MICROCIRCULATION IN CRITICALLY ILL CHILDREN

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Microcirculation in critically ill children

Microcirculatie in ernstig zieke kinderen

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INTRODUCTION AND OUTLINE OF THE THESIS

CHAPTER 1

The microcirculation of the critically ill pediatric patient

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INTRODUCTION

Hemodynamic monitoring is the cornerstone of critical care, especially when the patient is unstable. It needs to be used with the perspective of tailoring treatment to physiology and the underlying disease process (1). Monitoring should be easy to apply and negative side effects should be limited. The results should be reliable and reproducible, not least because we also need to monitor response to therapy when cardiovascular insufficiency has been identified. One of the primary goals of hemodynamic monitoring is to alert the physician to impending cardiovascular crisis before organ or tissue injury ensues.

In general, the adequacy of circulatory stability is judged by clinical assessment of parameters that we can measure, e.g., blood pressure, urine output, heart rate and serum lactate concentration. However these are indirect clinical markers of systemic blood flow and, as such, they are unreliable estimates of overall hemodynamic status, during critical illness, irrespective of the experience of the assessing clinician (2, 3). The logic is obvious when one considers that since blood pressure is a regulated variable, a normal blood pressure does not necessarily reflect hemodynamic stability or perturbation (4).

Early recognition of hemodynamic instability in combination with an understanding of the often complex underlying pathophysiology is therefore essential. The clinical art is, first, to monitor the right parameters and, secondly, apply the right target values, which can vary according to age or underlying pathology. In critical illness these are not necessarily the same as normal values in health (5). Pediatric intensivists and anesthesiologists should be familiar with age-appropriate normal values and the physiological differences between adults and children.

Cardiovascular physiology of the pediatric patient

Differences in growth and development, as well as the pathophysiological response to illness mean that children should not be regarded as small adults and data obtained from adults cannot be easily extrapolated to children. Different body proportions, a higher metabolic rate, and lack of compensatory reserve for respiratory or circulatory threats are examples of factors that should influence ones approach to critically ill children.

The cardiovascular system changes markedly at birth due to dramatic alterations in blood flow patterns. Under normal circumstances, the fetal circulation, with its reduced perfusion of the lungs and intra- and extra-cardiac shunts between the pulmonary and systemic circulations, transitions rapidly to an adult circulation. The precipitous fall in pulmonary vascular resistance and corresponding increase in pulmonary blood flow leads to increased left atrial filling and closing of the intra-atrial connection (foramen ovale). The left ventricular preload rises and the cardiac output increases to meet metabolic demands (6). In the fetus and newborn the left ventricle is flattened and the right ventricle is dominant. Newborn babies have less compliant ventricles and there-

fore have compromised diastolic function. They have a reduced response to inotropes, volume loading, and increased sensitivity to afterload. The immature heart has reduced contractile reserve and a depressed contractile response to exogenous administration of catecholamines.

After the separation of the systemic circulation from the low pressure placental circuit, systemic vascular resistance (SVR) and left ventricular afterload rise steeply in concert with each other. Hence, neonates at birth have little inotropic reserve. They also have reduced preload reserve in comparison with older children and adults. After the transition from fetal to neonatal circulation, the SVR starts to drop and stroke volumes and cardiac output rise (7). Stroke volume index (SVI) and cardiac index (CI) continue to increase until the age of 5 years. SVI is stable and CI decreases slightly beyond the age of 5 years. The SVR decreases in the first decade, with the major increase followed by progressive decrease, occurring in the first 48 hours after birth (7). In infancy and childhood the myocardium adapts progressively to its new loading conditions and develops increased reserve to β adrenergic stimulation.

The compensatory mechanisms to hemodynamic disease appear to differ in children compared with adults. Adults with septic shock have decreased ejection fraction and increased cardiac output (CO) through ventricular dilatation and increased heart rate (8). Children with septic shock do not have ventricular dilatation (9). Therefore, the most important way of increasing CO in young children is to increase heart rate. However, there is a problem here. An adult can increase resting heart rate from 60 to 100 beats per minute, but a proportionate increase in an infant from 140 to 220 beats per minute is not sustainable. In adults with septic shock, the hyperdynamic state ('warm shock') is the hallmark of cardiovascular pathophysiology. In children the response is more heterogeneous (10). Only a small percentage of children who present with septic shock exhibit a hyperdynamic state, with diminished SVR that responds to vasopressor support without a decrease in CI (10). The main presentation in pediatric septic shock is a hypodynamic state ('cold shock') with low CO and a high SVR (11, 12).

Studies in man with septic shock suggest that low CO and/or low SVR is detrimental to organ perfusion and survival. Children with septic shock, who maintain a CI between 3.3 and 6.0 L/min/m^2, seem to have higher survival rate compared with those who have CI outside this range (12).

Hemodynamic monitoring of the pediatric patient

An important goal of hemodynamic monitoring is to be able to detect inadequate tissue perfusion and oxygenation at an early stage, long before it becomes detrimental. As a consequence, the monitor should prompt and guide resuscitation.

Three key components in the physiology of oxygen delivery can be identified: 1) uptake of oxygen in the lung; 2) transport and delivery of oxygen from the lung to the tissues; and 3) oxygen uptake and utilization by the tissues.

At present, devices available for bedside monitoring give limited information about these processes. Oxygen saturation (SO_2), measured by continuous pulse oximetry (SpO_2) and arterial blood gas analysis (SaO_2) provides information about the oxygen content of the blood (CaO_2), providing we also know the hemoglobin concentration. This covers oxygen uptake. Oxygen delivery (DO_2) and utilization are more difficult to assess. DO_2 is the product of CaO_2 and CO. However, at regional level, vascular resistance is a major factor. There is evidence that low CO is associated with increased morbidity and mortality (10). Assessment of CO by means of clinical estimation is inaccurate (3), and invasive measurements in children are little used (13, 14). As an alternative to invasive measurement of CO in adults, central or mixed venous oxygen saturation (SvO_2) has been used as a surrogate for the adequacy of CO. In children with complex congenital heart disease or intra- and / or extracardiac shunts SvO_2 is not a useful measure. Central venous or mixed venous oxygen saturation is also invasive and carries risks in small children. Volumetric indices of cardiac function (e.g., M-Mode, femoral artery thermodilution catheter) are used extensively in the adult intensive care unit (ICU) in order to derive oxygen delivery. However, in the pediatric ICU, intra- and extracardiac shunts may change their validity and relevance. Again, the risks accompanying the insertion of the catheters (e.g. arterial thrombosis) outweigh the potential benefits. Echocardiographic evaluation of CO is not consistently reliable because even in the hands of experienced operators the variation in measurements between and within individuals is large (15).Taken together, evaluation of CO is much less straightforward in children, as it is in adults.

In order to assess tissue perfusion, a variety of measures are followed, including capillary refill time (CRT), temperature, and serum lactate concentration. In circulatory failure there is a hierarchy in regional blood flow with diversion away from skin and muscles towards vital organs such as heart, brain, and kidneys. Thus monitoring skin perfusion could be an early marker of increased sympathetic activity and hypoperfusion. CRT is a useful clinical parameter during the acute assessment and resuscitation of dehydrated children (16, 17). It is non-invasive, easy to use, and cheap. Its use in the pediatric ICU however might be of limited value. The correlation with global hemodynamics is poor (18). Only a weak correlation exists between severely prolonged CRT and SVI and serum lactate concentration (18). In addition, confounding factors such as fever and use of vasoactive medications should be considered. Nonetheless, a dramatic change in this parameter should alert the clinician to the need for a more detailed hemodynamic assessment of the patient.

Lactate metabolism and the prognostic value of high serum lactate concentration in the ICU patient are well documented (19-21). However, the relationship between lactate

and tissue perfusion is not always well defined (22-24); possibly due to the fact that measured lactate is not only the result of the balance between anaerobic production and clearance, but that it may also arise from sources other than hypoxic tissues (25). Overall, these macrocirculatory parameters are currently considered as insensitive markers of tissue perfusion (26). Ideal hemodynamic monitoring should provide information about whether cells are receiving sufficient oxygen to sustain cellular mitochondrial production of ATP. Two key elements are DO_2 and the removal of waste products such as carbon dioxide (CO_2). Important factors that determine DO_2 are CO, blood hemoglobin concentration, SO_2 of the hemoglobin molecule, and convection and diffusion of oxygen from arterioles to cells. In critical illness, DO_2 is often deranged, and many of the therapeutic interventions in the pediatric ICU (e.g., fluid administration, blood transfusion, inotropes, mechanical ventilation) are, ultimately, used to improve DO_2. At present, no real-time monitoring tool for use at the bedside is available for tracking DO_2.

The microcirculation as an essential hemodynamic compartment

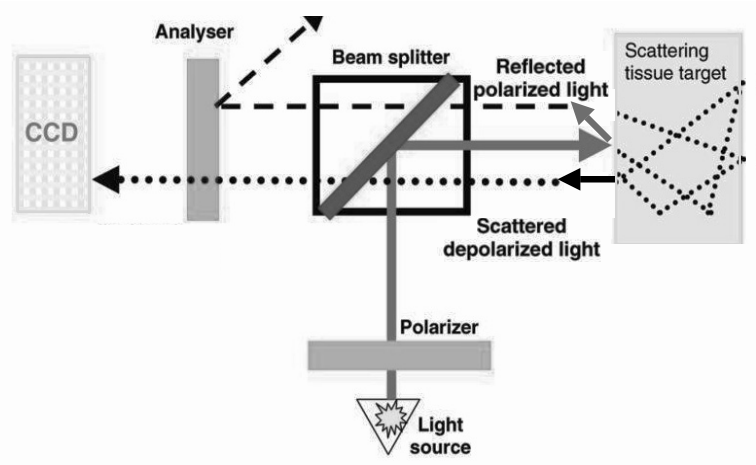
Circulatory shock is defined as failure of the cardiovascular system to maintain effective tissue perfusion, causing cellular dysfunction and subsequent acute organ system failure if not restored promptly. Although it is the macrocirculation that distributes blood flow throughout the body, it is the microcirculation that is the critical component of the cardiovascular system that ensures regional blood flow to individual tissues. Optimal macrocirculation, however, is the obvious prerequisite for adequate microcirculatory perfusion. Nevertheless, restoration of global hemodynamics does not always mean that adequate regional tissue perfusion is achieved, especially in conditions of impaired autoregulation such as occurs during critical illness. Previous studies in adults with septic shock have shown that indices of microcirculatory blood flow can serve as early indicators of tissue hypoperfusion, and therefore provide timely information about the potential onset of multi-organ failure (27-29). In health, microvascular perfusion is controlled locally so that tissue blood flow and substrate delivery is maintained, despite changes in arterial pressure (30). The lower limit of such flow-autoregulation – from first principles based on mean arterial pressure and other factors in Poiseuille's equation – varies between organs, patients, disease state, metabolic activity, and associated vasoactive therapies. Thus, there is no absolute threshold in blood pressure that defines adequate organ perfusion among organs, between patients, or in the same patient over time (31). However, because arterial pressure is a primary determinant of organ blood flow, hypotension is always pathological. Measuring the adequacy of microcirculatory blood flow as a direct indicator of the success of the cardiovascular system to provide adequate oxygen and nutrients to the cells, can be regarded as an important extension of the measurement of systemic hemodynamic variables (32). However, several issues need to be addressed. These are: 1) the reliability and reproducibility of the measure-

ment; 2) the identification of the most relevant microcirculatory parameters which need to be determined; and 3) the prognostic value of these parameters in guiding therapy.

Bedside measurement of the pediatric microcirculation

The microcirculation plays a crucial role in the interaction between blood and tissue, both in physiological and pathophysiological states. Analysis of alterations in microvascular blood flow therefore provides a unique perspective of disease processes at microscopic level (33). Orthogonal Polarization Spectral (OPS) imaging is the first hand-held imaging device that allows bedside visualization of the microcirculation. OPS imaging is based on the optical technique introduced by Slaaf et al. (34), where green polarized light is used to illuminate the tissue area of interest, which at the bedside is usually the buccal or sublingual mucosa. The green light is absorbed by hemoglobin within the red blood cells (RBCs) in the microcirculation. The reflected light is detected by an orthogonally placed analyzer which filters out surface reflections in order to produce a high-contrast reflected light image of flowing RBCs within the microcirculation (35) (Figure 1).

Figure 1. The CYTOSCAN E-II consists of a probe with a small lens attached to a halogen light source with a polarizing filter (selecting at a wavelength of 540 nm ± 10 nm) and a dichromic mirror. The light travels from the lens to the tissue of interest. Reflected light is filtered out by the analyzer and light that penetrates the tissue will be depolarized and can pass the analyzer to reach the CCD camera. The light is absorbed by hemoglobin, yielding an image of the illuminated vessels in negative contrast with a resolution of 1 pixel ≈ 1 µm.



OPS imaging has been assessed in several studies (36-40). Quantification of images is now standardized (41), reproducible, and validated (42). The parameters that are used to quantify the images include: the microvascular flow index (MFI), a measure of convective flow; and the functional capillary density (FCD) or vessel density index (VDI), for

diffusion distance and the heterogeneity index (HI).The MFI (42) is an average flow score, based on the integer score per quadrant that ranges from no flow to continuous flow. This score is calculated for the different types of vessels, i.e. small (<25 µm), medium (26-50 µm) and large (51-100 µm). For the FCD calculation (35), the assessor needs to trace the path of the moving RBCs within the capillaries (i.e., vessels <10 µm) using a software program (Capiscope version 3.7.1.0, KK Technology 1993-2000). A functional capillary is defined as a capillary that has at least one RBC moving through it during the observation period. Dividing the length of the perfused capillaries by the area of interest gives the density. In order to calculate the VDI the assessor draws a grid on the computer screen field-of-view composed of three equidistant horizontal and three equidistant vertical lines. Vessel density is calculated as the number of vessels crossing the lines divided by the total length of the lines. Assessment of the HI (29) involves evaluating three to five mucosal sites and measuring the MFI in each quadrant, taking the difference between highest MFI minus the lowest site MFI, and then dividing the number by the mean flow velocity.

A variety of techniques have been used to assess the microcirculation of critically ill patients (43). OPS imaging has been applied in adults in various clinical settings. Little is known about the microcirculation in children and infants. A few studies in newborns and in infants have used videophotometric microscopy or laser Doppler to evaluate blood cell velocity in the nailfold capillaries of the thumb (44-46). OPS imaging of the skin has been used in premature and term infants(47-50). These observations show that the microcirculation in premature infants can be quantified and RBC velocity can be measured.

OUTLINE OF THESIS

The general aims of this thesis were to assess the feasibility of OPS imaging of the buccal microcirculation in children and to investigate the effect of treatments used during intensive care treatment on microcirculatory hemodynamics. The hypothesis was that the microcirculation of critically ill children is altered.

Developmental changes of the microcirculation in young infants are described in chapter two. In chapter three I present a report of the OPS technique applied in a child with meningococcal sepsis. It shows the feasibility of the technique. Changes of the microcirculation in response to treatment with a vasodilator were observed in the first 24 hours after admission.

Treatments used in the pediatric ICU, such as inhaled nitric oxide (iNO) and extracorporeal membrane oxygenation (ECMO) are likely to influence microvascular flow. iNO is used frequently in neonatal and pediatric ICU, especially in neonates with pulmonary

hypertension (51). It is administered via the endotracheal tube in order to improve ventilation perfusion mismatch. It is known to be a pulmonary vasodilator. In chapter four the results of investigations of the effect of iNO on a distal microvascular bed (the buccal microcirculation) are presented. Neonates with severe respiratory failure, who were treated with ECMO were studied and compared with neonates who were not supported by ECMO. The results are shown in chapter five and chapter six. Sepsis is associated with severe alterationsin the microcirculation. The time course of these changes is presented in chapter seven.

In chapter 8 the key observations from the studies are summarized along with conclusions and future perspectives in regards to clinical practice and research into the microcirculation. Last, a summary is given in Chapter 9.

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NORMAL MICROCIRCULATION AND FEASIBILITY OF THE TECHNIQUE

CHAPTER 2

Functional capillary density decreases after the first week of life in term neonates

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ABSTRACT

Background

Changes in the microcirculation have been recognized to play a crucial role in many disease processes. In premature neonates functional capillary density (FCD) decreases during the first months of life.

Objective

The aims of this study were to obtain microcirculatory parameters in term neonates and older children who did not present with compromised respiration or circulation and to determine developmental changes in the microcirculation in young children.

Method

This single-center prospective observational study was performed at a level III university children's hospital. Subjects eligible for inclusion were children up to the age of 3 years who did not have any respiratory compromise, circulatory compromise or signs of dehydration. The buccal mucosa of 45 children was assessed, using orthogonal polarization imaging (OPS).

Results

We found a significantly higher FCD in neonates younger than 1 week, compared to older children. The median FCD was 8.1 cm/cm^2 (range 7.3-9.4) for 0- to 7-day-old neonates (n = 12), 6.9 cm/cm^2 (range 4.7-8.7) for 8- to 28-day-olds (n = 10), 7.3 cm/cm^2 (range 6.1-8.8) for 1- to 6-month-olds (n = 19) and 6.7 cm/cm^2 (range 6.5-9.2) for 3-year-olds (n=4). After the first week, there was no significant correlation between age and FCD.

Conclusion

FCD of the buccal mucosa decreases after the first week of life.

INTRODUCTION

The microcirculation plays a crucial role in the interaction between blood and tissue in both physiological and pathophysiological states. Analysis of microvascular blood flow alterations gives a unique perspective to study disease processes at the microscopic level (1). Measurement of tissue perfusion is of great importance in intensive care medicine, mostly because critically ill patients with abnormal tissue perfusion may develop multiple organ failure, a complication associated with high morbidity and mortality. Macrocirculatory parameters such as blood pressure, heart rate and hemoglobin oxygen saturation are rather insensitive markers of tissue perfusion (2).

A variety of techniques has been used to assess the microcirculation of critically ill patients. Orthogonal polarization spectral (OPS) imaging is a technique for bedside visualization of the microcirculation in which polarized light illuminates the area of interest, is reflected by the background, and is absorbed by the hemoglobin of the flowing red blood cells in the microcirculation (3). Specific optic filtration allows the elimination of light reflected at the surface of the tissue to produce high contrast reflected light images of the microcirculation. Therefore, in contrast to conventional intravital microscopy, there is no need to inject contrast or fluorescent dyes. This technique is safe, non-invasive and allows imaging of mucous membranes and surfaces of solid organs. The introduction of OPS imaging to clinical medicine has opened a new field of monitoring of the microcirculation for the investigation of the pathophysiological processes of various disease states (4).

