressure, mm Hg								
Hypotension +	127 [109-151]	110 [87-122]	122 [116-139]	111 [108-137]	121 [105-158]	133	139	0.16, <0.001, <0.001
Hypotension -	123 [110-132]	118 [109-129]	125 [113-129]	135 [111-141]	128 [108-137]	130 [115-149]	112 [109-114]	
For changes								0.008, <0.001, <0.001
Diastolic blood pressure, mm Hg								
Hypotension +	61 [57-69]	53 [46-63][p]	55 [53-61]	53 [53-59]	54 [49-66]	65	69	0.58, <0.001, <0.001
Hypotension -	58 [49-60]	55 [51-57]	57 [50-61]	62 [52-63]	58 [51-62]	59 [52-64]	56 [53-59]	
For changes								0.06, <0.001, <0001

Mean arterial pressure, mmHg								
Hypotension +	86 [75-98]	74 [60-82]	75 [71-92]	74 [71-79]	79 [63-103]	92	96	0.78, <0.001, <0.001
Hypotension -	78 [72-85]	73 [70-84]	78 [72-83]	80 [75-88]	77 [75-83]	79 [74-87]	76 [73-79]	
For changes								0.03, <0.001, <0.001
Pulse pressure, mmHg								
Hypotension +	61 [54-66]	48 [41-68]	68 [61-78]	59 [55-63]	67 [56-92]	68	70	0.02, <0.001, <0.001
Hypotension -	69 [62-75]	66 [53-74]	66 [56-81]	79 [48-90]	74 [46-88]	74 [56-93]	56 [55-56]	
For changes								0.01, <0.001, 0.007
Systemic vascular resistance, dyn.s.cm^{-5}								
Hypotension +	1028 [668-1247]	989 [703-1190]	959 [770-1163]	1273 [768-1455]	1174 [535-1576]	1282	1310	0.20, <0.001, <0.001
Hypotension -	825 [664-1034]	761 [642-1082]	928 [789-1113]	1111 [847-1177]	1158 [826-1246]	1166 [946-1256]	994 [868-1120]	
For changes								0.74, <0.001, <0.001
Peripheral perfusion index, AU								
Hypotension +	0.8 [0.4-1.4]	0.6 [0.3-0.8]	0.6 [0.1-0.7]	0.3 [0.3-0.8]	0.4 [0.1-0.8]	0.6	0.6	0.001, <0.001, 0.09
Hypotension -	2.7 [1.4-4.6]	1.4 [0.9-2.3]	1.5 [0.6-2.4]	2.1 [1.4-2.4]	1.6 [0.9-2.1]	0.8 [0.7-2.2]	0.7 [0.7-0.7]	
For changes								0.17, <0.001, 0.12
$T_{skin-diff}$, °C								
Hypotension +	3.1 [0-5.0]	2.2 [-1.3-4.2]	2.6 [1.1-5.5]	4.1 [1.4-5.9]	5.1 [2.4-5.5]	6.9	7.4	0.11, 0.02, 0.08
Hypotension -	0.5 [-0.8-4.6]	0.6 [-1.8-3.8]	0.1 [-2.7-6.8]	-1.4 [-5.4- -0.8]	-1.4 [-5.4- -0.5]	-0.7 [-3.6- 3.7]	3.0 [0.7-5.3]	
For changes								0.01, 0.15, 0.11
Tissue oxygen saturation, %								
Hypotension +	78 [70-89]	78 [68-87]	83 [66-88]	84 [70-88]	83 [60-88]	78	80	0.18, <0.001, 0.14
Hypotension -	79 [78-85]	80 [77-85]	81 [77-83]	85 [81-88]	85 [80-85]	78 [76-82]	80 [79-80]	
For changes								0.06, <0.001, 0.14

Median [IQR]. AU, arbitrary unit

DISCUSSION

Our results suggest that progressive fluid withdrawal by CVVH in critically ill AKI patients is poorly tolerated by development of hypotension in patients with less increases in SVR upon falls in SV and CO than in non-hypotensive patients with even larger falls in the latter, independently of sepsis or use of norepinephrine. Although PPI generally parallels CO and SV, only a low baseline and early, but low, decreases in PPI with relatively large CO/SV decreases predict hypotension. Finally, less overhydrated patients tolerate CVVH-induced fluid withdrawal less well.

Routinely and continuously monitored blood pressure is often used to guide the rate of fluid withdrawal during CVVH and occurrence of hypotension often urges to slow down or stop withdrawal. Our study suggests that the occurrence of hypotension results from insufficient ability to compensate by vasoconstriction for the fluid withdrawal-induced blood volume and flow reductions. In the patients who did not develop hypotension, the PPI decreased while SVR increased during fluid withdrawal, suggestive of peripheral vasoconstriction lowering PPI but maintaining blood pressure. Conversely, patients developing fluid withdrawal-induced hypotension tended to have higher baseline SVR and thus less ability to further vasoconstrict during a fall in blood flow. Therefore, the only difference at baseline between the patients who developed hypotension and the patients who did not was a lower PPI, suggesting that PPI reflects basal vasomotor tone, even though decreases of SV and CO were, as expected, accompanied by decreases in PPI in our patients, irrespective of hypotension. Our results thus partly agree with those obtained by van Genderen et al. in healthy volunteers.[10] They demonstrated that in healthy volunteers subjected to progressive reductions in central blood volume using lower body negative pressure, PPI decreases with the onset of central hypovolemia.

The $T_{skin-diff}$ increased most in hypotensive patients, but baseline values did not predict hypotension. In a previous study there is a good relationship between $T_{skin-diff}$ and PPI in anesthetized patients to identify the initiation if thermoregulatory vasoconstriction.[11] A possible explanation for lack of similarity in hypotension prediction in our study is that it takes more time for skin temperature to decrease as a result of peripheral vasoconstriction, in contrast to PPI which may reflect real time changes in peripheral vasomotor tone. The unchanged StO_2 is in accordance with a study from De Blasi et al.[12] They demonstrated that during hemodialysis in patients with end stage kidney disease StO_2 remained unaltered. The slight increases rather than decreases in StO_2 could be explained by the fact that the value represents a global reflection of oxygenation in all vascular compartments and does not directly reflect changes in vasomotor tone.

