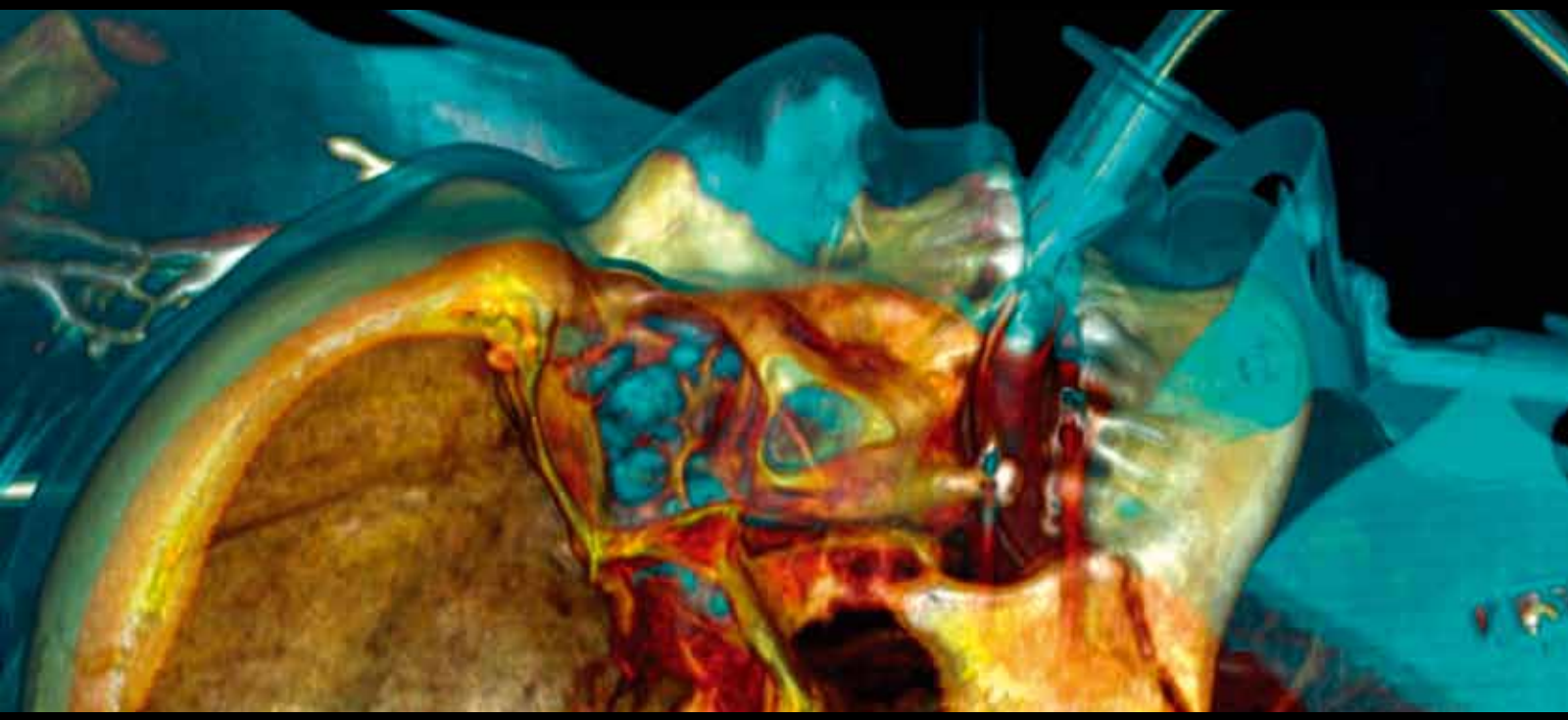
Critical Care Research and Practice

Microcirculation

Guest Editors: Michael Piagnerelli, Can Ince, and Arnaldo Dubin



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Editorial Board

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Editorial
Microcirculation

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The microcirculation is the part of the circulation where oxygen, nutrients, hormones, and waste products are exchanged between circulating blood and parenchymal cells. The microcirculation includes not only all the vessels with a diameter $<100\,\mu$m but also the interactions between blood components (circulating cells, coagulation factors), the vessels lined by the endothelium, and the glycocalyx.

Over the last decade, especially since the development of new techniques such as orthogonal polarized spectral (OPS) and sidestream dark field (SDF) imaging, we have been able to assess alterations in the microcirculation of critically ill patients at the bedside [1–4]. From the various studies, it is clear that all components of the microcirculation are altered early in critical illness, especially during sepsis. Persistence of these alterations is associated with increased morbidity and a poor outcome [2, 4, 5]. Interestingly, these alterations are not correlated with systemic hemodynamics [6], making monitoring the microcirculation of particular interest for titrating potential therapies.

So, should we all be assessing the microcirculation at the bedside and use it to guide therapy in all critically ill patients? Unfortunately, we are not yet ready for this step! Indeed, several questions need to be answered before we try to modulate the microcirculation with any therapeutic intervention. In this special issue, several recent studies in this field are published to try and provide some responses to these remaining questions.

Before microcirculatory monitoring can become widespread, it needs to be standardized: first, in terms of imaging the sublingual microcirculation, and second, in terms of quantifying the alterations observed. N. A. R. Vellinga et al. suggest the development of a picture database from 36 intensive care units (ICUs) worldwide. These authors called their network: microSOAP (Microcirculatory Shock Occurrence in Acutely ill Patients). The aim of this multicenter, observational study was to collect 500 images from critically ill patients and to estimate the prevalence of microcirculatory alterations in ICU patients, related to conventional clinical and hemodynamic variables. Moreover, this database could serve as a source for further investigations.

Despite a roundtable involving experts in the field [7], scoring of microcirculatory alterations remains controversial [8, 9] and could limit the expansion of this technique. Indeed, if different scoring techniques are used in different studies, it is difficult to compare studies and patients. In this special issue, M. O. Pozo et al. compared different methods of calculating the microvascular flow index (MFI). This index is commonly used to semiquantitatively characterize the velocity of microcirculatory perfusion as absent, intermittent, sluggish, or normal [7, 10]. Three approaches are described to compute the MFI: (1) the average of the predominant flow in each of the four quadrants (MFI by quadrants), (2) direct assessment during bedside video acquisition (MFI point of care), and (3) the mean value of the MFIs determined

in each individual vessel (MFI vessel by vessel). In this study, performed by analyzing 100 pictures from septic patients, the best correlations were between the MFI vessel by vessel and RBC velocity (r^2 : 0.61, $P < 0.0001$) and between the MFI vessel by vessel and the fraction of perfused small vessels (r^2 : 0.96, $P < 0.0001$). Although MFI measurement reflects the magnitude of microvascular perfusion, the different approaches are not interchangeable. As noted by the authors, however, although the MFI vessel by vessel approach may seem to be preferable, it is time consuming and does not facilitate use of the technique at the bedside.

Also in this issue, E.–S. Tripodaki et al. and A. P. C. Top et al. introduce new pieces into the puzzle of the relationship of the microcirculation and systemic hemodynamics. First, Tripodaki et al. evaluate the relationship of muscle microcirculation to systemic parameters and outcome after cardiac surgery. The authors studied the microcirculation using near-infrared spectroscopy (NIRS) and the vascular occlusion technique. They observed good correlations between NIRS-derived variables and cardiac output, lactate, and mortality. These relationships have been described previously in septic shock [11] but this is the first time they have been demonstrated in cardiac surgery patients. The dependence of the microcirculation on systemic hemodynamics is a controversial issue. In septic shock, sublingual microvascular perfusion is independent of either cardiac output or blood pressure [4]; therefore, the microcirculation may behave as an independent compartment of the cardiovascular system. Increasing blood pressure with norepinephrine, however, did affect the sublingual microcirculation showing that some dependency is still present [12].

In this context, G. Hernandez et al. investigated the microcirculation of a particular critically ill septic population: septic patients with arterial hypotension without elevated lactate concentrations. In an earlier study, these authors showed that persistent sepsis-induced hypotension without hyperlactatemia was associated with less severe organ dysfunction and a very low mortality risk (5.2 versus 17.4% for patients with lactate concentrations >2.5 mmol/L) [13]. In the present study, the authors used an SDF imaging device to study the microcirculation of 45 of these patients. There were relatively few abnormalities in this population, as shown by a median MFI value of 2.4 and a median percentage of perfused vessels of 87.3%. This study tends to support the notion that patients with persistent sepsis-induced hypotension without hyperlactatemia exhibit a distinctive clinical and physiological profile within the spectrum of septic shock. This subject should be addressed in future studies.

A. P. C. Top et al. studied the behavior of the sublingual microcirculation after the start of ECMO therapy in neonates with severe respiratory failure. ECMO usually induces an improvement in hemodynamics and an immediate decrease in vasopressor needs. Nevertheless, beneficial cardiovascular effects after ECMO were not evident in this study as shown by unchanged blood pressure and no changes in infusions of vasoactive or inotropic drugs. Simultaneously, the sublingual microcirculation failed to improve and the alterations present at baseline remained present. In contrast, a group of patients on mechanical ventilation, with similar derangements at baseline, showed a decrease in microvascular perfusion over time. These findings suggest that ECMO could have a delayed effect on the microcirculation and thus prevent a further deterioration in microvascular flow. Unfortunately, the lack of cardiac output measurements precludes a fuller understanding of these results.

Finally, after works on measurements of the microcirculation at the bedside, studies on other compounds of the microcirculation, such as the glycocalyx or red blood cells (RBCs), are reported. Enzymatic degradation of the glycocalyx induces vascular leakage *ex vivo*, so S. A. Landsverk et al. investigated enzymatic treatment in an *in vivo* whole body hamster model. In addition to looking at the effects of degradation of the glycocalyx on endothelial leakage, these authors also investigated the potential effects of this process on the microcirculation. After injection of hyaluronidase, they measured plasma volume and functional capillary density as markers of the microcirculation. Enzyme treatment did not induce changes in plasma or albumin volumes, but reduced functional capillary density. There was no correlation between plasma hyaluronan concentrations and plasma volume or microcirculatory disturbances, despite a 50–100 fold increase in plasma hyaluronan. To explain their results, the authors suggest that impaired mechanotransduction associated with vasoconstriction, mainly due to loss of hyaluronan from the endothelial glycocalyx, was a possible mechanism [14]. Another possibility is the increased RBC rigidity at higher hyaluronan concentrations [15].

In another article, Y. Serroukh et al. comprehensively review the alterations in the erythrocyte membrane that occur in sepsis. This issue is potentially important to explain the microcirculatory abnormalities in sepsis. The authors discuss the alterations in the components of the RBC membrane that have previously been described. This membrane is essential for RBC deformability and rheology, and changes in the membrane and its complex interactions could significantly affect the microcirculation. Although clinical evidence is limited, RBC rheologic alterations in sepsis and their effects on blood flow and oxygen transport may have important implications, and improved understanding of the underlying mechanisms is important. Consequently, this review not only contributes to our understanding of current knowledge but also provides a framework for future research.

In conclusion, this special issue highlights the disturbances in the microcirculation in critically ill patients and presents some answers to important questions concerning methodology or particular populations of patients. These articles provide some additional pieces to the complex puzzle of optimizing treatment of the critically ill patient!

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Research Article

Impact of Enzymatic Degradation of the Endothelial Glycocalyx on Vascular Permeability in an Awake Hamster Model

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Background. The inside of the endothelium is covered by a glycocalyx layer, and enzymatic degradation of this layer induces vascular leakage *ex vivo*. We hypothesized that enzymatic degrading of the glycocalyx in an *in vivo*, whole body model, would induce plasma leakage and affect the microcirculation. *Methods*. Golden Syrian hamsters were divided into an enzyme (hyaluronidase) and a control group. Mean arterial pressure (MAP), heart rate (HR), hematocrit (Hct), base excess (BE), and plasma volume were obtained before, 45 and 120 min after enzyme/saline treatment. Plasma volume was evaluated by the distribution volume of indocyanine green and the microcirculation by functional capillary density (FCD). The enzymatic effect was determined by measuring plasma levels of hyaluronan (HA). *Results*. There were no differences in MAP, HR, Hct, and BE between the two groups. Enzyme treatment did not induce changes in plasma volume but reduced FCD. There was a 50–100-fold increase in plasma HA, but no relationship was found between HA levels and plasma volume or FCD. *Conclusion*. Vascular leakage was not confirmed in an *in vivo*, whole body model after degradation of the endothelial glycocalyx. The microcirculation was affected, but no relationship between plasma levels of HA and FCD was seen.

1. Introduction

Increased vascular permeability is often seen in surgical and critical care patients [1] and has, among other mechanisms, been attributed to damage of the endothelial glycocalyx. The importance of an intact glycocalyx layer to prevent vascular leakage has been demonstrated by experimental degradation of this layer in isolated hearts or blood vessels, and *in vivo* using genetically modified mice predisposed to atherosclerosis [2–5]. In these experimental models, different approaches to preserve the glycocalyx has also been evaluated [6, 7]. Hyperglycemia, ischemia, and inflammation are associated with degradation of the endothelial glycocalyx [8, 9]. Based on this knowledge, clinical recommendations have been given for the preservation of the glycocalyx during surgery, to avoid pathological fluid and protein shifts [10]. As far as we are aware of, increased vascular permeability with decreased plasma volume or tissue edema after enzymatic degradation of the glycocalyx has not been demonstrated in an *in vivo*, whole body (wild-type) model. A previous study performed in our laboratory found no reduction in plasma volume after enzymatic degradation of the glycocalyx in hamsters, whereas the microcirculation was affected, demonstrated by a reduction of functional capillary density (FCD) [11]. The effect of hyaluronidase on the endothelial glycocalyx was not evaluated in that study and the plasma volume tracer used, Dextran 40 kDA, has been criticized [12].

The aim of the present study was to evaluate plasma leakage and impairment of the microcirculation by enzymatic degrading the endothelial glycocalyx in an awake hamster model. A plasma volume tracer, indocyanine green (ICG), suitable for repetitive measurements was used, and as vascular leakage is time dependent, the observation period was extended. Changes in plasma volume and FCD could then be related to enzymatic effects measured by the total amount of hyaluronan (HA) released into the circulation.

2. Materials and Methods

2.1. Animals. Male Golden Syrian hamsters, 6-7 weeks old, weight 58–66 gr (Charles River Laboratories, Boston, MA), were used in a hamster window chamber model. The protocol was approved by the local animal subjects committee and was in accordance with the Guide for the Care and Use of Laboratory Animals (National Research Council, 1996). The hamster window chamber model allows the study of the skin microcirculation, to infuse drugs and to collect blood samples without the influence of anesthesia [13]. The implantation of the chamber window and catheters in the carotid artery and jugular vein (PE50/PE10) were performed in two separate procedures during anesthesia (Nembutal, 50 mg/kg, intraperitoneal injection, Abbott, Abbott Park, IL). The complete surgical technique is previously described [13]. Experiments were performed 48 hours after the implantation of the catheters, without any influence of anesthetic drugs.

2.2. Hemodynamic Parameters and Hematology. Continuous blood pressure was obtained from a carotid artery catheter during the experimental period, giving mean arterial pressure (MAP) and heart rate (HR) (Biopac, Santa Barbara, CA; Spectramed Pressure Transducer). Hemoglobin level was determined spectrophotometrically (B-Hemoglobin, Hemocue, Stockholm, Sweden) and hematocrit was measured from centrifuged arterial blood samples using heparinized capillary tubes. Analysis of PaO_2, $PaCO_2$, base excess and pH was determined from arterial blood, collected in heparinized capillary tubes (Blood Chemistry Analyzer 248, Bayer, Norwood, MA).

2.3. Plasma Levels of Albumin. Vascular permeability has previously also been measured using transcapillary escape rate of radioactive labeled albumin [14]. An increased leakage of albumin due to degradation of the endothelial glycocalyx could reduce the total amount of plasma albumin. Plasma levels of albumin were determined (Vetscan VS2, Abaxis Inc. Union City, CA) before and 45 min after enzyme or saline was given, in a separate group of hamsters ($n = 6$).

2.4. Distribution Volume of Indocyanine Green. ICG has been used as a method to measure plasma volume [15]. The advantage of ICG relates to its low toxicity, rapid distribution, and clearance allowing for repetitive measurements [16]. Whole blood was used to determine dye absorption. A calibration curve was made from two known concentrations of dye in blood from one hamster. The circulation time of ICG was based on multiple samples from pilots. Baseline absorption was obtained by taking 10 μL samples from the arterial line before each measurement. 0.1 mg of ICG, diluted in 0.1 mL sterile water, was then given intravenously. 10 μL blood was then collected from the arterial line at 3, 4, 5, and 6 min after the injection of the dye and placed in a cuvette with 100 μL deionized water before each measurement. To reduce dye contamination and minimize blood loss, the length of the arterial line was adjusted so that taking 2 drops of blood before each sample would ensure that the sample taken was from the circulation, and not from the catheter. In addition, the tip of the catheter was cleaned between each sample. The cuvette was analyzed in a spectrometer (Lambda 20, Perkin Elmer, Waltham, MA), absorption measured at 800 and 880 nm. Mixing of the cuvette, timing from obtaining samples to analysis was performed similarly each time. A monoexponential extrapolation was performed to calculate absorption at time zero. Measurements were included only if $r^2 \geq 0.9$.

2.5. Functional Capillary Density (FCD). FCD was evaluated microscopically as the number of capillary vessel with erythrocytes passing in the visual field during one minute. 10 visual fields were counted and the average value calculated. Each visual field was identified in a way that repeating measurements could be obtained at the same location.

2.6. Plasma Levels of Hyaluronan. Plasma levels of hyaluronan were determined by hyaluronan—Enzyme Linked Immunsorbent Assay kit (HA-ELISA) (Echelon Bioscience Inc., Salt Lake City, UT). Arterial blood was collected at baseline, 45, 60, and 120 min after hyaluronidase was given, using a heparinized capillary tube. After centrifuging, 10 μL of plasma was obtained with a micropipette and then transferred to an Eppendorf tube and stored at -80°C, until analysis.

2.7. Experimental Setup and Protocol. 14 animals were divided in two groups, receiving either Streptomyces hyaluronidase (Sigma-Aldrich, St. Louise, MO) or saline. In a separate group of animal ($n = 6$), hyaluronidase or saline was given to determine the impact on albumin levels. On the day of the experiment, the hamster was placed in a restraining tube with a longitudinal slit for the chamber window. The anesthetized animal was made to adapt to the new environment for 30 min. Baseline values of MAP, HR, hematology, including levels of HA in plasma and the distribution volume of ICG were then obtained. A bolus of 100 units of hyaluronidase (0.1 mL) or a similar volume of saline was infused after baseline measurements. This represents time zero, in the time line shown in Figure 1. The distribution volume of ICG was measured at 45 and 120 min. FCD was obtained at 30 and 60 min and before the distribution volume of ICG at 120 min. Hemodynamic parameters and hematology were obtained at 45, 60, and 120 min, together with blood samples for HA measurement.

2.8. Data Analysis and Statistics. Values are given as mean and standard deviation, unless, otherwise, stated. Data on distribution volume of ICG- and FCD are also given as relative values to baseline. A value of 1.0 then refers to zero change from baseline. In Figures 2(b) and 3, data are shown as box plots. The horizontal line within the box represents median value. The upper and lower limit of the box represents the 75th and 25th% percentile and the upper and lower whisker represents the 95th and 5th%. Comparisons within groups were performed with a one-way ANOVA, with post

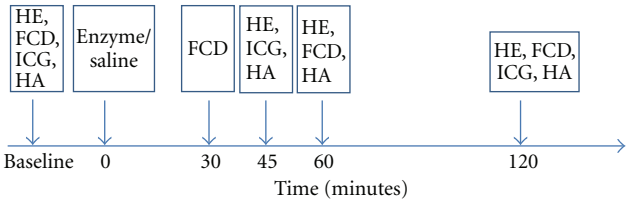

Figure 1: The time line shows the experimental setup. Baseline registrations includes hemodynamic and hematologic data (HE), functional capillary density (FCD) the distribution volume of indocyanine green (ICG) and plasma hyaluronan (HA). Enzyme or saline was given at time zero. Following measurements are indicated with an arrow.

hoc analyses performed with the Bonferroni's multiple-comparison tests. Comparisons between the groups at each time point regarded were performed with an unpaired t-test. All statistics were calculated using GraphPad Prism 4.01 (GraphPad Software, San Diego, CA). Changes were considered statistically significant if $P < 0.05$.

3. Results

A total of 20 animals were used in this study. An enzyme group ($n = 7$, weight 63.4 ± 2.3 g) received Streptomyses hyaluronidase and a control group ($n = 7$, weight 61.4 ± 3.6 g) was given the same amount of saline. There was an estimated blood loss of 0.35 mL, and an infusion of 0.6 mL saline between each measurement of the distribution volume of ICG. The impact of enzymatic degradation of the glycocalyx on plasma albumin levels was tested in a separate group of animals ($n = 6$).

3.1. Hemodynamic Parameters and Hematology. There was no significant difference between the groups at the three different time points. Within the groups, there were no differences in pH, BE, PO_2, and PCO_2. There was trend to lower MAP from baseline to 120 min in the enzyme group and a similar, but significant reduction in the control group. HR was decreased between 60 and 120 min in the control group. Hct was reduced significantly from baseline values to 120 min in both groups. Data are shown in Table 1.

3.2. Levels of Albumin after Hyaluronidase/Saline. In the separate group of hamsters ($n = 6$), there was no difference in levels of plasma albumin before and at 45 min in the group with enzyme treatment (4.5 ± 0.3 gm/dL versus 4.5 ± 0.2 gm/dL) or in animals receiving saline.

3.3. Distribution Volume of Indocyanine Green. No differences at baseline values were found between groups. 45 min after enzyme or saline treatment, no changes in distribution volume were found (relative values to baseline 0.99 ± 01 versus 0.98 ± 0.05, $P = 0.8$). After 120 min, there was a trend in reduction of ICG distribution volume in the enzyme group, but there was no significant difference between the two groups (relative values to baseline 0.93 ± 0.16 versus 1.01 ± 0.11, $P = 0.25$). Data are shown as absolute values and relative values to baseline in Figure 2.