The OPS imaging technique has been applied in adults in various clinical settings, mainly in sepsis. Little is known about the microcirculation in children and infants. A few studies in newborns and in infants have used videophotometric microscopy or laser Doppler to evaluate blood cell velocity in the nailfold capillaries of the thumb (5, 6). More recently, the group of Genzel-Boroviczeny applied OPS imaging in premature and term infants to study the microvascular perfusion of the skin (7, 8). This transdermal application of OPS imaging proved useful in premature infants as a non-invasive procedure and provided quantitative data of microvascular diameter and red blood cell velocity. The functional capillary density in the skin of premature infants decreased significantly over the first month of life and correlated with decreases in hemoglobin and incubator temperature (7).

We published data on the buccal microcirculation in critically ill neonates (8). Most research in adults is done in the sublingual region. This region is difficult or impossible to access in younger children because of the size of the probe, and measurements in this area are not as well tolerated. In children above the neonatal age the quality of transcutaneous measurements is poor because the skin is thicker. The vasculature of the skin is also influenced by environmental factors such as temperature (9). The skin

microcirculation has a specific function, namely thermoregulation. This function is not shared by other microcirculatory beds, which limits generality. In this study, the buccal mucosa was chosen as the site of investigation for several reasons. It shares a similar embryonic origin with the splanchnic mucosa, making it a relevant bed to study in pathological conditions (10). It is readily accessible and has become an important site for microcirculatory studies (2).

The aim of the present study was to determine developmental changes in microcirculation by obtaining microcirculatory parameters in term neonates and children up to the age of 3 years who did not present with compromised respiration or circulation. These data may serve as reference data for critically ill patients.

METHODS

Setting and Patients

This single-center prospective observational study was performed at the surgical ward and the surgical intensive care unit of a level III university children's hospital. Subjects eligible for inclusion were children up to the age of 3 years who did not present with any respiratory or circulatory compromise or signs of dehydration. These were mainly children with congenital malformations or children admitted for small surgical procedures. The age, gender, weight, underlying diagnosis and medication of all patients were recorded in a case report form. Written informed consent was obtained from the parents or guardians. Children with fever, signs of infection, congenital heart disease or lung disease were excluded.

Technique

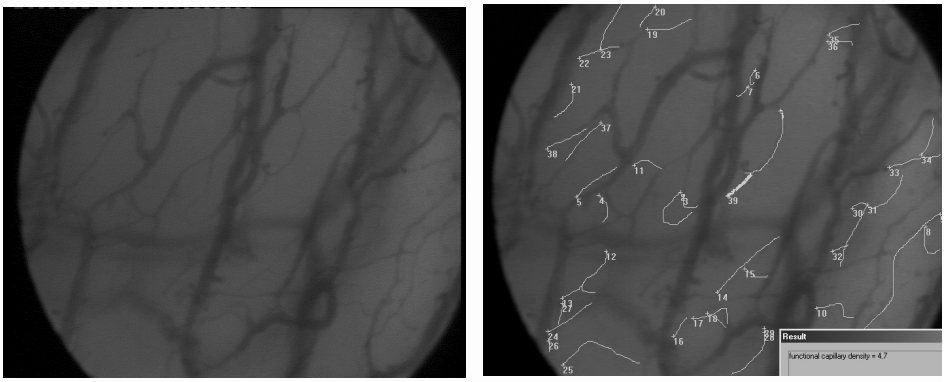
The microvascular network was studied with a CYTOSCAN E-II Backfocus type device (Cytometrics, Philadelphia, PA) using a 5 x objective. Data were recorded on a Sony DSR-20P digital video recorder. Segments of 5 seconds were selected and analyzed offline blindly by the first author. Quantification of the images was accomplished using functional capillary density (FCD) (3). The images created were analyzed with the Capiscope software (version 3.7.1.0, KK Technology 1993-2000). For calculation of the FCD, the operator traced out the path of the moving red blood cells within the capillaries. Dividing the total length of the perfused capillaries by the area of the field of view gives the functional capillary density (cm/cm^2).

Procedure

The microcirculation was assessed in the buccal mucosa. The inside of the cheek is readily accessible and intervention was well tolerated in this age group. Moreover, qualitatively good images could be obtained from this site.

In case the patient was scheduled for surgery, measurements were performed either before or at least one day after the surgery when there were no signs of postoperative pain or discomfort. All subjects had fasted for at least 30 minutes before the measurement. First, saliva was gently removed with gauze. The lens of the OPS imaging device was covered with a disposable sterile cap and was applied to the buccal mucosa without pressure (Figure 1). Images from three different regions were obtained and stored on digital videotapes. Segments of 5 seconds were selected and computer captured in AVI format. Images had to meet quality criteria, including adequate focus, the absence of saliva and the presence of visible capillaries. Segments that did not meet these criteria were discarded. For every child FCD values of the different video segments were averaged.

Figure 1. Example of an image (left) and a tracing of the capillaries (right) to determine the FCD.



Statistical Analysis

The data were analyzed using SPSS 15.0. Pearson's product moment correlation coefficient was calculated to establish the linear relationship between postnatal age and FCD. Patients were divided into four age groups: 0-7 days, 7 days- 28 days, 1-6 months and 2-3 years old. For comparison between groups the non-parametric Mann-Whitney test was applied.

Results

A total of 45 patients were included (15 female, 30 male). The age ranges within the different groups were 0 - 4 days (median 2 days), 9 - 22 days (median 15 days), 36 - 169 days (median 77 days) and 1151 - 1416 days (median 1228 days). Table1 shows the diagnoses, the number of patients and the FCD per group.

Table 1: Diagnoses, number of patients and gender per age group

Age group	Diagnosis	Number	Gender [male/female]	FCD [cm/cm^2]
0-7 days	Total	12	5/7	8.1 (7.3-9.4)
	Gastro intestinal	9		
	Urogenital	1		
	Meningomyelocele	1		
	Sacrococcygeal teratoma	1		
8-28 days	Total	10	6/4	6.9 (4.7-8.7)
	Gastro intestinal	9		
	Aplasia cutis	1		
1 - 6 monthsmonithmonths	Total	20	15/4	7.3 (6.1-8.8)
	Gastro intestinal	17		
	Urogenital	2		
	Sacrococcygeal teratoma	1		
3 years	Total	4	4/0	6.7 (6.5-9.2)
	Gastrointestinal	3		
	Subcutaneous abscess	1		

FCD is presented as median (range)

The median FCD was 8.1 cm/cm^2 (range 7.3-9.4) for 0- to 7-day-old neonates (n = 12), 6.9 cm/cm^2 (range 4.7-8.7) for 8- to 28-day-olds (n = 10), 7.3 cm/cm^2 (range 6.1-8.8) for 1- to 6-month-olds (n = 19), and 6.7 cm/cm^2 (range 6.5-9.2) for 3-year-olds (n = 4). (Figure 2). Figure 3 shows an example of an image.

Figure 2. Example of measurement technique in one of the patients.

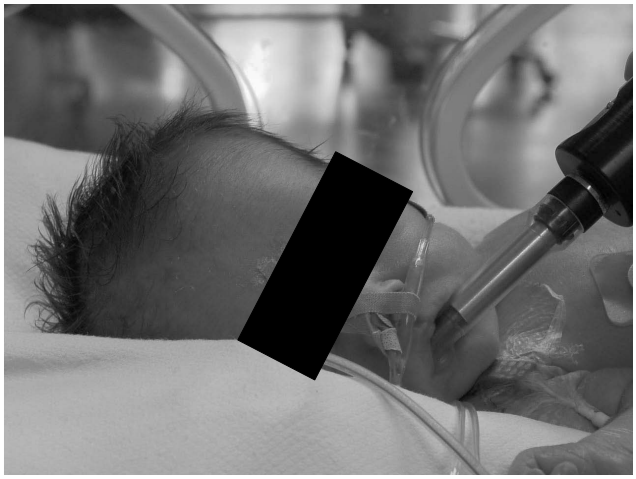
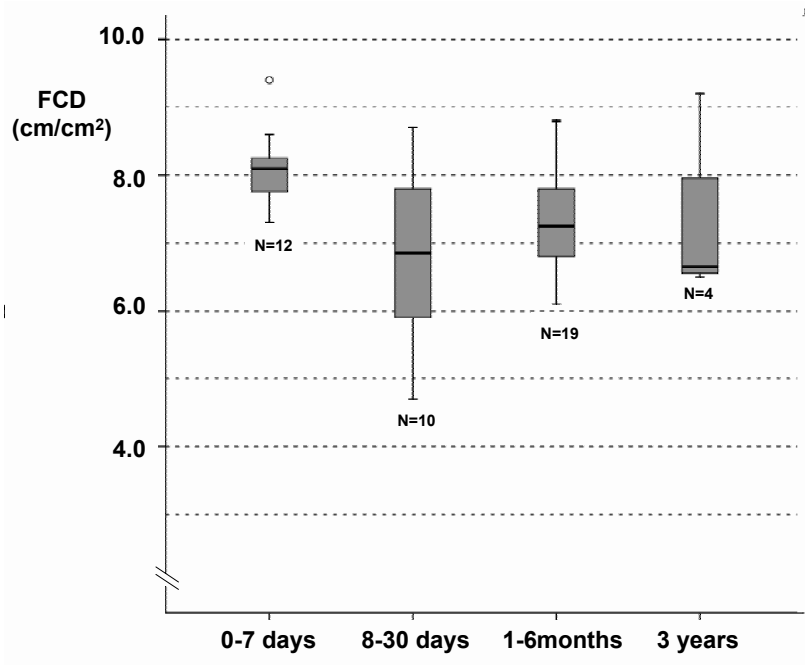


Figure 3. Boxplot showing functional capillary density (FCD) for different age groups (0-7 days, n = 12), (8-30 days, n = 10), (1-6 months, n = 19), (3 years, n = 4). FCD for neonates younger than 1 week was higher compared to older children. ($p < 0.05$)



DISCUSSION

The main finding of this study was the FCD of hospitalized infants was highest in the first week of life in term infants. This observation is consistent with findings from a study in premature and term neonates that employed this technique for measurements of the skin (7). Given that these were measurements in the skin, we cannot compare the results other than to conclude that postnatal reduction in FCD may be a generalized phenomenon. It would seem that developmental changes of the microcirculation in early postnatal life are related to adaptation after birth rather than post-conceptional age. It is believed that an adult pattern of microvasculature in the skin is reached by the age of 3 months (8). An important factor in this is cooling; therefore, it is unclear whether the same would apply for other microvascular beds.

The higher FCD in the first week of postnatal life may be related to the notably higher cardiac output in the first week (9). This is likely because the circulatory alterations that occur following birth impose an added workload (higher volume load and pressure load) on the left ventricle, whereas the pressure load on the right ventricle decreases. The added presence of a 2- to 3-fold increase in oxygen consumption at birth due to the

work of breathing, increased gastrointestinal function with feeding and especially the low temperature environment could be compensated for by higher systemic blood flow. Moreover, the high levels of fetal hemoglobin reduce the oxygen extraction level (9). Over the first few postnatal days resting cardiac output is highest and progressively falls afterwards over a 6- to 8-week period (9).

Several other cardiovascular changes found during the initial weeks following birth could account for the reduction in FCD observed here. Wu et al. found an increase in skin peripheral vascular resistance during the first 7 days of life and the neonatal period that correlated with a decreasing blood flow using electrocapacitance plethysmography (10). This technique, however, gives information about total blood flow and does not distinguish between flow and vessel density. Beinder et al. suggested that myogenic activity in skin arterioles increases with advancing age, a mechanism that might explain the reduced functional capillary density observed here (11). Finally, Hb level is closely correlated to FCD (7, 12) and has been found to decrease during the 5- to 6-week postnatal period. Although we did not find a progressive drop in FCD after the age of 1 week, decreasing Hb levels may have played a role in the developmental change in FCD during the first month of life. In this study, we were not able to correlate Hb levels with FCD.

Microvascular abnormalities can be frequently observed in critically ill patients and may play an important role in the pathogenesis of organ dysfunction. Especially due to the limitations of monitoring cardiac output and tissue perfusion (13, 14) in young children, this non-invasive technique might become an important additive bedside tool to the currently used monitoring tools in critically ill neonates and children.

The technique was well tolerated in children under the age of 6 months or over the age of 3 years. In children between 6 months and 2 years of age, it was often impossible to perform measurements because these patients were not compliant. Therefore, subjects in this age range could not be included in our study because it was too difficult to obtain stable images over a long period of time that fulfilled the quality criteria (15). This is because the probe is very sensitive to movements.

To our knowledge, this study presents the first observations of the buccal microcirculation in children with a presumably normal cardiovascular system. These data can serve as reference data for critically ill children and are relevant for future studies of the microcirculation in children. Our study also shows the feasibility of the technique in young children. The application in children who are not sedated is limited by the lack of compliance in younger age groups. Therefore, we recommend using this technique in sedated patients. This technique is a promising bedside tool for future clinical application. Further research is needed to investigate older age groups to determine if the trend observed in our study is limited to the neonatal period or whether developmental changes can be observed throughout childhood.

CONCLUSION

FCD of the buccal mucosa decreases after the first week of neonatal life. More research is needed to investigate the development of vascular resistance and other cardiovascular parameters after birth, which might underlie the developmental changes observed in the microcirculation of children.

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MICROCIRCULATION IN DISEASE AND THE EFFECT OF TREATMENT

CHAPTER 3

Microcirculation in meningococcal septic shock

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ABSTRACT

We report the case of a 3-year-old boy with meningococcal septic shock in whom we evaluated the microcirculation in an early phase of the treatment. Meningococcal septic shock can lead to multiple organ failure and death. Persistent disturbances in the microcirculation, despite recovery of global hemodynamics, may account for such deterioration. The microvascular network of the oral mucosa was studied. The functional capillary density was 3.2 on admission to the unit and improved to 7.2 after fluid resuscitation, inotropes and vasodilatation. Clinical recovery was good.

This in vivo observation of the microcirculation during the acute resuscitation of a child with septic shock suggests that the microcirculation improves by recruitment of capillaries.

INTRODUCTION

Sepsis and septic shock require emergency treatment with antibiotics, appropriate fluid resuscitation and if needed vaso-active drugs. Microvascular failure may persist, however, even when macrocirculatory parameters, such as blood pressure, are restored. Tissue dysoxia and organ failure may then be the result (1).

The treatment of septic shock aims to restore global hemodynamic function, but may not prevent deterioration of the microcirculation. Septic shock causes shunting of microcirculatory weak units and disturbs tissue oxygenation (2). Thus, resuscitation should target the oxygenation of the microcirculation. In this context, administration of vasopressors for restoring blood pressure to improve systemic oxygen delivery may have adverse effects and even cause more distress because it promotes shunting. Changes in the microcirculation cannot be predicted from changes in heart rate, blood pressure and pulse oxymetry (3). A new minimally invasive technique, Orthogonal Polarization Spectral (OPS) imaging allows imaging of the microcirculation in mucus membranes and the surface of solid organs.

In this report we demonstrate how OPS imaging revealed microcirculatory alterations in a child with meningococcal septic shock who received vasodilatory therapy.

CASE

A previously healthy 3-year-old male, who became acutely ill, unresponsive and febrile (41.4 °C), was admitted to the Intensive Care Unit after developing petechiae on his upper abdominal wall. The diagnosis of Neisseria meningitides type B sepsis was later confirmed. He received a total fluid amount of 40ml/kg and the maximum dose of dobutamine was 20 ug/kg.min, norepinephrine 1.0 ug/kg.min, and nitroglycerin 1.0 ug/kg.

METHODS

The microvascular network of the oral mucosa was studied with a CYTOSCAN E-II Backfocus type device (Cytometrics, Philadelphia, PA). A 5x objective was used. All data were visualized on a Philips black and white monitor and recorded on a Sony DSR-20P digital video recorder. The measurements were performed in a standardized way. Images from three different regions were obtained and stored on digital videotapes. Segments of 5 seconds were selected and captured in AVI (audio video interleave) format. Segments that did not meet quality criteria (4) were discarded. Simultaneously global hemodynamic parameters were recorded.

In accordance with the guidelines of our hospital medical ethical review board, informed consent is waived when standard therapy is monitored by non-invasive techniques.

The functional capillary density (FCD) of the vessels was determined by tracing out all of the red blood cell perfused capillaries. The total length of the capillaries (vessels, smaller than 10 µm) with flowing cells per field of view was measured using specialized software (Capiscope, version 3.7.1.0, KK technology 1993-2000) and expressed in cm/cm^2.

The Microcirculatory flow index (MFI) was applied to qualitatively assess perfusion. The MFI is based on the determination of the predominant type of flow in four quadrants. Flow is characterized as absent (0), intermittent (1), sluggish (2) or normal (3). The values of the four quadrants are averaged. Statistical analysis of the data was performed using SPSS 15.0. The significance of differences between scores was tested with the Wilcoxon signed rank test.