This study has several limitations that should be acknowledged. First, we have not included a control group on CVVH without fluid withdrawal. Therefore we cannot definitely exclude the effects on the measured parameters by CVVH itself. However it seems likely that initiation of CVVH itself gives a hemodynamic challenge, our patients had several hours of CVVH before start of the study. It therefore seems unlikely that initiation of CVVH has influenced the measured effects. Secondly, we have used a somewhat arbitrary decrease in SBP of ≥20 mmHg as cut off value to define hypotension. This is based on the international criteria for intradialytic hypotension in patients with end stage kidney disease on chronic hemodialysis.[9] We used this value because there are no well-defined criteria for hemodynamic instability following fluid withdrawal in the

critically ill. We have not withdrawn fluids in every patient until overt hemodynamic instability occurred (i.e. a decrease of MAP below 60 mmHg and increase in HR), for safety reasons. Hence, fluid withdrawal was discontinued before 2000 mL was reached in 7 of 13 patients in the absence of development of hypotension; conversely it was discontinued in all patients developing hypotension except for two in whom it was considered important to try to continue withdrawal. Thirdly, our results in this proof of principle study are based on a small population.

In conclusion, intolerance to fluid withdrawal is reflected by hypotension induced by a decrease in CO and lack of increase in SVR. Although changes in CO/SV are paralleled by changes in PPI, only baseline (or early unchanged) PPI and early decreases in CO/SV are able to predict fluid withdrawal-induced hypotension during CVVH in critically ill patients with AKI.

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PART D
SUMMARY AND CONCLUSIONS

10

SUMMARY AND CONCLUSIONS

INTRODUCTION

In this thesis we studied the value of noninvasive microcirculatory assessment to recognize and treat critically ill patients, in addition to the traditionally measured systemic hemodynamic parameters. With the availability of advanced technologies to measure microcirculatory perfusion, the role of the microcirculation in pathophysiological processes has emerged. With the current noninvasive techniques, microcirculatory perfusion can easily be evaluated at the bedside and has therefore been proposed as a target for therapies were systemic hemodynamic parameters lack sensitivity. This thesis is driven by problems commonly encountered in the clinical setting where microcirculatory perfusion is thought to play a pathophysiological role and/or possibly could replace more invasive (hemodynamic) measurements.

Part A (respectively, **chapters 1**, **2A** and **2B**) of this thesis provides an overview of the literature concerning microcirculatory perfusion in critically ill patients at the start of our research. **Chapter 2A** focusses mainly on the anatomy and function of several microvascular beds and describes disturbances found in the microcirculation during sepsis. **Chapter 2B** describes several methods available to monitor the microcirculation and the disturbances found in shock of various etiologies.

LASER SPECKLE IMAGING TO MEASURE MICROCIRCULATION

In part B we describe the use of Laser speckle imaging (LSI) to study microcirculatory perfusion. The full field laser technique provides real time images of blood flow. A granular or speckle pattern is produced when an area illuminated by laser light is imaged onto a camera. If the scattering particles are in motion, a time varying speckle pattern is generated at each pixel in the image. Using this approach, two dimensional maps of blood flow can be obtained with very high spatial and temporal resolution. At the time when we started our research, LSI had been used mainly in experimental research and only scarcely in patients. However whether LSI is indeed sensitive to changes in capillary perfusion had not been validated before. Therefore in **Chapter 3** LSI is compared to sidestream dark field (SDF) imaging, a technique capable of quantitatively measuring capillary red blood cell velocity, with the aim to validate LSI for the assessment of microvascular perfusion. For this purpose, simultaneous LSI and SDF measurements were performed on the human nail fold during gradual occlusion of the upper arm circulation to modify nail fold perfusion under controlled circumstances. To determine the ability of LSI to detect rapid changes in tissue perfusion during reactive hyperemia a vascular occlusion test was performed. Additionally, a hyperthermic challenge was applied to measure tissue perfusion using LSI under conditions of maximal functional capillary density. The main finding was that changes in perfusion as measured using LSI correlated well with changes in capillary red blood cell velocities. We have furthermore shown that LSI is capable of measuring tissue perfusion in high temporal and spatial resolution and that this technology can be used to assess microvascular reactivity to ischemic (low flow) and hyperthermic (high flow) challenges.

With the knowledge that LSI adequately reflects microcirculatory blood flow we used the LSI in an experimental model to investigate a commonly encountered clinical problem. In patients undergoing esophagectomy with reconstruction of a gastric tube, impaired anastomotic healing

caused by insufficient local blood flow is frequently seen. This can result in leakage and stenosis of the gastro-esophageal anastomosis. This insufficient local blood flow is attributed to poor arterial inflow and venous congestion. Therefore in **chapter 4** we describe the results of an experimental study on the effect of perfusion pressure in a pig model for gastric reconstruction. We hypothesized that increasing mean arterial pressure would improve blood flow, measured with LSI, at the anastomotic site of the gastric tube. A gastric tube was reconstructed in 9 pigs. LSI and thermographic imaging were used to measure blood flow, at the base, medial part, future anastomotic site, and top of the gastric tube. Measurements were repeated at every stepwise increase of mean arterial blood pressure (MAP) from 50 to 110 mm Hg. Besides MAP, global hemodynamics did not change throughout the experiment. The blood flow in the top of the gastric tube was significantly lower than the flow in the base and medial part of the gastric tube at all levels of MAP. Increasing MAP did not have a significant effect on blood flow at any location in the gastric tube.

In **chapter 5** we present a clinical study aimed to investigate whether LSI would be suitable for mapping functional brain areas during neurosurgical tumor removal procedures. For this purpose, LSI measurements were performed on brain tissue in patients undergoing awake neurosurgery. To determine the ability of LSI to detect local changes in cortical microcirculatory perfusion, patients were asked to perform a motor task, and the results were compared with standard electrocortical stimulation mapping data. After opening the dura mater, patients were woken up, and LSI was set up to image the exposed brain area. Patients were instructed to follow a rest-activation-rest protocol in which activation consisted of the hand-clenching motor task. Subsequently, exposed brain areas were mapped for functional motor areas by using standard electrocortical stimulation (ECS). Changes in the LSI signal were analyzed offline and compared with the results of ECS. The changes in laser speckle perfusion, as a measure of cortical microvascular blood flow when performing a motor task, correlated with the ECS mapping data. This correlation was demonstrated objectively by significant changes in laser perfusion blood flow in areas determined to be functional motor areas by ECS during hand clenching as compared with control regions (offline analyses). Furthermore, we saw visible changes in the color-coded laser speckle images when the motor task was performed during surgery. A specific advantage of LSI is the ability to superimpose morphological images of the cortex surface on functional perfusion images, allowing direct and detailed neurosurgical identification of functional locations on the brain during surgery. In addition, it is a rapid-response imaging modality, which allows direct and instant assessment of cortical perfusion changes to motor or speech activity. Other functional imaging modalities that might be used during awake craniotomy for functional neurosurgery, such as infrared thermography and Doppler imaging, do not have such advantages.