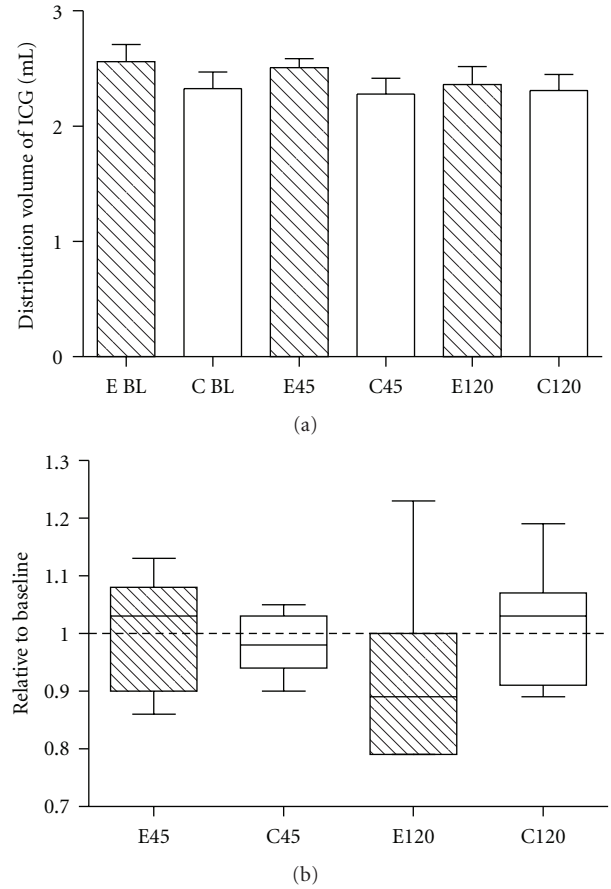

Figure 2: Effect of enzymatic degradation of the glycocalyx on the distribution volume of indocyanine green (ICG). (a): There was no difference within or between the enzyme group (E-shaded bars) and the control group (C-open bars), at baseline (BL) and after 45 or 120 minutes. (b): There is no difference in values relative to baseline for the distribution volume of ICG after 45 minutes: Enzyme group (E45) and control group (C45), and after 120 minutes, enzyme group (E120) and control group (C120).

3.4. Functional Capillary Density. FCD was significant lower in the enzyme group than in the control group at 30 and 45 minutes. There was nonsignificant trend ($P = 0.07$) at 120 minutes. Data are shown as values relative to baseline in Figure 3.

3.5. Plasma Levels of HA. There was a 50–100 folds increase of HA levels after hyaluronidase treatment. Plasma levels of HA are shown in Figure 4.

3.6. Relation between Levels of Hyaluronan and Distribution Volume ICG. There was no significant relationship between plasma volume and FCD at 45 and 120 min (Figures 5(a) and 5(b)) as the confidence intervals for the regression lines were not significantly different from zero.

Table 1: Hemodymanical parameters and hematology.

	Baseline		60 minutes		120 minutes	
	Enzyme	Control	Enzyme	Control	Enzyme	Control
MAP	114 (5)	111 (6)	109 (5)	104 (9)	106 (9)	99 (5)*
HR	459 (16)	468 (17)	455 (22)	473 (18)	454 (16)	448 (16)*
Hct	49 (2)	50 (2)	47 (2)	47 (2)	45 (2)**	45 (2)***
Ph	7.37 (0.03)	7.35 (0.04)	7.35 (0.04)	7.32 (0.04)	7.35 (0.02)	7.32 (0.03)
P_aO_2	56.7 (9.9)	54.4 (5.7)	61.2 (5.9)	59.5 (8.9)	57.1 (10.9)	59.2 (7.4)
P_aCO_2	56.6 (7.5)	59.7 (3.89)	56.1 (7.6)	61.3 (6.6)	55.7 (5.2)	59.2 (2.7)
BE	6.8 (2.8)	6.9 (2.1)	4.4 (1.6)	4.7 (2.3)	4.4 (1.6)	3.9 (2.0)

Values are given as mean and standard deviation. MAP: mean arterial pressure, HR: heart rate, Hct: hematocrit, BE: base excess. Significant differences were seen within the groups from baseline to 120 minutes (MAP and Hct) and from 60 to 120 minutes in the control group (HR). $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

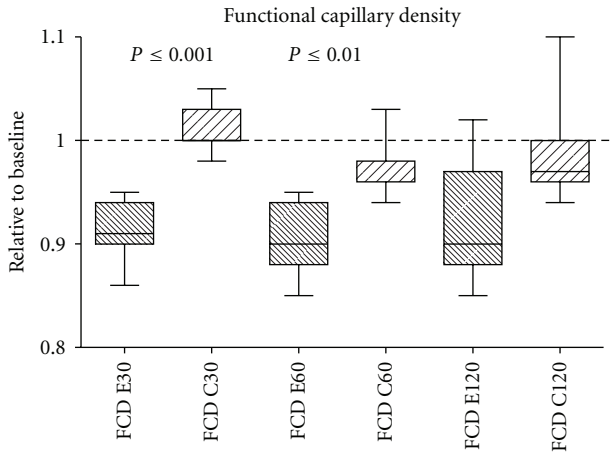

Figure 3: Effect of enzymatic degradation of the glycocalyx on functional capillary density (FCD). Data shown as values relative to baseline. Significant changes were found, between the enzyme group (E30) and control group (C30) after 60 minutes (E60, C60). After 120 minutes (E120, C120) there was a non significant trend ($P = 0.07$).

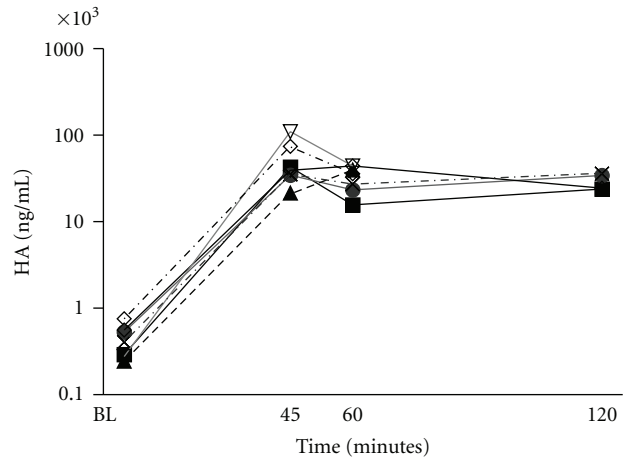

Figure 4: Plasma levels of hyaluronan (HA) for the 7 animals treated with hyaluronase displayed on a logarithmic axis. Plasma level of HA from each animal is indicated by a single line based on measurements obtained at baseline (BL), after 45, 60, and 120 minutes. At 120 minutes, HA was measured in 4 animals.

3.7. Relation between Plasma Levels of HA and Distribution Volume of ICG and FCD. There was no significant relationship between levels of HA and the distribution volume of ICG at 45 and 120 min (Figures 6(a) and 6(b)) or with FCD (Figures 6(c) and 6(d)) . The confidence intervals for all the regression lines were not significantly different from zero.

4. Discussion

The main finding in this study was that enzymatic degradation of the endothelial glycocalyx in an *in vivo*, whole-body model, did not induce leakage of plasma or albumin. The microcirculation was affected, demonstrated by a significant reduction of FCD. However, there was no clear relationship between the amount HA released into the circulation and the distribution volume of ICG or FCD.

The clinical consequences of increased vascular permeability are tissue edema and hypovolemia, associated with reduced tissue oxygenation and impairment of organ function. Thus, increased vascular permeability represents a significant clinical challenge. Increased attention has been given to the endothelial glycocalyx and its role in regulating vascular permeability [17]. Previous studies have been performed in a variety of experimental settings, from cultured cells, *ex vivo* isolated organs or vessels, genetically modified animals, mainly focusing on the permeability of proteins and tracers within the glycocalyx layer. Conflicting results on glycocalyx thickness from *in vitro* and *ex vivo* studies also indicate that the integrity of this structure is dependent on experimental conditions [18]. Surprisingly, few studies have addressed the clinical consequences of increased vascular permeability, such as plasma leakage and tissue edema. Despite this, experimental studies have been used as evidence for clinical recommendations in fluid therapy [10, 19]. Interestingly, the authors of these two reviews draw different conclusions based on the same experimental literature regarding the use of colloids.

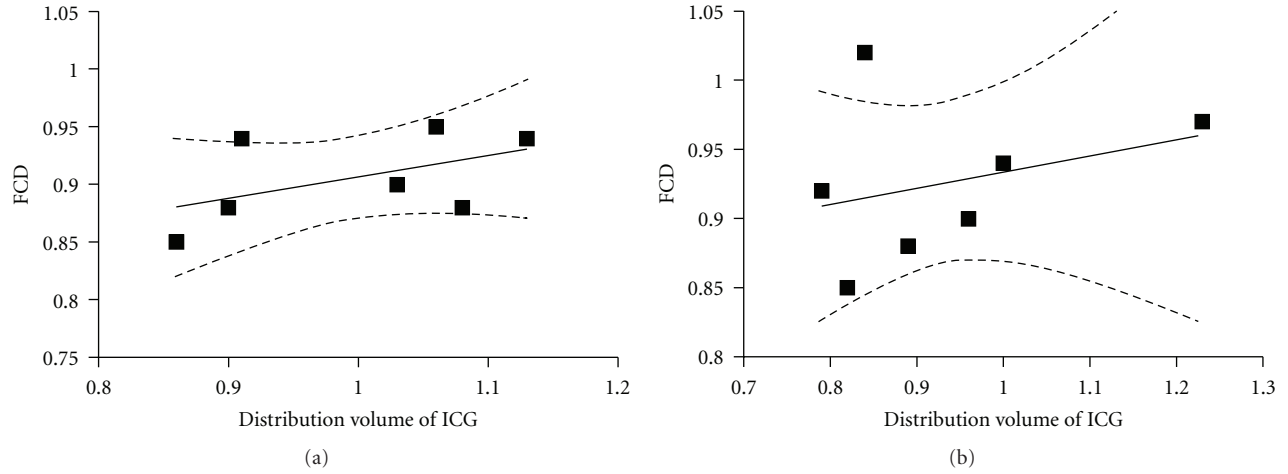

Figure 5: (a): Correlation between functional capillary density (FCD) and the distribution volume of indocyanine green (ICG) at 45 minutes (a) and 120 minutes (b). FCD obtained at 30 minutes was used for the correlation at 45 minutes. The confidence intervals for the regression line (the two dotted lines) indicates the lack of correlation at any of the time points.

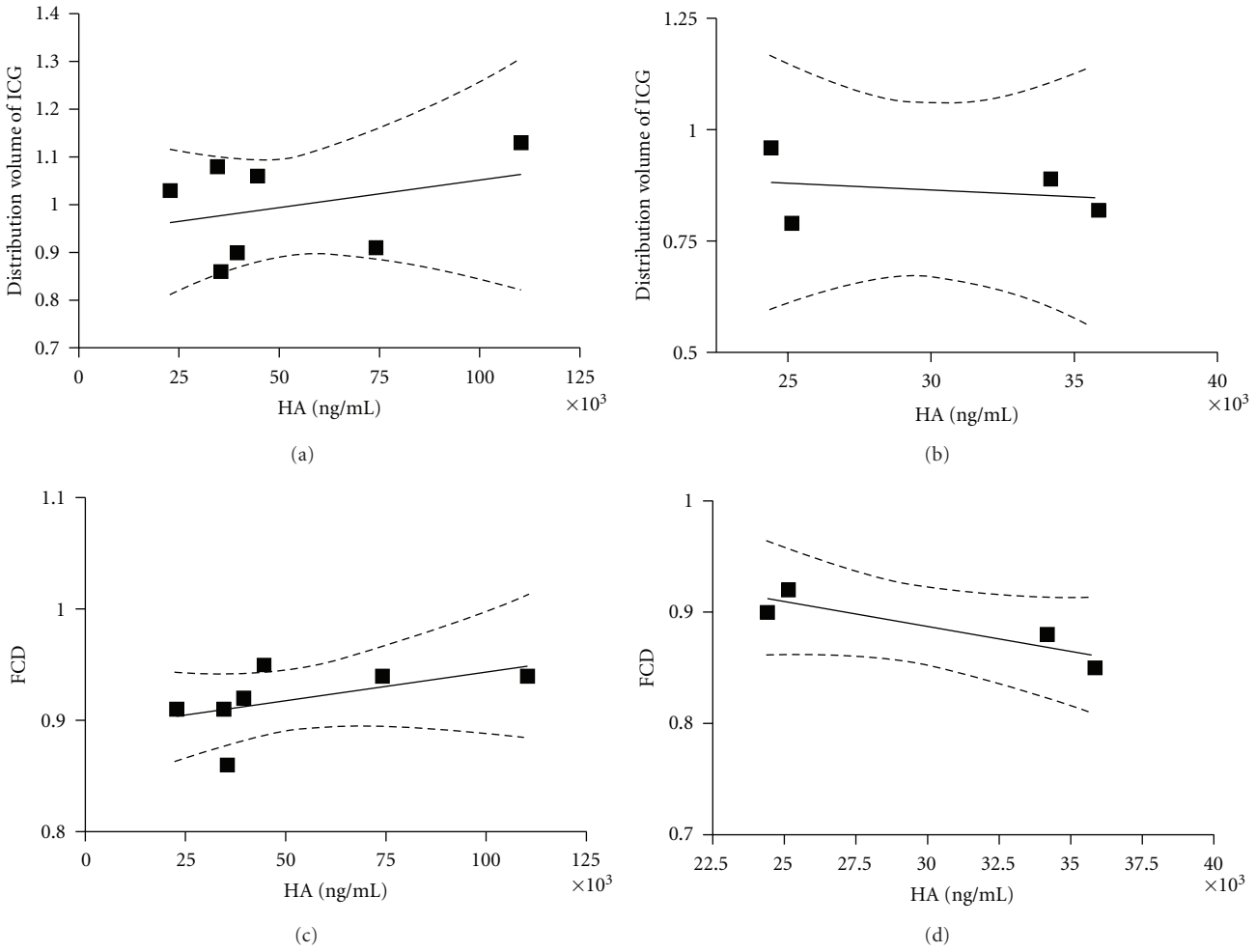

Figure 6: Correlation between plasma hyaluronan (HA) and the distribution volume of indocyanine green (ICG) after 45 and 120 minutes ((a) and (b)). The correlation between functional capillary density (FCD) is shown after 45 and 120 minutes ((c) and (d)). The confidence interval for the regression lines are marked as dotted lines and indicate no correlation.

The fact that enzymatic degradation had no impact on the distribution volume of ICG was supported by laboratory and hemodynamic data. There was no difference in blood pressure, heart rate, Hct or BE between the enzyme group and the control group indicating an increased vascular leakage after hyaluronidase treatment.

Our findings are in accordance with a previous study in our laboratory, using the same animal model, but with a different plasma volume tracer, Dextran 40 kDA [11]. These two studies are in contrast to the vascular leakage leading to myocardial edema seen *ex vivo* [4] or reduced plasma volume and proteinuria after 4 weeks of hyaluronidase treatment in Apolipoprotein E-deficient mice [5]. The intensity of the hyaluronidase treatment of the isolated heart or the duration of treatment of the genetically modified vascular wall could explain these conflicting findings.

We found that FCD was reduced after enzymatic treatment. FCD is one of the most important indicator of tissues perfusion and has been shown to predict survival after shock both in clinical and animal studies [20–22]. Reduction of the circulating plasma volume is one of many factors known to affect FCD. However, in our study, there was no relation between FCD and the distribution volumes of ICG (Figure 5). Previously, several mechanisms for the relationship between FCD and the degradation of the endothelial glycocalyx have been proposed. Impairment of mechanotransduction associated with vasoconstriction, mainly due to loss of hyaluronan from the endothelial glycocalyx [23] was suggested as the most likely mechanism in a study by Zuurbier et al. [24]. However, when microcirculatory vessels diameter and flow was measured before and after hyaluronidase treatment, this was not confirmed [11]. In a study by Luquita et al. [25], increased erythrocyte rigidity was seen with elevated levels of plasma HA. Although there was no clear relationship between FCD and HA levels in the present study, the levels of HA was 50–100 times higher in all animals. Elevated levels of HA are also related to increased viscosity [11]. Thus, increased levels of HA might contribute to reduced FCD seen in our study.

Detection of components from the glycocalyx, such as syndecan, heparan sulfate, and HA, has previously been used as evidence for enzymatic degradation [9, 26]. By measuring HA in the animals together with the distribution volume of ICG and FCD, we evaluated the relationship between these parameters. The distribution volumes of ICG were similar after 45 min in both group, but with a larger variation seen in the enzyme group compared to the saline group. The standard deviation was almost twice. This pattern was also seen in FCD, although not as clear. A large variation in FCD, arteriolar and venular diameter, and velocity, was also seen in a previous study [11]. As seen in Figure 6, there was no relationship between either the distribution volume of ICG, or FCD and levels of HA. Thus, variability found in both studies cannot be explained by a different response of hyaluronidase to produce HA.

There are several other factors that can influence our observations. The endothelial glycocalyx is influenced by a wide range of stimuli. The two-step surgical procedure, the implantation of the window chamber one day, and then the catheters two days before the experiment could induce a postoperative response impairing the whole endothelial glycocalyx in both groups, thus reducing the difference after enzyme treatment.

Many baseline values of HA in our study were high, but reasonable based on the hamsters young age and the reduction of food intake postoperatively. Hamster usually drops 3–5 grams after surgery [27]. The surgery and postoperative alterations could also contribute. The increase in plasma levels of hyaluronan after enzymatic treatment was substantial. Even though others have found large increase of glycocalyx components after shedding [9], it is possible that the increase in circulating HA could originate from other sources than the endothelial glycocalyx [28]. Most of HA are found in the extracellular matrix, and the size of the hyaluronidase molecule would allow penetrating the capillary wall. Thus, it is likely that a considerable amount of HA found could be a product of the extracellular matrix. The high plasma levels of HA 2 hours after the enzyme bolus probably reflect that there is no reincorporation of HA into the glycocalyx, and that the plasma levels exceed the capacity of the liver to metabolize HA [29, 30].

Hyaluronidase only degrades parts of the glycocalyx, and the remaining structure could be capable of preventing plasma leakage. As the endothelial glycocalyx regenerates slowly [31], and plasma and protein leakage are time dependent, the time frame of the present study, although longer compared to the previous study [11], could have been too short. Thus, extending the time frame, combining several enzymes [32], including additional experimental methods such as detecting leakage of ICG into to perivascular space using intravital fluorescence microscopy, could be an approach to demonstrate vascular leakage in an *in vivo,* whole-body model in the future.

5. Conclusion

Enzymatic degradation of the endothelial glycocalyx with hyaluronidase does not induce plasma leakage in awake hamsters in a two hours' time frame, but reduces FCD. No relationship between changes in plasma volume or FCD to the amount of HA released into the circulation after enzyme treatment was found.

Summary Statements

Enzymatic degradation of the endothelial glycocalyx with hyaluronidase does not decrease plasma volume in an awake hamster model.

Conflict of Interests

There is no conflict of interest for any of the authors. Presented in part at the American Society of Anesthesiologists annual meeting 2010, San Diego, CA.

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Clinical Study

Microcirculation and Macrocirculation in Cardiac Surgical Patients

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Background. The aim of our study was to investigate the relationship between microcirculatory alterations after open cardiac surgery, macrohemodynamics, and global indices of organ perfusion. *Methods*. Patients' microcirculation was assessed with near-infrared spectroscopy (NIRS) and the vascular occlusion technique (VOT). *Results*. 23 patients undergoing open cardiac surgery (11 male/12 female, median age 68 (range 28–82) years, EuroSCORE 6 (1–12)) were enrolled in the study. For pooled data, CI correlated with the tissue oxygen consumption rate as well as the reperfusion rate ($r = 0.56$, $P < 0.001$ and $r = 0.58$, $P < 0.001$, resp.). In addition, both total oxygen delivery (DO_2, mL/min per m^2) and total oxygen consumption (VO_2, mL/min per m^2) also correlated with the tissue oxygen consumption rate and the reperfusion rate. The tissue oxygen saturation of the thenar postoperatively correlated with the peak lactate levels during the six hour monitoring period ($r = 0.50$, $P < 0.05$). The tissue oxygen consumption rate (%/min) and the reperfusion rate (%/min), as derived from the VOT, were higher in survivors compared to nonsurvivors for pooled data [23 (4–54) versus 20 (8–38) $P < 0.05$] and [424 (27–1215) versus 197 (57–632) $P < 0.01$], respectively. *Conclusion*. Microcirculatory alterations after open cardiac surgery are related to macrohemodynamics and global indices of organ perfusion.

1. Introduction

Cardiac surgery is characterized by microcirculatory alterations and reduced organ perfusion, due to a combination of the surgery itself, the anesthesia, the hypothermia, the hemodilution, the microemboli formation that occur during the procedure [1, 2], and mainly the intense systematic inflammatory response that develops and peaks the first twenty four hours postoperatively [3]. Peripheral blood flow and oxygen supply can also be affected postoperatively by a low cardiac output state. This can occur in patients with compromised systolic or diastolic ventricular function but also as a result of myocardial "stunning" due to ischemia-reperfusion injury of the heart. If left without intervention, it can also lead to tissue damage and organ failure [4]. It has been observed that microcirculatory derangements may be present despite systemic hemodynamics being within satisfactory goals [5, 6].

Near-infrared spectroscopy (NIRS) is a noninvasive, bed side easily applicable tool that has been used to provide an estimate of tissue oxygenation in health and in disease [7, 8]. By performing a vascular occlusion technique (VOT) NIRS can be used at rest [9, 10] and during interventions [11, 12] for the evaluation of the microcirculation.

We hypothesized that the microcirculatory alterations after cardiac surgery as assessed by NIRS technology are related to macrocirculatory indices.

The aim of our study was to investigate a possible relationship between NIRS derived parameters and macrohemodynamics, as well as global indices of organ perfusion, in cardiac surgical patients.

2. Materials and Methods

2.1. Study Design. We conducted an observational study at Evangelismos hospital, a 1000-bed tertiary hospital. Patients undergoing planned cardiac surgery with cardiopulmonary bypass were included in the study. The study was approved by the Scientific Council and the Ethics Committee of our Hospital and informed consent was obtained from all patients. Before the operation, in-hospital mortality risk was predicted with the EuroSCORE [13]. Data collection included near-infrared spectroscopy (NIRS) measurements upon routine admission to the cardiac Intensive Care Unit (cICU) postoperatively and every two hours for a six hour monitoring period as well as hemodynamic measurements.