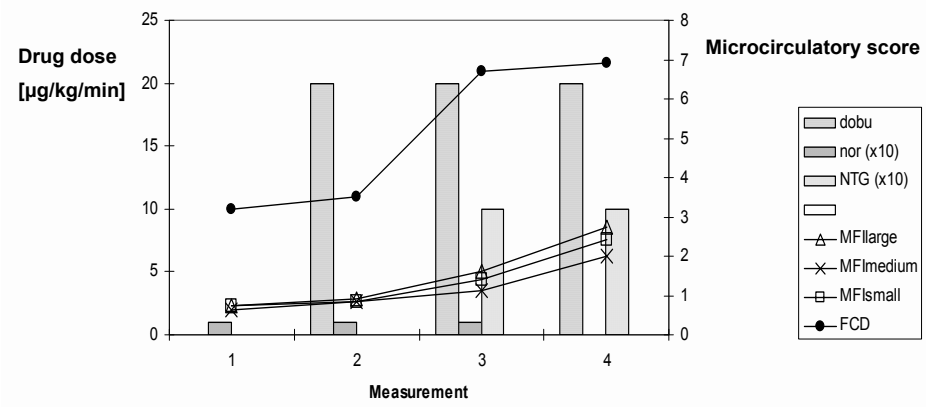
RESULTS

Table 1 shows vital signs and microcirculatory observations. The first measurement was performed after administration of 40ml/kg fluid and norepinephrine 0.1mcg/kg/min. The FCD was 3.2 cm/cm2; the MFI for large, medium and small vessels was respectively 0.75 / 0.63 / 0.75. OPS revealed impaired microvascular blood flow patterns, especially in the capillaries, as well as sludging of red blood cells. A second measurement was performed after administration of dobutamine 20mcg/kg/min. The FCD was now 3.5 cm/cm2; the MFI values were slightly higher, although not significantly, at 0.92 / 0.83 / 0.83 (large /medium/small vessels, respectively). Between these two measurements the heart rate increased from 153 to 173; the blood pressure improved from 71/35 mm Hg (mean 49 mm Hg) to 97/33 mm Hg (mean 57 mm Hg). The urine output was unchanged (1.2-1.4ml/kg/h). The third measurement, after the administration of 1 µg/kg/min nitroglycerin (NTG), yielded an FCD of 6.7 cm/cm2. The blood pressure was 77/42 mm Hg (mean 54 mm Hg) and the heart rate had risen to 181. The urine output had increased to 2.3ml/kg/h. Norepinephrine infusion was discontinued before the fourth measurement; the FCD was 6.9 and the MFI increased significantly for all vessel types 2.75 / 2.00 / 2.42 (large/medium/small). The blood pressure at that point was 102/46 mm Hg (mean 62 mm Hg) and the heart rate was 163. The urine output was 2.8ml/kg/h. Amounts of vasoactive drugs and microcirculatory parameters are shown in figure 1.

Table 1. Macrocirculatory and microcirculatory parameters

Measurement	1	2	3	4
Hours after admission	0.5	1.5	8	25.5
Heart rate (beats/min)	153	173	181	163
Respiratory rate(breaths/min)	27	37	25	28
Systolic Blood Pressure (mm Hg)	71	97	77	102
Diastolic Blood Pressure (mm Hg)	35	33	42	46
Mean Arterial Pressure (mm Hg)	49	57	54	62
Urine output (ml/kg/hr)	1.2	1.4	2.3	2.8
Serum lactate (mmol/l)	5.0	4.7	4.0	2.3
pH	7.39	7.24	7.41	7.44
FCD (cm/cm^2)	3.2	3.5	6.7	6.9
MFI large	0.75	0.92	1.63	2.75
MFI medium	0.63	0.83	1.13	2.00
MFI small	0.75	0.83	1.41	2.42

Figure 1. diagram showing improvement of functional capillary density (FCD) and microcirculatory flow index (MFI) after introduction of vasodilators.



DISCUSSION

In this child with septic shock, OPS imaging revealed a compromised microcirculation in the first hours of resuscitation, despite apparent improvement in global haemodynamic parameters after fluid boluses and start of norepinephrine. OPS imaging enables to visualize perfusion on a microcirculatory level in real time. After administration of dobutamine the heart rate and blood pressure went up, but microcirculatory perfusion did not seem to change. It was only after the administration of vasodilators that the microcirculatory parameters, in particular vessel density, improved. After discontinuation

of vasopressors the vessel density did not change and the microvascular flow further improved. All this suggests that initial vaso-active agents may affect the microcirculatory shunting. Blood flow and recruitment of vessels at microcirculatory level improved remarkably after the administration of vasodilators. Data in adults reported by Spronk et al (5) support the idea of a role for vasodilators in the improvement of the microcirculation. They showed that nitroglycerin could play a pivotal role in restoration of tissue perfusion. However, because that study was an observational one, it is impossible to tell whether pharmacologic effects or rather the natural course of the disease led to the observed improvement.

Active recruitment of the microcirculation with vasodilators could in fact be a primary target. This would restore the microcirculatory blood flow and thus match mitochondrial demands. Protecting the microcirculation by opening hypoperfused regions has been the key for improved tissue oxygenation in sepsis. Our study proved the feasibility of this new technique to visualize microcirculatory flow in real time. Further research should focus on the effect of disease on the microcirculation and to explore effects of treatments on the microcirculation.

CONCLUSION

OPS imaging is a feasible technique to evaluate the microcirculation and provides new insights in the dysfunctional microcirculation in septic shock in children. This technique could open the way for guided-therapy in selected patients using the microcirculation as a non-invasive biomarker.

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CHAPTER 4

Inhaled Nitric Oxide improves systemic microcirculation in infants with hypoxemic respiratory failure

Ped Crit Care Med 2011

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ABSTRACT

Background:
Inhaled NO (iNO) improves outcome in infants diagnosed with persistent pulmonary hypertension of the newborn (PPHN), by improving pulmonary blood flow and oxygenation. It reduces pulmonary vascular resistance, without fall in systemic blood pressure. iNO is also utilized in the treatment of acute hypoxemic respiratory failure in children and adults. It is felt to improve regional ventilation perfusion by regional selective pulmonary vasodilation.

Objectives:
To investigate the effect of iNO on the systemic microcirculation. We hypothesized that iNO improves the systemic microcirculation.
Design:
Pilot study

Setting:
Intensive care unit of a level III university children's hospital.

Patients:
Consecutive ventilated patients who were treated with iNO (20ppm) were enrolled in this study. Eight patients (6 boys, 2 girls) were included; 5 were diagnosed with congenital diaphragmatic hernia, 1 with PPHN, 1 with ARDS and 1 with bronchiolitis. The median age was 0 months (range 0 – 38 months).

Interventions:
iNO administration

Measurements and main results:
The microcirculation was assessed in the buccal mucosa within one hour before and within one hour after the start of iNO, using Orthogonal Polarization Spectral imaging. The median Functional Capillary Density (FCD) before the iNO was started was 4.0 cm/cm2 (range1.8 – 5.6) and improved to (4.9 cm/cm2 (range 2.8 – 6.6) (p=0.017)) after the start of iNO.

Conclusion:
Inhaled NO improves the systemic microcirculation in children with hypoxemic respiratory failure.

INTRODUCTION

Inhaled Nitric oxide (iNO) is frequently used therapy in neonatal and pediatric intensive care, especially in neonates with pulmonary hypertension (1). Persistent pulmonary hypertension of the newborn (PPHN) describes the state where pulmonary vascular resistance fails to fall after birth, resulting in a persistence of fetal circulation. As a consequence there is cyanosis due to right-to-left shunting of blood across the foramen ovale and the arterial duct (2). PPHN is diagnosed in 0.2 percent of live-born term infants. The management consists of identifying and treating the underlying cause (e.g. congenital, sepsis, meconium aspiration syndrome), as well as general supportive measures, such as sedation and analgesia; maintenance of adequate systemic blood pressure; drugs to increase pulmonary vasodilatation and decrease pulmonary vascular resistance; maintenance of acid-base balance with supportive mechanical ventilation to increase blood and tissue oxygenation, and normalize blood pH (3, 4). High-frequency oscillatory ventilation and iNO are frequently used in combination to provide a mode of mechanical ventilation that does not impair myocardial function and corrects reversible pulmonary hypertension (5).

Nitric oxide (NO) is a major endogenous regulator of vascular tone. INO has been investigated as treatment for PPHN in several studies including a number of randomized controlled trials (6, 7). It appears to improve outcome in hypoxemic infants and reduces the need for ECMO (8). Oxygenation improves in approximately 50% of infants receiving iNO (8). It affects the pulmonary microcirculation through vasodilation and reducing pulmonary vascular resistance, without effect on systemic global hemodynamics. It improves ventilation-perfusion matching (9). It decreases right to left shunting and improves oxygenation by improving the ratio of pulmonary to systemic vascular resistance. INO is thought to replace endogenous NO, which is normally produced by endothelial NO synthase.

The classical views of NO as short-lived paracrine agent, which specifically acts within the lung, are now yielding to a broader paradigm, in which remote delivery of NO is recognized. The exact mechanism is still under some debate, but is widely accepted that red blood cells (RBCs) and specific plasma proteins play a major role in the transport and delivery of NO to distant tissues (10-12). Hemoglobin (Hb)-O2 saturation and local redox conditions influence the local provision of NO bioactivity (13). Thereby coupling local oxygen gradients and microcirculatory blood flow regulation, by vasodilation or vasoconstriction (13).

In this study, we have examined the systemic microcirculation, by OPS imaging of the buccal mucosa, in infants with hypoxemic respiratory failure and PPHN. We hypothesized that iNO in this patient group would recruit the buccal microcirculation.

METHODS

This study took place in the Intensive Care Unit of Erasmus MC Sophia Children's Hospital Rotterdam, which serves as a level III referral center and ECMO facility in the Netherlands. During the study period, eligible consecutive mechanically ventilated patients treated with iNO were recruited and enrolled in this study. Patients with an oxygenation index (OI) greater than 20 were included. In accordance with the guidelines of the medical ethical review board of our hospital, informed consent was waived when standard therapy is monitored by non-invasive techniques.

All patients underwent echocardiography before the start of iNO. Patients were defined as having PPHN if the estimated pulmonary pressure (from the tricuspid valve regurgitation jet) was more than half the mean systemic arterial pressure. The microcirculation of the buccal mucosa was assessed before and after the start of iNO: within one hour before (T0) and one hour after (T1). Measurements were performed using our standard protocol (14). To evaluate the microcirculation, the CYTOSCAN E-II Backfocus type device was applied in order to capture images of the microcirculation. Video segments of 3 different areas of the buccal mucosa were obtained and the scores were averaged (14, 15).

The exclusion criteria were change of dose of inotropic or vasopressive drugs between the two measurements, administration of fluid bolus between the two measurements, congenital heart disease or pre-existing cardiomyopathy and severe blood loss.

General Management

All patients were mechanically ventilated. All patients received inotopes (dobutamine and /or dopamine) and if needed vasopressors (norepinephrine) to maintain a normal mean arterial pressure and to improve pulmonary perfusion. Sedation and muscle relaxants were given according to our standard protocol (midazolam, morphine and vecuronium). Nitric Oxide gas (Nederlandse Technische Gasmaatschappij BV), from a 1000 ppm cylinder, was introduced in the afferent limb of the ventilator circuit. The gas flow of the ventilator was adjusted to yield the target concentration of iNO. NO and nitrogen dioxide concentration in the inspired gas were measured by electrochemical monitors (Siemens).

MICROCIRCULATORY ANALYSIS

Images were analyzed for vessel density by an investigator (AT), who was blinded for the clinical course and order of the images. The Functional Capillary Density (FCD) was determined by tracing out all of the red blood cell perfused capillaries. In this way the

total length of the capillaries (vessels, smaller than 10 µm) with flowing cells per field of view was measured using specialized software (Capiscope, version 3.7.1.0, KK technology 1993-2000) and expressed in cm/cm^2 (16).

STATISTICAL ANALYSIS

Statistical analysis of data was performed using SPSS 15.0. Significant difference between scores (before and after iNO) was tested with the Wilcoxon signed rank test. The level of significance was set at $p < 0.05$. Patients who clinically responded to iNO were compared with patients who did not respond. A responder was defined as a patient who showed a greater than 20% improvement in PaO2 (17).

RESULTS

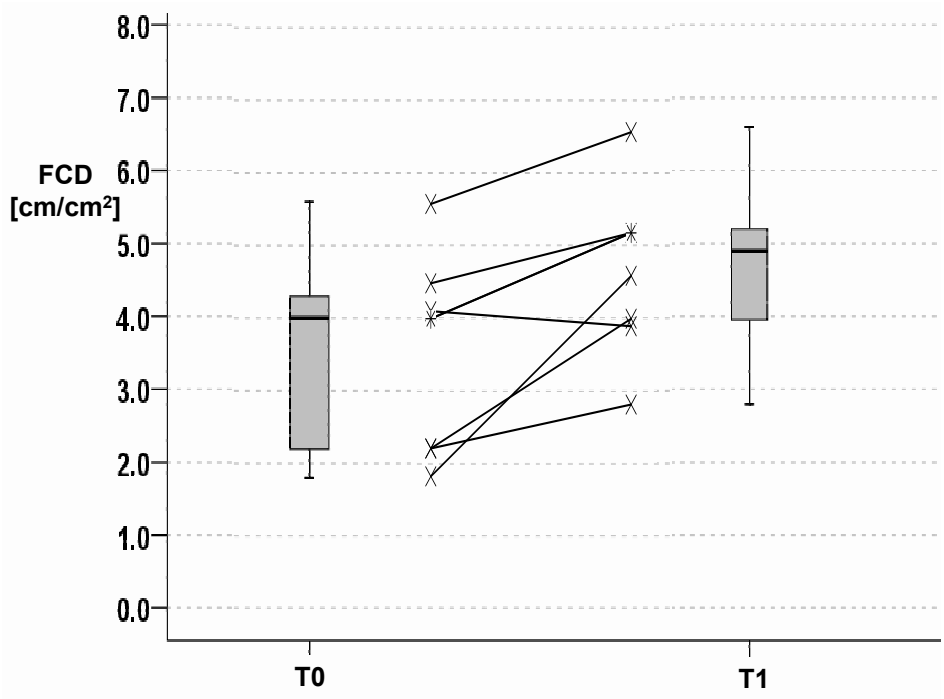
During the study, 11 patients were eligible for inclusion. Three subjects were excluded; one because of a cardiac malformation and two patients because the dose of vaso-active medication was adjusted between the two measurements. All neonates had PPHN diagnosed by a difference in pre- and post-ductal oxygen saturation and echocardiography. In the two older patients, the echocardiogram showed no signs of pulmonary hypertension. Table 1 shows background data and oxygenation and microcirculatory values. Only one patient responded clinically to iNO. Despite the treatment with iNO all neonatal patients needed treatment with ECMO after a median of 14 hours (range 5-85 hours). No changes in mechanical ventilation (except in FiO2), vaso-active medication or sedation were made between the first and the second measurement. Mean airway pressure remained unchanged between the two measurements. Last, global hemodynamics (heart rate, blood pressure) remained stable between the two measurements. The FCD increased from 4.0 cm/cm2 (range1.8 – 5.6) to 4.9 cm/cm2 (range 2.8 – 6.6) (p=0.017) (Figure 1). There was no difference between the responder and non-responders. Despite an improvement of FCD, microcirculatory restoration was not complete. Normal values vary according to age (18). Reference values for the studied group would lie above 6.0 cm/cm2 for all patients.

Table 1

Patient	Gender	Age [days]	Diagnosis	Hematocit [l/l]	OI pre-iNO	OI post-iNO	pO2 pre- iNO [mmHg]	Δ pO2 [%]	FCD pre-iNO [cm/cm2]	FCD post-iNO [cm/cm2]
1	male	0	CDH	0.43	70	63	28.5	0	4.0	5.2
2	male	0	CDH	0.59	31	30	58.9	0	2.2	4.0
3	male	1	CDH	0.62	55	38	33.0	15	4.1	3.9
4	female	0	CDH	0.41	33	61	60.0	-27	4.0	5.2
5	female	0	CDH	0.45	80	100	22.5	-4	5.6	6.6
6	female	24	MAS	0.26	23	30	86.0	-20	1.8	4.6
7	male	49	bronchiolitis	0.33	23	14	55.5	30	4.5	5.2
8	male	1165	ARDS	0.28	25	20	72.8	16	2.2	2.8

CDH = Congenital diaphragmatic hernia, ARDS = Acute respiratory distress syndrome, MAS = meconium aspiration syndrome, OI = oxygenation index.

Figure 1. Box plot illustrating the increase of the FCD between T0 (before) and T1 (after the start of iNO)



DISCUSSION

The main finding of this study is that iNO improves the FCD in a distal microvascular bed in hypoxemic respiratory failure. There was no effect on global hemodynamic parameters.

INO has been shown to reduce pulmonary vascular resistance and improve pulmonary hypertension and oxygenation in patients with PPHN, ARDS or after cardiac surgery (19-21). Several studies have investigated the effect of iNO distal to the pulmonary vascular bed. It does not affect systemic blood pressure (19, 20, 22).

Two mechanisms could explain the improvement in FCD of the buccal mucosa. Firstly, because iNO is a pulmonary vasodilator, it reduces right ventricular afterload and will therefore improve myocardial function and cardiac output. A shortcoming of our study was that central venous O2 saturation or other surrogate markers of cardiac output were not available. However based on the large body of evidence in the adult literature showing that microcirculation is relatively independent of global hemodynamic parameters (23, 24), it is unlikely that the improvement in macrocirculation explains the observed increase in microcirculatory perfusion. Secondly, and more likely, the improvement could be the result of remote NO bioactivity in the systemic microvasculature and impact on its perfusion directly.

Interactions between Hb and NO are very complex. Hb binds NO, forming S-nitrosohemoglobin (SNO-Hb), which is currently believed to be major mechanism behind the transport and delivery of NO bioactivity. When Hb is oxygenated in the lung, O2 triggers an allosteric structure transition of Hb and act as an electron acceptor necessary for SNO formation in Hb. Deoxygenation of Hb promotes the decoupling of SNO from Hb, which provokes vasodilation (11). Through the influence of Hb O2 saturation, redox conditions and other allosteric effectors on the local NO bioavailability (13), microcirculatory blood flow is regulated according to metabolic demands.

Cannon et al (25) showed restoration of vascular resistance and blood flow when endothelial NO synthesis was blocked. They suggest several mechanisms of vascular transport of bioactive NO by RBCs and plasma during iNO. They state that when Hb O2 saturation and tissue PO2 are very low NO release from RBCs occurs, which is consistent with mechanisms proposed in reviews of more recent literature (10, 11, 13). The production of SNO-Hb is now considered superior to methemoglobin and heme-iron nitrosyl hemoglobin under physiologic conditions (10).

This mechanism could be a plausible explanation for the observed recruitment of the microcirculation. Given the short time span between the two observations, lactate or urine output were not monitored and were deemed inaccurate to reflect change in tissue perfusion.

In the healthy state and under pathologic conditions, NO maintains microcirculatory homeostasis by regulating microvascular tone, leukocyte adhesion, platelet aggregation, microthrombi formation, and microvascular permeability (26-28). When the microvasculature sustains an insult (e.g. sepsis) the NO molecule becomes vital to maintaining microcirculatory patency, integrity and function (29). Nitric oxide is especially attractive as a candidate therapy to treat microcirculatory dysfunction in sepsis because in theory it could recruit microcirculatory flow. In clinical studies of sepsis using OPS imaging, the sepsis-induced impairment of sublingual microcirculatory blood flow was reversed with topical administration of acetylcholine (suggesting that the endothelium was still NO-responsive) (30), and intravenous nitroglycerin (a NO donor) (31).