MICROCIRCULATION AND ITS RELATION TO VOLUME STATUS

PART C focusses on several microcirculatory parameters in relation to volume and/or preload status. Theoretically administration of fluids can augment microcirculatory perfusion by its effects on systemic hemodynamic parameters (increasing cardiac output and perfusion pressure) and

by its effects on rheological parameters (decreasing microvascular viscosity). Administration of fluids however can also result in adverse effects on microcirculatory oxygen extraction by resulting in hemodilution and tissue edema, increasing the oxygen diffusion distance. In the critically ill, non-invasively obtained microcirculatory perfusion abnormalities measured sublingual or cutaneous are associated with a poor outcome, relatively independent of global hemodynamics. They can be ameliorated by fluid infusion and, in fact, perfusion abnormalities may be surrogate indicators of fluid responsiveness. In order to study this observed discrepancy between global and microvascular hemodynamics we hypothesized that sympathetic activation that may result in peripheral vasoconstriction may explain this discrepancy in the critically ill. Changes in sympathetic tone concomitantly with changes in volume status may confound measurements of microvascular perfusion abnormalities to reflect global hemodynamics and resultant tissue oxygenation remote from the sublingual or cutaneous microvasculature.

In **chapter 6** we tested the hypothesis that the site and type of microvascular perfusion response to a cardiac preload challenge depends on sympathetic tone alterations. Therefore we focused on effects of increasing preload on microvascular perfusion parameters with and without altering sympathetic activity in healthy volunteers. We aimed to investigate which of the microvascular parameters can be used to truly reflect a preload challenge and resultant increase in forward flow, so called fluid responsiveness, by studying effects of two postural changes known to establish a preload challenge but with different effects on the autonomic nervous system. We used the sublingual and cutaneous microvascular beds because these are the most easily accessible and most studied microvascular beds in critically ill patients. Fifteen healthy volunteers underwent a postural change from head up tilt (HUT) to supine position, diminishing sympathetic tone, followed by a 30° passive leg raising (PLR), with unaltered tone. Prior to and after the postural changes, SV and CO were measured as well as sublingual microcirculatory perfusion (sidestream dark field imaging), skin perfusion and oxygenation (laser Doppler flowmetry and reflectance spectroscopy). HUT to supine posture change increased CO, SV and pulse pressure, while heart rate, systemic vascular resistance and mean arterial decreased, in the responders (≥ 10% increase in CO). In the latter, microvascular flow index, laser Doppler flow, microvascular hemoglobin oxygen saturation and concentration increased. During PLR, only CO, SV and sublingual functional capillary density increased in responders (≥ 5% increase in CO), whereas systemic vascular resistance decreased less than from HUT to supine. When cardiac preload and forward flow increase with diminishing sympathetic activity (i.e. postural change from HUT to supine) microvascular perfusion indices in skin as well as the sublingual area increase. When only preload and flow increase in a much lesser degree than HUT to supine, without altering sympathetic activity (i.e. PLR test) sublingual functional capillary density is increased. Therefore our results indicate that sublingual functional capillary density is the best parameter to use when evaluating fluid responsiveness independent of reflex activity of the autonomic nervous system.

In **chapter 7** we further explore the relationship between systemic hemodynamic and microvascular parameters in an experimental setting. This chapter describes the effect of cardiac output resuscitation on different microvascular perfusion and peripheral perfusion variables during different endotoxemic and obstructive shock states. We hypothesized that

only in endotoxemic shock, microvascular and peripheral perfusion abnormalities would persist after the resuscitation of systemic hemodynamics to pre-shock levels and that these abnormalities would be remedied with additional resuscitation of cardiac output in a different magnitude for the different peripheral tissues and the different regional perfusion variables. In this controlled experimental study, fourteen fasted anesthetized mechanically ventilated domestic pigs were randomly assigned to the endotoxemic (n = 7) or obstructive shock (n = 7) model. Systemic hemodynamic and regional perfusion parameters were obtained at baseline, during greater than or equal to 50% reduction of CO (T1), after initial resuscitation to baseline (T2), and after optimization of CO (T3). Regional perfusion was assessed in the sublingual, intestinal, and muscle vascular beds at the different time points. Hypodynamic shock (T1) simultaneously decreased all regional perfusion variables in both models. In the obstructive model, these variables returned to baseline levels at T2 and remained in this range after T3, similar to CO. In the endotoxemic model, however, the different regional perfusion variables were only normalized at T3 associated with the hyperdynamic state at this point. The magnitude of changes over time between the different vascular beds was similar in both models, but the endotoxemic model displayed greater heterogeneity between tissues. Resuscitation of CO to pre-shock levels produced a full recovery of the peripheral circulation in obstructive but not in endotoxemic shock. Apparently, the relationship between the systemic circulation and different microvascular and different peripheral perfusion variables is dependent on the underlying cause of circulatory shock. Our data show that different microvascular and peripheral perfusion variables can be used to assess the adequacy of hemodynamic resuscitation during different types of shock. Supranormal optimization of CO is needed to restore these different variables in sepsis but might still not lead to full recovery of tissue oxygenation. Further research is required to assess the reproducibility of our findings in a clinical setting and further elucidate the relationship between systemic and different peripheral circulation and oxygenation variables as targets for systemic therapeutic interventions during the early phase of septic shock.

In **chapter 8** we studied whether parameters of tissue perfusion and oxygenation are able to predict and monitor fluid responsiveness, in critically ill, septic patients considered hypovolemic on clinical grounds after initial resuscitation. Therefore, critically ill, septic patients underwent infusion of 250 mL of colloids, after initial fluid resuscitation. Prior to and after fluid infusion, systemic hemodynamics, sublingual microcirculatory perfusion and skin perfusion and oxygenation were measured. In responders to fluids (defined as ≥5 or 10% increase in SV upon fluids), sublingual microcirculatory and skin perfusion and oxygenation increased, but only the increase in cardiac output, mean arterial and pulse pressure, microvascular flow index and relative Hb concentration and oxygen saturation were able to monitor a SV increase. Our proof of principle study demonstrates that non-invasively assessed tissue perfusion and oxygenation is not inferior to invasive hemodynamic measurements in monitoring fluid responsiveness. However skin reflectance spectroscopy may be more helpful than sublingual microcirculatory parameters. This may help fluid management in septic patients, even though an outcome benefit has yet to be demonstrated.