2.2. Anesthesia, Surgery, and CBP Management. Before induction of anesthesia, an arterial line was placed into the radial artery. Anesthesia was intravenously induced with midazolam, fentanyl, hypnomidate or propofol, and cis-atracurium, and it was maintained with sevoflurane, or propofol, fentanyl and cis-atracurium. After tracheal intubation, the lungs were volume controlled with a tidal volume of 8–10 mL/kg resulting in an end-tidal CO_2 concentration between 4 and 5%, using an O_2-air mixture with an inspiratory O_2 concentration of 40%. A positive-end expiratory pressure (PEEP) of 5 cm H_2O was applied. Nonpulsatile CPB was established through a standard median sternotomy with aortic root and right heart cannulation. Surgery was performed under temperatures ranging between 30 and 32°C. Anticoagulation was established with intravenous heparin (3 mg/kg) given 10 minutes before initiation of CPB with target-activated clotting time being at least 440 seconds. After aortic cross clamping, 1000 cc of cold blood cardioplegia were administered, and this was repeated every 20 min thereafter. At the end of CPB, the patient was rewarmed, and heparin was reversed with intravenous protamine sulphate (4 mg/kg). After the operation, patients were admitted to the cICU where they remained until they were hemodynamically stabile and extubated, when they returned to the ward.

2.3. Macrohemodynamic Monitoring. In the cICU cardiac output (CO) was monitored with the thermodilution technique with the insertion of a pulmonary artery catheter (19 patients) or with the pulse contour analysis method (4 patients). Arterial and central venous pressure was monitored invasively in all cases. During the postoperative period, patients were resuscitated according to the following parameters [4]: mean arterial pressure (MAP) $\geq$ 60 mmHg, central venous pressure between 8 and 12 mmHg, cardiac index (CI) $\geq$ 2.2 L/min/m^2, hemoglobin concentration (Hb) between 8 and 10 g/dL, $SvO_2 \geq 65\%$, $SaO_2 \geq 96\%$, blood glucose $\leq$ 160 mg/dL.

Total oxygen delivery (DO_2), total oxygen consumption (VO_2), and the oxygen extraction ratio were calculated for every patient at each measurement.

2.4. Microcirculatory Assessment and Analysis. Near-infrared spectroscopy is a noninvasive method for continuous monitoring of tissue oxygenation. Although visible light is unable to penetrate biological tissue for more than 1 cm because it is strongly absorbed and scattered by tissue constituents (mainly water), light in the near-infrared region can easily reach much deeper biological structures. In mammalian tissue, only three compounds change their spectra when oxygenated: hemoglobin, myoglobin, and cytochrome aa3 [14]. As the absorption spectra of oxyhemoglobin and deoxyhemoglobin differ, a modified Beer-Lambert's law can be used to detect their relative concentrations within tissues. StO_2 reflects the ratio of oxygenated hemoglobin to total hemoglobin. Because NIRS measurements are performed regardless of the diastolic or systolic phase and as only 20% of blood volume within tissue microcirculation is intra-arterial, spectroscopic measurements are primarily indicative of the venous oxyhemoglobin concentration. In our study, thenar tissue oxygen saturation (StO_2) was measured using wide-gap second derivative NIRS (InSpectra; Hutchinson Technology). This technology provides an estimate of the hemoglobin saturation (StO_2) in the microvasculature of muscle tissue, comprising the arteriolar, capillary, and venular compartments, according to principles described previously [15–19].

The measurements at each time point were made while a vascular occlusion technique was applied. After an initial resting StO_2 value had been recorded on the thenar, a pneumatic cuff placed above the elbow was rapidly inflated to 50 mmHg above the patient's systolic arterial blood pressure and maintained for 3 minutes, after which it was released. Signal acquisition proceeded during the occlusion period and until StO_2 values were again stabilized following cuff release. The vascular occlusion derived curves were stored using InSpectra software. StO_2 curves were analyzed offline blindly and in random order (InSpectra Analysis Program, version 2.0; Hutchinson Technology; Hutchinson, MN; running in MatLab 7.0; The MathWorks; Novi, MI). The first degree slope of the hemoglobin desaturation curve during stagnant limb ischemia reflects the tissue oxygen consumption rate (%/min), and the slope of the increase of StO_2 after the release of the brachial vascular occlusion is indicative of the reperfusion rate (%/min) [20–22].

The first NIRS measurement was performed upon cICU admission (H_0), and then at two (H_2), four (H_4), and six hours (H_6) postoperatively. At each time point, arterial and venous blood samples were drawn for the measurement of arterial and venous blood gases, lactate concentration, and $ScvO_2$.

3. Statistical Analysis

All continuous variables are presented as median (range) or mean $\pm$ standard deviation. Analysis of variance (repeated measures ANOVA) and subsequent Bonferroni test were used to establish differences in microcirculatory parameters and global hemodynamic variables between the successive measurement periods. Pearson bivariate correlation was used to study the correlation of various parameters of the microcirculation with hemodynamic indices. The level of significance was set at <0.05.

Table 1: Demographic data and cardiovascular risk factors. Data are presented as absolute numbers (percentage) or median (range), as appropriate.

Age	68 (28–82)
Gender	11 Male/12 Female
BMI (kg/m^2)	27.1 (19–34.9)
Standard EuroSCORE	6 (1–12)
Logistic EuroSCORE (%)	5.4 (1.2–39.4)
Preoperative Risk Factors (No)	
Diabetes mellitus	3 (13)
Hypertension	13 (57)
Peripheral vascular disease	4 (17)
Dyslipidemia	7 (30)
Previous MI	4 (17)
Current smoking	6 (26)
EF (%)	60 (40–65)
Type of surgery (No)	
Coronary artery bypass grafting (CABG)	4
Aortic/Mitral valve replacement (AVR/MVR)	8
Atrial septum defect closure	1
CABG and AVR	2
Ascending aorta replacement	1
Ascending aorta replacement and MVR	1
Bentall procedure	4
MVR and tricuspid valve repair	2
CPB duration (min)	155 (60–226)
Aortic cross clamp time (min)	89 (32–156)

AV: aortic valve, BMI: body mass index, CABG: coronary artery bypass grafting, CPB: cardiopulmonary bypass, MI: myocardial infarction, MV: mitral valve, TV: tricuspid valve.

4. Results

4.1. Study Population. Twenty three patients undergoing open cardiac surgery (11 male/12 female) of a median age of 68 (range 28–82) years were enrolled in the study. The patients had a median EuroSCORE of 6 (range 1–12). The surgical procedure performed is seen in Table 1. Patients were observed for 40 days postoperatively and 5 died within this period (time until death: median 14 days, range 5–39).

Upon routine admission to the cICU postoperatively, patients' circulation was supported with noradrenaline (1 patient), dobutamine (4), both (15), and levosimendan (1). One patient was supported with noradrenaline, adrenaline and levosimendan and one patient was not on inotropes/vasopressors upon cICU admission. All patients were sedated and mechanically ventilated throughout the six hour monitoring period.

The microcirculatory indices and the trend of the macrohemodynamics during the six-hour monitoring period postoperatively are presented in Tables 2(a) and 2(b).

4.2. Relationship between Microcirculation Parameters and Macrohemodynamics. For pooled data, a statistically significant correlation was found between cardiac index (CI) (L/min/m^2) and microcirculatory parameters obtained by performing the vascular occlusion technique. Specifically, CI correlated with the tissue O_2 consumption rate (%/min) as well as the reperfusion rate (%/min) ($r = 0.56$, $P < 0.001$ and $r = 0.58$, $P < 0.001$ resp., Figures 1(a) and 1(b)). This relationship remained significant when controlled for patients' temperature. In addition, both total oxygen delivery (DO_2, mL/min per m^2) and total oxygen consumption (VO_2, mL/min per m^2) also correlated with NIRS-derived parameters and specifically with the tissue O_2 consumption rate and the reperfusion rate ($r = 0.42$, $P < 0.001$, and $r = 0.43$, $P < 0.001$ for DO_2 and the tissue O_2 consumption rate and the reperfusion rate, resp.) and ($r = 0.50$, $P < 0.001$ and $r = 0.43$, $P < 0.001$ for VO_2 and the tissue O_2 consumption rate and the reperfusion rate resp., Figures 2(a) and 2(b)). This relationship also remained significant when controlled for patients' temperature.

4.3. Relationship between Microcirculation Parameters and Global Indices of Organ Perfusion. When comparing VOT-derived microcirculatory indices amongst measurements with lactate levels up to 4 mg/dL (including 4) and lactate levels greater than 4, the first group had higher values of tissue O_2 consumption rate and reperfusion rate (26 ± 11 versus 19 ± 13, $P < 0.05$ and 459 ± 237 versus 259 ± 240, $P < 0.001$, resp.).

The first measurement of the tissue oxygen saturation of the thenar (StO_2 (%)) immediately after cICU admission (H_0) postoperatively correlated with the peak lactate levels during the six hour monitoring period ($r = 0.50$, $P < 0.05$).

It is interesting to note the strong correlation between the tissue O_2 consumption rate and the reperfusion rate ($r = 0.79$, $P < 0.001$).

The tissue oxygen consumption rate was higher in survivors to hospital discharge compared to nonsurvivors (median 23 (range 4–54) versus 20 (8–38) $P < 0.05$), for pooled data (Figure 3(a)). Similarily, the reperfusion rate was higher in survivors compared to nonsurvivors (424 (27–1215) versus 197 (57–632) $P < 0.01$), for pooled data (Figure 3(b)).

5. Discussion

5.1. Relationship between Microcirculation Parameters and Macrohemodynamics. In our study, a significant relationship was found between patients' cardiac index and thenar tissue oxygen consumption rate as well as reperfusion rate. This is the first study to our knowledge that correlates NIRS and VOT-derived microcirculatory indices with macrohemodynamics in cardiac surgery patients. In a population of critically ill patients with septic shock, Payen et al. found a significant relationship between cardiac output and the reperfusion slope [23]. This relation may simply show that systemic flow influences peripheral StO_2. Increase in cardiac index leads to increased regional perfusion and the subsequent improvement of microcirculatory indices.

Tissue oxygenation, tissue oxygen consumption rate, and the reperfusion slope gradually increased during the six

Table 2: (a) Microcirculation parameters assessed after cICU admission (H0) and every 2 hours for 6 hours (H2, H4, H6) in survivors and nonsurvivors combined. Data are presented as median (range). Microcirculation parameters assessed after cICU admission (H0) and every 2 hours for 6 hours (H2, H4, H6) in survivors (S) and nonsurvivors (NS). Data are presented as median (range). (b) Global Hemodynamics, pressure, lactate concentration, and temperature parameters assessed after cICU admission (H0) and every 2 hours for 6 hours (H2, H4, H6) in survivors and nonsurvivors combined. Data are presented as median (range). Global Hemodynamics, pressure, lactate concentration, and temperature parameters assessed after cICU admission (H0) and every 2 hours for 6 hours (H_2, H_4, H_6) in survivors (S) and nonsurvivors (NS). Data are presented as median (range).

(a)

	In cICU, after admission (H_0), and every 2 hours up to 6 hours								
	H_0		H_2		H_4		H_6		P
Number of patients	23		21		18		17		
Thenar StO_2 (%)	85 (71–98)		88 (78–98)		89 (76–98)		89 (79–98)*		<0.001
O_2 consumption rate (%/min)	17 (5–44)		23 (4–50)*		25 (4–45)		28 (11–54)*§§††		<0.001
Reperfusion rate (%/min)	203 (27–732)		350 (50–1215)**		467 (63–760)**		577 (123–1120)**		<0.01
	S	NS	S	NS	S	NS	S	NS	
Thenar StO_2 (%)	85 (71–98)	88 (80–90)	88 (78–98)	90 (80–92)	89 (76–98)	91 (80–93)	89 (81–98)	92 (79–93)	ns
O_2 consumption rate (%/min)	17 (5.1–44.2)	12.7 (8–24.9)	22.9 (3.8–50)	23.3 (9.4–26.8)	26.5 (4.2–44.8)	22 (12.5–30)	29.4 (11.2–53.9)	24 (11,3–38)	ns
Reperfusion rate (%/min)	236 (27–732)	148 (57–676)	362 (50–1215)	331 (73–457)	477 (63–760)	456 (168–589)	607 (123–1120)	312 (180–632)	ns

*$P < 0.01$ from H_0, **$P < 0.05$ from H_0, §$P < 0.01$ from H_2, §§$P < 0.05$ from H_2, †$P < 0.01$ from H_4, ††$P < 0.05$ from H_4.

(b)

	H_0	H_2	H_4	H_6	P
CO (L/min)	4.7 (2.4–11.4)	4.7 (2.3–10.5)	4.7 (3.3–9.2)	5.1 (2.4–11.6)	ns
CI (L/min/m^2)	2.5 (1.5–5.7)	2.6 (1.2–5.3)	2.7 (2–4.7)	2.9 (1.6–5.8)	ns
PCWP (mmHg)	11 (4–27)	10.5 (5–22)	13 (4–20)	11 (3–21)	ns
CVP (mmHg)	9 (0–15)	8.5 (3–16)	10 (1–14)	11 (0–14)	ns
SVR (dynes-s/cm^5)	1339 (89–2200)	1125 (594–1967)	1154 (558–1941)	1067 (564–1901)	ns
PVR (dynes-s/cm^5)	214 (84–564)	206 (76–672)	182 (60–956)	220 (63–328)	ns
SvO_2 (%)	71 (55–79)	71 (54–76)	69 (50–75)	71 (50–75)	ns
$ScvO_2$ (%)	74 (65–85)	76 (59–87)	69 (49–78)	72 (48–85)	ns
Hb (g/dL)	10.9 (8.7–14.9)	10.7 (8.3–14.4)	10.7 (9–15.3)	10.7 (8.3–13.6)	ns
Lac (mg/dL)	2.2 (0.9–7)	3 (1.3–9.1)*	3.1 (0.7–9.9)*	3.6 (0.7–9.7)*	<0.05
MAP (mmHg)	79 (66–109)	73 (52–100)	76 (57–99)	77 (54–99)	ns
MPP (mmHg)	24 (13–33)	23 (14–35)	24 (17–61)	24 (15–36)	ns
Central temp (°C)	37.1 (35.6–38.8)	37.4 (36.2–39.1)*	37.6 (36.9–38.8)*§	37.6 (37.2–38.4)*§	<0.001
Periph temp (°C)	36.7 (35–38.7)	36.9 (35.3–38.5)**	37 (36–38.5)*§	37.2 (36.2–38.4)*§	<0.001
Central-Periph (°C)	0.4 (0–1.3)	0.4 (0–0.9)	0.4 (0–1.4)	0.4 (0–1)	ns
DO_2 (mL/min/m^2)	411 (193–721)	411 (137–737)	398 (278–660)	429 (264–750)	ns
VO_2 (mL/min/m^2)	114 (61–214)	110 (87–221)	116 (91–237)	129 (83–208)	ns
O_2 ER (%)	27 (21–57)	29 (21–71)	29 (25–50)	28 (21–50)	ns

(b) Continued.

	H_0		H_2		H_4		H_6		P
	S	NS	S	NS	S	NS	S	NS	ns
CO (L/min)	5 (3.1–11.4)	3.1 (2.4–4.7)	5.1 (3.3–10.5)	3.1 (2.3–3.9)	4.7 (3.5–9.2)	4.4 (3.3–4.7)	5.6 (4.3–11.6)	4.3 (2.4–4.4)	0.058
CI (L/min/m^2)	2.5 (1.8–5.7)	1.8 (1.5–2.5)	2.8 (1.9–5.3)	1.8 (1.2–2.1)	2.7 (2–4.7)	2.3 (2.2–2.5)	3.1 (2.5–5.8)	2.3 (1.6–2.3)	0.064
PCWP (mmHg)	12 (4–27)	10 (8–10)	12 (8–22)	9 (5–10)	13 (4–20)	11 (8–11)	14 (3–21)	10 (9–11)	ns
CVP (mmHg)	9 (0–15)	8 (5–10)	9 (4–16)	8 (3–11)	10 (1–13)	9 (8–14)	11 (0–14)	8 (8–12)	ns
SVR (dynes-s/cm^5)	1144 (89–2001)	1717 (1548–2200)	1113 (594–1506)	1504 (1128–1967)	1152 (558–1941)	1358 (1139–1525)	1006 (564–1327)	1580 (1325–1901)	0.074
PVR (dynes-s/cm^5)	204 (84–479)	294 (103–564)	192 (76–301)	277 (167–672)	200 (60–956)	182 (170–286)	221 (63–296)	205 (163–328)	ns
SvO_2 (%)	71 (55–79)	68 (42–72)	71 (54–76)	59 (29–73)	70 (50–75)	66 (63–74)	71 (50–75)	71 (66–77)	ns
$ScvO_2$ (%)	74 (60–87)	70 (45–73)	75 (61–87)	59 (29–82)	71(49–82)	66 (60–69)	72 (48–85)	64(60–68)	0.035
Hb (g/dL)	11.3 (8.7–14.9)	10.9 (9.4–12.1)	10.9 (8.3–14.4)	10.2 (8.3–11.9)	11 (9–15.3)	9.9 (9.1–10.5)	10.7 (8.3–13.6)	11.9 (10.2–12.4)	ns
Lac (mg/dL)	1.9 (0.9–7)	2.9 (1.2–6.6)	2.6 (1.3–9.1)	5.5 (2.1–7.7)	3.2 (0.7–9.9)	3 (1.9–8.2)	3.7 (0.7–9.7)	2.1 (1.7–6.6)	0.076
MAP (mmHg)	81 (66–109)	74 (70–107)	75 (60–100)	66 (52–84)	75 (57–99)	76 (65–98)	76 (54–93)	79 (66–99)	ns
MPP (mmHg)	25 (13–33)	23 (14–29)	26 (20–35)	18 (14–25)	25 (17–61)	21 (20–21)	26 (15–36)	20 (19–21)	ns
Central temp (°C)	36.9 (35.7–38.8)	37.3 (35.6–37.7)	37.4 (36.2–37.1)	36.7 (36.5–38.1)	37.7 (37–38.8)	37 (36.9–37.1)	37.6 (37.2–38.4)	37.6 (37.5–37.6)	ns
Periph temp (°C)	36.7 (35.3–38.7)	36.9 (35.2–37.2)	37 (35.9–38.5)	36.4 (36.1–37.2)	37.2 (36.2–38.5)	36.7 (36.6–36.8)	37.2 (36.4–38.4)	37.2 (37.1–37.2)	ns
Central-Periph (°C)	0.2 (0–1.3)	0.45 (0.4–0.5)	0.5 (0–0.8)	0.4 (0.3–0.9)	0.5 (0–1.4)	0.3 (0.2–0.3)	0.4 (0–1)	0.5 (0.4–0.5)	ns
DO_2 (mL/min/m^2)	457 (292–721)	255 (193–416)	417 (281–737)	252 (137–340)	421 (335–660)	316 (278–361)	431 (350–750)	356 (323–389)	ns
VO_2 (mL/min/m^2)	118 (76–214)	107 (61–113)	118 (94–221)	96 (87–109)	117 (99–238)	93 (91–117)	136 (97–208)	96 (83–109)	ns
O_2 ER (%)	27 (21–43)	31 (27–57)	29 (21–45)	40 (25–70)	29 (25–50)	34 (25–37)	28 (24–50)	28 (21–34)	ns

CI: cardiac index, CO: cardiac output, CVP: central venous pressure, DO_2: whole-body oxygen delivery, Hb: hemoglobin, Lac: lactate, MAP: mean arterial pressure, MPP: mean pulmonary pressure, O_2ER: oxygen extraction ratio, PCWP: pulmonary capillary wedge pressure, PVR: pulmonary vascular resistance, $ScvO_2$: central venous oxygen saturation, SvO_2: mixed venous oxygen saturation, SVR: systematic vascular resistance, temp: temperature, VO_2: whole-body oxygen consumption. $^*P < 0.01$ from H_0, $^{**}P < 0.05$ from H_0, $^{\S}P < 0.01$ from H_2, $^{\S\S}P < 0.05$ from H_2, $^{\dagger}P < 0.01$ from H_4, $^{\dagger\dagger}P < 0.05$ from H_4.

hour monitoring period. However, a statistically significant increase was not established for more global indices of the patients' circulatory and oxygenation status, such as the CI, SvO_2, DO_2, and VO_2.

The relationship between DO_2 and VO_2 and the tissue oxygen consumption rate and reperfusion rate can be interpreted by the fact that what happens regionally (i.e., at the site of the thenar muscle) is indicative of what happens globally. This relationship is not absolute though, as DO_2 and VO_2 are dependent on multiple parameters.

5.2. Relationship between Microcirculation Parameters and Global Indices of Organ Perfusion. The vascular occlusion-derived microcirculatory parameters and specifically the tissue oxygen consumption rate and the reperfusion rate were lower in the group with higher lactate levels. The SIRS which develops postoperatively and leads to microcirculatory alterations may also lead to increased lactate levels. The severity of microvascular alterations, as assessed by orthogonal polarization spectral imaging, also correlated with peak lactate levels after cardiac surgery in a study by de Backer et al. [1]. In the previously mentioned study by Payen et al., the reperfusion slope correlated with lactate levels [23]. The relationship between the thenar StO_2 (%) immediately after cICU admission (H_0) postoperatively and the peak lactate levels during the six-hour monitoring period suggests that the microvascular alterations were associated with impaired cellular oxygenation.

It is worth mentioning the strong correlation between the tissue oxygen consumption rate and the reperfusion rate. A similar finding was noted by Payen et al. in the previously mentioned study [23]. The greater the tissue oxygen consumption rate, the faster oxygen is consumed in a given time period, which in turn leads to greater muscle ischemia and subsequent increased release of vasodilating substances. After the cuff is released, this may then lead to a faster reperfusion rate.