To our knowledge, this is the first observation of the effect of iNO on the systemic microcirculation in pediatric patients.

CONCLUSION

Inhaled NO improves the systemic microcirculation in children with hypoxemic respiratory failure.

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CHAPTER 5

Changes in buccal microcirculation following extracorporeal membrane oxygenation in term neonates with severe respiratory failure

Crit Care Med 2009; 37(3):1121-1124
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ABSTRACT

Objectives

Extracorporeal membrane oxygenation (ECMO) is known to improve cardiorespiratory function and outcome in neonates with severe respiratory failure. In this study we tested two hypotheses: 1) neonates with severe respiratory failure exhibit alterations of the microcirculation, 2) after ECMO therapy these microcirculatory alterations are improved.

Design

Single-center prospective observational study

Setting

Intensive care unit of a level III university children's hospital.

Patients

Term neonates receiving veno-arterial ECMO. Control patients with and without respiratory failure.

Measurements and main results

The microcirculation was assessed in the buccal mucosa, using Orthogonal Polarization Spectral imaging, before and after ECMO. Functional capillary density was lower in patients with severe respiratory failure before ECMO (n=14) compared with control patients (n=10) ($p<0.01$). Functional capillary density had increased significantly after ECMO ($p<0.01$).

Conclusion

Microcirculatory parameters are depressed in neonates with severe respiratory failure and improve significantly following ECMO treatment.

INTRODUCTION

Extracorporeal membrane oxygenation (ECMO) is a cardiopulmonary bypass technique used as life support in selected newborns and children with acute reversible cardio respiratory failure when conventional management is not successful Worldwide, over 23,000 neonates have been treated with ECMO for respiratory problems, and the overall survival rate was 77% (1).

Monitoring tissue oxygenation is of great importance in intensive care medicine, as critically ill patients with abnormal tissue perfusion and oxygenation may develop multiple organ failure, a complication associated with high morbidity and mortality. So far, it is mainly macrocirculatory parameters such as blood pressure, cardiac output and hemoglobin saturation that are routinely measured. Regrettably these parameters are rather insensitive markers of tissue oxygenation. Orthogonal Polarization Spectral imaging (OPS imaging) allows direct visualization of the microcirculation (2). This non-invasive technique was recently studied in adults in various clinical settings, mainly sepsis, and showed microcirculatory alterations to be associated with poor outcome (3). These alterations appeared to be responsive to vasodilators (4). So far little is known about the microcirculation in children and infants. Genzel-Boroviczeny et al. studied the microvascular perfusion of the skin in preterm and term infants (5,6,). A literature search of PubMed yielded no reports on the microcirculation in critically ill newborns with severe respiratory failure.

We hypothesize that patients with severe respiratory failure, and therefore compromised cardiovascular function, show microcirculatory alterations and these alterations improve after ECMO therapy.

MATERIALS AND METHODS

Setting and Patients

This single-center prospective observational study was performed at an intensive care unit of a level III university children's hospital. Consecutive newborn patients receiving veno-arterial ECMO (VA-ECMO) were enrolled between 2004 and 2006. All patients in this study had pulmonary hypertension and therefore compromised cardiovascular function and were treated with VA-ECMO, according to our unit specific policy. To determine potential alterations of the microcirculation in patients with severe respiratory failure we compared this group to patients not receiving any form of respiratory or circulatory support who served as controls. As these patients soon are transferred it is impossible to continue measurements. Additionally the ECMO group was compared to a group of patients admitted to the same ICU, with severe respiratory failure, who did not

receive ECMO treatment. (Ventilated control group). This group consisted of neonates with congenital diaphragmatic hernia. These children were measured several consecutive days after admission. The first measurement was taken as a control for the before ECMO measurement and the last measurement was taken to evaluate the changes over time without ECMO treatment. Children with congenital heart disease were excluded.

In accordance with the guidelines of the medical ethical review board of our hospital, informed consent is waived when standard therapy is monitored by non-invasive techniques. Demographic data and clinical parameters before and after ECMO, like the vasopressor score (7) are shown in Table1. Patients in the study group had severe cardiorespiratory failure and hypoxemia despite adequate conventional treatments such as mechanical ventilation, sedation, muscle paralysis, vaso-active drugs, and nitric oxide inhalation. All patients met the established entry criteria for ECMO (8) and received ECMO treatment, according to a standard protocol. ECMO flow was weaned on the guidance of changes in arterial pO2 and signs of pulmonary hypertension, until a flow rate of 50ml/min was reached. ECMO was then discontinued by clamping the external circuit. Decannulation followed when the patient's clinical condition was satisfactory. If a patient's condition did not improve within the first couple of days on ECMO we started vasodilators to accelerate the weaning process.

Technique

OPS imaging (2), was used to visualize the microvascular network of the buccal mucosa. The measurements were done with a CYTOSCAN E-II Backfocus type device (Cytometrics, Philadelphia, PA) using the x 5 objective. All data were recorded on a Sony DSR-20P digital video recorder. Segments of 5 seconds were selected and analyzed off-line blindly by the first author. Quantifications of the images were accomplished as described previously (2). The total length of the capillaries (vessels, smaller than 10 µm) with flowing cells per field of view was measured using specialized software (Capiscope, version 3.7.1.0, KK technology 1993-2000) and expressed as functional capillary density (FCD (cm/cm^2)).

Procedure

The microcirculation was assessed within 24 hours before start and within 48 hours after discontinuation of ECMO. In the study period control subjects were measured once.

Before the measurements, saliva was gently removed with gauze. The lens of the OPS imaging device was covered with a disposable sterile cap and was applied to the buccal mucosa without pressure. This was best accomplished by placing a hand on the patient or on the bed for stabilization. The tip of the probe is inserted into the mouth and placed on the buccal surface. When an image appears the probe is retracted slightly prior to gently placing it on the surface of the mucosa in a way that the probe just makes contact

with the mucosa. Images from 3 different regions were obtained and stored on digital videotapes. Segments of 5 seconds were selected and captured in AVI format. Images had to meet quality criteria, such as adequate focus, the absence of saliva and the presence of visible capillaries. Segments that did not meet these criteria were discarded. For every measurement point FCD of the different video segments was averaged. If only one segment was good enough, this score was taken.

Statistical Analysis

The data were analyzed using SPSS 14.0. For comparison of two groups (e.g. patients before or after ECMO therapy and controls) the Mann-Whitney test was applied. Significance of difference between scores (before and after ECMO) was tested with the Wilcoxon signed rank test. The level of significance was set at $p < 0.05$.

Results

In the study period 41 patients were eligible. In 16 patients one of the measurements could not be done for logistic reasons. Two patients died immediately after ECMO, due to therapy withdrawal. Approximately 20% of all the captured video segments was judged as unfit for reliable analysis. In 9 cases one of the measurements was unfit in terms of image quality and these were rejected.

Video segments from 14 patients with respiratory failure, 9 boys and 5 girls, were eligible for analysis. The underlying diseases were congenital diaphragmatic hernia (n =7), meconium aspiration syndrome (n =5), and persistent pulmonary hypertension of the neonate (n = 2). Table 1 shows relevant clinical parameters. The median duration of the ECMO therapy was 141 (range 60 to 253) hours. Median time between the measurement and start of ECMO was 1.5 (range 1 to 24) hours and between discontinuation and the final measurement 4.5 (range 1 to 47) hours. Before ECMO 4 patients were treated with magnesium sulphate and none received Sildenafil, after ECMO 8 patients received magnesium sulphate and 3 were given Sildenafil in a dose of 5-10mg/kg/day.

The non-ventilated control group consisted of 5 boys and 5 girls. Diagnoses of these patients were esophageal atresia (n=4, including 2 patients with also anal atresia), meconium plug syndrome (n=2), duodenal atresia (n=1), meningomyelocele (n=1), omphalocele (n=1), sacrococcygeal teratoma (n=1). The ventilated control group consisted of 10 patients diagnosed with congenital diaphragmatic hernia, 5 boys and 5 girls. In one of the patients only one measurement could be done, so for the second measurement n=9. During the first measurement none of these patients were treated with magnesium sulphate and none received Sildenafil, During the second measurement one of these patients received magnesium sulphate and one was given Sildenafil in a dose of 8 mg/kg/day.

Table 1. Clinical parameters of patients before and after ECMO therapy and of controls

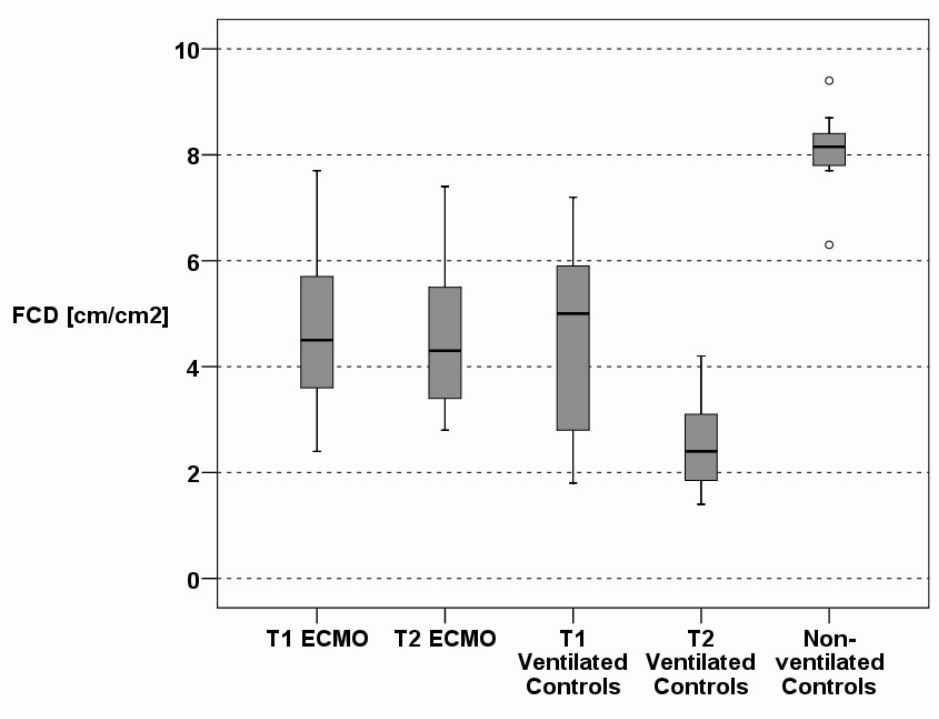
	ECMO group		Control groups		
	Before ECMO n = 14 Median (Range)	**After ECMO n = 14 Median (Range)**	**Non ventilated n = 10 Median (Range)**	**Ventilated, first measurement n = 10 Median (Range)**	**Ventilated, last measurement n = 9 Median (Range)**
Age [days]	0 (0-12)	6 (3-18)	1 (0-4)	0 (0-5)	7 (1-12)
Gestational age [weeks]	39 (36-42)		40 (38-42)	38 (37-39)	38 (37-39)
Birth weight [kg]	3.5 (1.9-5.1)		3.3 (2.5-4.1)	3.6 (1.8-5.4)	3.0 (1.8-5.4)
Mean Airway Pressure [cmH2O]	17 (14-27)	15 (10-19)[a]		13 (8-17)[a]	10 (8-14)[a]
Ventilation Mode [HFO vs. CMV]	10 vs. 4	3 vs. 11		7 vs. 3	2 vs. 7
Nitric Oxide (n)	9	1		5	2
Oxygenation Index	31 (5-86)	6 (2-15)[a]		7 (3-25)[a]	4 (2-7)[a]
Heart Rate [/min]	179 (120-209)	143 (121-158)[b]	139 (100-163)[b]	148 (113-193)	141 (113-172)[b]
Mean Blood Pressure [mmHg]	46 (29-77)	48 (36-64)	47 (32-65)	44 (32-60)	51 (42-60)
Pulse pressure [mmHg]	25 (11-40)	26 (18-35)	24 (16-31)	19 (12-36)	25 (15-28)
Vasopressor score	28 (0-180)	11 (0-30)[b]		65 (0-80)	10 (0-35)[b]
Midazolam [mcg/kg/hr]	100 (0-200)	250 (0-400)[b]		100 (0-200)	200 (0-300)
Morphine [mcg/kg/hr]	18 (10-40)	20 (5-400)		13 (0-23)	10 (0-20)
Paralysis [number of patients]	7	0		0	2
Hb [mmol/l]	9.2 (8.2-12.5)	7.0 (6.0-9.2)[a]	10,5 (6,7-13,6)	9.8 (6.2-11.0)	7.9 (6.1-10.8)

compared with before ECMO [a] $p \leq 0.01$, [b] $p < 0.05$
Abbreviations
HFO High Frequency Oscillation, CMV Conventional Mandatory Ventilation

Microcirculatory parameters

Functional capillary density (FCD) was significantly lower in patients with severe respiratory failure before ECMO therapy (n=14) compared to control patients (n=10) with a median of 4.5 (range 2.4 to 6.2) vs. 8.1 (range 6.6 to 9.4) respectively, ($p<0.01$). FCD increased significantly after ECMO therapy, median 6.8 (range 4.4 to 9.0), ($p<0.01$) but remained lower than the FCD of the control group ($p=0.05$). There was no difference in FCD between the ventilated control group and the ECMO group, before ECMO ($p=0.59$). FCD in the ventilated group was 5.1 (range 1.8 to 8.0). In the ventilated group the FCD did not improve (FCD 3.4 (range 1.2 to 5.1) ($p=0.18$), in contrast to the ECMO group. (Figure 1)

Figure 1. Box plot of Functional Capillary Density of newborns with severe respiratory failure. Before extracorporeal membrane oxygenation (ECMO) the functional capillary density (FCD) is significantly lower than the FCD of non ventilated control patients ($p<0.01$) and it is not different from the FCD of ventilated control patients at the same stage of the admission. After ECMO the FCD significantly improves ($p<0.01$). No improvement in FCD is seen in the ventilated control group after the same time course.



DISCUSSION

This is the first study to show that patients with severe respiratory failure have depressed microcirculation. In this study we focus on a specific group of patients who receive ECMO treatment after meeting criteria for ECMO treatment (8). The circulation and oxygenation of these patients are disturbed (1) indicating a compromised cardiovascular function. Reflecting this condition, these patients' microcirculatory parameters were significantly reduced before ECMO. Although macrocirculatory parameters like blood pressure were adequate, perfusion at the microcirculatory level was diminished. Studies in adults have shown that macrocirculatory parameters can not adequately reflect the perfusion at tissue level and that the circulation at microcirculatory level has a prognostic value (3,4).

The functional capillary density improved significantly following ECMO, as hypothesized, in parallel to improved clinical condition of patients. Improvement was achieved even though the Hb level, which has been described to promote a higher FCD (6), was

significantly lower after ECMO. FCD is highest in the first week after birth (9). Therefore, the improvement found, is most likely not to be ascribed to normal developmental changes, but rather to the clinical therapy of which ECMO was an important component. Although the effect of other factors like vasodilators can not be ruled out, this study describes changes in the microcirculation in a group of patients who received ECMO treatment following our standard of care. Because the vasopressor score at the second measurement, as well as the time interval between the measurements is comparable for the ECMO and the ventilated control group and the mean airway pressure is lower in the control group, we consider the clinical improvement in at the second measurement comparable. Therefore, this will not explain the difference in development of the FCD.

ECMO therapy gives time to restore normal pulmonary oxygenation to neonates with severe respiratory failure who do not respond to maximal conventional therapy. If lung rest does not achieve spontaneous clinical improvement, these patients receive phosphodiesterase inhibitors per our unit policy. Therefore, after ECMO many patients were given vasodilators, which may have contributed to the improvement seen in microcirculatory parameters.

The improvement in FCD after ECMO correlates well with an improvement in clinical condition. After ECMO patients needed less cardiac support and vasopressors, which may also have benefited the microcirculation. As the study sample was small, we were not able to perform a multi-variate analysis. Therefore, further research is needed done in larger samples to determine the influence of different variables.

CONCLUSION

Microcirculatory parameters are depressed in term neonates with severe respiratory failure and improve significantly following ECMO treatment.

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CHAPTER 6

Extracorporeal membrane oxygenation preserves the microcirculation in neonates with severe respiratory failure

Submitted

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ABSTRACT

Purpose

Extracorporeal membrane oxygenation (ECMO) is known to improve cardiorespiratory function and outcome in neonates with severe respiratory failure. We tested the hypothesis that veno-arterial ECMO (VA-ECMO) therapy improves the microcirculation in neonates with severe respiratory failure.

Methods

This single-center prospective observational study took place in an intensive care unit of a level III university children's hospital. Twenty-one term neonates, who received VA-ECMO treatment, were studied. The microcirculation was assessed in the buccal mucosa, using Orthogonal Polarization Spectral imaging, within 24 hours before (T1) and during the first 24 hours of ECMO treatment (T2). Data were compared to data of a ventilated (N=7) control group that was measured with the same time interval and a single measurement of a non-ventilated control group (N=10).

Results

The initiation of ECMO provides cardiac output and therefore has an impact on the macrocirculation, reflected by a significantly decreased vasopressor score. The microcirculation remained preserved in the ECMO treated group (functional capillary density (FCD) did not change; median 4.5 (range 2.4-7.7) at T1 and 4.3 (range 2.8-7.4) at T2 (p=0.47)), while it deteriorated in those patients solely treated with mechanical ventilation. The FCD in this group decreased from median 5.0 (range 1.8-7.2) at T1 to median 2.4 (range 1.4-4.2) at T2, (p=0.03).

Conclusion

VA-ECMO preserves the microcirculation in neonates with severe respiratory failure.

INTRODUCTION

Extracorporeal membrane oxygenation (ECMO) is a cardiopulmonary bypass technique used as life support in selected newborns and children with acute reversible cardiorespiratory failure when conventional management is not successful. Worldwide, over 24,000 neonates have been treated with ECMO for respiratory problems (1).