Taking in to account that parameters of tissue perfusion and oxygenation were able to monitor fluid responsiveness, we questioned whether these parameters could be of help in

guiding fluid withdrawal in patients with possible fluid overload. Therefore in **Chapter 9** we investigated whether non-invasive peripheral perfusion indicators prior to and during fluid withdrawal were able to predict central hypovolemia during progressive fluid withdrawal by CVVH in critically ill patients with AKI. Baseline PPI was lower in hypotensive than non-hypotensive patients and subsequent PPI values paralleled blood flow. A baseline PPI ≤0.82 AU predicted hypotension with a sensitivity of 70%, and a specificity of 92% (AUC 0.80 ± 0.11, P=0.004). Most hemodynamic values decreased in time, particularly in the hypotensive group, except for blood flow which decreased more in the non-hypotensive patients. Progressive and generalized vasoconstriction was more pronounced in the non-hypotension group. In conclusion, intolerance to fluid withdrawal is reflected by hypotension induced by a decrease in CO and lack of vasoconstrictor reserve. Although changes in CO/SV are paralleled by changes in PPI, only baseline PPI and immediate decreases in CO/SV are able to predict fluid withdrawal-induced hypotension during CVVH in critically ill patients with AKI.

CONCLUSION

Hemodynamic monitoring is essential in the care of every critically ill patient. One of the main goals of hemodynamic support is to preserve tissue perfusion. It is however known that tissue perfusion is more related to microcirculatory perfusion than systemic hemodynamic perfusion. Monitoring of the microcirculation has long been difficult. Due to the recent technologic advances it is nowadays possible to easily assess, microcirculatory perfusion at the bedside of critically ill patients.

In this order laser speckle imaging technique seems a promising tool to study several physiological and pathophysiological microcirculatory processes especially in settings where direct contact with the studied tissue is unwanted (e.g. during (neuro)surgical procedures). However whether LSI could replace currently used techniques needs further exploration. Furthermore microcirculatory imaging in critically ill patients admitted to the intensive care could be used to guide fluid administration and fluid withdrawal. Using a videomicroscopic device, such as the SDF-imager, fluid responsiveness can be monitored during fluid administration. Moreover, hypotension can be predicted when using peripheral perfusion index during fluid withdrawal. These findings demonstrate that additional microcirculatory imaging could be used to early identify specifically those patients in need of additional (fluid) therapy and open the perspective of microcirculation based fluid resuscitation. However, because we studied rather small sample sizes, it is difficult to discuss whether systemic hemodynamic monitoring can be replaced for microcirculatory monitoring. Although, combining both seems to have sufficient additional value.

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SAMENVATTING EN CONCLUSIES

INTRODUCTIE

In dit proefschrift hebben we onderzocht wat de waarde van niet-invasieve microcirculatoire monitoring is bij het herkennen en het behandelen van ernstig zieke patiënten naast de traditioneel gemeten systemische hemodynamische parameters. Met het beschikbaar komen van nieuwe technologieën om de microcirculatie te beoordelen is de rol van de microcirculatie in pathofysiologische processen opgekomen. Met de huidige niet-invasieve technieken is het mogelijk om microcirculatoire doorbloeding in de klinische praktijk aan het bed te evalueren. Mogelijk kan het zelfs gebruikt worden als een eindpunt van bepaalde behandelingen waar de systemische hemodynamische parameters niet gevoelig genoeg zijn. Dit proefschrift is voor het grootste deel gericht op problemen die we regelmatig in de klinische praktijk tegenkomen, waarvan gedacht wordt dat de microcirculatoire doorbloeding een belangrijke pathofysiologische rol speelt of waarvan gedacht wordt dat microcirculatoire monitoring meer invasieve (hemodynamische) monitoring zou kunnen vervangen. In dit deel zal ik onze belangrijkste bevindingen samenvatten en de implicaties voor de klinische praktijk beschrijven.

Part A (**hoofdstukken 1**, **2A** en **2B**) van dit proefschrift beschrijft de beschikbare literatuur met betrekking tot de microcirculatoire doorbloeding in ernstig zieke patiënten ten tijde van de start van ons onderzoek. **Hoofdstuk 2A** richt zich in het bijzonder op de anatomie en functie van de verschillende microcirculatoire vaatbedden en beschrijft de afwijkingen van de microcirculatoire doorbloeding die gevonden worden tijdens sepsis. **Hoofdstuk 2B** beschrijft de verschillende methoden die beschikbaar zijn voor het monitoren van de microcirculatie en vat de afwijkingen die in de verschillende oorzaken van shock gevonden worden samen.

LASER SPECKLE IMAGING VOOR HET METEN VAN DE MICROCIRCULATIE

In part B beschrijven we het gebruik van Laser Speckle imaging (LSI) voor het bestuderen van de microcirculatoire doorbloeding. Deze techniek is in staat om direct en continu doorbloeding in beeld te brengen. Een granulair of speckle patroon wordt geproduceerd wanneer een gebied dat geïllumineerd wordt door laserlicht op een camera geprojecteerd wordt. Als de verstrooiende deeltjes in beweging zijn ontstaat er een in de tijd variërend speckle patroon. De temporele en spatiële variaties in intensiteit van dit speckle patroon bevatten informatie over de beweging van de verstrooiende deeltjes. Het meten van de spatiële karakteristieken van de intensiteit fluctuaties geeft kwantitatieve informatie over "flow". Het gebruik van deze techniek geeft een tweedimensionale kaart van de doorbloeding met hoge spatiële (nauwkeurigheid van de verandering met betrekking tot de locatie) en temporele resolutie (nauwkeurigheid van de veranderingen in de tijd).