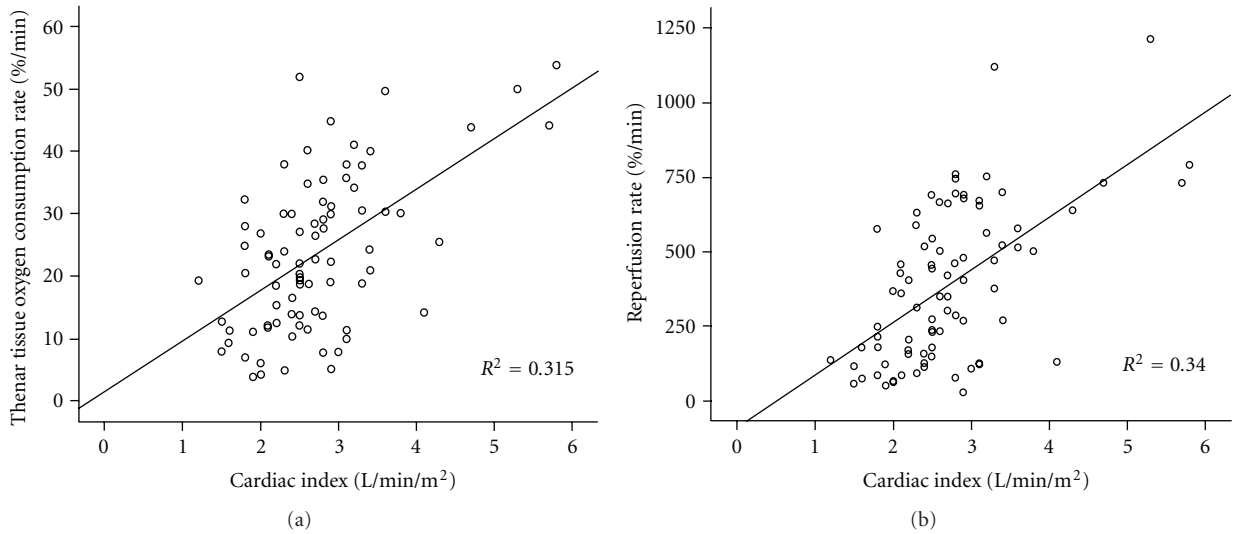

Figure 1: (a) Scattergram of the thenar tissue O_2 consumption rate (%/min) during a 3 min vascular occlusion technique and cardiac index (L/min/m^2, $r = 0.56$, $P < 0.001$, $n = 82$) for pooled data. (b) Scattergram of the reperfusion rate (%/min), indicative of the endothelial function after cuff release following a 3 min vascular occlusion technique and cardiac index (L/min/m^2, $r = 0.58$, $P < 0.001$, $n = 82$) for pooled data.

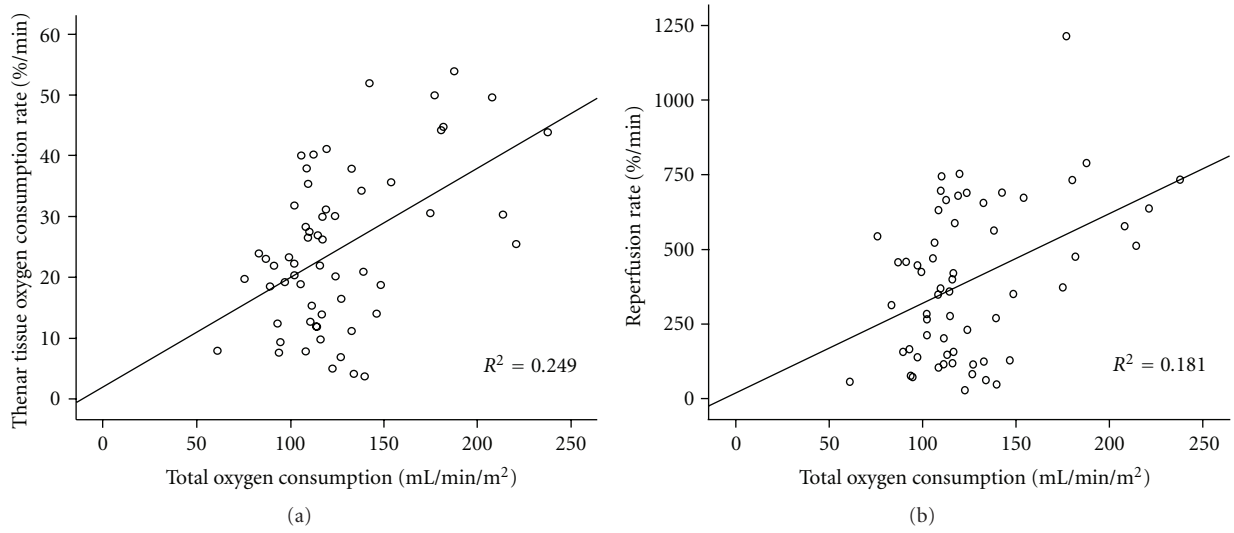

Figure 2: (a) Scattergram of the thenar tissue O_2 consumption rate (%/min) during a 3 min vascular occlusion technique and total oxygen consumption (mL/min/m^2, $r = 0.50$, $P < 0.001$, $n = 60$) for pooled data. (b) Scattergram of the reperfusion rate (%/min), indicative of, not or the endothelial function after cuff release following a 3 min vascular occlusion technique and total oxygen consumption (mL/min/m^2) ($r = 0.43$, $P < 0.001$, $n = 60$) for pooled data.

An interesting observation—which was not in the aim of the study- that needs confirming, is the relationship between VOT derived parameters and patient outcome. In the previously mentioned study by Payen et al. [23], a relationship between the reperfusion slope and survival was found. Specifically, the reperfusion slope was significantly lower in nonsurvivors compared to survivors. Microcirculatory abnormalities have been associated with organ dysfunction and impaired outcome in cardiogenic as well as in septic shock [24, 25]. After major abdominal surgery Jhanji et al. reported that microvascular abnormalities were present in patients who subsequently developed postoperative complications, whereas microcirculation was intact in patients with an uneventful postoperative course [26]. It is interesting to note that in the aforementioned study global hemodynamic variables could not separate the two groups of patients.

There are two different mechanisms—although not, necessarily, mutually exclusive—which can affect microcirculation and tissue perfusion in these patients, leading to organ dysfunction and poor outcome.

The first is the postpump or postperfusion syndrome. During CPB the blood is brought into direct contact with a

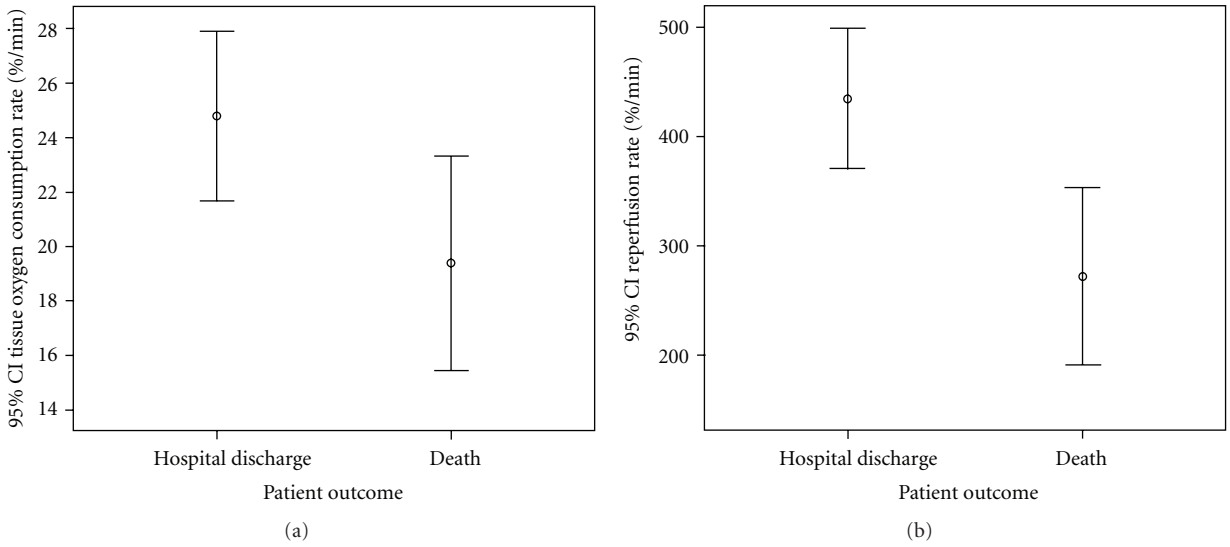

Figure 3: (a) Error bar of the thenar tissue O_2 consumption rate (%/min) during a 3 min vascular occlusion technique in patient' measurements who survived to hospital discharge and patients who died, for pooled data ($P < 0.05$). (b) Error bar of the reperfusion rate (%/min) during a 3 min vascular occlusion technique in patient' measurements who survived to hospital discharge and patients who died, for pooled data ($P < 0.01$).

large artificial surface, pulsatile flow is converted to laminar flow, the heart is exposed to global cold ischemia with cardioplegic protection and the patient's body temperature is lowered by several degrees. In addition CPB causes endotoxemia, due to bacterial translocation from the gut. These features in combination with the surgical trauma contribute to an intense inflammatory reaction characterized by systemic endothelial and leukocyte interaction with widespread and local release of inflammatory mediators and activation of the complement system and the coagulation cascade. The microcirculation can be compromised due to microthrombi formation, neutrophil accumulation, swollen endothelial cells, as well as loss of the physiological vasodilation. The syndrome manifests clinically four to six hours postoperatively, with low peripheral resistance and hypotension, tachycardia and lactic acidosis, and results in tissue cell injury and MOD [3].

Peripheral blood flow and oxygen supply can also be affected postoperatively by a low cardiac output state. This can occur in patients with compromised systolic or diastolic ventricular function and also as a result of myocardial "stunning" due to ischemia-reperfusion injury of the heart. It is characterized by a CI less than 2.2 L/min/m^2, cardiac filling pressures exceeding 20 mmHg, SVR exceeding 1500 dynes-s/cm^5, and heart rate above 100/min. If left without intervention, it can also lead to tissue damage and organ failure [4].

In case of a low output state, the SvO_2 decreases significantly, reflecting an elevated O_2ER (up to 50–60% or more) and oxygen-supply dependency of tissue metabolism; patients also exhibit peripheral vasoconstriction with cool extremities and an amplified central to peripheral temperature difference [27], unlike our observations.

StO_2 reflects the dynamic balance between the regional oxygen delivery and oxygen utilization. As the oxygen consumption rate increases gradually postoperatively, a similar/greater rise of the tissue oxygen flow should exist to allow for the stable/increased StO_2. Thus, NIRS technology identifies tissue oxygenation status in its dynamic equilibrium and progression, which, the global oxygenation indices (DO_2, VO_2) estimated with the use of the Swan-Ganz catheter, fail to demonstrate.

All together, these data suggest that microvascular alterations in the postoperative period are associated with macrohemodynamics and may play a role in the development of postoperative organ dysfunction. Future studies are needed in cardiac surgery in order to further investigate the relationship between microcirculatory alterations postoperatively and patient outcome.

5.3. Limitations. A limitation of our study is the small number of patients included, as well as the lack of measurements at all time points for each patient. An additional limitation is that the statistical analysis included pooled data. A more extensive monitoring period, past the six hours, would have been useful in increasing our understanding of microcirculatory alterations and their trend after cardiac surgery with cardiopulmonary bypass. It would also have been useful to study a more homogeneous group of patients as the CABG patients are more likely to suffer from systemic cardiovascular disease as opposed to valve surgery patients.

6. Conclusion

The microcirculation, as assessed by near-infrared spectroscopy and the vascular occlusion technique, after cardiac surgery with cardiopulmonary bypass is related to macrohemodynamics and global indices of organ perfusion.

Before incorporating microcirculatory parameters into clinical algorithms, a better understanding of the link between systemic hemodynamics and microvascular perfusion is needed, as well as a clarification of the relationship between microcirculatory alterations and organ failure in cardiac surgery.

Abbreviations

AV: Aortic valve
BMI: Body mass index
CABG: Coronary artery bypass grafting
CI: Cardiac index
cICU: Cardiac ICU
CO: Cardiac output
CPB: Cardiopulmonary bypass
CVP: Central venous pressure
DO_2: Total oxygen delivery
Hb: Hemoglobin
Lac: Lactate
MAP: Mean arterial pressure
MI: Myocardial infarction
MPP: Mean pulmonary pressure
MV: Mitral Valve
NIRS: Near-infrared spectroscopy
O_2 ER: Oxygen extraction ratio
PCWP: Pulmonary capillary wedge pressure
PVR: Pulmonary vascular resistance
$ScvO_2$ Central venous oxygen saturation
SIRS: Systematic inflammatory response syndrome
$StO_{2:}$ Tissue oxygen saturation
$SvO_{2:}$ Mixed venous oxygen saturation
SVR: Systematic vascular resistance
Temp: Temperature
TV: Tricuspid valve
$VO_{2:}$ Total oxygen consumption
VOT: Vascular occlusion technique.

Conflict of Interests

The authors declare there is no conflict of interests.

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Clinical Study

The Microcirculation Is Unchanged in Neonates with Severe Respiratory Failure after the Initiation of ECMO Treatment

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Purpose. Venoarterial extracorporeal membrane oxygenation (VA-ECMO) is known to improve cardiorespiratory function and outcome in neonates with severe respiratory failure. We tested the hypothesis that VA-ECMO therapy improves the microcirculation in neonates with severe respiratory failure. *Methods*. This single-center prospective observational pilot 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 included. The microcirculation was assessed in the buccal mucosa, using Orthogonal Polarization Spectral imaging, within 24 hours before (T1) and within the first 24 hours after initiation of ECMO treatment (T2). Data were compared to data of a ventilated control group ($N = 7$). *Results*. At baseline (T1), median functional capillary density (FCD), microvascular flow index (MFI), and heterogeneity index (HI) did not differ between the ECMO group and the control group. At T2 the median FCD was lower in the control group (median [range]: 2.4 [1.4–4.2] versus 4.3 [2.8–7.4] cm/cm^2; P value <0.001). For MFI and HI there were no differences at T2 between the two groups. *Conclusion*. The perfusion of the microcirculation does not change after initiation of VA-ECMO treatment in neonates with severe respiratory failure.

1. 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 [1, 2]. Worldwide, over 24,000 neonates have been treated with ECMO for respiratory problems [1–3].

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 [1, 2, 4]. The institution of venoarterial ECMO (VA-ECMO) partly takes over oxygenation, and carbon dioxide removal and thereby allows ventilator settings to be reduced and restores circulation [4].

The institution of an ECMO circuit in neonates results in an expansion of the circulating volume by approximately factor 2.5. In VA-ECMO, the heart is bypassed and flow in the systemic circulation is generated mostly by the ECMO pump, producing nonpulsatile 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 [4, 5].

In neonatal patients with severe respiratory failure, who meet the criteria for ECMO treatment [4], the circulation and oxygenation are severely compromised. Reflecting this condition, these patients' microcirculatory parameters are significantly reduced before VA-ECMO [6]. At the time when the patient no longer needs ECMO, the microcirculatory

parameters are improved, correlating well with an improvement in clinical condition [6]. After VA-ECMO initiation, circulation and oxygenation generally improve rapidly and patients show a decrease in the need for vasoactive medication. Direct effects of artificial, nonpulsatile 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.

2. Materials and Methods

2.1. Patients. Neonatal patients (aged ≤28 days) admitted to our intensive care unit 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 noninvasive techniques.

Patients in the study group had severe cardiorespiratory failure and hypoxemia 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 [4]. Starting ECMO treatment in a newborn implies a massive increase of the circulating volume (the priming volume of the used system is ±350 mL, which is about 1.5 times the circulating volume of a newborn baby). The ECMO system was primed with a combination of Ringer's lactate, packed red blood cells and albumen. Bicarbonate and calcium were added based on bloodgas analysis of the priming fluid. 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 pO_2 and signs of pulmonary hypertension.

In addition to the microvascular measurements, 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, with severe respiratory failure, who did not receive ECMO treatment. In the 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.

2.2. Procedures. The microcirculation was assessed within 24 hours before start of ECMO (T1) and within 24 hours after start of ECMO (T2). OPS imaging [7] 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, USA), 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 [6]. 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 were discarded [6, 8]. For every measurement, the functional capillary density (FCD), microvascular flow index (MFI), and heterogeneity index (HI) of the different video segments were averaged. If only one segment met the quality criteria, this score was taken. (This was the case for 2 ECMO patients at T2 and 1 control patient at T1).

2.3. Microcirculatory Analysis. Quantification of the images was performed as described previously [6, 7]. To investigate vessel density, the images were analyzed with the Capiscope software program (version 3.7.1.0, KK Technology 1993–2000). For the 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 through it, during the observation period. Dividing the length of the perfused capillaries by the area gives the functional capillary density value expressed in cm/cm^2.

The flow pattern was studied using the MFI, and the HI [8]. For MFI the predominant type of flow for small, medium, and large vessels in every quadrant of the images was determined, as described before by Boerma et al. [9]. For every measurement, the scores for the different video segments were averaged. If only one segment met the quality criteria, this score was taken. HI was calculated as the highest site flow velocity minus the lowest site flow velocity, divided by the mean flow velocity of all sites per measurement [8].

2.4. Statistical Analysis. The data were analyzed using SPSS 17.0. Continuous data are presented as median and range, discrete data as number and percentage. The intergroup differences at T1 were assessed using the Mann Whitney test. Changes over time were assessed using analysis of covariance (ANCOVA) with the T2 measurement as outcome variable, the groups as factor, and the T1 measurement as covariate. In this way, differences at T2 are corrected for the baseline measurements. The level of significance was set at $P < 0.05$.

3. Results

During the study period, 31 VA-ECMO patients were eligible for inclusion. Twenty-one patients were included in the study. Four patients were missed for inclusion due to logistic reasons (a 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 [6]. The excluded ECMO patients did not differ from the included ECMO patient group for gestational age, postnatal age, diagnosis, duration of ECMO treatment, or mortality. In the control group, four patients were missed for inclusion

Table 1: Demographic data.

		ECMO $N = 21$	Controls $N = 7$
Gestational age [weeks]		39.0 (34.4–42.5)	38.1 (38.0–39.3)
Birth weight [kilograms]		3.1 (2.3–5.1)	3.0 (3.0–3.8)
Gender [males] (%)		12 (57)	4 (57)
Diagnosis [n] (%)	CDH	10 (48)	7 (100)
	MAS	5 (24)	
	PPHN	5 (24)	
	CCAM	1 (5)	
Survival [n] (%)		18 (86)	7 (100)

Continuous data are presented as medians and range, discrete data as number and percentage. CDH: congenital diaphragmatic hernia, MAS: meconium aspiration syndrome, PPHN: persistent pulmonary hypertension of the neonate, CCAM: congenital cystic adenomatoid malformation.

and seven patients had to be excluded due to insufficient quality of the images. Demographic data are presented in Table 1, clinical data in Table 2, and microcirculatory data obtained by SDF are presented in Table 3.

At baseline (T1), median FCD did not differ between the ECMO group and the control group (median [range]: 4.5 [2.4–7.7] versus 5.0 [1.8–7.2] cm/cm^2, P value = 0.811) (Figure 1). ANCOVA analysis indicated that at T2 the median FCD was 1.9 cm/cm^2 lower in the control group than it was in the ECMO group (median [range]: 2.4 [1.4–4.2] versus 4.3 [2.8–7.4] cm/cm^2; P value <0.001). For MFI and HI, there was neither a difference at T1 nor a difference at T2 between the two groups (see Table 3 for absolute MFI values and HI values per vessel type as well as the associated P values).

At baseline, the disease severity indices oxygenation index (median [range]: 31 [5–94] versus 5 [3–13]; P value = 0.004) and the PELOD score (median [range]: 20 [11–31] versus 11 [11–20]; P value = 0.006) were more unfavourable for the ECMO patients than for the control patients. The heart rate was higher in the ECMO patients (median [range]: 180 [120–220] versus 138 [113–191] bpm; P value = 0.046), whereas the mean arterial blood pressure and the pulse pressure did not differ. The need for vasoactive medication as indicated by the vasopressor score did not differ between the two groups at T1. Mean airway pressure (median [range]: 18 [12–27] versus 14 [9–16] cm H_2O; P value = 0.019) and the median dosage of inhaled nitric oxide (median [range]: 20 [0–40] versus 0 [0–19] ppm; P value = 0.012) were both higher in the ECMO patients than in the control patients.

At T2, ANCOVA analysis indicated that there was no difference in OI between the ECMO group and the control group. The heart rate and the mean arterial blood pressure did not differ. Pulse pressure was lower in the ECMO patients than in the control patients (median [range]: 10 [0–33] versus 24 [15–32]; P value <0.001). The vasopressor score did not differ at T2, nor did the mean airway pressure. Regarding the dosage of inhaled nitric oxide, ANCOVA analysis indicated that the need for more inhaled nitric oxide in the ECMO patients at T1 had disappeared at T2.

All patients in the control group survived. Three patients in the ECMO-treated group (2 diagnosed with CDH, 1 with CCAM) did not survive, due to recurrent and therapy-resistant pulmonary hypertension. Subanalysis showed that neither FCD nor MFI, nor HI differed between the ECMO survivors and the ECMO nonsurvivors at T1 and at T2.

4. Discussion

The main finding of this study was that there was no change in microcirculatory parameters after the start of VA-ECMO therapy in patients with severe respiratory failure. In both the ECMO and the control group, the FCD at T1 was significantly lower than FCD values of neonates without any respiratory or cardiovascular problems (who served as a control group in a previous study [6]). The FCD in those patients was 8.1 cm/cm^2 (range, 6.6–9.4). MFI values in both study groups were relatively high and HI values relatively low, in contrast to observations in patients with sepsis. There was no difference in MFI and HI between the two groups at T1 and T2. Deterioration of the FCD 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 to the patients in the ventilated control group (Oxygenation Index and PELOD score in ECMO group significantly higher), ECMO succeeded to better microcirculatory support compared to solely conservative treatment with mechanical ventilation and pharmacologic support.

Thus, ECMO seems to prevent a further deterioration of microcirculatory perfusion. The start of ECMO instigates an instant improvement in oxygenation, which makes vasopressors and the use of high mean airway pressures instantly redundant. No correlation between the vasopressor score or the main airway pressure and FCD was found.

Deterioration of microvascular perfusion in patients in the ventilated control group was not correlated with mortality. This is in contrast with observations in patients with severe sepsis [10–12]. The underlying pathophysiology in patients in our study is different from sepsis. Therefore, data from patients with sepsis cannot be extrapolated to this patient group. Both patient groups revealed a relatively normal flow pattern and selectively affected vessel density. At this stage, it is not clear if this could be explained by their specific hemodynamic pattern. Patients in this study suffered from hypoxic respiratory failure, mainly due to failure of adequate feto-neonatal transition of the circulation.

Table 2: Macrocirculatory data.