ECMO therapy gives time to restore normal pulmonary oxygenation in neonates with severe respiratory failure who do not respond to maximal conventional therapy and is regarded as a bridge to recovery. The institution of veno-arterial ECMO (VA-ECMO) partly takes over oxygenation and carbon dioxide removal and thereby allows ventilator settings to be reduced and restores circulation.

The institution of an ECMO circuit in neonates results in an expansion of the circulating volume by approximately factor 3. In VA-ECMO the heart is bypassed and flow in the systemic circulation is generated mostly by the ECMO pump, producing non-pulsatile flow. Especially during high ECMO flow rate (120-200 ml/kg/min) this results in disturbance of the physiologic blood flow, which can be represented by a flattening of the arterial pulse waves on invasive blood pressure monitoring.

In neonatal patients with severe respiratory failure, who meet the criteria for ECMO treatment (2), the circulation and oxygenation are severely compromised. Reflecting this condition, these patients' microcirculatory parameters are significantly reduced before ECMO (3). At the time when the patient no longer needs ECMO, the microcirculatory parameters are improved, correlating well with an improvement in clinical condition (3). Direct effects of artificial, non-pulsatile ECMO flow on the microcirculation are still not completely understood.

Based on clinical observations and the instant decrease of need for vasoactive medication after the start of ECMO therapy, we hypothesize, that microcirculatory alterations observed in neonates with severe respiratory failure improve with the initiation of ECMO therapy.

MATERIALS AND METHODS

Patients

Neonatal patients (aged ≤ 28 days) admitted to our ICU and treated with VA-ECMO were enrolled in this study. Patients were treated with ECMO, according to our unit specific policy. Patients suffering from congenital heart disease were excluded.

In accordance with the guidelines of the medical ethical review board of our hospital, informed consent was waived when standard therapy is monitored by non-invasive techniques. Patients in the study group had severe cardiorespiratory failure and hy-

poxemia despite adequate conventional treatments such as mechanical ventilation, sedation, muscle paralysis, vasoactive drugs, and nitric oxide inhalation. All patients met the established entry criteria for ECMO (2). Initially the aimed ECMO flow rate was 150-200 ml/kg/min and after 24 hours weaning of the flow was started under guidance of changes in arterial pO2 and signs of pulmonary hypertension.

In addition to the microvascular measurement, patient's demographic and clinical parameters, such as gender, birth weight, gestational age, postnatal age, diagnosis, ECMO flow, heart rate, blood pressure, mean arterial blood pressure, body temperature, administered medication, hemoglobin and hematocrit levels were recorded. Data were compared to data of control subjects, who were not ventilated, and to a group of patients with severe respiratory failure, who did not receive ECMO treatment. The non-ventilated group consisted of patients who were admitted to the same unit and did not receive any form of respiratory or circulatory support. This group served as a control group in a previous study (3). As these patients soon were transferred they were only measured once. In the ventilated control group patients were measured several consecutive days after admission. The first two measurements on consecutive days were taken to serve as control for T1 and T2 and to evaluate the changes without ECMO treatment.

Procedure

The microcirculation was assessed within 24 hours before start of ECMO (T1) and within 24 hours after start of ECMO (T2). OPS imaging (4) was used to visualize the microvascular network of the buccal mucosa. The measurements were done with a CYTOSCAN E-II Backfocus type device (Cytometrics, Philadelphia, PA), using the 5x objective.

Before the measurements, saliva was gently removed with gauze. The lens of the OPS imaging device was covered with a disposable sterile cap and was applied to the buccal mucosa without pressure, as described before (3). Images from 3 different regions were obtained and stored on digital videotapes, using a Sony DSR-20P digital video recorder. Segments of 5 seconds were selected and captured in AVI (audio video interleave) format. Video segments that did not meet quality criteria (3) were discarded. For every measurement, the FCD of the different video segments was averaged. If only one segment met the quality criteria, this score was taken.

Microcirculatory Analysis

Quantification of the images was performed as described previously (3, 4). To investigate vessel density the images were analyzed with the Capiscope software program (version 3.7.1.0, KK Technology 1993-2000). For the Functional Capillary Density (FCD) calculation, the analyst is required to trace out the path of the moving red blood cells within the capillaries (vessels, smaller than 10 μm). A functional capillary is defined as a capillary that has at least one red blood cell moving trough it, during the observation period.

Dividing the length of the perfused capillaries by the area gives the functional capillary density value expressed in cm/cm2

Statistical Analysis

The data were analyzed using SPSS 15.0. Significance of difference between scores (T1 vs.T2) was tested using the Wilcoxon signed rank test. For comparison of two groups, the Mann-Whitney test was applied. The level of significance was set at $p < 0.05$.

RESULTS

During the study period, 31 VA-ECMO patients were eligible for inclusion. Twenty-one patients were included according to the protocol. Four patients were missed for inclusion due to logistic reasons (researcher was not contacted in time or no investigator or camera available). Six patients were excluded because their video segments did not meet the quality criteria (3). The excluded group did not differ from the included patient group for gestational age, postnatal age, diagnosis, duration of ECMO treatment or mortality. The diagnoses of the ECMO patients were congenital diaphragmatic hernia (CDH) (n=10), meconium aspiration syndrome (n=5), persistent pulmonary hypertension of the neonate (n=5) and congenital cystic adenomatoid malformation (CCAM) of the lung (n=1). The group existed of 9 female and 12 male patients, with a median gestational age of 39.6 (range 34.4-42.5) weeks and a median birth weight of 3.1 (range 2.3-5.1) kg. In the non-ventilated group (n=10), the median age was 1 day (0-4), the median gestational age 40 weeks (38-42), and the median birth weight 3.3 kg (2.5-4.1). The ventilated control group consisted of neonates with CDH (n=7). Their median age was 1 day (0-6), their median gestational age 38.1 weeks (38-39.3), and their median birth weight 3.0 kg (3.0-3.8).

After the start of ECMO, macrocirculatory parameters were stable, while the dose of vasoactive drugs could be significantly reduced (Table1). The FCD did not change significantly. The FCD before ECMO (T1) was 4.5 (range 2.4-7.7) and after the start of ECMO therapy (T2) 4.3 (range 2.8-7.7), ($p = 0.47$). In the ECMO group, the FCD on T1 was not statistically significantly different from the FCD of ventilated control patients ($p=0.81$). In this group the median FCD decreased from 5.0 (range 1.8-7.2) to 2.4 (range 1.4-4.2) ($p = 0.03$). On T2, the FCD was significantly lower in the ventilated control group compared to the ECMO group ($p=0.002$). The FCD in the non-ventilated control group was significantly higher than in the other groups. (Figure 1)

All patients in the control group survived. Three patients in the ECMO treated group (CDH n=2, CCAM n=1) did not survive, due to recurrent and therapy resistant pulmonary hypertension.

Table 1. Macro- and microcirculatory values

	T1 ECMO	T2 ECMO	T1 Ventilated Controls	T2 Ventilated Controls
Age [days]	1 (0-12)	1 (0-12) [a]	1 (0-6)	1 (0-7) [a]
Time interval to or from start ECMO flow [hours]	2 (0.5-24)	2 (0.5-24)	-	-
Heart Rate [/min]	180 (120-220)	150 (106-198)	138 (113-191) [d]	129 (110-160)
Mean Blood Pressure [mmHg]	49 (29-77)	49 (35-86)	44 (32-60)	52 (41-63)
Pulse pressure [mmHg]	19 (10-40)	10 (0-33) [a]	25 (12-36)	24 (15-32) [c]
Vasopressor score	40 (0-140)	10 (0-108) [a]	15 (0-75)	19 (0-66)
Dopamine [µ/kg/min]	10 (0-20)	0 (0-20)	10 (0-21)	16 (0-21) [c]
Dobutamine [µ/kg/min]	10 (0-20)	5 (0-20)	10 (0-20)	5 (0-20)
Norepinephrine [µ/kg/min]	0.1 (0-1.0)	0 (0-0.9)	0 (0-0.4)	0 (0-0.3)
Inhaled Nitric Oxide [ppm]	20 (0-40)	0 (0-0) [a]	0 (0-19)	0 (0-20) [d]
Hemoglobin [mmol/l]	9.2 (6.9-12.6)	8.7 (6.7-12.0)	8.7 (7.4-11.0)	8.5 (7.8-10.8)
Hematocrit [l/l]	0.45 (0.32-0.62)	0.41 (0.31-0.56)	0.43 (0.38-0.53)	0.40 (0.34-0.53)
Temperature [degrees Celsius]	37.4 (34,4-38.6)	36.9 (35.9-38.4)	37.3 (36.7-38.4)	36.8 (36.5-37.3)
ECMO flow [ml/kg/min]	-	140 (110-210)	-	-
FCD [cm/cm2]	4.5 (2.4-7.7)	4.3(2.8-7.4)	5.0 (1.8-7.2)	2.4 (1.4-4.2) [b,c]

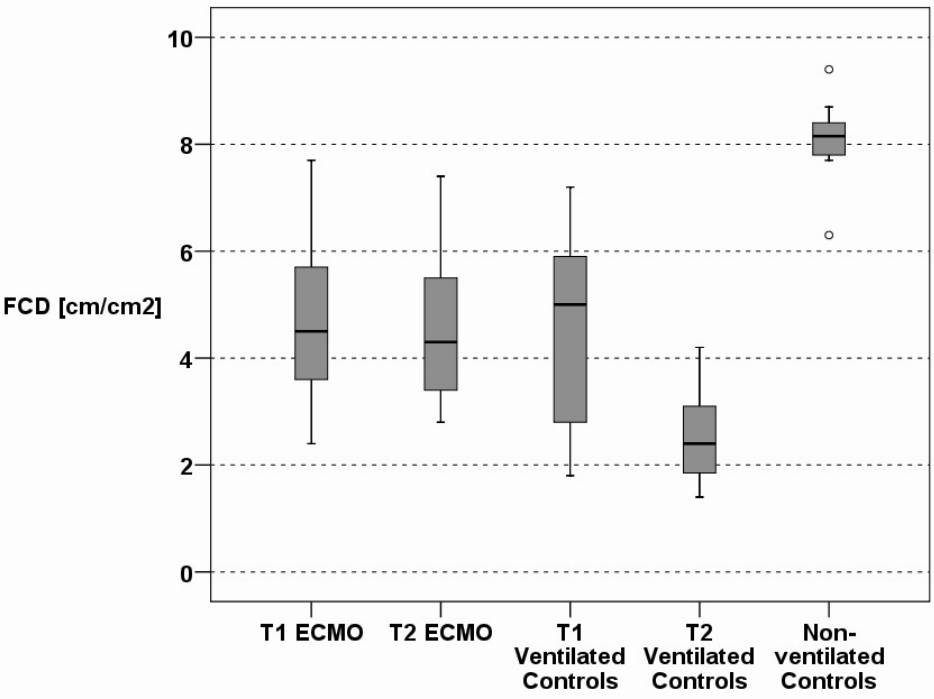
Data are presented as median and range. Comparison of T1 and T2 within groups was performed with the Wilcoxon signed ranks test. For comparison between groups the Mann-Whitney test was applied.
Compared toT1 within group [a] $p \leq 0.01$, [b] $p < 0.05$
Compared ECMO group [c] $p \leq 0.01$, [d] $p < 0.05$

DISCUSSION

The main finding of our study was that there was no change in microcirculatory parameters after the start of VA-ECMO therapy. Nevertheless, it seems to preserve the microcirculation, while deterioration was observed in patients with severe respiratory failure, who did not receive ECMO treatment. Despite the fact that patients in the ECMO group were more severely ill, in comparison with the patients in the ventilated control group (OI in ECMO group significantly higher), ECMO succeeded to better microcirculatory support compared to solely conservative treatment with mechanical ventilation and pharmacologic support, where there was a deterioration. ECMO resulted in stabilizing macro- and micro- circulation, and a reduced vasopressor score, whereas in the ventilated control group, the macrocirculation was stabilized but more vasopressor was given, resulting in a deterioration of the microcirculation.

Thus, ECMO seems to prevent a further deterioration of microcirculatory perfusion. An important confounding factor is the change in vasoactive medication between T1 and T2, because vasopressors have been suggested to have a negative effect on perfusion of the microcirculation, despite increase of cardiac index (5, 6). Although the change in

Figure 1. Box plot of the functional capillary density (FCD) of newborns with severe respiratory failure who were treated with extracorporeal membrane oxygenation (ECMO) (n=21), before (T1 ECMO) and within the first 24 hours after the start of ECMO (T2 ECMO), ventilated controls (n=7) and non-ventilated controls (n=10).



vasopressor score between T1 and T2 in the control group is not statistically significant, there is a tendency towards more vasopressive medication and less vasodilators on T2, which could possibly explain the reduction of FCD in this group. Another factor could be cardiac output (CO). All patients had pulmonary hypertension. This can compromise the pulmonary venous return and preload of the left ventricle, and therefore influence global hemodynamics. No measures of CO were available in this study, so this can not be verified.

Although ECMO provides CO and bypasses the lung, it does not restore microcirculatory tissue perfusion instantly. This is in contrast to observations in adults showing an improvement of microvascular flow secondary to improvement of cardiac output (7). This observation suggests a lack of correlation between CO and microcirculatory perfusion. This is consistent with a large quantity of evidence in the adult literature showing that microcirculation is relatively independent of global hemodynamic parameters (8). Optimal macrocirculation, however, is the obvious prerequisite for adequate microcirculatory perfusion. It is unclear why there was no improvement of the microcirculation, while the macrocirculation was restored. During cardiopulmonary bypass (CPB) in adults,

microcirculatory alterations have been described before (9-12). We found one report on microcirculatory alterations during CPB in neonates where OPS was used, which shows a reduction in vessel density during CPB (13).

In previous observations our group found that after treatment with ECMO, at a time when the patient was recovering and no longer needed ECMO (within 48 hours after discontinuation), the microcirculatory parameters had improved (3). Based on the instant restoration of the macrocirculation, it was expected that microcirculatory alterations observed in neonates with severe respiratory failure, would improve with the initiation of ECMO therapy.

Factors that could prevent further recruitment of the microcirculation at this point could be the primary disease process, which seems to cause a further deterioration of tissue perfusion, or direct effects of EMCO flow. The initiation of ECMO causes inflammation, triggering of the catecholamine system resulting in vasoconstriction and formation of microscopic thromboemboli or gaseous microemboli (14). There is an inflammatory reaction aggravated by the exposure of blood to the artificial components of the extracorporeal circuit and by the surgery necessary for cannulation (15). To which degree ECMO contributes to the inflammatory response is unknown. It is suggested that the inflammatory reaction in response to extracorporeal circuits is remarkably similar to the reaction that occurs during severe inflammatory response syndrome (SIRS) (14). A marked inflammatory reaction is recognized from early after the start of ECMO and frequently persists for several days (16-19). ECMO related SIRS is thought to occur due to contact with the surface of the artificial circuit (20). Possible other ECMO related mechanisms are reperfusion injury on initiation of ECMO and, in case of VA-ECMO, non-pulsatile flow generated by the ECMO pump (17). Additionally, severe respiratory failure and subsequent hypoxemia for which ECMO is required, the banked blood used for circuit priming and, possibly, the surgical procedure needed for cannulation could be factors that aggravate SIRS (16, 19).

Flow disturbances are well known in sepsis and SIRS (21, 22). Inflammation leads to a variety of alterations on the microvascular level, of which the most important are microvascular thrombosis, shunting of blood, increased capillary permeability and an altered oxygen metabolism (14). These factors could explain the persistently reduced capillary density. Although blood of ECMO recipients is constantly heparinized and special filters are used, the possibility of gaseous microemboli or microscopic thromboemboli formation cannot be excluded. By occluding the microcirculation these microemboli could therefore be of influence on the observed low FCD.

Disturbance of physiologic flow also triggers the catecholamine system leading to vasoconstriction and altered tissue perfusion (14). Although the mechanism behind is not completely understood, Agati et al (23-25) reported that in cardiac patients on CPB non-pulsatile flow seemed to affect the microcirculation and organ perfusion in a

more negative way than pulsatile flow did. The beneficial effects of pulsatile VA-ECMO systems are still debated (26-28). More research is needed to explore the effect of above-mentioned factors on the microcirculation. Folllow-up investigations of the microcirculation are necessary and comparison of survivors and non-survivors within the group that received ECMO treatment. In this way, the prognostic value of microcirculatory parameters can be determined.

Limitations

There were some limitations to our study. First, the lack of cardiac output (CO) measurements limits the possibility to relate microvasular observations to global hemodynamics. Changes in CO could possibly play a role in the decrease of FCD between T1 and T2 in the control group. In children, it is not routine to measure mixed venous saturation and cardiac output. A prerequisite for adequate CO monitoring is a tool that is accurate, is easy to use, and has an acceptable risk-benefit profile. These three factors have constituted the major hurdle to bedside pediatric cardiac output measurement to date (29). The reliability of echocardiography evaluation of cardiac output in children is debatable because even in the hands of experienced operators the inter- and intra-individual variation is large (30), which is why we did not use this form of assessment. In addition, especially in neonates intra-and extracardiac shunts complicate the use of these measurements. Second, this study is observational and not randomized controlled, which skews outcome data. If children in the control group had disposed progressive respiratory and/or circulatory failure, they would have received ECMO treatment. From an ethical perspective, randomization for this type of treatments is unacceptable.

CONCLUSION

The initiation of ECMO preserves the microcirculation in neonates with severe respiratory failure.

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CLINICAL VALUE OF MONITORING THE MICROCIRCULATION

CHAPTER 7

Persistent low microcirculatory vessel density in non-survivors of sepsis in Pediatric Intensive Care

Crit Care Med 2010

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ABSTRACT

Objectives
To investigate the time course and predictive value of microvascular alterations in children with severe sepsis.

Design
Single-center prospective observational study.

Setting
Intensive Care unit of a level III university children's hospital.

Patients
Patients with septic shock, requiring the administration of fluid and vasopressor agents and /or inotropes after the correction of hypovolemia, who were intubated and ventilated, were included.

Interventions
None.