Ten tijde van de start van ons onderzoek werd LSI met name gebruikt in experimentele studies en alleen sporadisch in patiënten. Het was op dat moment nog onduidelijk of LSI ook sensitief de veranderingen in doorbloeding op capillair niveau kan weergeven. Met deze reden hebben we in **hoofdstuk 3** LSI vergeleken met sidestream dark field (SDF) imaging met als doel LSI te valideren voor het beoordelen van microvasculaire perfusie. SDF is een techniek die reeds gevalideerd is voor het kwantitatief beoordelen van de stroomsnelheid van erythrocyten

in capillairen. Tijdens deze studie werden LSI en SDF metingen uitgevoerd op het nagelbed van de vinger tijdens graduele occlusie van de circulatie van de bovenarm om de capillaire doorbloeding van het nagelbed gecontroleerd te veranderen. Om te bepalen of LSI in staat is om snelle veranderingen in weefseldoorbloeding weer te geven tijdens reactieve hyperemie werd tevens een vasculaire occlusietest uitgevoerd. Als laatste werd een hyperthermische test uitgevoerd om te beoordelen of LSI in staat is adequaat capillaire perfusie weer te geven bij maximale functionele capillaire dichtheid. De belangrijkste bevinding van deze studie is dat de waarden gemeten met LSI goed correleren met de verandering in snelheid van capillaire doorbloeding gemeten met SDF tijdens gecontroleerde afname van (micro)vasculaire doorbloeding. Daarnaast blijkt uit deze studie dat LSI in staat is met hoge spatiële en temporele resolutie de weefseldoorbloeding te meten en dat deze techniek gebruikt kan worden om microvasculaire reactiviteit na ischemie (lage flow) en hyperthermie (hoge flow)te beoordelen.

Met de kennis dat LSI adequaat de microcirculatoire doorbloeding kan weergeven, hebben we deze techniek gebruikt in een experimenteel model van een veel voorkomend klinisch probleem. Bij patiënten die een oesofagectomie met reconstructie van een buismaag ondergaan is de verminderde genezing van de anastomose mogelijk het gevolg van onvoldoende doorbloeding van de gastro-oesophageale anastomose. Dit kan uiteindelijk leiden tot lekkage en stenose van de anastomose. Het gebrek aan voldoende doorbloeding van de anastomose wordt meestal toegeschreven aan zowel matige arteriële doorbloeding als veneuze congestie. In **hoofdstuk 4** beschrijven we de resultaten van een experimentele studie naar het effect van het verhogen van de perfusiedruk op de doorbloeding van de buismaag in een varkensmodel. Onze hypothese was dat het verhogen van de gemiddelde arteriële druk (MAP) de doorbloeding, gemeten met LSI, van de anastomose van de buismaag zou verbeteren. In negen varkens werd een buismaag aangelegd. LSI en thermografische imaging werden gebruik om de doorbloeding van de basis, het mediale deel, de toekomstige anastomose en de top van de buismaag te meten. De metingen werden herhaald bij elke stapsgewijze toename van de MAP van 50 tot 110 mmHg. Behoudens de MAP waren er geen veranderingen in de systemische hemodynamische parameters gedurende het experiment. De doorbloeding van de top van de buismaag was significant lager dan de basis en mediale deel van de buismaag voor elke waarde van MAP. Verhoging van de MAP had geen significant effect op de doorbloeding op welke locatie van de buismaag dan ook.

In **hoofdstuk 5** presenteren we een studie met het doel uit te zoeken of LSI geschikt is voor het lokaliseren van functionele hersengebieden tijdens neurochirurgische operaties. We hebben LSI-metingen uitgevoerd op hersenweefsel van patiënten die een wakkere craniotomie ondergingen. Om te bepalen of LSI in staat is om lokale veranderingen in corticale microcirculatoire doorbloeding te meten werden patiënten gevraagd om een motorische taak uit te voeren, waarbij de resultaten werden vergeleken met de standaard uitgevoerde electro-corticale stimulatie (ECS) mapping. Na het openen van de hersenvliezen werden de patiënten weer wakker gemaakt en werd de LSI opgesteld. Patiënten werden gevraagd om op geprotocolleerde wijze de hand samen te knijpen. Vervolgens werden het geëxposeerde brein door middel van electro-corticale stimulatie (ECS) onderzocht op functionele motorische delen nodig voor het samenknijpen van de hand. Verandering in het LSI signaal werden offline

geanalyseerd en vergeleken met de resultaten van de ECS. De verandering in het LSI signaal (als maat van de corticale microcirculatoire doorbloeding) ten gevolge van de motorische taak correleerde goed met de ECS resultaten. Dit was eveneens duidelijk peroperatief te beoordelen door zichtbare veranderingen in de kleur gecodeerde LSI beelden tijdens het uitvoeren van de motorische taak. Een specifiek voordeel van de LSI is het feit dat het in staat is de functionele perfusie te projecteren op het morfologische beeld van het brein. Hierdoor is het mogelijk om direct (hoge temporele resolutie) en gedetailleerd (hoge spatiële resolutie) de functionele hersengebieden te identificeren tijdens neurochirurgische ingrepen.

DE MICROCIRCULATIE IN RELATIE TOT VOLUMESTATUS

Part C richt zich op de verschillende microcirculatoire parameters en de relatie tot volume en/of preload status. Theoretisch zorgt toediening van vocht voor verbetering van de microcirculatoire doorbloeding door het effect op de systemische hemodynamische parameters (verhoging van hart minuut volume en perfusiedruk) en door de reologische effecten (verlaging van microcirculatoire viscositeit). Toediening van vocht kan daarentegen ook nadelige effecten hebben op de microcirculatoire zuurstofextractie wanneer het gepaard gaat met een vergroting van de zuurstof diffusie afstand, door weefseloedeem en hemodilutie.

In ernstig zieke patiënten zijn afwijkingen in de microcirculatoire doorbloeding van de huid en sublinguaal geassocieerd met een slechte uitkomst, welke relatief onafhankelijk blijken te zijn van de globale hemodynamische parameters. Het is wel mogelijk om de afwijkingen in de microcirculatoire perfusie te verbeteren met therapieën die ook effect hebben op systemische hemodynamische parameters. Mogelijk zou de discrepantie tussen de systemische hemodynamische parameters en de microcirculatoire parameters verklaard kunnen worden door activatie van het autonome zenuwstelsel wat leidt tot vasoconstrictie. Gelijktijdige verandering in sympathische tonus en volumestatus zouden het effect op de microcirculatoire afwijkingen kunnen beïnvloeden.