	T1 ECMO $N = 21$	T2 ECMO $N = 21$	T1 Controls $N = 7$	T2 Controls $N = 7$	P value at baseline*	P value over time†
Age [days]	1 (0–12)	1 (0–12)	1 (0–6)	1 (0–7)	0.694	NA
Time to or from start ECMO [hours]	2 (0.5–24)	2 (0.5–24)	—	—	NA	NA
Time to or from ICU admission [hours]	2.5 (0.3–55.4)	6.4 (2.3–82.7)	12.4 (1.0–145.3)	33.5 (17.9–173.5)	NA	NA
Time between SDF measurements [hours]	4.0 (1.3–39.2)		26.8 (13.0–32.3)		0.005	
Heart rate [beats/min]	180 (120–220)	150 (106–198)	138 (113–191)	129 (110–160)	0.046	0.387
Mean blood pressure [mmHg]	49 (29–77)	49 (35–86)	44 (32–60)	52 (41–63)	0.264	0.727
Pulse pressure [mmHg]	19 (10–40)	10 (0–33)	25 (12–36)	24 (15–32)	0.559	<0.001
Vasopressor score	40 (0–140)	10 (0–108)	15 (0–75)	19 (0–66)	0.410	0.136
Dopamine [mcg/kg/min]	10 (0–20)	0 (0–20)	10 (0–21)	16 (0–21)	NA	NA
Dobutamine [mcg/kg/min]	10 (0–20)	5 (0–20)	10 (0–20)	5 (0–20)	NA	NA
Norepinephrine [mcg/kg/min]	0.1 (0.0–1.0)	0.0 (0.0–0.9)	0.0 (0.0–0.4)	0.0 (0.0–0.3)	NA	NA
Mean airway pressure [cm H_2O]	18 (12–27)	11 (7–21)	14 (9–16)	13 (8–16)	0.019	0.357
Inhaled nitric oxide [ppm]	20 (0–40)	0 (0-0)	0 (0–19)	0 (0–20)	0.012	0.002
Oxygenation index	31 (5–94)	2 (1–21)	5 (3–13)	3 (0–7)	0.004	0.520
PELOD	20 (11–31)	—	11 (11–20)	—	0.006	—
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)	0.336	0.978
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)	0.514	0.384
Fluid amount administered [mL/kg]	—	—	63 (10–145)	54 (26–112)	NA	NA
Fluid balance [mL/kg]	—	—	33 (6–139)	26 (−25–56)	NA	NA
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)	NA	NA
ECMO flow [mL/kg/min]	—	140 (110–210)	—	—	NA	NA

Data are presented as median and range.

*Intergroup differences at T1 were assessed using Mann-Whitney test. †For the time-dependent variables differences at T2 were assessed using ANCOVA with the baseline measurement as covariate.

NA: not assessed, —: not relevant, ECMO: extracorporeal membrane oxygenation, ICU: intensive Care Unit, PELOD: pediatric logistic organ dysfunction.

Table 3: Microcirculatory values.

	T1 ECMO $N = 21$	T2 ECMO $N = 21$	T1 Controls $N = 7$	T2 Controls $N = 7$	P value at baseline*	P value over time†
FCD [cm/cm^2]	4.5 (2.4–7.7)	4.3 (2.8–7.4)	5.0 (1.8–7.2)	2.4 (1.4–4.2)	0.811	<0.001
MFI Large	2.76 (2.50–3.00)	2.88 (2.34–3.00)	2.92 (2.50–3.00)	3.00 (2.63–3.00)	0.266	0.367
MFI Medium	2.67 (2.13–3.00)	2.75 (2.13–3.00)	2.75 (2.38–3.00)	2.81 (2.50–3.00)	0.254	0.411
MFI Small	2.75 (2.06–3.00)	2.75 (2.08–3.00)	2.88 (2.44–3.00)	2.90 (2.63–3.00)	0.574	0.090
HI Large	0.10 (0.00–0.30)	0.09 (0.00–0.40)	0.09 (0.00–0.29)	0.00 (0.00–0.26)	0.951	0.2406
HI Medium	0.14 (0.00–0.60)	0.11 (0.00–0.35)	0.10 (0.00–0.51)	0.00 (0.00–0.27)	0.736	0.2421
HI Small	0.18 (0.00–0.73)	0.09 (0.00–0.37)	0.09 (0.00–0.40)	0.00 (0.00–0.17)	0.579	0.0971

Data are presented as median and range.
*Intergroup differences at T1 were assessed using Mann-Whitney test. †For the time-dependent variables, differences at T2 were assessed using ANCOVA with the baseline measurement as covariate.
FCD: functional capillary density, MFI: microvascular flow index, HI: heterogeneity index.

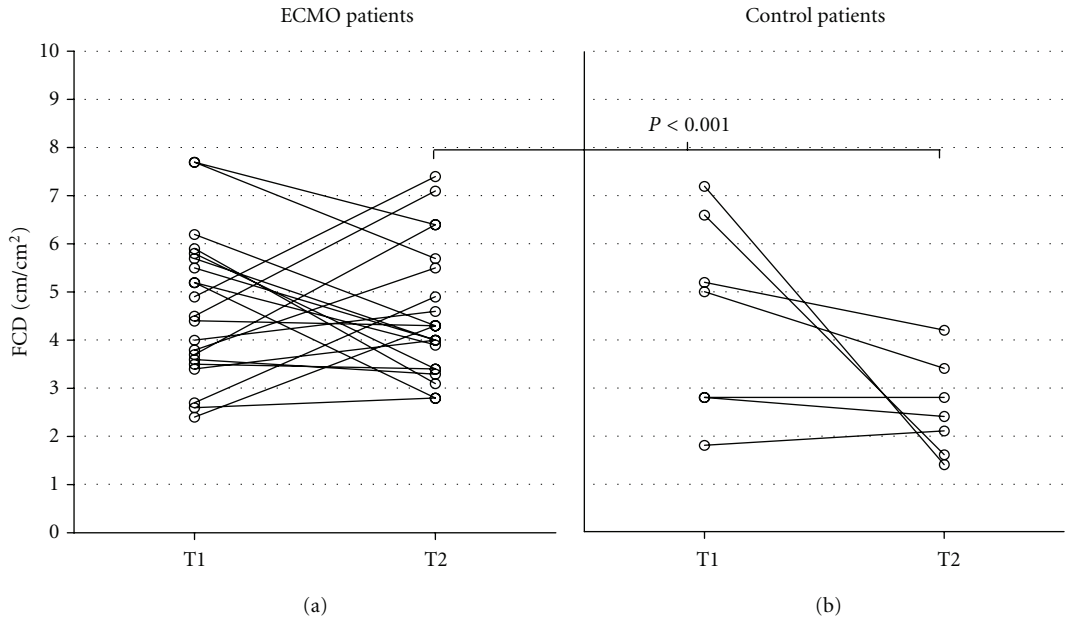

Figure 1: Diagram showing the functional capillary density (FCD). (a): ECMO patients, (b): ventilated control patients. No difference in median FCD was seen at T1 between the two groups: 4.5 cm/cm^2 (range 2.4–7.7) versus 5.0 cm/cm^2 (range 1.8–7.2), P value = 0.811. At T2, FCD was higher in ECMO group than in the control group: 4.3 cm/cm^2 (range 2.8–7.7) versus 2.4 cm/cm^2 (range 1.4–4.2), P value <0.001.

Typically, these patients display a hemodynamic pattern with persistent pulmonary hypertension of the neonate (PPHN), which is clinically characterized by a persistent high pulmonary vascular resistance and an abnormal vascular response, leading to worsening of gas exchange and shunting (intracardiac, extracardial, and intrapulmonary) and right ventricular failure. PPHN occurs as a primary disease or in association with abnormal lung development, for example, in congenital diaphragmatic hernia and is a critical determinant of morbidity and mortality [13].

All patients had pulmonary hypertension, assessed by echocardiography and differences in the pre- and postductal oxygen saturation (due to shunting through persistent fetal pathways such as the ductus arteriosus). This can compromise the pulmonary venous return and preload of the left ventricle and, therefore, influence global hemodynamics. No measures of cardiac output (CO) were available in this study, so this cannot be verified.

During cardiopulmonary bypass (CPB) in adults, microcirculatory alterations have been described before [14–17]. We found one report on microcirculatory alterations during CPB in neonates where OPS was used, which shows a reduction in vessel density during CPB [18].

The circulatory volume increases by about 150%, when a newborn is attached to an ECMO circuit. Therefore, it is necessary that the system is primed with blood products. The addition of these products is titrated against normal values for the age. Thus, with ECMO, blood is transfused, which could improve the microcirculation [19]. However, there was no increment in the hemoglobin level, to support this. With the attachment of the system, a large amount of fluid is administered, which could influence the perfusion of

the microcirculation [20]. Due to the relatively large amount of circulating volume in the system, it is difficult to comment on volume expansion in the patient in absolute numbers. During cannulation and shortly afterwards extra fluid was administered on discretion of the treating physician, based on clinical judgment and following standard unit policies and procedures.

Disturbance of physiologic flow also triggers the catecholamine system leading to vasoconstriction and altered tissue perfusion [21]. Although the mechanism behind this is not completely understood, Agati et al. [22–24] reported that in cardiac patients on CPB nonpulsatile flow seemed to affect the microcirculation and organ perfusion in a more negative way than pulsatile flow did. No correlation between ECMO flow and FCD was seen in our study.

All in all, the initiation of ECMO therapy instigates many changes in the homeostasis of the critically ill patient. It is difficult to unravel the complex processes that take place and to assess separate factors, in order to understand the effect of the different components of the treatment. Nowadays, the importance of microcirculatory improvement is recognized [25, 26]. With this paper, we have shown that the current way of using ECMO treatment stabilizes the microcirculation, but does not restore microvascular density. More research is needed to explore the different factors that have influence on the microcirculation. In addition, follow-up investigations of the microcirculation are necessary as well as comparison of survivors and nonsurvivors within the group that received ECMO treatment. In this way, the prognostic value of microcirculatory parameters can be determined.

There were some limitations to our study. First, the lack of CO measurements limits the possibility to relate microvascular 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, mixed venous saturation and cardiac output are not routinely measured. 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 [27]. The reliability of echocardiography evaluation of cardiac output in children is debatable because even in the hands of experienced operators the inter- and intraindividual variation is large [28].

Second, the control group consisted entirely of patients with CDH, while the ECMO group also contained patients with severe respiratory failure and pulmonary hypertension due to other causes. Patients with CDH suffer from a specific hemodynamic pattern, based on a structural congenital abnormality [13]. This could possibly have different implications on the development of global hemodynamics and the microcirculation.

Unfortunately, the exact amounts of priming fluids and fluids, given during or shortly after the cannulation procedure prior to T2, are not well documented. In addition, 12 of the 21 ECMO patients were first measured within 2 hours of IC admission. In these patients, no reliable data on the amount of fluid administration prior to admission was available. Therefore, we are unable to provide reliable data for fluid balance, fluid amount, and type of fluids administered for ECMO patients in this study.

In this pilot study, the microcirculation was assessed before and after the start of ECMO; therefore, long-term effects of ECMO could not be evaluated. In addition, the median time interval for the subsequent SDF measurements in the ECMO group was shorter than that of the control group. The earlier microcirculatory evaluation in the ECMO group might be of influence on our results.

Finally, 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.

5. Conclusion

The perfusion of the microcirculation does not change after initiation of VA-ECMO treatment in neonates with severe respiratory failure.

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Review Article

Alterations of the Erythrocyte Membrane during Sepsis

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Erythrocytes have been long considered as "dead" cells with transport of oxygen (O_2) as their only function. However, the ability of red blood cells (RBCs) to modulate the microcirculation is now recognized as an important additional function. This capacity is regulated by a key element in the rheologic process: the RBC membrane. This membrane is a complex unit with multiple interactions between the extracellular and intracellular compartments: blood stream, endothelium, and other blood cells on the one hand, and the intracytoplasmic compartment with possible rapid adaptation of erythrocyte metabolism on the other. In this paper, we review the alterations in the erythrocyte membrane observed in critically ill patients and the influence of these alterations on the microcirculatory abnormalities observed in such patients. An understanding of the mechanisms of RBC rheologic alterations in sepsis and their effects on blood flow and on oxygen transport may be important to help reduce morbidity and mortality from severe sepsis.

1. Introduction

The microcirculation, which includes all vessels with a diameter $<100\,\mu$m, blood cells (red blood cells-RBCs-, white blood cells-WBCs- and platelets), endothelium, and microparticles, plays a central role in tissue oxygenation because it is across the walls of the microvessels that oxygen (O_2) diffuses from the blood to the cells within each tissue. Alterations at this circulatory compartment level are frequently observed in critically ill patients, especially in those with sepsis [1–5], and persistence of these alterations is associated with a poor outcome [4, 6].

RBCs were formerly considered as a simple container (the membrane) for one important cytoplasmic protein: haemoglobin. The membrane is considered as one of the key determinants of RBC deformability, alongside cell geometry and cytoplasmic viscosity. Understanding the relationship between the different components of the membrane (lipids-50% of molecular weight, proteins- 40%- and carbohydrates-10%-) (Figure 1) [6–8] helps explain the deformability process. All these components may be altered in sepsis, through a direct effect of bacteria, or via enzymes and/or reactive oxygen species (ROS) produced by WBCs and/or platelets. However, few studies have evaluated membrane alterations during sepsis.

Interestingly, several recent studies have reported an important role of the RBC in modulating the microcirculation. For these reasons, RBCs are no longer considered merely as "dead cells" without a nucleus and only a membrane and haemoglobin to transport O_2 and CO_2, but as living cells capable of modulating the microcirculation in response to various stimuli.

This review first reports the alterations in RBC membrane components and biochemistry observed during sepsis and, second, the possible contribution of these altered RBCs to the microcirculatory abnormalities observed during sepsis (Table 1 for summary).

2. The RBC Membrane

The RBC membrane is considered as a key element in RBC rheology, especially deformability. It is composed of proteins (52% of the molecular weight), lipids (30%), and carbohydrates (8%) with complex interactions among these

Table 1: Main modifications of the RBC membrane observed during sepsis.

Membrane components	Model	Modifications reported	Effects	References
Proteins	Human RBCs	Membrane glycophorin A content increased during sepsis	Desialylation facilitates glycophorin A fixation	[9]
	Human serum of patients with meningococcemia	No changes in serum glycophorin A during the first 36 hours		[10]
	Mice RBCs with sepsis induced by caecal ligature and perforation	Increased band 3/α-spectrin ratio	Associated altered RBC deformability	[11]
		Phosphorylation of the band 3 and anion transporter capacity	No effects on anion transporter capacity	[12]
	Human RBCs	Decreased RBC proteins in septic and non-septic patients	No difference between septic and nonseptic patients	[13]
Lipids	Human RBCs	Increased membrane phosphatidylserine exposition	Increased entry of calcium → increased eryptosis ?	[14]
	Human RBCs	Increased membrane lipid peroxidation	Modifications of RBC lipid organization	[15]
	Rat RBCs	Controversial results on membrane lipid peroxidation	Effects on membrane fluidity?	[16, 17]
Carbohydrates	Human RBCs	Decreased sialic acid membrane content	Inverse relationship between spherical shape and decreased sialic acid membrane content. Stimulation of RBC glycolysis (increased lactate, 2,3-diphosphoglycerate)	[9]

elements. The membrane and these interactions have been reviewed elsewhere [6–8]. Modifications of RBC membrane components could alter RBC rheology and probably also RBC biochemistry. Here we review the alterations of the RBC membrane that have already been described during sepsis.

2.1. Proteins. Few works have studied modifications of the protein part of the RBC membrane during sepsis. Nieuwland et al. [10] measured, in the sera of patients with meningococcaemia, the concentrations of microparticles derived from leucocytes (granulocytes and monocytes), endothelial cells, and platelets. As controls, these authors measured microparticles containing glycophorin A—a major integral protein of the RBC membrane (Figure 1). These authors observed increased blood concentrations of the different microparticles from WBCs, platelets, and endothelial cells during the first 36 hours of sepsis, but no modifications in glycophorin A. These data suggest that the RBC membrane glycophorin A content remains constant, at least during the early stage of sepsis [10].

In a mouse model of septic shock induced by caecal ligation and puncture, Spolarics et al. [11] studied the effects of sepsis on RBC glucose-6-phosphodehydrogenase (G-6-PD) knock-out mice. This enzyme, the first in the pentose phosphate pathway, enables the synthesis of NADPH for glutathione production and antioxidant defenses [8, 18]. These authors observed increased haemolysis in the "knock-out" mice, with significant alterations in RBC deformability. To explain these results, Spolarics et al. [11] hypothesised that the RBC membrane becomes unstable because of an increased band 3/α-spectrin ratio, suggesting an alteration of the membrane integral/peripheral protein ratio (Figure 1). Interestingly, this band 3/α-spectrin ratio was also increased in the group of septic "sham" mice with decreased deformability but without haemolysis compared to "knock-out" G6PD mice [11].

The same authors [12] continued their studies on sepsis and the RBC membrane, especially band 3. In the same animal model, they observed that sepsis induced a significant increased in tyrosine phosphorylation of band 3. This phosphorylation modified the link between band 3 and other proteins of the membrane, but without effects on the anion transport capacity of band 3 [12].

Because of interspecies differences in membrane composition, rheology, and biochemistry, these observations

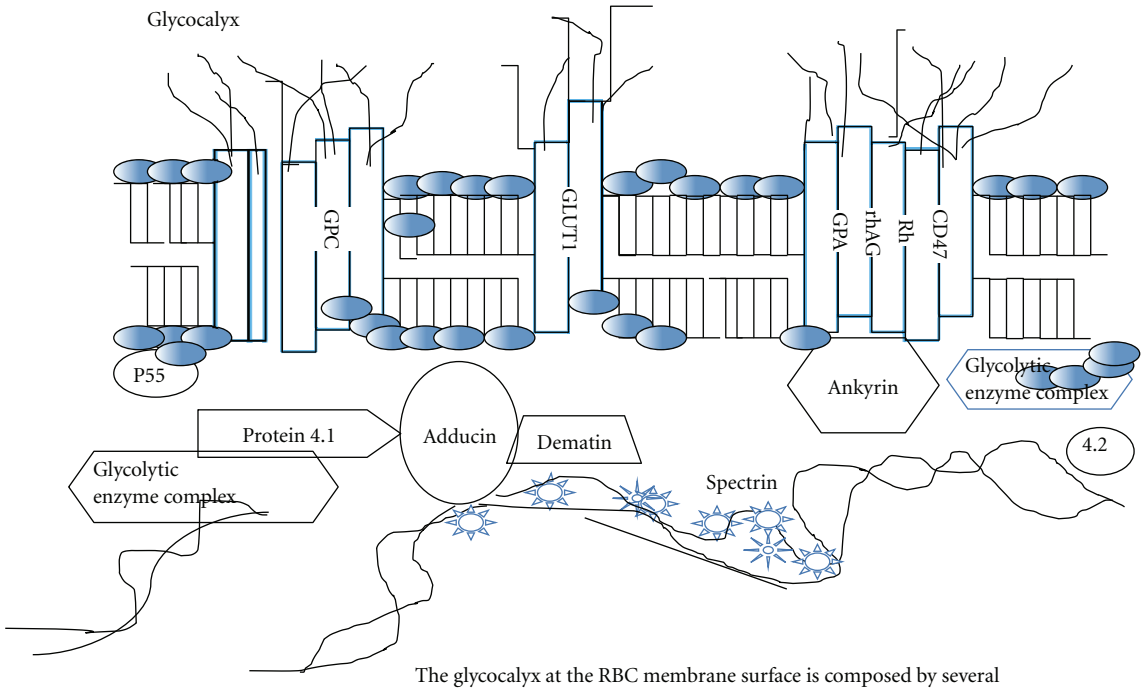

Figure 1: Schematic representation of the red blood cell membrane.

need confirmation in humans. Piagnerelli et al. [13] studied RBC membrane proteins from healthy volunteers and from patients with and without sepsis within 24 hours of ICU admission, and on day 3 for the septic patients. Procedures included screening for alterations in RBC membrane proteins using cryohaemolysis and separation of RBC membrane and skeletal proteins using polyacrylamide gel electrophoresis in the presence of sodium dodecylsulfate. The majority of RBC membrane protein ratios, including band 3/spectrin, were more elevated in critically ill patients (nonseptic and septic) than in volunteers, but RBC membrane skeletal protein content was similar in septic and nonseptic patients [13]. There were no significant differences in cryohaemolysis results among groups. The authors concluded that there were differences in the RBC membrane protein content between critically ill patients within 24 hours of ICU admission and healthy volunteers, but no differences in membrane protein content in septic patients compared to non-septic patients, suggesting that sepsis per se does not alter the RBC membrane protein content.

ROS can also alter the protein part of the membrane and thus the deformability. Uyesaka et al. [19] observed that incubation of RBCs with O_2^- induced a rapid and important degradation of RBC membrane proteins (band 3 and spectrin) with the formation of a new protein band in the membrane. This new organization of the protein part of the membrane may decrease RBC deformability [19].

On the other hand, the RBC membrane participates in ROS synthesis in hypoxic conditions. Kiefmann et al. [20] recently demonstrated in a rat model of isolated and perfused lungs that H_2O_2 was produced by RBCs as a result of autooxidation of haemoglobin located on the membrane. RBCs then transported ROS to endothelial cells. In response, endothelial cells increased intracytosol Ca^{2+}-inducing P-selectin expression on the plasma membrane favouring leucocyte adhesion in venules and capillaries. All these processes contribute to inflammation [20].

2.2. Lipids. The lipid portion of the human RBC membrane may be altered during sepsis. Todd et al. studied, using spectroscopy, the effects of endotoxins on the viscosity of the lipid part of the RBC membrane [21]. They noted an increased viscosity of these lipids without modifications in mean corpuscular volume or mean corpuscular haemoglobin concentration, suggesting no loss of this portion of the RBC membrane [21]. Interestingly, Kempe et al. [14] observed that incubation of RBCs from healthy volunteers with plasma from septic patients induced phosphatidylserine expression on the RBC membrane surface suggested by fixation of annexin V in flow cytometry [14]. These results were identical when they incubated RBCs with the supernatant

of bacteria cultures [14]. The physiopathologic mechanism to explain these results, suggested by Kempe et al. [14], is the formation of membrane ceramide, which could induce a significant increase in RBC Ca^{2+} concentrations. This effect in turn stimulates K^{+}-Ca^{2+} channels, which, with Cl^{-} channels, drives KCl out of the cells. All of these modifications induce cellular dehydration. Indeed, according to these authors, sepsis induces eryptosis—programmed RBC death—in contrast to apoptosis for nuclear cells [22].

Lipid peroxidation of the RBC membrane also seems to be increased during sepsis. Huet et al. [15] observed this effect by measuring ROS production, using thiobarbituric acid-malondialdehyde solutions, by RBCs from septic patients already on the first day of severe sepsis. In rats with sepsis induced by caecal ligature and perforation, Baskurt et al. also observed significantly increased lipid peroxidation [16], but these results were not confirmed in a study by Bateman et al. [17].