Measurements and main results
The microcirculation was assessed in the buccal mucosa, using Orthogonal Polarization Spectral imaging, within 24 hours after admission and subsequent measurements were performed every 24 hours for three days. The measurements were discontinued when the patient was extubated.
There were no significant differences in the Functional Capillary Density (FCD) or Microvascular Flow Index (MFI) for all vessel types between survivors and non-survivors on day 1. In the survival group the FCD increased significantly between day 1 and day 2 from 1.7 cm/cm2 (0.8-3.4) to 4.3 cm/cm2 (2.1-6.9) (p=0.001). FCD values in non-survivors did not change (day 1: 3.2 cm/cm2 (0.8-3.8), day 2: 1.9 cm/cm2 (1.0-2.1)). The median FCD on days 2 and 3 were significantly lower in non-survivors (1.9 cm/cm2 (1.0-2.1) vs. 4.3 cm/cm2 (2.1-6.9) (p=0.009) and 1.8 cm/cm2 (1.0-2.0) vs. 4.7 cm/cm2 (2.1-8.6) (p=0.01) respectively). The MFI for all vessel types improved in survivors and did not change in non-survivors. Differences in MFI values between survivors and non-survivors were not significant.

Conclusion
Persistent microcirculatory alterations can be prognostic for survival in children with septic shock.

INTRODUCTION

Sepsis and septic shock requires emergency treatment with antibiotics, appropriate fluid resuscitation and, if needed, vaso-active drugs (1). Despite major improvements in the management of patients with severe sepsis, patients can develop multiple organ failure (MOF) and will subsequently die.

Microvascular alterations are frequent in patients with sepsis (2). Indirect evidence indicates that improving the microcirculation may prevent or ameliorate sepsis-induced organ failure (3). This state can be very critical as there is a great risk for developing multiple organ dysfunction syndrome (MODS) (4).

With the advances of pediatric intensive care therapy, overall mortality in children has been reduced to approximately 10% (1, 5). Unlike adults, children who die of shock usually do so because of decreased cardiac output with high or normal systemic vascular resistance (6). Mortality rates improve with an early, aggressive fluid resuscitation as well as vasopressor and inotropic support if signs of shock persist (7, 8).

Clinical parameters that are routinely used for the evaluation of treatment of children with sepsis are mainly focused on the macrocirculatory part of the cardiovascular system. However, restoration of the systemic hemodynamics does not eliminate the possibility that there is still deterioration of the microcirculation and organ dysfunction and MOF can still frequently occur (9, 10). In septic shock microcirculatory weak units become shunted and tissue oxygenation is disturbed (11). Thus, an adequate resuscitation procedure should target the oxygenation of the microcirculation. Changes in the microcirculation cannot be predicted from changes in heart rate, blood pressure and pulse oxymetry (2, 12).

Orthogonal Polarization Spectral (OPS) imaging allows imaging of mucus membranes and the surface of solid organs and can be applied at the bedside to investigate the human microvasculature (13, 14).

Major microvascular blood flow alterations in patients with severe sepsis are described in adults (2). These alterations included a decreased vascular density and were more severe in non-survivors than in survivors but were not affected by the global hemodynamic state or vasopressor agents. More and more, alterations of the microcirculation are assumed to play a crucial role in sepsis (3, 15). Left uncorrected, these processes will lead to cellular dysfunction followed by organ failure and death (3, 10, 15-17). Weidlich et al. described decreased vessel density of the skin in neonates in the 5 days prior to the onset of sepsis (18). To our knowledge, no reports on the microcirculation in children during sepsis are available in literature.

In this study, we performed daily measurements using the OPS technique in patients with septic shock to investigate the time course of microvascular alterations.

METHODS

This study took place in the Intensive Care Unit of Erasmus MC Sophia Children's Hospital Rotterdam. In accordance with the guidelines of the medical ethical review board of our hospital, informed consent is waived when standard therapy is monitored by non-invasive techniques. Patients with septic shock, as defined by the American College of Critical Care Medicine (19), requiring the administration of fluid and vasopressor agents and /or inotropes after the correction of hypovolemia, who were intubated and ventilated, were included. Subjects were measured within 24 hours after admission and subsequent measurements were performed every 24 hours for three days. The measurements were discontinued when the patient was extubated. Demographic data (sex, age, weight) and the Pediatric Risk of Mortality (PRISM) score (20) were recorded for all subjects. During each measurement global hemodynamic parameters vaso-active drug dose expressed as vasopressor score (21) were recorded as well. Exclusion criteria: treatment with Extra Corporal Membrane Oxygenation (ECMO), congenital heart disease or pre-existing cardiomyopathy and severe blood loss.

General Management

All patients had an arterial and central venous catheter. Treatment for septic shock was standardized, including fluid resuscitation and inotropes (dobutamine and /or dopamine) and vasopressors (norepinephrine and, if needed, epinephrine as well) to maintain a normal mean arterial pressure, according to the guidelines for management of sepsis (22). All patients were mechanically ventilated. Sedation was given following a unit specific protocol (with midazolam and morphine) and was increased according to individual needs.

Imaging

To evaluate the microcirculation, the CYTOSCAN E-II Backfocus type device (Cytometrics, Philadelphia USA) was applied to acquire images of the microcirculation. The buccal mucosa was studied and video segments were obtained in a standardized way, as described previously (23).

Microcirculatory analysis

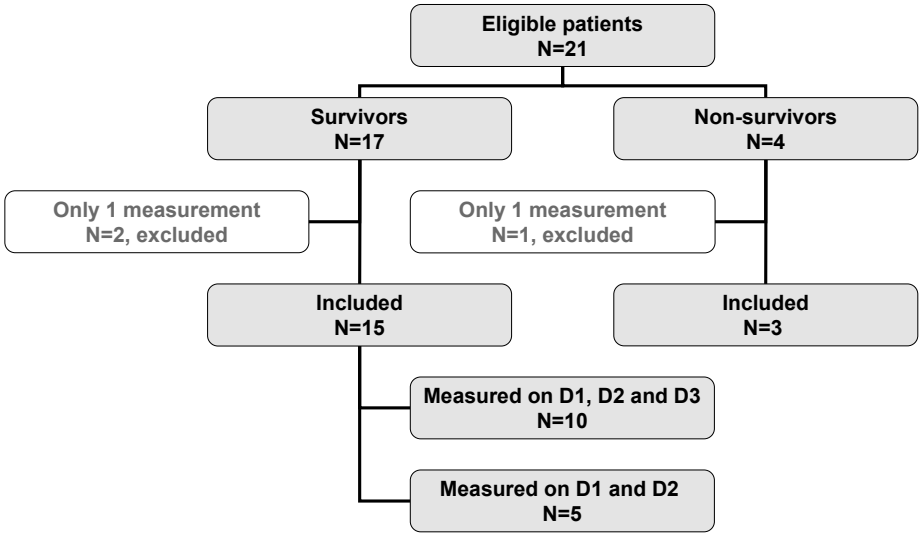
Images were analyzed for vessel density by the first author and flow by the third author, who were blinded for the clinical course and order of the images. The functional capillary density (FCD) was determined by tracing out all of the red blood cell-perfused capillaries. In this way the total length of the capillaries (vessels, smaller than 10 µm) with flowing cells per field of view was measured using specialized software (Capiscope, version 3.7.1.0, KK technology 1993-2000) and expressed in cm/cm^2 (13). Semiquantitative flow

scoring was performed using the Microcirculatory Flow Index (MFI), a method published by Boerma et al. (24). This score is based on the determination of the predominant type of flow in 4 quadrants of the screen. Flow is characterized as absent (0), intermittent (1), sluggish (2) or normal (3). The values of the 4 quadrants are averaged.

Statistical Analysis

Statistical analysis of the data was performed using SPSS 15.0. For comparison of two groups (survivors and non-survivors) the Mann-Whitney test was applied. Survivors were defined as patients who were alive at day 28 after admission. The significance of differences between scores (day 1, day 2 and day 3) was tested with the Wilcoxon signed rank test. The predictive value of change in FCD values (ΔFCD) on survival was calculated using a receiver operator characteristic (ROC) curve and the area under the curve (AUC) was computed. The level of significance was set at $p < 0.05$. Data are presented as the median (and the range).

Figure 1. Flow diagram of patients included in the study



RESULTS

Twenty-one consecutive patients were studied (Figure 1). Four patients died, one of whom died within 48 hours, before a second measurement could be done. Seventeen patients survived. Two patients were only measured once; 1 patient because she was extubated within 24 hours, and, therefore, the measurements were discontinued, whereas 1 patient could not be measured on day 2 due to logistical reasons. The 3 patients

that missed follow-up measurements (2 survivors and 1 non-survivor) were excluded. These patients were not different from the included patients in terms of microcirculatory values or clinical parameters. Background data are shown in Table 1. Five patients could not be measured on the third day due to logistical reasons (e.g., no investigator available, technical problems with camera). All non-survivors died due to MOF. Global hemodynamic parameters are shown in Table 2.

Table 1. Back ground data

Patient	Sex	Age [months]	Weight [kg]	Cause of sepsis	PRISM score	Survival
1	Male	45	18.0	Meningococcal sepsis	40	Yes
2	Male	26	12.7	Bowel perforation	20	Yes
3	Male	67	4.2	St. Aureus sepsis	30	Yes
4	Female	178	60	Neutropenia	31	No
5	Male	8	9	Urosepsis	29	Yes
6	Male	18	10	Meningococcal sepsis	31	Yes
7	Male	0	2.8	Bowel obstruction	19	Yes
8	Male	6	22	Bowel obstruction	17	Yes
9	Male	107	40	Neutropenia	13	Yes
10	Male	31	13	Neutropenia, Immune deficiency	18	No
11	Male	146	33	Pneumonia ARDS	19	Yes
12	Female	1	1.3	Necrotizing Enterocolitis	40	Yes
13	Female	89	25	Meningococcal sepsis	20	Yes
14	Female	184	45	Bowel perforation	26	Yes
15	Female	3	2.5	Necrotizing Enterocolitis	18	No
16	Female	190	58.0	Toxic Shock Syndrome	3	Yes
17	Male	38	12.6	Sepsis-meningitis	30	Yes
18	Female	189	55.0	Bowel perforation	20	Yes

Table 2. Macrocirculatory parameters and catecholamine doses

	Survivors			Non-survivors		
Day	**1 (n=15)**	**2 (n=15)**	**3 (n=10)**	**1 (n=3)**	**2 (n=3)**	**3 (n=3)**
Heart rate [beats/min]	169 (121-210)	150 (124-194)	142 * (101-175)	169 (168-173)	167 (163-170)	155 (129-180)
Mean arterial blood pressure [mm Hg]	60 (27-88)	65 (39-89)	66 (43-86)	54 (32-66)	66 (42-72)	74 (65-74)
Diastolic blood pressure [mm Hg]	45 (21-74)	52 (32-73)	54 (32-76)	52 (32-73)	45 (32-55)	56 (48-60)
Systolic blood pressure [mm Hg]	85 (30-136)	99 (56-145)	100 (57-141)	99 (56-145)	112 (59-125)	95 (92-111)
Dopamine [mcg/kg/min]	0 (0-27)	0 (0-27)	0 (0-27)	0 (0-5)	0 (0-5)	0 (0-5)
Dobutamine [mcg/kg/min]	5 (0-20)	13 (0-21)	5 ! (0-21)	20 (0-21)	20 (0-20)	10 (0-20)
Norepinephrine [mcg/kg/min]	0.1 (0-1.0)	0.1 (0-1.0)	0 *! (0-1.0)	0.4 (0.4-1.2)	0.5 (0.3-1.4)	0.4 (0.3-1.4)
Epinephrine [mcg/kg/min]	0 (0-0.7)	0 (0-0.9)	0 (0-0.2)	0 (0-0.1)	0 (0-0.4)	0 (0-0.4)
Fluid bolus crystalloids [ml/kg]	0 0-36	0 0-44	0 * 0-20	10 0-32	9 0-10	0 0-0
Fluid bolus colloids [ml/kg]	12 0-340	0 * 0-56	0 * 0-10	12 0-48	13 0-20	25 0-42
Vasopressor Score	10 (0-52)	15 (0-52)	8 * (0-47)	24 (14-43)	25 (18-38)	18 (14-38)
Body temperature [°Celsius]	38.1 (34.5-39.8)	37.5 (36.0-40.7)	37.5 (36.4-38.5	36.7 (36.1-36.7)	37.1 (36.7-37.7)	37.1 (35.6-39.3)
Lactate [mmol/l]	3.3 (1.0-11.6)	2.4 * (0.8-19)		11 (1.4-15)	9.2 (1.8-15)	14.2 (1.9-14.4)
Urine output [ml/kg/h]	1.5 (0-4.0)	2.8 (0-7.3)	2.9 * (1.5-5.9)	0.3 (0.3-2.3)	0.5 † (0.5-1.0)	0.7 (0.1-5.8)
Hemoglobin [mmol/l]	6.0 (3.1-8.1)	6.6 (5.8-12.3)	6.3 (5.2-8.1)	6.6 (6.0-8.1)	5.4 (5.1-7.4)	6.0 (5.8-7.9)

	Survivors			Non-survivors		
Day	**1 (n=15)**	**2 (n=15)**	**3 (n=10)**	**1 (n=3)**	**2 (n=3)**	**3 (n=3)**
pH	7.32 (7.20-7.47)	7.39 * (7.21-7.51)	7.42 * (7.30-7.49)	7.19 † (7.14-7.24)	7.31† (7.26-7.34)	7.35 (7.11-7.55)
pCO2 [kPa]	4.4 (3.4-7.4)	4.7 (4.1-8.6)	5.5 (4.9-7.3)	7.2 (3.4-7.5)	5.6 (2.8-6.4)	5.5 (4.2-6.3)
Base Excess [mmol/l]	-9 (-16 - -3)	-3 (-8- 7)	2 (-3 - 9)	-8 (-20- -6)	-6 (-14 - -3)	-3 (-14 - 6)

Global hemodynamic parameters of survivors and non-survivors and dose of vaso-active medication. For the age range, the normal mean blood pressure ranges from 35-85 mmHg with diastolic blood pressure from 25-75 mmHg and systolic blood pressure from 50-140 mmHg. For comparison of survivors and non-survivors the Mann-Whitney test was applied. The significance of differences between day 1, day 2 and day 3, within groups, was tested with the Wilcoxon signed rank test. Global hemodynamics and the dose of vaso-active medication were not significantly different between survivors and non-survivors.
Data are presented as median and range
* Significantly different from day 1 ($p<0.05$)
! Significantly different from day 2 ($p<0.05$)
† Significantly different from survivors on the same day ($p<0.05$)
Vasopressor score= ([dopamine x 1] + [dobutamine x 1] + [epinephrine x 100] + [norepinephrine x 100] + [phenylephrine x 100]) (21)

Microcirculatory parameters

Microcirculatory parameters are shown in Table 3. All patients who had at least two measurements were included. Changes in microvascular scores were analyzed. There were no significant differences in the FCD or the MFI for all vessel types between survivors and non-survivors on day 1. In the survival group, the FCD increased significantly between day 1 and day 2 from 1.7 cm/cm2 (0.8-3.4) to 4.3 cm/cm2 (2.1-6.9) (p=0.001). The FCD in non-survivors did not change (day 1: 3.2 cm/cm2 (0.8-3.8) and day 2: 1.9 cm/cm2 (1.0-2.1)) (Figure 2). In the survivors the ΔFCD was 2,3 (-0.4-4.4). Non-survivors had a lower ΔFCD (-1.3 (-1.7-0.2)) (p=0.01). The Microvascular Flow Index (MFI) improved in all vessel types in survivors (day 1: small 2.00 (0.33-2.75), medium 1.92 (0.08-2.5), large 2.13 (0.67-3.00), day 2: small 2.63 (2.06-3.00) (p=0.001) medium 2.62 (1.66-3.00) (p=0.004), large 2.83 (2.00-3.00) (p=0.01). MFI values in non-survivors did not change (day 1: small 2.75 (1.00-2.88), medium 2.38 (0.75-3.00), large 2.75 (1.63-3.00), day 2: small 2.17 (2.00-2.33), medium 2.00 (1.25-2.33), large 2.50 (1.92-3.00).

The median FCD values on days 2 and 3 were significantly lower in non-survivors, 1.9 cm/cm2 (1.0-2.1) vs. 4.3 cm/cm2 (2.1-6.9) (p=0.009) and 1.8 cm/cm2 (1.0-2.0) vs. 4.7 cm/cm2 (2.1-8.6) (p=0.01), respectively. Differences in the MFI between survivors and non-survivors were not significant.