In **hoofdstuk 6** testen we de hypothese dat de locatie en het type microvasculaire respons op een preload test afhangt van verandering in sympathische tonus. Het doel van deze studie was uit te zoeken welke van de microvasculaire parameters gebruikt kunnen worden om "fluid responsiveness" te beoordelen in gezonde vrijwilligers. We hebben onderzocht wat het effect is van het verhoging van de preload met en zonder veranderingen in de sympathische activatie op de microcirculatoire doorbloeding van gezonde vrijwilligers. Hiervoor hebben we de effecten van twee verschillende houdingsveranderingen bestudeerd die beide een preload toename geven, maar daarentegen een ander effect hebben op de sympathische tonus. Als microvasculair vaatbed hebben we gekozen voor het sublinguale en het cutane vaatbed aangezien deze met de huidige technieken het best toegankelijk zijn en bij ernstige zieke patiënten het meest bestudeerd worden. Vijftien gezonde vrijwilligers ondergingen een houdingsverandering van recht op staand naar liggende positie (*HUT to supine*) (met behulp van een tilt tafel) waarbij sympathische tonus afneemt. Dit werd gevolgd door een 30°C *passive leg raising* (PLR) waarbij sympathische tonus gelijk zal blijven. Voor en na de houdingsveranderingen werd het hartminuutvolume (HMV), het slagvolume (SV) en de sublinguale en cutane microcirculatoire doorbloeding gemeten. De houdingsverandering van *HUT*

to supine verhoogde in de responders (≥ 10% stijging van het HMV) het SV en de polsdruk, terwijl de hartfrequentie, de totale perifere vaatweerstand en MAP daalde. In de responders verbeterde de microvasculaire flow index, de laser Doppler flow en de microvasculaire hemoglobine saturatie en concentratie. Tijdens de PLR steeg in de responders (≥ 5% stijging van het HMV), het SV en de sublinguale functionele capillaire dichtheid. De PLR zorgde voor een daling van de totale perifere weerstand; echter in mindere mate dan bij de *HUT to supine* houdingsverandering. Wanneer de cardiale preload en het HMV stijgen, terwijl de sympathische activiteit vermindert, (*HUT to supine* houdingsverandering)dan verbetert de microvasculaire perfusie van zowel de huid als het sublinguale vaatbed. Wanneer de cardiale preload en het HMV in mindere mate stijgen, zonder dat er verandering optreedt van de sympathische activiteit (de PLR test), dan verbetert alleen de sublinguale functionele capillaire dichtheid. Dit lijkt dan ook de beste parameter om fluid responsiveness, onafhankelijk van de activiteit van het autonome zenuwstelsel, te beoordelen.

In **hoofdstuk 7** onderzoeken we de relatie tussen de systemische hemodynamische en microcirculatoire parameters in een experimentele situatie. In dit hoofdstuk wordt het effect van resuscitatie van het HMV op verschillende microcirculatoire en perifere perfusie parameters beschreven tijdens endotoxemische en obstructieve shock. Onze hypothese was dat alleen tijdens endotoxemische shock de afwijkingen in de microcirculatoire en perifere perfusie zouden persisteren na het resusciteren van het HMV naar pre-shock waarden. Daarnaast vroegen wij ons af of deze afwijkingen van de microcirculatoire en perifere perfusie zouden normaliseren wanneer het HMV verder verhoogd zou worden.

In deze gecontroleerde experimentele studie werden veertien gesedeerde mechanisch beademde varkens toegewezen aan een endoxemische (n=7) en een obstructief (n=7) model. Systemische hemodynamische en regionale perfusie parameters werden verkregen tijdens baseline, wanneer er een ≥ 50% daling was van het HMV (T1), na de initiële resuscitatie naar baseline waarden (T2) en na optimalisatie van het HMV (T3). De regionale perfusie werd beoordeeld in het sublinguale, intestinale en spiervaatbed tijdens de verschillende tijdspunten. Tijdens de hypodynamische shock (T1) waren in beide modellen alle regionale perfusie parameters simultaan verlaagd. In het obstructieve shock model normaliseerde de variabelen naar baseline waarden tijdens de initiële resuscitatie (T2) en deze bleven, evenals het HMV, normaal tijdens tijdstip T3. Echter in het endotoxemische model normaliseerde de regionale perfusie parameters pas tijdens tijdstip T3. Dit ging samen met een hyperdynamisch HMV. Tevens was er in het endotoxemische model een grotere mate van heterogeniteit tussen de verschillende vaatbedden.

Resuscitatie van het hartminuutvolume naar pre-shock waarden zorgt voor een volledig herstel van de perifere circulatie tijdens obstructieve shock, maar niet tijdens endotoxemische shock. Dit wijst erop dat de relatie van de systemische en verschillende microcirculatoire en perifere perfusie variabelen afhankelijk is van de onderliggende oorzaak van shock. Tevens wijzen onze resultaten op het feit dat de verschillende microcirculatoire en perifere perfusie variabelen gebruikt kunnen worden om de volledigheid van de hemodynamische resuscitatie te beoordelen. Supranormale optimalisatie van het hartminuutvolume lijkt nodig om de verschillende microcirculatoire variabelen te herstellen tijdens sepsis, maar hoeft niet te leiden tot volledig herstel van de weefseloxygenatie. Verder lijkt dit te gelden voor de effecten van vroege resuscitatie van septische shock, echter voor

de resuscitatie later in het beloop van de sepsis is dit nog onduidelijk. Om dit uit te zoeken is verder onderzoek nodig; met name in de klinische situatie.

In **hoofdstuk 8** beschrijven we het onderzoek naar welke parameters van weefselperfusie en oxygenatie in staat zijn "fluid responsiveness" te voorspellen en te monitoren in ernstig zieke septische patiënten. Deze patiënten zijn bestudeerd na de initiële resuscitatie en op klinische gronden beoordeeld als hypovolemisch. Ernstig zieke septische patiënten kregen een 250 ml toediening van vocht, in de tijdsperiode na de initiële resuscitatie. Voor en na de vochttoediening werden de systemische hemodynamische, sublinguale, cutane microcirculatoire en oxygenatie parameters gemeten. In responders (gedefinieerd als een ≥ 5% of ≥10% stijging van het SV na toediening van vocht) steeg de sublinguale microcirculatoire perfusie en de microcirculatoire perfusie en oxygenatie van de huid. Echter, de parameters die in staat waren om een stijging van het SV na vochttoediening te monitoren waren: het HMV, de MAP, de polsdruk, de microcirculatoire flow index en de relatieve hemoglobine zuurstof-saturatie en -concentratie. De non-invasief bestudeerde parameters van weefselperfusie en oxygenatie waren niet inferieur aan de invasief gemeten systemische hemodynamische variabelen in het monitoren van fluid responsiveness. Echter, de perfusieparameters van de huid lijken in deze patiënten een iets beter hulpmiddel dan de sublinguaal gemeten microcirculatie. Dit zou nuttig kunnen zijn in het bepalen van het vochtbeleid, echter om dit definitief te kunnen zeggen zal een voordeel op uitkomst aangetoond moeten worden.