2.3. Carbohydrates. Carbohydrates are a minor component of the RBC membrane, representing only 8% of the molecular weight of the human RBC membrane. The RBC glycocalyx (Figure 1) is dominated by the carbohydrate domains of glycolipids and integral glycoproteins. These oligosaccharides contain, in addition to neutral hexoses, pentoses and N-acetylhexosamines, fully ionised sialic acid. Sialic acid (sialon in Greek word: saliva), less commonly called neuraminic acid, is the designation given to a family of over 40 naturally occurring 9-carbon keto sugars acids derived from N-acetylneuraminic acid (Neu5AC) [23]. The most abundant derivative of sialic acid present in humans and in RBCs is N-acetylneuraminic acid [23] (SA).

Glycophorin A, the most important transmembrane protein, is highly glycosylated, with approximately 60% of its weight attributable to carbohydrates. Most of the carbohydrate is in the form of 15 O-glycosidically linked tetrasaccharides. The two SA residues of each of these many O-glycosidically linked oligosaccharides account for 60% to 90%, depending on the species, of the negative charge of the RBC membrane surface [24] and account for the fact that RBCs normally repel each other and do not aggregate [6, 7].

Piagnerelli et al. [9] observed, already within the first 24 hours of ICU admission, a significant inverse relationship between RBC shape, assessed by a flow cytometry technique, and the RBC membrane SA content in critically ill patients with and without sepsis. They observed a more spherical shape in RBCs from septic compared to non-septic patients and healthy volunteers, associated with a decreased RBC membrane SA content. To exclude the possible loss of membrane during the inflammatory process, they measured the fixation of an antiglycophorin A antibody to the RBC membrane glycophorin A content. They observed an increased fixation of glycophorin A in RBCs from septic compared to nonseptic patients and to healthy volunteers [9]. These results excluded loss of the membrane and confirmed decreased RBC membrane SA content in septic patients facilitating links between antibody-glycophorin A. These results were in agreement with the results of Nieuwland et al. [10]. Interestingly, non-septic patients also had modifications of the RBC shape (more spherical) and decreased membrane SA content compared to RBCs from healthy volunteers. This group of patients is an "intermediate" population exhibiting a moderate inflammatory process that could alter the RBC membrane and shape. To explain these modifications of the RBC membrane SA content, Piagnerelli et al. suggested a possible increased activity and/or concentration of an enzyme, neuraminidase, which leaks SA during the inflammatory process [9]. Indeed, these authors also studied the desialylation process on one circulating protein—transferrin—in critically ill patients with and without sepsis [25]. In humans, circulating transferrin is represented by different glycoforms. The largest representative is tetrasialotransferrin, which binds 4 SA; the smallest representative is disialotransferrin, 2 SA, accounting for less than 1% of the concentration. Transferrin is considered as a "negative" acute phase protein, the concentrations of which decreases with the inflammatory process [26].

Because of the long half-life, approximately 16 days in humans, modifications of the SA pattern that were measured in patients are due to changes in a blood degradation rather than in synthesis. In patients admitted to the ICU with and without sepsis, these authors [25] observed increased concentrations of disialotransferrin in septic (18.3% [1.3–30.5]) compared to non-septic patients (0.7% [0.5–0.9]) and healthy volunteers (0.9% [0.5–1.1]; $P < 0.05$). They also measured increased concentrations of protein-bound SA and free SA concentrations in the septic patients. To prove that these modifications in SA metabolism occur rapidly in sepsis, the time course of the free SA concentrations was also measured in a model of septic shock induced by ligature and caecal perforation in sheep. An increased concentration of free SA was observed after 15 hours of sepsis in this animal model [25].

All these modifications could be explained by increased concentration and/or activity of neuraminidase. To demonstrate this hypothesis, the same group measured the neuraminidase activity in critically ill patients with and without sepsis [27]. They observed significantly increased neuraminidase activity in septic compared to nonseptic patients and healthy volunteers. To assess the effects of decreased RBC membrane SA content on deformability assessed by flow cytometry, these authors incubated neuraminidase from *Clostridium perfringens* at several concentrations (0.125, 0.25, and 0.5 U/mL) with RBCs from healthy volunteers and measured the free SA concentrations in the supernatant. After 2 hours of incubation with the higher concentrations of neuraminidase, these investigators observed the same modifications in RBC shape as had been observed in septic patients [9, 27]. Moreover, the RBC membrane contains a neuraminidase linked by a phosphatidylinositol link [28]. Incubation of RBCs from healthy volunteers with phosphatidylinositol phospholipase C (PIPLC) reproduced the same alterations in RBC shape and increased SA concentrations as observed in septic patients [27]. These data suggest a possible liberation of RBC membrane neuraminidase during sepsis. Nevertheless, several sources of neuraminidase have been reported: RBC membrane as described above and in other studies [29–31], WBCs [32–34], platelets [35], bacteria

[36–39], and viruses [40, 41]. Indeed, some studies have shown that RBCs are able to recycle the free SA released by neuraminidase [42, 43] through a cytosolic sialate pyruvate lyase that specifically and reversibly catalyses the cleavage of SA to form N-acetylmannosamine and pyruvate [42, 43].

3. Links between RBC Alterations and the Microcirculation

Although several studies performed in animal models of sepsis and in human sepsis have reported alterations in RBC rheology [44–56], no studies have demonstrated the effects of altered RBCs on the microcirculation during sepsis. Several studies have reported the deleterious effects of transfused altered RBCs in sham animals. In sedated rats, Simchon et al. [57] showed the effects of transfused RBCs altered by incubation with glutaraldehyde and neuraminidase. These authors analysed the clearance of altered RBCs fixed by Cr^{51} and In^{111}. They observed a decrease, by approximately 70%, of the altered RBCs in a few minutes in areas (liver, spleen, lungs, and kidneys) where the reticuloendothelial system was the most represented. Moreover, the blood flow neuraminidase RBC/control RBC ratio, measured by the microsphera technique (15 μm, with a value of 1 as normal range), was markedly decreased for the spleen (0.4 ± 0.05), for the liver (0.66 ± 0.06), for the lungs (0.78 ± 0.03), and for the kidneys (0.78 ± 0.09). These data suggest a deleterious effect of neuraminidase-altered RBC deformability on blood flow [57].

Lux et al. [58] studied the effects of RBCs with deformability altered by different concentrations of glutaraldehyde on the rat pulmonary circulation. These authors observed an increased pulmonary arterial pressure related to the severity of the RBC deformability alterations. Baskurt in a rat model of isolated perfused leg demonstrated the same effect of altered RBCs on vascular resistance. They measured an increase in the resistance of up to 78% with the more altered RBC suspensions [59]. Cabrales [60] compared, in a model of isovolaemic haemodilution in the hamster, the effects of RBCs altered by glutaraldehyde, compared with Dextran 6% 70-kDA, and "fresh" RBCs, on the cutaneous microcirculation observed by the "window chamber." He observed a decrease in flow and in diameter, especially in the arteriolar part of the microcirculation, with transfusion of altered compared to fresh RBCs, without modifications in blood viscosity. Interestingly, microvascular density was significantly decreased in the rats transfused with altered RBCs with decreased arteriolar, tissues, and venular PO_2 [60].

There are several possible hypotheses to explain the effects of altered RBCs on tissue oxygenation. First, low flow rates lead to the depletion of arteriolar O_2 and lowering of arteriolar blood PO_2. The same effect was observed in the venular part of the circulation as a consequence of the lowered flow velocity after exchange with rigid RBCs leading to low PO_2. Thus, the residence time of the RBCs within the vessel critically influences the amount of O_2 that diffuses into the surrounding tissue, affecting O_2 delivery to the capillary network. The altered RBC is associated in part with increased surface area-to-volume ratio, supporting the concept of decreased O_2 uploading by rigid RBCs in the lung. This effect, in combination with the associated reduced arteriolar flow and functional capillary density, should explain why tissue and venular PO_2 values are significantly reduced. Finally, decreased functional capillary density could be explained by microvascular vasoconstriction due to decreased ATP released by altered RBCs. Nevertheless, these hypotheses remain speculative, especially in septic conditions where decreased ATP release by altered RBCs has never been studied.

The studies discussed above suggest deleterious effects of altered RBCs on the microcirculation, but the mechanisms have not really been evaluated. Moreover, these studies were not performed in septic models.

4. Relationship between Alterations in RBC Rheology, Microcirculation, and Outcome

Few investigations have studied RBC rheology and the microcirculation in septic patients. Donadello et al. [61] showed in 64 septic patients that worsened RBC deformability at day 3, as assessed by the laser-assisted optical rotational cell analyzer (LORCA, Mechatronics Instruments BV, AN Zwaag, Netherlands), was associated with a poor outcome. In contrast, RBC aggregation did not change over time in these patients [61]. Further studies investigating the microcirculation and RBC rheology in the same patients and the relationship of these aspects with mortality are needed in the future.

In conclusion, components of the RBC membrane are modified during sepsis and may contribute to the observed alterations in RBC rheology. A better understanding of these processes could help identify strategies to improve RBC rheology and, thus, the microcirculation in this particular population of patients.

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Clinical Study

Study Design of the Microcirculatory Shock Occurrence in Acutely Ill Patients (microSOAP): An International Multicenter Observational Study of Sublingual Microcirculatory Alterations in Intensive Care Patients

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Objective. Sublingual microcirculatory alterations are associated with an adverse prognosis in several critical illness subgroups. Up to now, single-center studies have reported on sublingual microcirculatory alterations in ICU patient subgroups, but an extensive evaluation of the prevalence of these alterations is lacking. We present the study design of an international multicenter observational study to investigate the prevalence of microcirculatory alterations in critically ill: the Microcirculatory Shock Occurrence in Acutely ill Patients (microSOAP). *Methods*. 36 ICU's worldwide have participated in this study aiming for inclusion of over 500 evaluable patients. To enable communication and data collection, a website, an Open Clinica 3.0 database, and image uploading software have been designed. A one-session assessment of the sublingual microcirculation using Sidestream Dark Field imaging and data collection on patient characteristics has been performed in every ICU patient >18 years, regardless of underlying disease. Statistical analysis will provide insight in the prevalence and severity of sublingual alterations, its relation to systemic hemodynamic variables, disease, therapy, and outcome. *Conclusion*. This study will be the largest microcirculation study ever performed. It is expected that this study will also establish a basis for future studies related to the microcirculation in critically ill.

1. Introduction

The microcirculation plays a pivotal role in oxygen delivery to the tissue [1]. It is believed to be a key player in several disease states, such as sepsis and shock. The development of Orthogonal Polarizing Spectral (OPS) imaging and more recently Sidestream Dark Field (SDF) imaging has enabled bedside imaging of the—predominantly sublingual—microcirculation [2, 3]. Main advantage of SDF/OPS imaging is the ability to visualize true capillary hemodynamics in a noninvasive way at the bedside, thereby providing functional information related to the microcirculation where oxygen delivery to the parenchymal cells takes place. With SDF/OPS imaging, the presence of microcirculatory alterations in different critical care patient subgroups, such as sepsis, heart failure, and major surgery, has been widely explored during the past decade [4–9]. These microcirculatory alterations appear to be associated with an adverse prognosis; they are more severe in nonsurvivors in comparison to survivors in sepsis and heart failure, and are associated with the

development of complications in abdominal surgery [4–12]. The aforementioned studies have all shown that microcirculatory alterations are apparent in the presence of more or less normal systemic haemodynamic parameters, thereby stressing the potential importance of the microcirculation as an additional target for resuscitation. Several interventions, ranging from vasoactive drugs and fluid therapy to circulatory assist devices, have been shown to have varying effects on their capacity to influence microcirculatory failure [7, 13–22].

Although a randomized controlled clinical trial (RCT) is considered as the highest level of evidence in medical research, the Empirics already realized the importanceh of observation for gaining a better understanding of diseases [23, 24]. Recent literature acknowledges the advantages of a solid observational study as a powerful tool to include large patient numbers with a variety of backgrounds, making the results easier to extrapolate to daily practice as opposed to RCTs with limited inclusion numbers due to stringent inclusion and exclusion criteria. This has especially been advocated for intensive care patients where several large RCTs fail to demonstrate beneficial effects of interventions. The heterogeneous nature of patients and applied therapy as well as uncertain underlying pathophysiology has been associated with this failure, emphasizing the need for more observational studies in intensive care patients to gain a better understanding of both patient characteristics and effects of interventions [25–29].

In microcirculatory research, the presence and significance of microcirculatory failure has been repeatedly demonstrated in single center studies with a limited number of patients in a variety of different ICU populations. However, a solid estimation of the prevalence of microcirculatory alterations in intensive care patients is not available as yet. Therefore, our aim was to conduct a multicenter observational study to map the prevalence of microcirculatory alterations in intensive care patients, irrespective of their underlying disease, to provide a solid basis for further (interventional) studies. The unique nature of this observational trial will be that it will not only observe the behavior of conventional clinical and hemodynamic variables but will also relate these to the behavior of a completely new unexplored physiological compartment in a multicentral international setting. In this paper we describe the trial design and methods we propose of evaluating the data.

2. Methods

Several large multicenter prevalence studies in critical care settings have been conducted, such as the Sepsis Occurrence in Acutely ill Patients (SOAP) study, the European Prevalence of Infection in Intensive Care (EPIC) study and the Columbian internet based Observatorio Nacional de Sepsis Pediátrica (ONASEP) [30–32]. We aimed for a similar study design.

2.1. Inclusion of Participating Centers. Out of 47 intensive care units (ICU's) that were invited to participate in this study (see Figure 1 for an overview), 36 ICU's decided to participate.

The list of participating centers is as follows:

(1) ICU, Medical Center Leeuwarden, Leeuwarden, The Netherlands

(2) ICU, Antonius Ziekenhuis, Nieuwegein, The Netherlands

(3) ICU, Onze Lieve Vrouwe Gasthuis, Amsterdam, The Netherlands

(4) ICU, Erasmus Medical Center, Rotterdam, The Netherlands

(5) ICU, Gelre Ziekenhuizen, Apeldoorn, The Netherlands

(6) Departamento de Medicina Intensiva, Hospital Clinico de la Pontificia, Universidad Católica de Chile. Santiago, Chili

(7) Departamento de Anestesiologia, Dor e terapia Intensiva, Hospital Sao Paulo, Universidade Federal de São Paulo, Sao Paulo, Brasil

(8) Servicio de Terapia Intensiva, Sanatorio Otamendi y Miroli, Buenos Aires, Argentina

(9) ICU, Hospital San Martín, La Plata, Argentina

(10) ICU, Hospital Español "Juan J Crotoggini," Montevideo, Uruguay

(11) ICU, Cooper University Hospital, Camden, USA

(12) ICU, Beth Israel Deaconess Medical Center/Harvard Medical School, Boston, USA

(13) Critical Care Medicine, University of Pittsburgh, Pittsburgh, PA, USA

(14) Critical Care Medicine, St. John's Mercy Medical Center, St Louis, Missouri, USA

(15) ICU, University of California, San Diego, USA

(16) Universitätsklinikum Jena, Friedrich-Schiller-University, Department of Internal Medicine I, Jena, Germany

(17) Department of Surgical Intensive Care, University Hospital Aachen, Aachen, Germany

(18) ICU, Royal London Hospital, London, UK

(19) ICU, Royal Free Hospital, London, UK

(20) ICU, The Royal Marsden Hospital, London, UK

(21) ICU, Derriford Hospital and Nuffield Health Plymouth Hospital, Plymouth, UK

(22) ICU, New Cross Hospital, Wolverhampton, UK

(23) ICU, RDE Hospital, Exeter, UK

(24) Critical Care Department, Joan XXIII University Hospital, Tarragona, Spain

(25) Department of Intensive Care Medicine, Waikato Hospital, Hamilton, New Zealand

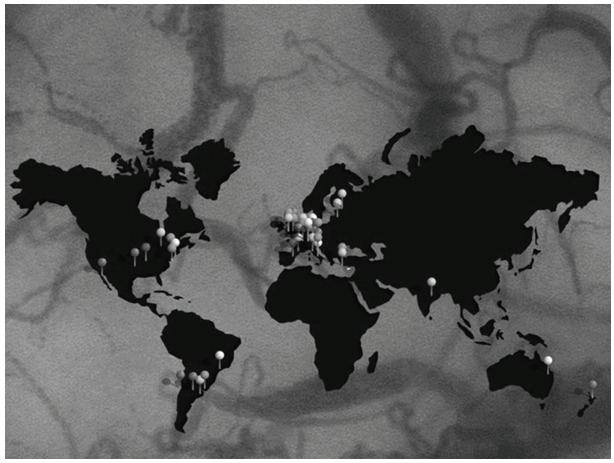

Figure 1: Overview of the ICU's that were invited to participate in the microSOAP.

(26) ICU, Kaunas University Hospital, Kaunas, Lithuania

(27) Clinica di Anestesia e Rianimazione, Azienda Ospedaliera-Universitaria Ospedali Riuniti, Ancona, Italy

(28) Dipartimento di Anestesia, Rianimazione e Terapia Intensiva, Azienda ULSS 9 Veneto, Treviso, Italy

(29) ICU, Santa Maria degli Angeli Hospital, Pordenone, Italy

(30) ICU, Royal Brisbane and Women's Hospital, Brisbane, Australia

(31) Departement d'Anesthesie-Reanimation, Hopital de Bicetre, Le Kremlin- Bicêtre, Paris, France

(32) Department of Anesthesiology, Critical Care et Samu, Hôpital Lariboisière, Paris, France

(33) ICU, University Hospital Basel, Basel, Switzerland

(34) Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand

(35) ICU, Hacettepe University, Ankara, Turkey

(36) ICU, Kosuyolu University, Istanbul, Turkey.

ICU's were selected based on SDF/OPS availability, established skills in OPS/SDF imaging as demonstrated in a separate teaching course, and/or publications in this field. For image quality check, centers were asked to provide a representative SDF/OPS video of a septic patient and a healthy volunteer, enabling feedback on image quality. During an investigators meeting in March 2011, the study was scheduled for the second week of September 2011. For logistic reasons, it was decided that centers could choose two or more consecutive days for performing measurements. To prevent overlap of patient inclusion, ICU's were divided into (virtual) units and measured as one unit per day. A medical steering committee was formed to oversee the study, including representatives from the different continents as well as the major centers in the participating countries. The medical steering consisted of, E. C. Boerma, MD, PhD; N. A. R. Vellinga, MD; M. Koopmans; A. Donati, MD; A. Dubin, MD, PhD; R. M. Pearse, MD, PhD; N. I. Shapiro, MD, MPH; J. Bakker, MD, PhD; C. Ince, PhD. The study is coordinated from Medical Center Leeuwarden by the principal investigator (E. C. Boerma, MD, PhD), a dedicated physician in charge of running the communication (N. A. R. Velinga, MD) and a research nurse (M. Koopmans). The study center ensures communication with and between study centers, coordinates study logistics, and manages data analysis.

2.2. Patient Selection. Every ICU patient ≥18 years, regardless of the underlying disease, was eligible for inclusion. Informed consent was obtained in accordance with local ethics approval. Participation in another study was no exclusion criterion, except when contradictory to local regulations. Patients <18 years or without informed consent were excluded, as well as patients with mucosal bleeding/injury or recent maxillofacial surgery that interfered with SDF/OPS imaging.

2.3. Ethics Approval. A study protocol was provided to participating centers. Every participating center obtained ethics approval according to local legislation. A copy of the ethics approval was sent to the study coordinator before start of the study. Written informed consent was obtained of all included subjects, unless the local ethics committee specifically allowed a waiver in this respect.

The study was registered at http://www.clinicaltrials.gov/ (NCT01179243). No (industry) sponsorship has been received for this investigator-initiated study, with the exception of a local hospital fund.

2.4. SDF/OPS Imaging. Sublingual OPS and SDF imagings are used for microcirculatory imaging at the bedside with the potential of quantification both at the bedside and offline [33–35]. In short, the OPS and SDF analogue cameras are incorporated in handheld devices, emitting polarized, respectively, stroboscopic green light, with a wavelength within the absorption spectrum of haemoglobin, thereby depicting erythrocytes as black cells. The area of visualization is approximately 1 mm^2. These techniques are described in detail elsewhere [2, 3]. Offline computer assisted analysis yields information on both convection and diffusion. Microvascular flow index (MFI) is calculated to describe convection in a semiquantitative way; the predominant flow in all quadrants of the SDF/OPS image is scored for different vessel sizes, using a scale ranging from 0 (no flow) to 3 (continuous flow). The averaged flow score yields the MFI for each image. MFI has been shown to correlate well with red blood cell velocity [36]. To obtain information on diffusion, several measures of functional capillary density are calculated, using a grid dividing the image into 16 segments. Every vessel crossing the grid is counted; furthermore, for each vessel crossing the grid, the type of flow using the MFI scale is used, a flow of 0 (no flow) or 1 (intermittent flow) is considered as nonperfused, whereas a flow of 2 (sluggish) or 3 (continuous) describes perfused vessels. By using these data, several measures of functional capillary density can be calculated, including proportion of perfused vessels (PPVs) and perfused vessel density (PVD). Dividing the numbers of

perfused grid crossings by the total number of grid crossings yields the PPV; the PVD is calculated as the number of perfused grid crossings divided by the total grid length. In the same way, total vessel density (TVD) can be calculated. A detailed description of MFI and measures of functional capillary density can be found elsewhere [34, 35].

2.5. Data Collection. The sublingual microcirculation was measured once in every patient. In line with internationally accepted consensus, 3 to 5 stable sublingual microcirculatory image sequences of 10–20 seconds were obtained for every patient [34, 35]. Along with the SDF/OPS imaging, data on demographics, reason for ICU admission, illness severity scores, haemodynamics, laboratory values, and treatment were collected. Afterwards, information on ICU/hospital length of stay and ICU/hospital mortality will be collected.

2.6. Internet-Based Study Equipment. The specifications of the internet platform which has been designed included (1) compliance with international guidelines on clinical research and data security, (2) fast and reliable uploading of clinical data and SDF/OPS images, and (3) facilitating adequate two-way communication. To facilitate communication, an e-mail server and an open access website (http://www.microcirculationstudies.org) have been developed. The website provided general study information and included a weblog and a frequently asked questions section to keep participants updated on the latest study news. For data exchange, a dedicated database has been developed, based on Open Clinica (OC) 3.1 open source (GNU LPGL license) clinical trial software [37]. OC is, amongst others, in compliance with 21 CFR Part 11 (FDA), ICH-GCP and the US Health Insurance Portability and Accountability Act of 1996 (HIPAA). It is a Java J2EE-based application that runs on both Linux and Windows servers. Several other large multicenter studies have used OC databases, for example, the European Surgical Outcomes Study (EuSOS) and the Fluid Expansion As a Supportive Therapy (FEAST) trial [38, 39]. OC allows customization of its database to meet study requirements. The electronic CRF is defined using a special Excel sheet, which is uploaded to the server to define the CRF in the database. For the OC database, a dedicated server is available. Every participating center can log in to a part of the database that is assigned to their ICU to fill out the electronic CRF. After completion of data collection, data will be exported to SPSS 18.0, IBM, New York, USA, for statistical analysis.