Table 3. Microvascular flow index on day 1-3

	Survivors			Non-survivors		
Day	**1 (n=15)**	**2 (n=15)**	**3 (n=10)**	**1 (n=3)**	**2 (n=3)**	**3 (n=3)**
MFI large	2.13 (0.67-3.00)	2.83 * (2.00-3.00)	2.71 * (2.13-3.00)	2.75 (1.63-3.00)	2.50 (1.92-3.00)	2.45 (2.40-2.50)
MFI medium	1.92 (0.08-2.5)	2.62 * (1.66-3.00)	2.44 (1.25-3.00)	2.38 (0.75-3.00)	2.00 (1.25-2.33)	1.58 (0.86-2.30)
MFI small	2.00 (0.33-2.75)	2.63 * (2.06-3.00)	2.54 (1.27-3.00)	2.75 (1.00-2.88)	2.17 (2.00-2.33)	1.62 (0.63-2.60)

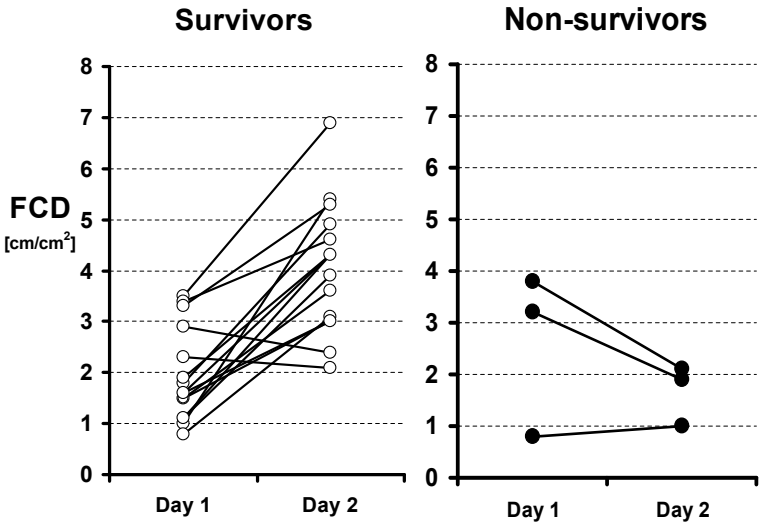
Data are presented as median and range

* Significantly different from day 1 ($p<0.05$)

† Significantly different from survivors on the same day ($p<0.05$)

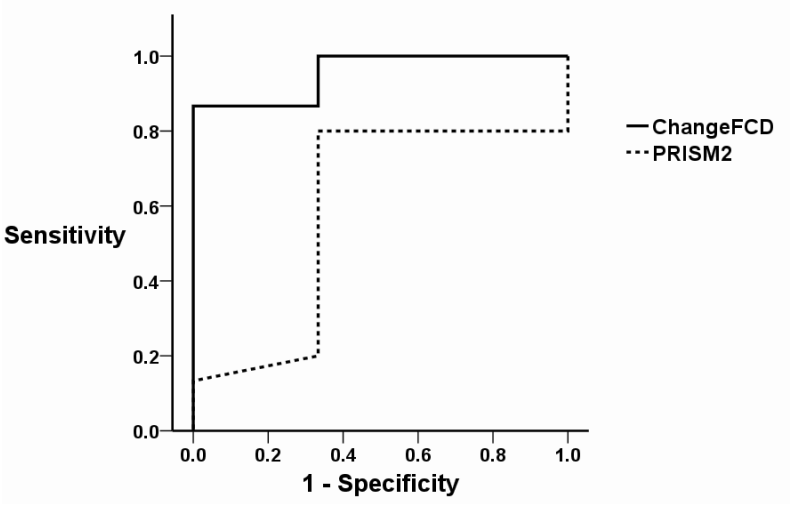
MFI Microvascular Flow Index

Figure 2. The Functional Capillary Density (FCD) improves in survivors (day 1:1.7 cm/cm^2 (0.8-3.4) and day 2: 4.3 cm/cm^2 (2.1-6.9) ($p=0.001$)). The FCD in non-survivors did not change (day 1: 3.2 cm/cm^2 (0.8-3.8) and day 2: 1.9 cm/cm^2 (1.0-2.1)). The median FCD on day 2 was lower in non-survivors, (1.9 cm/cm^2 (1.0-2.1) vs. 4.3 cm/cm^2 (2.1-6.9) ($p=0.009$))



A ROC curve showed that the ΔFCD is predictive for survival. A ΔFCD of 0.7, the best cut-off had a sensitivity of 87% and a specificity of 100%. The area under the curve was 0.956 (95% CI 0.853-1.058). For PRISM the area under the curve was 0.59 (95%CI 0.209-0.969), which is statistically significantly less sensitive and specific than the ΔFCD measured by the ROC curve (Figure 3).

Figure 3. Receiver operator characteristic curve for the change in Functional Capillary Density (ΔFCD) within the first 2days of septic shock in children. The best cut-off is 0.7 Area under the curve (AUC) for ΔFCD =0.956 (95% CI 0.853-1.058). The ROC curve for PRISM shows a low sensitivity and specificity. It has an area under the curve of 0.59 (95%CI 0.209-0.969) and is significantly less sensitive and specific than ΔFCD measured by the ROC curve.



DISCUSSION

The main finding of our study is that in children with septic shock, despite normalization of the systemic hemodynamic variables, persistence of microcirculatory alterations is associated with a worse outcome. Although microcirculatory parameters were similar in survivors and non-survivors on the first day, our study demonstrates recruitment of the microcirculation in the survivors of septic shock, but not in the non-survivors. Despite a restored macrocirculation, which was similar in all groups, 3 of the 18 patients did not survive and the improvement of the microcirculation appeared to be a distinctive feature. Capillary density increased over time in the survivors but not in the non-survivors. Due to the small number of patients in the non-survival group, it is difficult to generalize based on our observation. Our finding is in accordance with observations in adults by Sakr et al. (17) and by Trzeciak et al. (25, 26). They found that microcirculatory alterations

were best correlated with the occurrence and severity of MOF and that persistent alterations in microvascular perfusion at later than 24 hours after the onset of shock were a good predictor of ICU mortality.

Circulatory shock is defined as a failure of the cardiovascular system to maintain effective tissue perfusion, causing cellular dysfunction and acute organ system failure if not promptly restored. Although it is the macrocirculation that distributes blood flow globally throughout the body, the microcirculation is a critical component of the cardiovascular system that is necessary for blood flow to individual tissues. It has been previously described in adults that indices of microcirculatory blood flow, in patients with septic shock, could serve as early indicators of tissue hypoperfusion and herald the onset of multi-organ failure and death (15, 25, 27).

Distinctive differences were found for vessel density. There were differences in development in MFI over the time as well, although these were less pronounced. There was no improvement in the MFI of the non-survival group, which might be because the initial MFI was high, so, therefore, it is difficult to observe improvement. This might also be the result of the small sample size.

Microcirculatory perfusion is regulated by an intricate interplay of neuroendocrine, paracrine, and mechano-sensory pathways (10). This regulated system can be further compromised by the following: decreased deformability of red blood cells and the consequent increased viscosity (28); increased percentage of activated neutrophils, with decreased deformability and increased aggregability due to upregulation of adhesion molecules (29); activation of the clotting cascade with fibrin deposition and the formation of microthrombi, leading to dysfunction of vascular autoregulatory mechanisms (30); or secondary enhanced perfusion of large arteriovenous shunts (31). These processes result in tissue dysoxia, either from impaired microcirculatory oxygen delivery or from mitochondrial dysfunction (32). New larger studies are needed to confirm the role of vessel density and to explore the influence of the above-mentioned factors.

Other indexes of impaired tissue perfusion also improved more rapidly in survivors. Urine output improved in survivors, while it did not increase in non-survivors. Arterial lactate concentrations decreased more rapidly in survivors. The lack of restoration of the microcirculation may explain these differences. Based on our findings, the prognostic markers for survival in children with septic shock should also include variables like lactate and urine output, however these are microcirculation dependent variables and FCD can be considered as a more direct visible hemodynamic marker.

After the first day, microvascular perfusion discriminated survivors and patients dying of MOF, while global hemodynamic measurements and the use of vaso-active drugs did not significantly differ. Previous studies reported that microcirculatory alterations can be observed in patients with septic shock (2, 15, 33). An important finding was that improvement of microcirculatory alterations, but not the global hemodynamic

or oxygenation variables, was related to survival. This relationship is illustrated by the area under the ROC curve (Figure 3). In accordance with observations in adults, global hemodynamic variables in our study were similar in survivors and non-survivors on days 2 and 3, despite the significantly altered tissue perfusion in non-survivors. In addition, it has been shown that improvement in microcirculatory perfusion in the initial hours following early goal directed therapy results in an improved sequential organ failure assessment (SOFA) score 24 hours later (34). The microcirculation is thus emphasized as an important player in the pathophysiology of shock and MOF, and therefore mortality (17). Microvascular recruitment can therefore be a possible target for resuscitation in patients with septic shock (11, 15, 25). Although the use of vasopressors (e.g. norepinephrine) was not significantly different between survivors and non-survivors in our study, the use of vasopressors under certain circumstances can influence microcirculatory variables (34).

There are several limitations and technical difficulties involved in performing the measurements that make them, in their present form, unsuitable for routine clinical use. Further studies are necessary to define potentially beneficial interventions, and studies that assess the microcirculation, like OPS and Sidestream Dark Field (SDF) Imaging, may be helpful in evaluating their effects on the microcirculation.

Limitations

There were some limitations to our study. First, in children, it is not routine to measure mixed venous saturation and cardiac output, as placement of Swan-Ganz catheter in children has never reached the application as in adults with septic shock. A prerequisite for adequate CO monitoring is a tool that is accurate, is easy to use, and has an acceptable risk-benefit profile. These three factors have constituted the major hurdle to bedside pediatric cardiac output measurement to date (35). Because we do not have continuous oxygen saturation measurement catheters for small children and because of the small size central venous line (CVL), we restrict the blood sampling of the line and we do not know the mixed venous saturation. In children, the reliability of echocardiography evaluation of cardiac output is debatable because even in the hands of experienced operators the inter- and intra-individual variation is large (36), which is why we did not use this form of assessment. Therefore, we have limited information about global hemodynamics. Thus, monitoring the microcirculation could be a valuable addition in the monitoring of critically ill children, given its non-invasive nature.

Second, the sublingual mucosa, which shares a similar embryonic origin with the digestive mucosa, may not reflect other microcirculatory beds (37). Nevertheless, as this study and others have shown, it is a sensitive location capable of predicting morbidity and mortality in advance of macroscopic hemodynamic parameters.

And finally, the possibility of correlating the observed values with lactate is limited by the fact that lactate was not measured in a significant number of the patients at the time of the measurement because we were dependent on the laboratory determinations taken on clinical indication by the treating team. Therefore, especially when high lactate levels were not anticipated, this issue was not investigated.

CONCLUSION

Persistent microcirculatory alterations can be prognostic for survival in children with septic shock. Imaging of the microcirculation may therefore open the way for guided-therapy. It can be valuable in addition to the limited techniques for monitoring hemodynamics in children and in selected patients using the microcirculation as a non-invasive biomarker. Recruitment of microcirculation may become an important therapeutic target in treatment of septic shock in the pediatric age group.

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SUMMARY AND FUTURE PERSPECTIVES

CHAPTER 8

General discussion

INTRODUCTION

Circulatory shock is an important problem in critical illness; it is a major cause of morbidity and mortality (1, 2). Shock is defined as the failure of the circulation to meet the metabolic demands of the tissues. Although it is one of the most seen and extensively studied problems in the intensive care unit, it remains an elusive process to monitor. The optimal method of hemodynamic monitoring to guide resuscitation and management of the critically ill patient is still controversial. The importance of tissue perfusion is well recognized, but current standard monitoring techniques often fail to elucidate local alterations in regional tissue perfusion. Global hemodynamic parameters are routinely used as primary endpoints in the treatment of shock (3). To date there are no tools, however, that could be used to routinely monitor tissue perfusion or tissue oxygenation at the bedside. Monitoring the microcirculation could narrow the gap between the information obtained by currently used methods and the information that is really needed.

Over the last decade, there has been considerable research into the role of the microcirculation in the pathophysiology of multiple organ failure during critical illness. The network of blood vessels that make-up the functional microcirculatory network provides the anatomical basis for supplying oxygen and nutrients to all cells in the body. It is the primary prerequisite for cell life. The microcirculation continuously adapts to the needs of the tissues by being responsive to tissue metabolic signals that, ultimately, will control vascular tone, and therefore regional blood flow.

Microvascular perfusion is dependent on sufficient cardiac output and systemic blood pressure. However, their relationship is very complex, determined by the underlying illness and possibly by treatments. Observations from laboratory preparations as well as bedside observations in patients have shown that, at a microvascular level, there is autoregulation (4, 5). That is, microvascular vessel density and flow, vessel heterogeneity, arteriolar-to-venous shunting are dissociated from systemic effects.

Most of the clinical research into the microcirculation has been carried out in adults with sepsis. The concept that dysfunction of the microcirculation contributes to the pathophysiology of sepsis is now widely accepted (6, 7). In fact, this has led to the idea that microcirculatory dysfunction can be regarded as the 'motor' that drives the development of multiple organ failure during sepsis (6). Very little work has been done in children. In this chapter, the current literature on direct observation of the microcirculation in pediatric patients will be reviewed. Taking the literature findings together with the results of the studies presented in this thesis, perspectives for future developments in monitoring the microcirculation will be discussed.

OBSERVING THE MICROCIRCULATION IN THE PEDIATRIC PATIENT

The improved successor of OPS imaging (8), sidestream dark field (SDF) imaging is based on the dark field illumination technique introduced by Sherman et al. (9). Transilluminating the tissue is not needed in this technique. Instead, SDF imaging uses a stroboscopic light-emitting, diode ring-based imaging device illuminating from the side of the field of view to avoid surface reflection, thereby providing better image quality of the microcirculation than does OPS imaging (10).

To date, eight studies have been published on the use of OPS or SDF imaging in children (Table 1). A few studies in newborns and infants have used videophotometric microscopy or laser Doppler to evaluate red blood cell (RBC) velocity in the nailfold capillaries of the thumb (11-13). The latter technique is not feasible for routine bedside use. OPS imaging of the skin of the upper arm has been used in premature and term infants (14-17). The microcirculation as well as RBC velocity could be quantified in premature infants. The most frequently used site for assessment in older children is the buccal mucosa.

Table 1. Pediatric studies of the microcirculation using OPS or SDF imaging

Author [ref]	Year	Age range	n	SDF/OPS	Site	Outcome
Genzel-Boroviczeny et al. (14)	2002	Preterm, 1-5 days	28	OPS	Skin	Feasibility study: RBC velocity increases from day 1-5 in premature neonates and correlates with decrease in hemoglobin
Genzel-Boroviczeny et al. (15)	2004	Preterm, 19-39 days	13	OPS	Skin	FCD improves 2 hours and 24 hours after blood transfusion
Kroth et al. (16)	2008	Preterm, 0-30 days	25	OPS	Skin	FCD decreases significantly over the first month of life
Top et al. (18)	2009	Term, 0-18 days	14	OPS	Buccal mucosa	FCD is reduced in neonates with severe respiratory failure and improves with veno-arterial extracorporeal membrane oxygenation
Weidlich et al. (19)	2009	Preterm, 0-30 days	10	OPS	Skin	FCD decreases 1 day before clinical signs of infection appear
Hiedl et al. (17)	2010	Preterm, 3-8 days	25	SDF		Patients with persistent ductus arteriosus have reduced FCD, which improves after treatment
Top et al. (20)	2010	0-3 years	45	OPS	Buccal mucosa	FCD of the buccal mucosa decreases after the first week of life
Top et al. (21)	2010	0-15 years	21	OPS	Buccal mucosa	Persistence of depressed FCD is associated with a worse outcome in children with septic shock

MICROCIRCULATION IN DISEASE AND THE EFFECTS OF TREATMENT

Microcirculatory abnormalities have been described in four different conditions or states. First, our clinical research group showed with OPS that microcirculatory values in critically ill neonates with severe respiratory failure were depressed and could be influenced by certain treatments (18), (chapters 4-6). Second, we have demonstrated alterations of the microcirculation in infants and children with sepsis (21), (chapters 3 and 7). In addition, others have reported alterations in the microcirculation of premature neonates with infection (19). Third, in premature neonates with patent ductus arteriosus (PDA), the functional capillary density (FCD) of the skin is reduced compared with neonates without PDA (17). The mechanism behind this is not clear. However, the left-to-right shunting through the PDA reduces the cardiac output (due to blood being shunted to the pulmonary circulation) may provide an explanation, seeing that there was no difference anymore after closure of the PDA (17). Lastly, Genzel-Boroviczeny et al. observed a direct effect of RBC transfusion on the microcirculation in premature infants, i.e. an increase of the FCD (15). RBC transfusion is a common intervention used to improve oxygen delivery to the tissues.

MICROCIRCULATION IN THE CRITICALLY ILL NEONATE WITH SEVERE RESPIRATORY FAILURE

We found depressed microcirculatory values in neonatal patients with severe respiratory failure with pulmonary hypertension. These patients had lower microvascular values compared with children without respiratory failure or signs of pulmonary hypertension. The alterations proved sensitive to different treatments (chapters 4-6). The question is whether these observations lie at the basis of the respiratory disease process or are merely epi-phenomena in the bigger picture of failure of the pediatric (micro-) circulation to adapt to post natal life.

The functional morphology of the cardiovascular system changes markedly at birth due to dramatic alterations in blood flow patterns. The fetal circulation is characterized by reduced perfusion of the lungs and intra- and extra-cardiac shunts between the pulmonary and systemic circulations. It develops and undergoes changes in the first few years of life but then rapidly transitions into an adult circulation under normal circumstances (Chapter 1).

Developmental changes in the structure of the microcirculation occur in the first few weeks of life in healthy neonates and children (16, 20). The FCD of the buccal mucosa decreases after the first week. After birth, there is a 2- to 3-fold increase in oxygen consumption because of increased work of breathing, and increased gastrointestinal func-

tion with feeding. Furthermore, high levels of fetal hemoglobin at this time of life reduce the level of oxygen extraction (22). These factors, taken together, can be compensated for by higher systemic and microcirculatory blood flow. The higher FCD in the first week of postnatal life may be related to higher cardiac output (22) and autoregulation of regional blood flow may have a role in this process (23).

Failure to produce normal physiologic changes of circulation after birth is not completely understood. Pathophysiologic mechanisms are inherent to certain birth defects (e.g. congenital diaphragmatic hernia (CDH)) and may cause persistent pulmonary hypertension of the neonate (PPHN) (24). These mechanisms differ from those underlying other causes of PPHN (e.g. meconium aspiration or infection).

Many of the patients with severe respiratory failure that we studied were diagnosed with CDH. In this birth defect, normal postnatal physiologic changes of circulation fail to occur. There is a high degree of lung hypoplasia and pulmonary vascular abnormalities (24). Newborns with CDH show a decreased pulmonary expression of inducible nitric oxide synthase (iNOS), which suggests a potential role in the pathogenesis of pulmonary hypertension (25). Nitric oxide (NO) is a key factor in the maintenance of the low normal pulmonary vascular resistance (PVR) in children as well as in adults (26). In health, NO maintains microcirculatory homeostasis (27-30), while causing injury under pathological conditions. Thus, the microvascular system of the lung plays an important role in the pathophysiology of CDH.

We found that neonates with CDH with severe respiratory failure had depressed microvascular perfusion values (chapters 4-6). It is unclear if these alterations correlate with alterations in the lung microvasculature. More research is needed to understand the full hemodynamic pattern in this patient group. For example, global hemodynamic monitoring by echocardiography and cardiac output monitoring should be combined with observations of the microcirculation, thus providing an integrative method of monitoring the functional state of the cardiovascular system. In addition, comparisons between patients with and without CDH could confirm the possibility of CDH being an intrinsic disease of the microcirculation.

PROGNOSTIC VALUE OF THE MICROCIRCULATION

OPS and SDF imaging have been used in adults in various clinical settings. The measurements derived from observation of the microcirculation in sepsis proved to have prognostic value (31-33). This prognostic value has also been shown for other disease states (34, 35). Altered microcirculatory parameters in adults with septic shock that do not resolve after 24 hours of admission are associated with poor outcome (32). Abnormal microcirculatory values also correlate with other measures of patient severity of illness

during sepsis, such as sequential organ failure assessment (SOFA) scores (33). Sublingual microcirculatory parameters were also found to be impaired in patients who develop nosocomial infections after major abdominal surgery (35). Lastly, De Backer et al. found that the degree of microvascular derangement in adults with cardiogenic shock was reflected in their survival (34).