Met de kennis dat sommige parameters van weefselperfusie en -oxygenatie in staat zijn om fluid responsiveness te monitoren, vroegen we ons af of deze parameters ook behulpzaam zijn bij het onttrekken van vocht in patiënten met mogelijke overvulling. In **hoofdstuk 9** hebben we onderzocht of niet-invasief gemeten parameters van perifere perfusie voor en tijdens het onttrekken van vocht, door niervervangende therapie, in staat zijn om centrale hypovolemie te voorspellen en te monitoren in ernstig zieke patiënten met nierfalen. Hieruit bleek dat de perifere perfusie index vóór het starten van het onttrekken, lager was in de patiënten die hypotensie tijdens het onttrekken ontwikkelde dan de patiënten die dat níet ontwikkelden. Ook volgde de daling van de perifere perfusie index tijdens het onttrekken de daling van het slagvolume. Een waarde van de perifere perfusie index van ≤0.82 AU kon redelijk sensitief en specifiek het optreden van hypotensie voorspellen. Verder bleek dat de patiënten die niet hypotensief werden tijdens het onttrekken van vocht, in staat waren tot verdere en meer gegeneraliseerde vasoconstrictie. Er lijkt dus een samenhang te zijn tussen de intolerantie van het onttrekken van vocht, door daling van het HMV gereflecteerd door het optreden van hypotensie, en de mate waarin het lichaam nog in staat is tot verdere vasoconstrictie.

CONCLUSIES

Hemodynamische monitoring is een essentieel onderdeel in de behandeling van ernstig zieke patiënten. Een van de belangrijkste doelen van hemodynamische ondersteuning is het behoud van weefselperfusie. Het is echter bekend dat weefselperfusie meer gerelateerd is aan microcirculatoire perfusie dan aan systemische hemodynamische perfusie. Het monitoren van

de microcirculatie is lang moeizaam geweest. Echter ten gevolge van recente technologische ontwikkelingen is het tegenwoordig mogelijk om op gemakkelijke wijze de microcirculatie aan het bed bij de ernstig zieke patiënt te beoordelen.

Uit onze onderzoeken blijkt dat laser speckle imaging een veelbelovende techniek is om verschillende fysiologische en pathofysiologische microcirculatoire processen te bestuderen. Deze techniek is met name veelbelovend in situaties waar direct contact met het te bestuderen weefsel onwenselijk is, zoals tijdens (neuro)chirurgische procedures. Het is echter nog te vroeg om te zeggen dat LSI de huidige gebruikte technieken kan vervangen.

Verder is het mogelijk om met microcirculatoire monitoring het beleid rondom vochttoediening en vochtonttrekking te bepalen. Met het gebruik van een videomicroscoop zoals de SDF imager kan fluid responsiveness beoordeeld worden. Tevens kan het optreden van hypotensie voorspeld worden door gebruik te maken van de perifere perfusie index tijdens het onttrekken van vocht. Deze bevindingen wijzen op een toegevoegde waarde van het monitoren van de microcirculatie bij het vroeg identificeren van patiënten die baat zullen hebben bij verder toediening van vocht en opent het perspectief naar een op de microcirculatie gebaseerde resuscitatie. Echter omdat in dit proefschrift slecht kleine populaties zijn onderzocht is het nog niet mogelijk om te zeggen dat het monitoren van de microcirculatie het monitoren van de systemische hemodynamiek kan vervangen. Echter het combineren van beiden lijkt veelbelovend.

PART E
APPENDICES

A

LIST OF PUBLICATIONS

PUBLICATIONS RELATED TO THIS THESIS

- **Klijn E**, Groeneveld ABJ, van Genderen ME, Betjes M, Bakker J, van Bommel J: Peripheral perfusion index predicts hypotension during fluid withdrawal by continuous veno-venous hemofiltration in critically ill patients. *Blood purification (in press)*
- **Klijn E**, Niehof S, Groeneveld ABJ, Lima A, Bakker J, van Bommel J: Postural changes in volunteers: sympathetic tone determines microvascular response to cardiac preload and output increases. *Clin Autonomic Res* (*in press*)
- **Klijn E**, van Velzen MH, Lima AP, Bakker J, van Bommel J, Groeneveld ABJ: Tissue perfusion and oxygenation to monitor fluid responsiveness in critically ill, septic patients after initial resuscitation: a prospective observational study. *J Clin Monit Comput* (*in press)*
- van Genderen ME, **Klijn E**, Lima A, de Jonge J, Sleeswijk VS, Voorbeijtel J, Bakker J, van Bommel J: Microvascular perfusion as a target for fluid resuscitation in experimental circulatory shock. *Crit Care Med* 2014; 42:e96-e105
- **Klijn E,** van Bommel J: Dynamische hemodynamische en microcirculatoire monitoring bij sepsis. *A&I* 2014; vol. 6, no. 3
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- **Klijn E**, Niehof S, de Jonge J, Gommers D, Ince C, van Bommel J: The effect of perfusion pressure on gastric tissue blood flow in an experimental gastric tube model. *Anesth Analg* 2010; 110:541-546
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- Den Uil CA, **Klijn E**, Lagrand WK, Brugts JJ, Ince C, Spronk PE, Simoons ML: The microcirculation in health and critical disease. *Prog Cardiovasc Dis* 2008; 51:161-170