A USB stick has been provided to each center with software developed specifically for this study. Its purpose is to provide the user a film editor so that captured film fragments can be edited to identify suitable clips for submission to the study center in Leeuwarden and to provide the needed communication protocols with the servers. The raw SDF/OPS image file can be imported in the image-editing program. By playing the SDF/OPS video, the user will be able to set a start mark and end mark at the appropriate points of the SDF/OPS clip, defining the part of the raw SDF/OPS file that will be used for subsequent analysis. The limit for the maximum clip length is set at 500 frames, that is, 20 seconds. In that way, we will be able to look for the part of the clip that is most suitable for analysis. The software on the USB stick automatically establishes an Internet connection with the central dedicated microSOAP study server using the required communication protocol and security settings. Backup copies of the clips are automatically stored on the USB stick. In case of failure of Internet connection, the USB stick containing the backup clips can alternatively be sent to the study coordinator by regular mail.

2.7. Data Analysis

2.7.1. Sample Size Calculation. Because this is the first extensive prevalence study on microcirculatory alterations ever done, with a primarily explorative character, a concise power calculation is virtually impossible. Based on previous studies, with a sample size between 25 and 50 patients, in heart failure, high-risk noncardiac surgery, sepsis, and paediatric ICU patients, significant correlations between the existence of microcirculatory alterations and parameters of morbidity and mortality could be established [6, 10, 12]. However, it is reasonable to assume that morbidity and mortality may be lower in a general ICU population. Therefore, we aim for a sample size ten times larger than previously reported in single-center subgroup studies. Since this is by far the largest cohort of in vivo microcirculatory research in humans ever done, practical limitations with respect to availability of SDF/OPS technique and skilled operators will undoubtedly play a significant role in the definitive sample size.

2.7.2. SDF/OPS Image Analysis. The SDF/OPS image analysis will be performed by the researchers appointed by the initiators of this study in accordance with internationally accepted guidelines using dedicated software [34, 35]. The analysis will be conducted blinded to the origin of the film clips. In a suitable subgroup, an automatic assessment method will be performed as described elsewhere to investigate the suitability of such an automatic software for evaluation and quantification of microcirculatory alterations [40].

Due to the demanding imaging technique, quality of SDF images may vary between centers [41]. However, up-to-date externally validated image quality scoring systems appear to be lacking. To ensure consistency in SDF analysis, SDF analysis will be performed by researchers appointed by the principal investigators, taking care for ongoing feedback and aiming for consensus. Since several reports from different research groups have reported excellent inter- and intraobserver agreement for the SDF image analysis, the steering committee decided beforehand that this would not be an extra topic of this study [5, 10, 34].

2.7.3. Statistical Analysis. Descriptive statistics will be used to describe the study population. Further statistical analysis will be conducted to relate the microcirculatory alterations to the severity of disease and other parameters. The primary

outcome measure is the prevalence of microcirculatory alterations. There is no consensus about the thresholds for a "normal" and an "abnormal" microcirculation. Several researchers have reported on values of several microcirculatory variables in healthy volunteers; MFI of capillaries (<20 μm) is reported to be 3.0 [2.9–3.0] (median [IQR]), 2.82 (0.1), and 2.97 (0.03) (mean(SD)) (IQR = interquartile range, SD = standard deviation) [5, 11, 42]. Therefore, one expects 95% of healthy subjects to have a small vessel MFI between 2.62 and 3 [11]. In healthy volunteers, PPVs (small vessels) well above 90% are described, whereas in septic patients, a capillary PPV of 78% (23%) is described [11, 42, 43]. In septic shock, norepinephrine dose >0.1 microgram/kg/min and a lactate >2 mmol/L were associated with a significantly lower PVD (12 [8–15] versus 14 [11–17] n/mm^2 for norepinephrine dose >0.1 microgram/kg/min and 10 [8–13] versus 14 [11–17] n/mm^2 for lactate >2 mmol/L), as well as a significantly lower PPV (80 [70–91] versus 100 [90–100]% for norepinephrine dose >0.1 microgram/kg/min and 82 [71–99] versus 93 [84–100]% for lactate >2 mmol/L) [44]. In uncomplicated major abdominal surgery, preoperative PPV (small vessels) was 89% (83–95) versus 79% (73–92) in patients who developed complications postoperatively [4]. In this study, ROC curves will be used to find cut off values of microcirculatory variables in relation to morbidity and mortality.

Secondary outcome parameters are the correlation between microcirculatory changes and macrohaemodynamic variables, correlations between microcirculatory changes, and length of ICU/hospital stay, mortality, and SOFA/APACHE II scores [45, 46]. Differences between several subgroups will be assessed using a t-test in case of normally distributed variables; in case of non-normally distributed variables, a nonparametric test will be chosen. Whenever applicable, forward stepwise logistic regression analysis will be used to test for associations between the severity of microcirculatory dysfunction and illness severity, mortality, and length of stay. In addition, the relation between microcirculatory alterations, applied therapy (e.g., fluid therapy and vasopressor therapy), and indicators of peripheral perfusion (e.g., lactate) will be explored. Furthermore, the geographical distribution of microcirculatory alterations will be assessed.

3. Discussion

This study will be by far the largest cohort of in vivo microcirculation research. We aim to provide insight in the worldwide prevalence and distribution of microcirculatory alterations. The questions we hope to answer are the following:

(1) Does the presence of microcirculatory alterations indicate impending bad outcome in terms of morbidity and mortality?

(2) Does the presence of microcirculatory alterations provide a more sensitive indicator of morbidity and mortality then conventional hemodynamic and oxygen derived parameters?

(3) Is the presence of microcirculatory alterations related to applied therapy, such as fluid therapy and vasopressors?

(4) Is there a difference between microcirculatory alterations in different patient (sub-) groups, and how are these geographically distributed, as well as over time?

We expect that the results of our study will make clinicians more aware of the presence and importance of microcirculatory alterations in daily practice, thereby leading to better identification of patients who are at risk of an unfavorable outcome. Furthermore, we hope to trigger researchers to develop methods enabling easier bedside evaluation of the microcirculation for detection of those at risk of "microcirculatory failure", as well as interventions aimed at ameliorating the microcirculation. Hopefully, by putting the microcirculation in a central position in future ICU practice, outcome of critically ill patients will be improved.

4. Conclusion

With an anticipated inclusion rate of approximately 500 patients, this study will provide the largest reported database of clinical in vivo microscopy in critically ill patients. We expect that this study will form a solid basis for a deeper understanding of the prevalence and meaning of microcirculatory alterations in intensive care patients and show the way forward to the design of a goal-directed interventional study based on the normalization of microcirculatory alterations in intensive care patients.

Conflict of Interests

C. Ince is the inventor of SDF technology, which is commercialized by MicroVision Medical. He has been a consultant for this company in the past, but he has broken all contact with this company for more than two years now, and he has no competing interests other than his commitment to promote the importance of the microcirculation in the care of critically ill patients.

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Research Article

Comparison of Different Methods for the Calculation of the Microvascular Flow Index

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The microvascular flow index (MFI) is commonly used to semiquantitatively characterize the velocity of microcirculatory perfusion as absent (0), intermittent (1), sluggish (2), or normal (3). There are three approaches to compute MFI: (1) the average of the predominant flow in each of the four quadrants ($MFI_{by\ quadrants}$), (2) the direct assessment during the bedside video acquisition ($MFI_{point\ of\ care}$), and (3) the mean value of the MFIs determined in each individual vessel ($MFI_{vessel\ by\ vessel}$). We hypothesized that the agreement between the MFIs is poor and that the $MFI_{vessel\ by\ vessel}$ better reflects the microvascular perfusion. For this purpose, we analyzed 100 videos from septic patients. In 25 of them, red blood cell (RBC) velocity was also measured. There were wide 95% limits of agreement between $MFI_{by\ quadrants}$ and $MFI_{point\ of\ care}$ (1.46), between $MFI_{by\ quadrants}$ and $MFI_{vessel\ by\ vessel}$ (2.85), and between $MFI_{by\ point\ of\ care}$ and $MFI_{vessel\ by\ vessel}$ (2.56). The MFIs significantly correlated with the RBC velocity and with the fraction of perfused small vessels, but $MFI_{vessel\ by\ vessel}$ showed the best R^2. Although the different methods for the calculation of MFI reflect microvascular perfusion, they are not interchangeable and $MFI_{vessel\ by\ vessel}$ might be better.

1. Introduction

The patency of microvascular perfusion is essential for the preservation of aerobic metabolism and organ functions. Although the microcirculation is a key component of the cardiovascular system, its behavior may differ from that of systemic circulation [1]. Despite the continuous developments in the monitoring of critically ill patients, the evaluation of the microcirculation remained as an elusive issue during many years. The introduction of the orthogonal polarization spectral (OPS) [2] and the sidestream dark field (SDF) [3] imaging devices has recently allowed the direct visualization of microcirculation at the bedside. Thereafter, different researchers described that septic patients showed sublingual microvascular alterations such as a decreased perfusion and increased heterogeneity [3–5]. These disorders were later found to be associated with the development of multiple organ failure and death [6]. Eventually, the microcirculation became used as a therapeutic target [7–9].

Some controversies, however, still remain about the proper evaluation of the microcirculation [10]. The magnitude of the microvascular perfusion is commonly evaluated by means of the microvascular flow index (MFI) [11]. The MFI is based on determination of the predominant type of flow. For this purpose, flow is characterized as absent (0), intermittent (1), sluggish (2), or normal (3). Subsequently, the MFI has been computed in three different ways. Originally, Boerma et al. calculated the MFI as the average of the predominant flow in each of the four quadrants ($MFI_{by\ quadrants}$) [11]. Then Arnold et al. reported that a determination of MFI during bedside video acquisition ($MFI_{point\ of\ care}$) gave a good agreement with the $MFI_{by\ quadrants}$ [12]. Finally, Dubin et al. used the mean value of the MFI determined in each individual vessel ($MFI_{vessel\ by\ vessel}$) [1, 8, 9]. This analysis is time consuming but tightly correlated with the actual red blood cell (RBC) velocity measured with a software both in experimental and clinical conditions [1, 13, 14].

Our hypothesis was that the agreement between the different methods to determine the MFI is poor and that the $MFI_{\text{vessel by vessel}}$ better reflects the microvascular perfusion than the other approaches.

2. Materials and Methods

This was a prospective observational study performed in a teaching intensive care unit. It was approved by the Institutional Review Board. Informed consent was obtained from the next of kin for all patients admitted to the study.

One hundred videos were obtained by a single operator (AD) from 25 patients with septic shock in different clinical and hemodynamic conditions. Their clinical and epidemiologic characteristics are shown in Table 1. All the patients were mechanically ventilated and received infusions of midazolam and fentanyl. Corticosteroids, propofol, and activated protein C were never used.

The microcirculatory network was evaluated in the sublingual mucosa by means of a SDF imaging device (Microscan, MicroVision Medical, Amsterdam, Netherlands) [3]. Different precautions were taken and steps followed to obtain images of adequate quality and to ensure good reproducibility. Video acquisition and image analyses were performed by well-trained researchers. After gentle removal of saliva by isotonic-saline-drenched gauze, steady images of at least 20 seconds were obtained while avoiding pressure artifacts through the use of a portable computer and an analog/digital video converter (ADVC110, Canopus Co, San Jose, CA, USA). Videoclips were stored as AVI files to allow computerized frame-by-frame image analysis. Adequate focus and contrast adjustment were verified and images of poor quality discarded. The entire sequence was used to characterize the semiquantitative characteristics of microvascular flow and particularly the presence of stopped or intermittent flow.

MFI was randomly and blindly determined in three different ways by a single researcher (MOP). First, a semiquantitative analysis by eye was performed in individual vessels. It distinguishes between no flow (0), intermittent flow (1), sluggish flow (2), and continuous flow (3) [11]. A value was assigned to each individual vessel. The overall score of each video is the average of the individual values ($MFI_{\text{vessel by vessel}}$). In addition, $MFI_{\text{by quadrants}}$ was calculated as the mean value of the predominant type of flow in each of the four quadrants. Finally, as an approximation to the real-time assessment at the bedside [12], $MFI_{\text{point of care}}$ was determined during a 20-second observation of a video sequence.

We also calculated the proportion of perfused small vessels as the number of vessels with flow values of 2 and 3 divided by the total number of vessels.

Quantitative RBC velocity of single vessels was measured through the use of space-time diagrams, which were generated by means of analysis software developed for the SDF video images [15]. This method of velocity determination consists of making diagrams of changes in grey-level values (e.g., flowing red blood cells) along the center line of a vessel segment being analyzed, as a function of time. In sequential images, the diagram of such an analysis consists of the y-axis, the distance traveled along the vessel segment and on the x-axis, time. This portrayal of the kinetics of sequential images generates slanted dark lines representing the movement of the red blood cells, the slopes of which give red blood cell velocity. This value is calculated as $v = \Delta s/\Delta t$, where Δs is the longitudinal displacement along the vessel centerline in time fragment Δt. We traced three center lines manually in the space-time diagram, and the average orientation was used to calculate the RBC velocity. The RBC velocity of each video was the average of all RBC velocities measured in single vessels in that video. The analysis was restricted to small vessels (i.e., vessels with a diameter $<20\,\mu$m).

Table 1: Clinical and epidemiologic characteristics of the patients.

Age, years	73 ± 10
Gender male, *n* (%)	14 (56)
SOFA score	10 ± 3
APACHE II score	25 ± 6
Actual mortality, %	
ICU mortality	48
30-day mortality	48
Hospital mortality	48
APACHE II predicted mortality, %	49 ± 20
Norepinephrine (μg/kg/min)	0.51 ± 0.41
Intra-abdominal	8 (32)
Respiratory	8 (32)
Urinary	6 (24)
Intravascular	3 (12)

Definition of abbreviations: SOFA, sepsis-related organ failure assessment; APACHE, acute physiology and chronic health evaluation.
Data are expressed as mean ± standard deviation or number (percentage).

2.1. Statistical Analysis. The agreement between the three methods for the determination of MFI was tested using the Bland-Altman method [16]. In addition, linear regression analysis was performed between MFIs and the fraction of perfused small vessels and between MFIs and RBC velocity.

3. Results

For the determination of $MFI_{\text{vessel by vessel}}$, 37 ± 9 small vessels per video were assessed. For the calculation of $MFI_{\text{by quadrants}}$, the four quadrants were analyzed in all videos. The red blood cell velocity was measured in 20 ± 8 small vessels per video.

Figure 1 shows the wide 95% limits of agreement among the different methods for determining MFI. The bias ± precision for $MFI_{\text{point of care}}$ and $MFI_{\text{by quadrants}}$ (0.03 ± 0.37) was lower than for the $MFI_{\text{point of care}}$ and $MFI_{\text{vessel by vessel}}$ (0.24 ± 0.65, $P = 0.005$) or $MFI_{\text{by quadrants}}$ and $MFI_{\text{vessel by vessel}}$ (0.21 ± 0.73, $P = 0.05$) comparisons.

RBC velocity significantly correlated with the three MFIs (Figure 2). Although, the $MFI_{\text{vessel by vessel}}$ method showed the highest R^2, the difference did not reach statistical significance.

The proportion of perfused small vessels exhibited significant correlations with the three methods used in the calculation of MFI (Figure 3). The $MFI_{\text{vessel by vessel}}$ showed

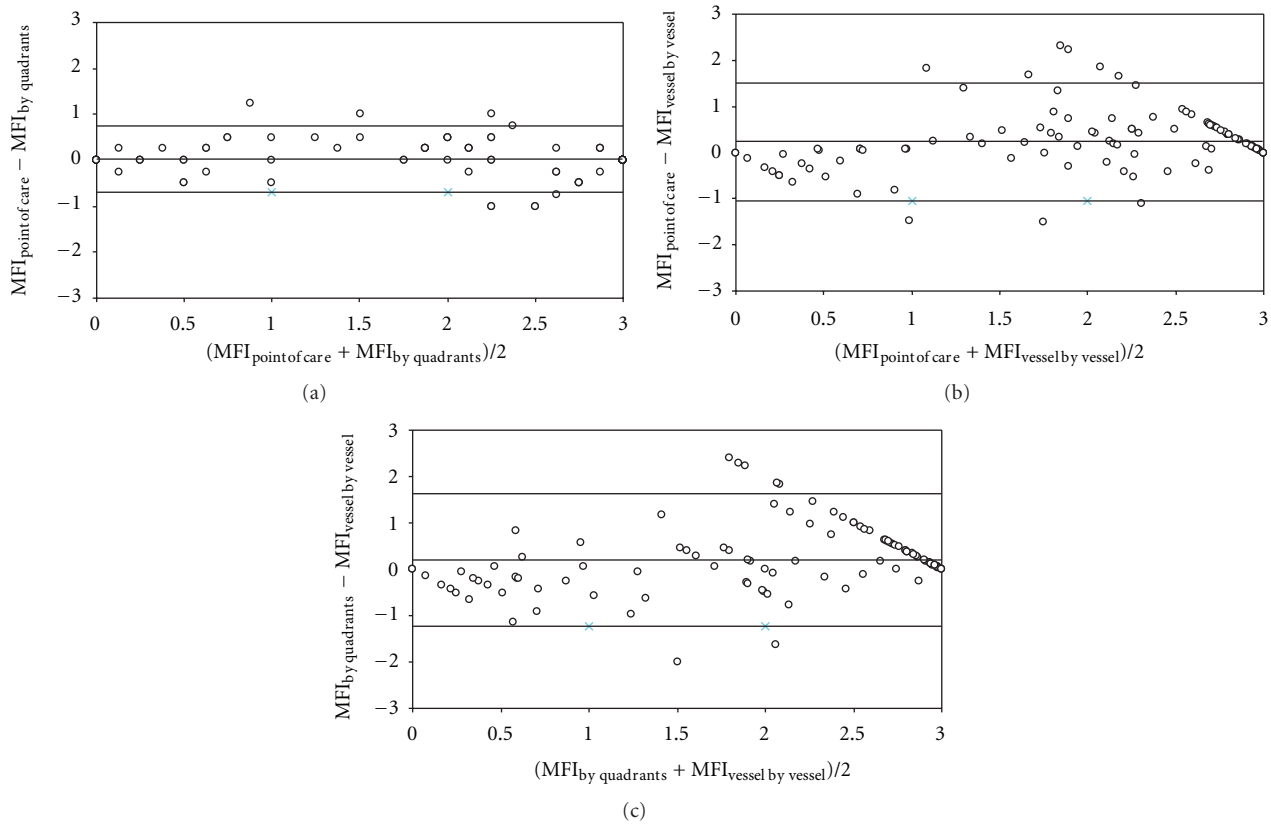

Figure 1: Bland and Altman analysis for the different methods used for the calculation of microvascular flow index (MFI). Panel (a): bedside point of care MFI ($MFI_{point\ of\ care}$) and MFI determined by quadrants ($MFI_{by\ quadrants}$). Panel (b): $MFI_{point\ of\ care}$) and MFI determined by vessel by vessel analysis ($MFI_{vessel\ by\ vessel}$). Panel (c): ($MFI_{by\ quadrants}$) and ($MFI_{vessel\ by\ vessel}$). Lines are bias and 95% limits of agreement.

the highest coefficient of determination, whose value was statistically higher than the other two ($P < 0.0001$ for both).

4. Discussion

Our results showed that each method used for the calculation of MFI was significantly correlated with the actual RBC velocity. Nevertheless, the agreement among the different MFIs was poor. The $MFI_{vessel\ by\ vessel}$ was the approach that had the best correlations with the RBC velocity and the proportion of perfused small vessels.

According to a recent consensus conference, the evaluation of the microcirculation should take into account the three different characteristics of density, perfusion, and flow heterogeneity. The question of which parameters are more appropriate to evaluate microcirculatory perfusion and density is still controversial. In particular, the discussion has mainly been focussed on the advantages versus the limitations of either the proportion of perfused vessels or the MFI [10]. Since the proportion of perfused vessels only distinguishes continuous from intermittent/stopped flow, the presence of a continuous but slow flow could be missed. The MFI does not provide information about functional density. Theoretically this index could be misleading if flow improves in perfused vessels, but the total number of perfused vessels also decreases. Moreover, the MFI is a categorical variable, so a change from 0 to 1 may have a different meaning in terms of tissue perfusion than a change from 2 to 3. Beyond these considerations, we found strong correlations between the proportion of perfused small vessels and the different approaches to MFI. The correlation with $MFI_{vessel\ by\ vessel}$, however, was the strongest and also exhibited very narrow 95% confidence intervals. These findings suggest a similar performance of both the proportion of perfused small vessels and MFI in the characterization of microcirculatory perfusion, especially when the $MFI_{vessel\ by\ vessel}$ is used.

We found statistically significant correlations between RBC velocity and the three measurements of MFI. Although the correlation with $MFI_{vessel\ by\ vessel}$ showed the best coefficient of determination, the difference between that r^2 value and the other two did not reach statistical significance. Probably, our study was underpowered for showing this difference.

The agreement between the different approaches to the MFI was poor. We found large 95% limits of agreements between them, whose range precludes any interchangeability. The 95% limits of agreement between $MFI_{point\ of\ care}$ and $MFI_{by\ quadrants}$ were lower than those found between the other MFIs, although still wide. Arnold et al. reported a similar bias ± precision for this Bland and Altman analysis (-0.031 ± 0.198). Nevertheless, they concluded that the agreement was good.