With regard to children, we found that persistent alterations of the microcirculation were associated with poor outcome in septic shock (21), (chapter 7). Persistent alterations proved to have a stronger predictive value for mortality than the pediatric risk of mortality (PRISM) model. In premature neonates, reduction of FCD of the skin can be the first sign of infection (19). Such alteration might be predictive of infection even before clinical suspicion arises. The observations in pediatric patients with septic shock and the prognostic value of change in microvascular alterations over time, are consistent with those in adults (32). Sepsis is now well recognized as having a major impact on the microcirculation. Given the relationship between persistent microcirculatory dysfunction and adverse outcome, targets for resuscitation in the future are likely to involve microcirculatory recruitment and 'defense' of tissue oxygenation. Several research groups are exploring new resuscitation strategies that focus on this area (7).

To date the literature on the prognostic value of microcirculatory observations is still scarce and mainly concerns sepsis. Sepsis is regarded as a disease of the microcirculation (6, 21, 31, 36-38), whereas in other diseases the role of the microcirculation has as yet not been extensively explored.

In our study about the effect of ECMO treatment (chapters 5 and 6), we compared critically ill neonates who received ECMO to those who did not receive this treatment. There was no difference in FCD between the two groups before the start of ECMO. The group that received ECMO treatment was regarded as sicker, but their condition was not reflected by microcirculatory values of the buccal mucosa. Therefore, these observations do not seem to have a predictive value for the need for ECMO.

The positive effect on the microcirculation during and after the treatment with ECMO did not seem to have prognostic value for outcome. However, the studies described in chapters 5 and 6 were not designed to compare outcomes. Patients in the ECMO treated group were sicker and therefore eligible for ECMO treatment. Further research is needed to investigate differences between survivors and non-survivors of ECMO treatment. It is obvious that microvascular parameters should not be interpreted separate from the disease specific context or the applied therapy. In addition, they should not be uncoupled from macrocirculatory parameters and other values that provide information about tissue oxygenation and adequate cell function, such as lactate.

FUTURE PERSPECTIVES

The fundamental role played by the microcirculation in regulating oxygen delivery supports the belief that alterations in microvascular perfusion are implicated in both organ dysfunction and multiple organ failure due to shock from a variety of etiologies (39). Clearly, maintenance of adequate tissue oxygenation should be considered as a fundamental objective of any intensive care resuscitation strategy. Ideally, we should be able to reliably, accurately and reproducibly measure oxygen supply and utilization in specific tissues at the bedside. Measuring microcirculatory flow as a prerequisite for tissue oxygenation and the integrity of the microcirculation can be highly valuable. As bedside assessment, it can be a sensitive marker for physiological circulatory derangement in shocked, critically unwell patients.

Although observations of the sublingual and buccal mucosa have been proven to be valuable, they may not necessarily correlate with the microcirculatory flow in other organs with their different microcirculation structure and function (31, 40, 41). A key characteristic of microcirculatory dysfunction in sepsis is the marked heterogeneity of the blood flow and hyperperfused capillaries adjacent to hypoperfused capillaries. To date, however, it is unclear if the same applies to other disease states. Functionally impaired focal microcirculatory units are believed to invoke local hypoxia, leading to reversible or irreversible tissue damage (42). Thus, the severity of these events could potentially dictate the extent of mitochondrial dysfunction leading to organ failure (32).

It is clear that observations of the microcirculation are valuable. They provide information about blood flow on tissue level and therefore insight into one of the crucial factors for oxygen delivery. This is in contrast to most macrocirculatory values which are based on pressure and do not provide information about flow. As stated in the introduction to this thesis, monitoring cardiac output in children has its limitations. Therefore, acquiring information about microcirculatory flow with a relatively non-invasive tool can be considered as a big step forward in the hemodynamic monitoring of the critically ill pediatric patient. Study of the microcirculation could also elucidate potential mechanisms of disease, something that is implied but not fully addressed in the current studies. Changes in local tissue perfusion do matter, even though global hemodynamic changes due to disease or in response to therapy can be more subtle.

Clinicians treating critically ill patients should realize that there is no straightforward relationship between macrocirculatory and microcirculatory hemodynamics, but that there are some independent determinants of flow in the different circulatory beds. In addition, there is clearly individual variability. A recent study examining the effect of varying perfusion pressure with norepinephrine on responses of the sublingual microcirculation found no effect on the population as a whole, but a fair amount of variation in individual responses, with patients reaching maximal perfusion at different mean

arterial pressures (43). This study also lends credence to the notion that evaluation of the perfusion of the microcirculation may be worthwhile as a clinical index of the adequacy of fluid resuscitation in individual patients (44).

The clinical art remains, firstly, to monitor the right parameters and, secondly, to apply the right target values, which can vary according to age or underlying pathology and choice of therapy. Optimal macrocirculatory values in critical illness are not necessarily the same as normal values in health (45). It is reasonable to assume that the same applies for microvascular parameters. In addition, the approach to improve microcirculation should be disease specific. More research is warranted to explore the microcirculation in its (patho-) physiologic context and the different factors influencing microcirculatory function. Randomized controlled trials are needed to investigate current treatment strategies and develop strategies to rescue the microcirculation.

To date, visualization of the buccal circulation is not quite ready for routine clinical application at the bedside. As it evolves, this technology needs to become more user friendly; there is room for improvement in both ease of acquisition and analysis to facilitate generation of reproducible and timely data to guide patient management (46).

CONCLUSION

The importance of microvascular perfusion is well appreciated, yet it remains elusive. Direct observation of the microcirculation could be a valuable addition to the arsenal of hemodynamic monitoring tools for the critically ill patient. Especially in the pediatric population, where other monitoring modalities are limited, this could be useful. If the current practical limitations for routine use of OPS and SDF imaging can be overcome, such monitoring modalities may improve outcome by directing clinicians to administering resuscitative therapies in a more timely and effective manner.

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Chapter 9

Summary

SUMMARY

The aims of this thesis were to assess the feasibility of orthogonal polarization spectral (OPS) imaging of the buccal microcirculation in children and to investigate the effect of disease and critical care treatments on microcirculatory hemodynamics. To fulfil these aims two groups of children were studied: those with presumably normal microcirculation and those who were critically ill with severe respiratory failure or with sepsis.

Routine hemodynamic monitoring in critically ill pediatric patients has limitations. Restoration of global hemodynamics does not always mean that adequate regional tissue perfusion is achieved, especially in conditions of impaired autoregulation such as occurs during critical illness. The microcirculation is an essential hemodynamic compartment and as such plays an important role in (patho-) physiology of the circulation. Taken together, monitoring the microcirculation, possibly, could be a valuable addition to the hemodynamic monitoring of the critically ill pediatric patient.

In **chapter 2**, the results of investigations in children with a presumably normal microcirculation are presented. This study shows the feasibility of the technique in young children. Developmental changes in the structure of the microcirculation occur in the first few weeks of life in healthy neonates. Consistent with a decrease of cutaneous functional capillary density (FCD) observed in premature infants in the first month of life, we found that FCD of the buccal mucosa decreases after the first week of neonatal life. The results of these observations can serve as reference data for future studies of the microcirculation in children.

In **chapter 3**, the first observations of the microcirculation of the buccal mucosa in a patient with meningococcal septic shock by using OPS imaging are described. It confirmed that the microcirculation was altered in a critically ill child with septic shock. Follow-up measurements were performed during the first 24 hours to observe effects of treatment on the microcirculation. After fluid resuscitation and the start of inotropes and vasopressors, the microvascular parameters were stable. After the start of nitroglycerin, a donor of nitric oxide (NO), which is a vasodilator, recruitment of capillaries was seen.

Inhaled Nitric oxide (iNO) is a frequently used therapy in neonatal and pediatric intensive care, especially in neonates with pulmonary hypertension. In **chapter 4**, the results of a study of the effect of iNO on the systemic microcirculation, by OPS imaging of the buccal mucosa, in infants with hypoxemic respiratory failure and PPHN are presented. The FCD of the buccal mucosa increased after the start of iNO, which supports the idea of a remote effect of iNO on distal vascular beds.

Chapter 5 shows the results of a study in neonates with severe respiratory failure, who received extracorporeal membrane oxygenation (ECMO) treatment. The microcirculatory values in critically ill patients with severe respiratory failure were found to be

depressed. After treatment with ECMO, at a time when the patient no longer needed ECMO, the microcirculatory parameters were improved.

Direct effects of initiation of ECMO therapy are described in **chapter 6**. Surprisingly, the microcirculation did not improve immediately after the start of ECMO treatment. Nonetheless, FCD was preserved in patients who received ECMO treatment, while a deterioration of microvascular values was observed in the control group. There are several potential explanations for the lack of improvement of the microcirculation immediately after the start of ECMO, such as side effects of the ECMO treatment, e.g. inflammatory response or disturbance of physiologic blood flow. Future studies should include folllow-up investigations and comparison of survivors and non-survivors. In this way, the prognostic value of microcirculatory parameters can be determined.

In **chapter 7**, the results of an observational study in pediatric patients with septic shock are described. These patients appeared to have reduced FCD, which is consistent with observations in adult patients. It was found that persistent alterations of the microcirculation were associated with poor outcome in children with septic shock. Lack of restoration of the altered microcirculation proved to have a stronger predictive value for mortality than the PRISM, a severity of illness score.

Chapter 8 contains the general discussion of this thesis. The currently available literature in addition to the contents of this thesis is reviewed and presented with future perspectives.

SAMENVATTING

In dit proefschrift werd de toepasbaarheid van orthogonal polarization spectral (OPS) imaging voor het visualiseren van de microcirculatie bij kinderen onderzocht. Daarnaast werd het effect van ernstige ziekte en behandeling op de doorbloeding van de microcirculatie geevalueerd. Hiervoor werden verschillende patiënten groepen bestudeerd, namelijk kinderen van wie aangenomen werd dat zij een normale microcirculatie hadden en kritisch zieke kinderen met ernstig respiratoir falen en kinderen met sepsis.

De standaard toegepaste monitorbewaking van kritisch zieke kinderen heeft beperkingen. Het corrigeren van macrocirculatoire parameters parameters, zoals bloeddruk, is niet altijd een garantie voor adequate doorbloeding van de organen en weefsels. Met name niet wanneer autoregulatie van de bloedcirculatie verstoord is zoals bij sommige ernstige ziekten. De microcirculatie speelt een belangrijke rol in de (patho-) fysiologie van de bloedcirculatie. Zodoende zou het monitoren van de microcirculatie , een mogelijk waardevolle aanvulling kunnen zijn op de huidig gebruikte monitorbewaking van kritische zieke kinderen.

In **hoofdstuk 2** worden de resultaten gepresenteerd van het bestuderen van de microcirculatie bij jonge kinderen, van wie aangenomen werd dat zij een normale microcirculatie hadden. In de eerste weken na de geboorte verandert de microcirculatie bij gezonde pasgeborenen. De belangrijkste bevinding was dat de functional capillary density (FCD) afneemt na de eerste levensweek. Dit onderzoek laat de toepasbaarheid van de techniek in kinderen zien. De resultaten kunnen gebruikt worden als referentiewaarden voor toekomstige studies naar de microcirculatie in kinderen.

Hoofdstuk 3 beschrijft een case report over een patiënt met shock op basis van meningococcen sepsis. Bij deze patient werd de microcirculatie met behulp van OPS imaging geëvalueerd. De eerste dag na opname werden OPS metingen verricht op verschillende tijdstip en het effect van de behandeling werd geëvalueerd. Na vochtsuppletie en de start van vasoactieve medicatie, waren microcirculatoire parameters stabiel. Na de introductie van nitroglycerine, een stikstofoxide (NO)- donor, werd toename van de vaatdichtheid in de microcirculatie waargenomen.

NO inhalatie (iNO) is een frequent toegepaste therapie in de neonatale en pediatrische intensive care, in het bijzonder in neonaten met pulmonale hypertensie. In **hoofdstuk 4** worden de resultaten gepresenteerd van een studie naar het effect van iNO op de systemische microcirculatie, gemeten in het wangslijmvlies door middel van OPS imaging, bij kinderen met hypoxemisch respiratoir falen en persisterende pulmonale hypertensie van de neonaat (PPHN). De FCD in het wangslijmvlies nam toe na de start van iNO. Dit ondersteunt het idee, dat NO via het bloed wordt getransporteerd en effect kan hebben op andere vaatbedden buiten de long.

Hoofdstuk 5 laat de resultaten zien van een studie bij neonaten met ernstig respiratoir falen, die behandeld werden met extracorporele membraan oxygenatie (ECMO). Deze patiënten hadden een verlaagde FCD bij aanvang van ECMO behandeling. Na afloop van de ECMO behandeling waren de microcirculatoire parameters verbeterd.

Directe effecten van ECMO behandeling bij kritische zieke neonaten met ernstig resppiratoir falen worden beschreven in **hoofdstuk 6**. In tegenstelling tot de verwachting, verbeterde de microcirculatie niet direct na de start van ECMO. FCD was stabiel in de patiënten die behandeld werden met ECMO, terwijl een verslechtering werd waargenomen in de controle groep, die bestond uit kinderen met ernstig respiratoir falen, die geen ECMO behandeling ontvingen. Het uitblijven van een herstel van de microcirculatie zou verklaard kunnen worden door een bijwerking van het ECMO systeem (bijvoorbeeld de inflammatoire reactie die ontstaat na de start of verstoring van fysiologische bloedstroom). Meer onderzoek is nodig om de ontwikkeling van microcirculatoire veranderingen tijdens deze behandeling in kaart te brengen en om de prognostische waarde van deze observaties te bepalen.

In **hoofdstuk 7** worden de resultaten van een observationele studie in pediatrische patiënten met septische shock beschreven. Deze patiënten bleken een verlaagde FCD te hebben, hetgeen in overeenstemming is met observaties in volwassen patiënten. Het persisteren van een verlaagde FCD bleek geassocieerd te zijn met een slechtere prognose in kinderen met septische shock. Het uitblijven van verbetering van de microcirculatie bleek een sterkere voorspellende waarde te hebben dan de PRISM-score (pediatric risk of mortality score).

Hoofdstuk 8 bevat de algemene discussie. Een overzicht van de beschikbare literatuur, in relatie tot de bevindingen in dit proefschrift, wordt besproken en aanbevelingen voor de toekomst worden gepresenteerd.

LIST OF ABBREVIATIONS

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ARDS	Acute respiratory distress syndrome
AUC	Area under the curve
AVI	Audio video interleave
CCAM	Congenital cystic adenomatoid malformation
CDH	Congenital diaphragmatic hernia
CI	Cardiac index
CMV	Conventional mandatory ventilation
CO	Cardiac output
CO2	Carbondioxide
CPB	Cardiopulmonary bypass
CRT	Capillary refill time
CVL	Central venous line
ΔFCD	Change in functional capillary density
ΔpO2	Change in oxygen pressure
DO2	Delivery of oxygen
ECMO	Extracorporeal membrane oxygenation
FCD	Functional capillary density
Hb	Hemoglobin
HFO	High frequency oscillation
HI	Heterogeneity index
ICU	Intensive care unit
INO	Inhaled nitric oxide
MAS	Meconium aspiration syndrome
MFI	Microvascular flow index
MODS	Multiple organ dysfunction syndrome
MOF	Multiple organ failure
NO	Nitric oxide
OI	Oxygenation index
OPS	Orthogonal polarization Spectral
PDA	Patent ductus arteriosus
pO2	Oxygen pressure
PPHN	Persistent pulmonary hypertension of the neonate
Ppm	Parts per million
PRISM	Pediatric risk of mortality
PVR	Pulmonary vascular resistance
ROC	Receiver operator characteristic
SaO2	Arterial oxygen saturation

SDF	Side stream darkfield
SIRS	Severe inflammatory response syndrome
SO2	Oxygen saturation
SOFA	Sequential organ failure assessment
SpO2	Pulse oxymetry
SPSS	Statistical Package for the Social Sciences
SVI	Stroke volume index
SvO2	Mixed venous oxygen saturation
SVR	Systemic vascular resistance
VA-ECMO	Veno-arterial extracorporeal membrane oxygenation
VDI	Vessel density index

LIST OF PUBLICATIONS

PUBLICATIONS

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Curriculum Vitae

CURRICULUM VITAE

The author was born in 1971 in Stad-Delden, the Netherlands. She went to secondary school (Voortgezet wetenschappelijk onderwijs (VWO)) in Hengelo. In 1991 she moved to Rotterdam to study medicine. During her study she went to Indonesia for a 5 month elective. She graduated from medical school in 1997. She started her career in pediatrics in St Joseph Hospital, in Veldhoven. In 1998 she returned to Rotterdam to work in the Erasmus Medical Center, Sophia Children's Hospital, where she trained as a pediatrician. After her registration in 2003, she started a fellowship in pediatric intensive care. As part of her training in Rotterdam, she electively worked 4 months in the Red Cross Children's Hospital, Cape Town, South Africa. After finishing her fellowship, she joined the staff of the Intensive Care of the Sophia's Children's hospital for almost 2 years. In 2008 she moved to the United Kingdom. Since then, she has been working in the Pediatric Intensive Care Unit of Cambridge University NHS Foundation Trust Hospital, Addenbrooke's Hospital.

MICROCIRCULATION IN CRITICALLY ILL CHILDREN

The aims of this thesis were to assess the feasibility of orthogonal polarization spectral (OPS) imaging of the buccal microcirculation in children and to investigate the effect of disease and critical care treatments on microcirculatory hemodynamics. To fulfil these aims two groups of children were studied: those with presumably normal microcirculation and those who were critically ill with severe respiratory failure or with sepsis.

Routine hemodynamic monitoring in critically ill pediatric patients has limitations. Restoration of global hemodynamics does not always mean that adequate regional tissue perfusion is achieved, especially in conditions of impaired autoregulation such as occurs during critical illness. The microcirculation is an essential hemodynamic compartment and as such plays an important role in (patho-) physiology of the circulation. Taken together, monitoring the microcirculation, possibly, could be a valuable addition to the hemodynamic monitoring of the critically ill pediatric patient.

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