PUBLICATIONS NOT RELATED TO THIS THESIS

- van Genderen ME, Engels N, van der Valk RJ, Lima A, **Klijn E**, Bakker J, van Bommel J: Early peripheral perfusion-guided fluid therapy in patients with septic shock. *Am J Respir Crit Care Med* 2015; 191:477-480
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- Lima A, van Genderen ME, **Klijn E**, Bakker J, van Bommel J: Peripheral vasoconstriction influences thenar oxygen saturation as measured by near-infrared spectroscopy. *Intensive Care Med* 2012; 38:606-611
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- Lima A, van Bommel J, Sikorska K, van Genderen ME, **Klijn E**, Lesaffre E, Ince C, Bakker J: The relation of near-infrared spectroscopy with changes in peripheral circulation in critically ill patients. *Crit Care Med* 2011; 39:1649-1654
- van Genderen ME, Lima A, de Geus H, **Klijn E**, Wijnhoven B, Gommers D, van Bommel J: Serum C-reactive protein as a predictor of morbidity and mortality in intensive care unit patients after esophagectomy. *Ann Thorac Surg* 2011; 91:1775-1779
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- van Bommel J, **Klijn E**: Stroke volume and passive leg raising predict volume responsiveness in ICU patients: who is actually responsive? *Crit Care* 2009; 13:423
- Den Uil CA, **Klijn E**, Brugts JJ, Lagrand WK, Spronk PE: Monitoring of the sublingual microcirculation in cardiac surgery using two-dimensional imaging. *Anesthesiology* 2008; 109:353-354

B

ACKNOWLEDGEMENTS - DANKWOORD

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Lieve Nanda, Lola, Esmee, Leonoor, Eric, Marthe, Aafke, Gerrie en Sanjeev, ik reageer nooit, ik bel (bijna) nooit, maar ik kom toch altijd...?

Lieve Loes en Aad, dank jullie wel dat jullie met zoveel plezier Finn altijd opvangen.

Lieve David, Daniël en Annemarie. Naarmate de jaren verstrijken wordt het alleen maar gezelliger met jullie (jullie maken me tegenwoordig in ieder geval minder belachelijk).

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C

PHD PORTFOLIO

PHD PORTFOLIO

Summary of PhD training and teaching activities

Erasmus MC Department: **Intensive Care**

Research School: **COEUR**

PhD period: **July 2007- December 2010**

Promotor: **Prof. dr. J. Bakker**
Prof. dr. A.B.J. Groeneveld

Supervisor: **Dr. J. van Bommel**

1. PhD TRAINING	Year	Workload (ECTS)
General courses		
- Biomedical English Writing and Communication	2008	4
- Biostatitistics for clinicians	2008	1
- Introduction in clinical research	2008	1
- Course in statistics in Intensive Care – prof. E. Lesaffre	2009	1
In-depth COEUR courses		
- Research seminars (Friday afternoon)	2007-2010	1.2
- Intensive Care Research	2010	1.5
Specific courses		
- Postgraduate course on microcirculation and mitochondrial dysfunction, Lisbon, Portugal	2008	1.5
- "Postgraduate course on Doppler-echocardiography in intensive care medicine", Brussels, Belgium	2010	1.5
Presentations		
- "The effect of passive leg raising and fluid therapy on peripheral perfusion" COEUR research seminar	2009	0.5
- "From macro- to microcirculation in critically ill patients" COEUR Intensive Care Research	2010	0.5
National conference – participation and presentation		
- Nederlandse Intensivistendagen (NVIC), Ede, Netherlands *Poster presentation*	2010	0.5
International conferences – participation and presentations		
- 28th ISICEM, Brussels, Belgium *Poster presentation*	2008	1.2
- 21th ESICM, Lisbon, Portugal *Poster presentation*	2008	1.2
- 29th ISICEM, Brussels, Belgium *Two poster presentations*	2009	1.2
- 22th ESICM, Vienna, Austria *Poster presentation*	2009	1.2
- 30th ISICEM, Brussels, Belgium *Poster presentation*	2010	1.2

-	23th ESICM, Barcelona, Spain *Poster presentation and oral presentation*	2010	1.5
-	24th ESICM, Berlin, Germany *Poster presentations*	2011	1.2
-	25th ESICM, Lisbon, Portugal *Poster presentation*	2012	1.2
Seminars and workshops			
-	PhD day 2009	2009	0.25
-	"Lactate in goal directed therapy", Rotterdam, the Netherlands	2010	0.25
-	PhD day 2010	2010	0.25
-	Intensive Care Adults research meetings (weekly)	2007-2010	1
-	Various intensive care (evening) symposia	2007-2010	0.5
2. TEACHING			
Lecturing			
-	The effect of stroke volume on parameters of peripheral perfusion	2009	1
-	Hemodynamics after esophagectomy	2009	1
-	Effects of a preload challenge on regional tissue perfusion in critically ill patients	2010	1
-	Volume regulation with CVVH	2010	1
Supervising practicals			
-	Various classes 'anaesthesia and intensive care' minor students	2009-2010	1
Supervising Master's theses			
-	Co-supervision of K. Moeksim	2009	1
-	Co-supervision of M. van Genderen	2010	1
Writing			
-	"Anesthesiologie en Intensive care" Contribution: "*Dynamische hemodynamische en microcirculatoire monitoring bij sepsis"*	2014	1

ECTS: European Credit Transfer and Accumulation System
1 ECTS credit represents 28 hours

COEUR: Cardiovascular Research School Erasmus University Rotterdam
ESICM: Annual Congress of the European Society of Intensive Care Medicine
ISICEM: International symposium on Intensive Care and Emergency Medicine

D

CURRICULUM VITAE

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Eva Klijn was born in Rotterdam on August 22, 1980. She attended secondary school at Melanchthon College in Rotterdam, where she graduated in 1998. In 2006 she obtained her medical degree at Erasmus University Rotterdam. Following graduation she worked for one year as a resident in Internal Medicine at the Sint Franscicus Gasthuis Rotterdam. During this residency she became interested in intensive care medicine. In 2007 she started a Ph.D.-project at the department of Intensive Care of the Erasmus Medical Centre. In 2011 she started her training in Internal Medicine under supervision of Prof.dr. J.L.C.M. van Saasse (Erasmus Medical Centre, Rotterdam) and Drs. A.P. Rietveld (Sint Franciscus Gasthuis, Rotterdam) in this latter hospital. In april 2014 she continued her speciality training in Internal Medicine at the Erasmus Medical Centre. In April 2015 she started her fellowship in Intensive Care Medicine under supervision of Prof. dr. J. Bakker (Erasmus Medical Centre, Rotterdam). She is living together with Wesley Hofman. They have a son, Finn Julius.