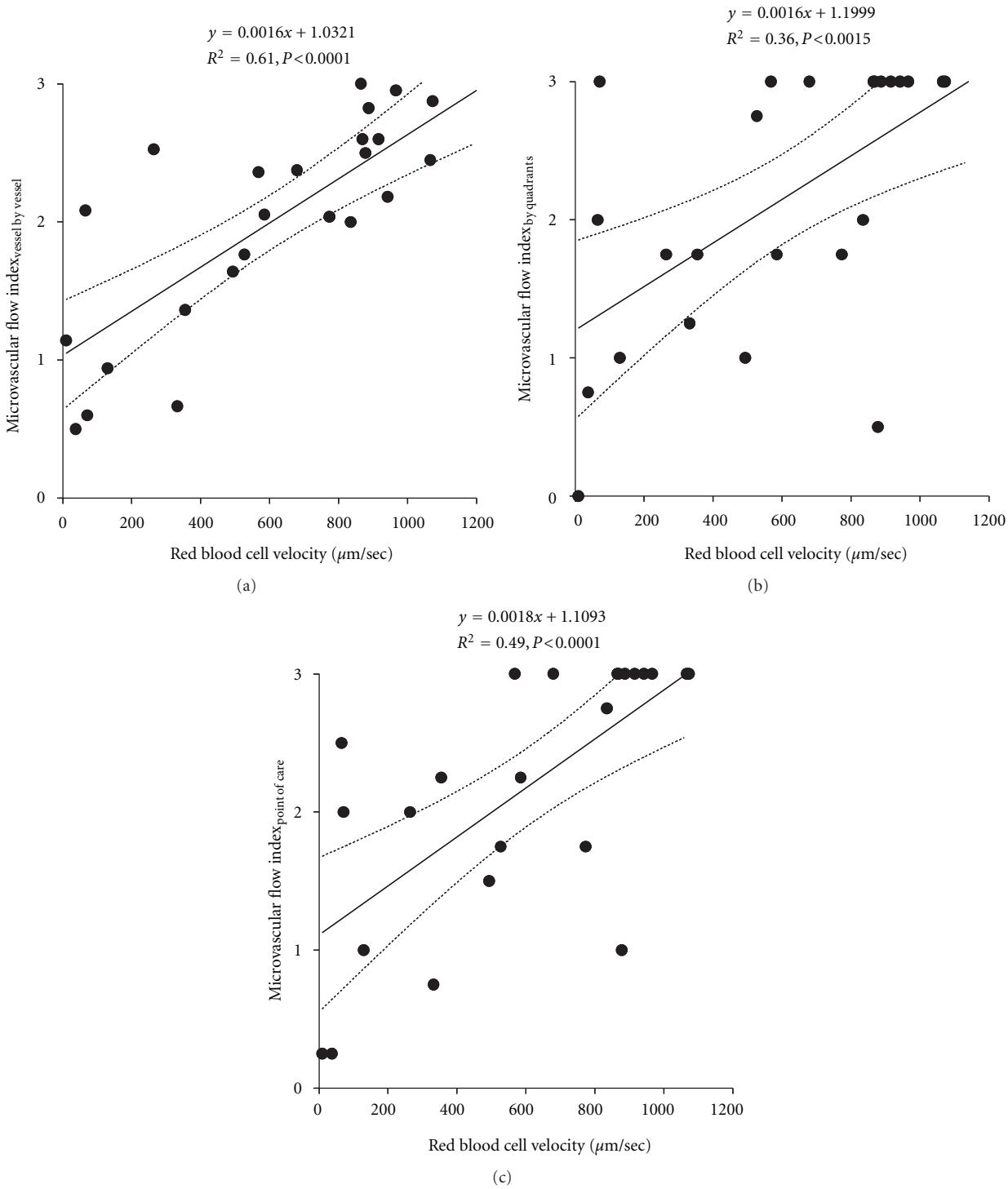

Figure 2: Correlations of the red blood cell velocity with the microvascular flow index determined by vessel by vessel analysis ($MFI_{\text{vessel by vessel}}$) Panel (a), the microvascular flow index determined by quadrants ($MFI_{\text{by quadrants}}$) Panel (b), and the bedside point-of-care microvascular flow index ($MFI_{\text{point of care}}$) Panel (c).

We found positive biases with $MFI_{\text{point of care}}$ versus $MFI_{\text{vessel by vessel}}$ and with $MFI_{\text{by quadrants}}$ versus $MFI_{\text{vessel by vessel}}$, meaning that $MFI_{\text{point of care}}$ and $MFI_{\text{by quadrants}}$ overestimate $MFI_{\text{vessel by vessel}}$. These biases could be anticipated since the two first methods use the predominant type of flow, either in the whole videomicroscopic area or in the quadrants. Accordingly, a high but not predominant proportion of small vessels with stopped or intermittent flow could be left unconsidered in the $MFI_{\text{point of care}}$ and $MFI_{\text{by quadrants}}$. In contrast, in

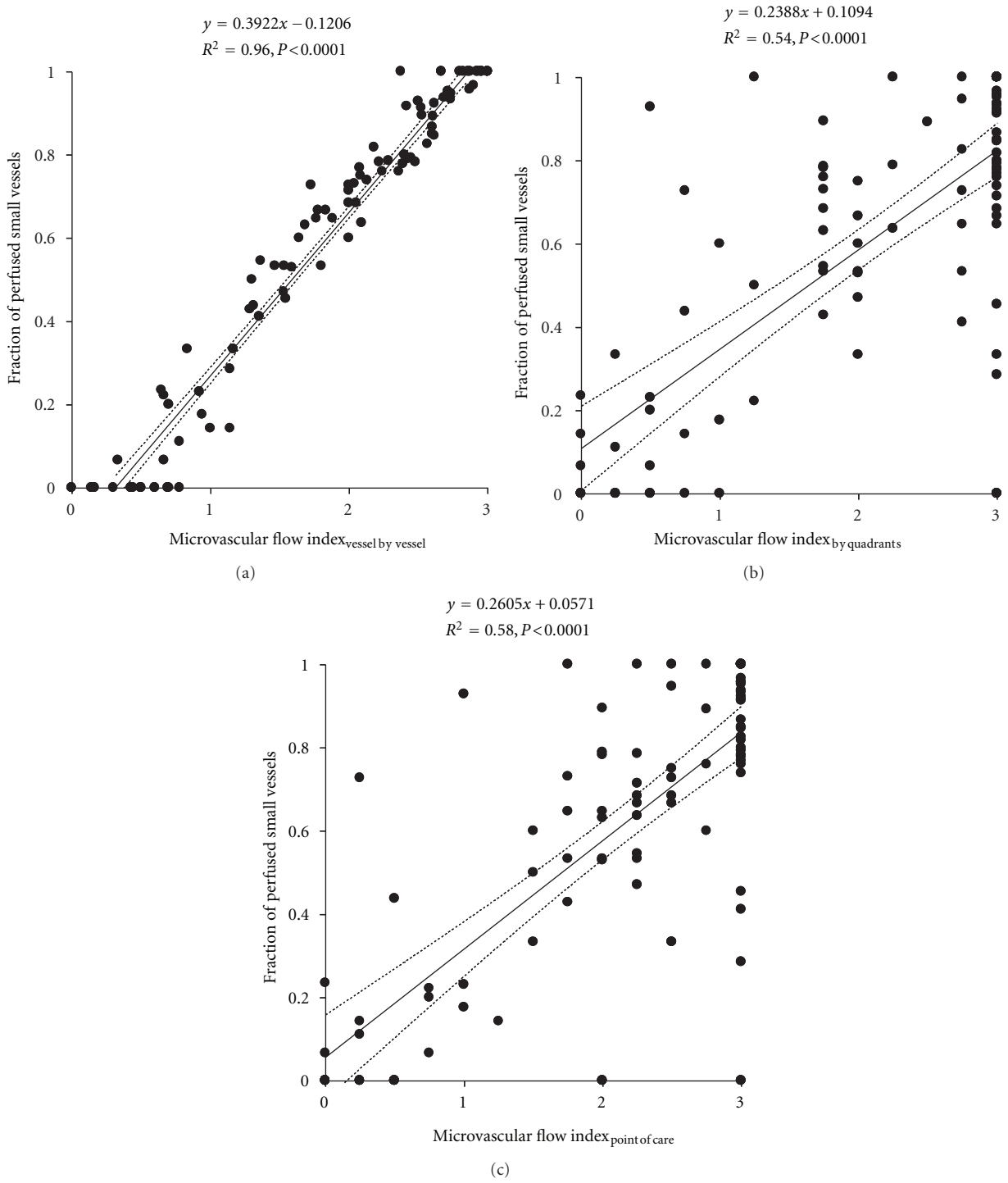

Figure 3: Correlations of the proportion of perfused small vessels with the microvascular flow index determined by vessel by vessel analysis ($MFI_{\text{vessel by vessel}}$) Panel (a), the microvascular flow index determined by quadrants ($MFI_{\text{by quadrants}}$) Panel (b), and the bedside point-of-care microvascular flow index ($MFI_{\text{point of care}}$) Panel (c).

the $MFI_{\text{vessel by vessel}}$, every vessel score is used in the final computation. For example, if 30% of the small vessels have stopped flow and 70% normal blood flow, the $MFI_{\text{vessel by vessel}}$ will be 2.1, while with the other two methods the predominant flow will be 3.

Although the methods are not interchangeable and $MFI_{\text{vessel by vessel}}$ probably better reflects the velocity of the perfusion, $MFI_{\text{by quadrants}}$ and $MFI_{\text{point of care}}$ were also significatively correlated with the proportion of perfused vessels and the RBC velocity.

This study has certain limitations. First, the $MFI_{point\ of\ care}$ used in this study was only a simulation of that used in the study of Arnold et al. [12]. We performed the $MFI_{point\ of\ care}$ during a 20 sec view of the video sequence but not during a real video acquisition. In addition, the strong correlation between $MFI_{vessel\ by\ vessel}$ and the proportion of perfused vessels could be partially explained by mathematical coupling. This problem can develop when two parameters, calculated from a shared variable, are subsequently correlated. If there is an error in the determination of the shared variable, it could be propagated in the calculation of those parameters. The resulting correlation could not be a real phenomenon but could be the expression of the methodological mistake. Mathematical coupling, however, is only applicable to artifactual relationships when there is a significant error in the measurement of the common variable. Another limitation is that the number of analyzed videos, especially those in which the RBC velocity was measured, was limited. Finally, we correlated the MFIs with other parameters of perfusion such as the proportion of perfused vessels and the RBC velocity but not with an actual measurement of microvascular flow.

In conclusion, although the different methods for the calculations of MFI reflect the magnitude of microvascular perfusion, they are not interchangeable. Even though the $MFI_{vessel\ by\ vessel}$ is time consuming, this method could arguably more precisely track the microcirculatory perfusion as suggested by its stronger correlations with other parameters of microvascular perfusion. Larger studies are needed to determine if these findings also imply advantages as an outcome predictor.

Disclosure

Dr. C. Ince has been a consultant in the past to MircoVision Medical maker of the sidestream dark field technology but as a result of irreconcilable differences has broken all contact with this company for >2 years. The remaining authors have not disclosed any potential conflicts of interests.

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Clinical Study

Persistent Sepsis-Induced Hypotension without Hyperlactatemia: A Distinct Clinical and Physiological Profile within the Spectrum of Septic Shock

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Introduction. A subgroup of septic shock patients will never develop hyperlactatemia despite being subjected to a massive circulatory stress. Maintenance of normal lactate levels during septic shock is of great clinical and physiological interest. Our aim was to describe the clinical, hemodynamic, perfusion, and microcirculatory profiles associated to the absence of hyperlactatemia during septic shock resuscitation. *Methods*. We conducted an observational study in septic shock patients undergoing resuscitation. Serial clinical, hemodynamic, and perfusion parameters were registered. A single sublingual microcirculatory assessment was performed in a subgroup. Patients evolving with versus without hyperlactatemia were compared. *Results*. 124 septic shock patients were included. Patients without hyperlactatemia exhibited lower severity scores and mortality. They also presented higher platelet counts and required less intensive treatment. Microcirculation was assessed in 45 patients. Patients without hyperlactatemia presented higher PPV and MFI values. Lactate was correlated to several microcirculatory parameters. No difference in systemic flow parameters was observed. *Conclusion*. Persistent sepsis-induced hypotension without hyperlactatemia is associated with less organ dysfunctions and a very low mortality risk. Patients without hyperlactatemia exhibit less coagulation and microcirculatory derangements despite comparable macrohemodynamics. Our study supports the notion that persistent sepsis-induced hypotension without hyperlactatemia exhibits a distinctive clinical and physiological profile.

1. Introduction

Although the physiologic basis of lactate generation during shock has been recently matter of debate and research, a perfusion-related mechanism is probably involved at least in early stages [1–3]. Recent clinical studies have confirmed the strong prognostic value of hyperlactatemia and its association to other hemodynamic and perfusion abnormalities in septic shock [4–6]. Either a single abnormal level or an impaired lactate clearance is related to morbidity and mortality.

More intriguingly, a subgroup of septic patients requiring prolonged vasopressor support, and thus classified as septic shock according to the 2001 Sepsis Definition Conference [7], will never develop hyperlactatemia despite being subjected to a massive circulatory stress [8, 9]. Moreover, we recently performed a retrospective analysis of 302 vasopressor-requiring septic patients, and demonstrated that the absence of hyperlactatemia was associated with a very low (7.7%) mortality risk as compared with that in patients presenting hyperlactatemia at some point during resuscitation (42%) [9].

The maintenance of normal lactate levels in a septic patient with circulatory dysfunction is of great clinical and physiological interest. In fact, since several potential mechanisms can induce hyperlactatemia, including low cardiac output, microcirculatory abnormalities, sustained hyperadrenergia with accelerated aerobic glycolysis, and hepatosplanchnic hypoperfusion, among others, it is likely that the absence of hyperlactatemia reflects a more adequate physiological response to stress [1, 9]. Indeed, the very low mortality associated to this condition supports the notion of a relatively preserved global homeostasis [9]. However, this statement is highly speculative and should be addressed in additional clinical and physiological research specifically focused on the determinants of lactate homeostasis during sepsis-related circulatory dysfunction.

Our aim was to describe the clinical, hemodynamic, perfusion, and microcirculatory profiles associated to the absence of hyperlactatemia during septic shock resuscitation as a hypothesis-generating study.

2. Patients and Methods

We conducted an observational study from April 2008 to October 2010, including all adult patients admitted to the ICU with a diagnosis of septic shock according to the 2001 Sepsis Definition Conference [7]. Under this definition, septic patients are considered in shock when presenting a volume-refractory hypotension and thus require vasopressors to sustain blood pressure.

All septic shock patients were treated with a periodically updated management protocol independently of their participation in this study, and their demographic and clinical data were registered in a prospective data set. The Institutional Review Board (IRB) of our University approved this study and waived the necessity of an informed consent because of the solely observational nature of the study design, and considering that it did not deviate from the best standard of care.

Patients requiring vasopressors to maintain mean arterial pressure (MAP) > 65 mmHg despite initial fluid loading [10] and committed to full resuscitation were considered eligible for this study.

Our local management algorithm for septic shock has been published elsewhere [9, 11–14]. Septic patients presenting a circulatory dysfunction at the emergency department (ED) or the pre-ICU service were subjected to vigorous fluid resuscitation and basal measurements of lactate (Radiometer ABL 735, Copenhagen Denmark). If developing persistent hypotension or hyperlactatemia, patients were transferred to the ICU as soon as a bed was available. In the meantime, and depending on the timing of ICU bed availability, a central venous catheter was inserted for measurement of central venous oxygen saturation. The mean transfer time from the ED to the ICU for septic shock patients in our university hospital is 48 minutes [14].

ICU-based resuscitation was aimed at normalizing macrohemodynamic and clinical and metabolic perfusion parameters. Invasive hemodynamic monitoring and mechanical ventilation (MV) were decided on an individual basis by attending physicians. Norepinephrine (NE) was used as the sole vasopressor and adjusted to the minimal dose to maintain the MAP target. Optimal fluid resuscitation was guided by dynamic predictors [15] or by a Starling curve approach when the former were not feasible. High-volume hemofiltration (HVHF) was indicated as a final salvage therapy in unresponsive patients [13]. Intra-abdominal pressure was monitored and treated according to recent guidelines [16]. Complementarily, a dedicated sepsis team performed a daily exhaustive reassessment of the adequacy of source control and participated in major decisions.

Perfusion assessment included metabolic (arterial lactate, central ($ScvO_2$) or mixed (SvO_2) venous O_2 saturation, central venous-to-arterial PCO_2 difference ($P(cv\text{-}a)CO_2$)) and peripheral perfusion parameters (capillary refill time, central-to-peripheral temperature gradient, skin mottling) at least every 6 h during the first 48 h of treatment. A patient with septic shock was subjected to at least 9 arterial lactate determinations (including the first pre-ICU assessment) during this period.

A patient was considered as resuscitated when normalization of both metabolic and peripheral perfusion parameters was achieved, while maintaining stable or decreasing NE requirements for at least 12 h. Patients were followed until hospital discharge or death. Baseline values were registered after arterial line and central venous catheter insertion. Sublingual microcirculatory assessments were performed within 6 h of ICU resuscitation in a subgroup of patients (see below).

We divided the whole cohort according to the presence or not of any abnormal lactate value during the resuscitation period and compared the resulting subgroups for differences in mortality and other relevant clinical and physiological variables. To be classified to the "normal" lactate subgroup, all lactate measurements including the pre-ICU determinations had to be in the normal range. Patients with at least one abnormal level were classified to the "hyperlactatemia" subgroup.

3. Lactate Determination

Lactate levels were measured in arterial blood using the hospital's central laboratory through a blood gas analyzer (Radiometer ABL 735, Copenhagen, Denmark). According to our laboratory standards, a range from 0.1 to 2.4 mmol/L was considered as normal. This cut-off was recently revalidated by Shapiro et al. [4].

3.1. Sublingual Microcirculation Imaging. Microcirculatory assessments were performed in all septic shock patients included after April 2010. At this point, proper training of staff in image acquisition was completed, thus allowing around-the-clock availability. A different investigator, who was blinded to clinical data, performed image analysis according to a recent consensus [17].

Sublingual microcirculation was assessed with sidestream dark field (SDF) videomicroscopy with a 5x lens (Microscan

for NTSC, Microvision Medical). At each time point, at least five 10–20 sec images were recorded. After removing saliva and oral secretions, the probe was applied over the mucosa at the base of the tongue. Special care was taken to avoid exerting excessive pressure on the mucosa, which was verified by checking ongoing flow in larger microvessels (>50 um). Analog images were digitalized by using the pass-through function of a digital video camera recorder (Sony DCR-HC96, for NTSC) and were recorded instantaneously and transformed to AVI format in a laptop with the aid of a commercial software (DVGate Plus 2.3, Sony Corporation).

According to recommendations of the cited consensus [17], image analysis consisted in flow (percentage of perfused vessels, PPV; microcirculatory blood flow, MFI), density (total vascular density, TVD; perfused vascular density, PVD) and heterogeneity parameters (MFI heterogeneity, Het MFI). Briefly, to determine MFI, the image was divided into four quadrants and the predominant type of flow is assessed in each quadrant and characterized as absent = 0, intermittent = 1, sluggish = 2, or normal = 3. Values of the 4 quadrants were averaged. MFI heterogeneity was calculated as Het MFI = (MFI max − MFI min) × 100/MFI mean. For TVD and PVD, a gridline consisting of 3 horizontal and 3 vertical equidistant lines was superimposed on the image. All vessels crossing the lines were counted and classified either as perfused (continuous flow) or nonperfused (no flow or intermittent flow) vessels. Next, densities were calculated as the total number of vessels (TVD) or the number of perfused vessels (PVD), divided by the total length of the gridline in millimeters. PPV was calculated as PVD/TVD × 100 [17].

4. Statistical Analysis

In order to accomplish our objectives, patients evolving with versus without hyperlactatemia were compared for differences in severity scores, organ dysfunctions, hemodynamic and perfusion parameters, microcirculatory abnormalities, and hospital mortality.

Numerical variables were compared using Mann-Whitney U test, and categorical variables were compared by chi-square goodness-of-fit test. Spearman's correlation was used for testing between continuous variables, due to nonnormal distribution of data. Logistic and multivariate regression was performed to determine variables independently associated with hyperlactatemia, microcirculatory abnormalities, and hospital mortality. SPSS software version 17.0 (Chicago, IL, USA) was used for statistical calculations. Results are expressed as percentages or median and interquartile range. A $P < 0.05$ was considered as statistically significant. All reported P values are two sided.

5. Results

A total of 124 patients were included in this study. The general characteristics of the cohort are shown in Table 1. Thirty-eight patients (31%) did not present hyperlactatemia during resuscitation and 86 (69%) did. Sepsis was caused more frequently by abdominal and respiratory sources. Surgical resolution of sepsis foci was necessary in 39%.

When comparing both subgroups, no difference in comorbidities was found (Table 1). Patients without hyperlactatemia presented lower severity scores, less MV requirements, and lower hospital mortality (Table 1). They also exhibited higher platelet counts and lower serum creatinine levels (Table 2).

In relation to hemodynamic and perfusion parameters, patients with persistent sepsis-induced hypotension without hyperlactatemia presented lower NE requirements, less positive fluid balances, and received dobutamine less frequently (Table 3). A pulmonary artery catheter was inserted in 9 patients without hyperlactatemia and in 38 with elevated lactate levels. No significant differences in cardiac index, pulmonary artery occlusion pressure, $ScvO_2$, and SvO_2 were observed.

A sublingual microcirculatory assessment was performed in 45 patients (36% of the whole cohort; see above), 14 without and 31 with hyperlactemia. This subset was comparable to the whole cohort in clinical, hemodynamic, and perfusion variables, and outcome. When comparing subgroups, patients without hyperlactatemia exhibited significantly higher PPV and MFI values (Table 4).

In the subset of patients in whom a sublingual microcirculatory assessment was performed, lactate levels exhibited a significant correlation with PPV (Spearman's Rho = 0.499, $P < 0.0001$) and MFI (Spearman's Rho = 0.497, $P < 0.0001$).

6. Discussion

Our results confirm that patients with persistent sepsis-induced hypotension without hyperlactatemia present a very low mortality risk. This condition is associated with less organ dysfunctions and intensity of ICU management. Age, comorbidities, sepsis source control, and macrohemodynamic parameters including cardiac output, were not related to the presence or absence of hyperlactatemia. Interestingly, patients without hyperlactatemia presented less severe microcirculatory abnormalities and higher platelet counts. Although our conclusions are to some extent speculative and basically hypothesis generating, these data support the notion that patients with persistent sepsis-induced hypotension without hyperlactatemia exhibit a distinctive clinical and physiological profile.

Sepsis involves a complex interaction between the coagulation and inflammatory systems at the endothelial and microvascular level [18, 19]. This may result in tissue hypoperfusion, thus inducing hypoxia-driven hyperlactatemia [20]. Moreover, disseminated intravascular platelet activation may occur, contributing to microvascular failure and organ dysfunction [21]. Thrombocytopenia is a marker of this process. On the other hand, several microcirculatory abnormalities, such as endothelial edema, leukocyte activation, red blood cells stiffness, platelet aggregation, and functional shunting, could also induce microvascular hypoperfusion and eventually hyperlactatemia [22].

In effect, patients without hyperlactatemia evolved with higher platelet counts, a trend to lower D-dimer levels

Table 1: General characteristics of the cohort and subgroups of patients.

	Total	Lactate < 2.5	Lactate ≥ 2.5
Number of patients	124	38	86
Age (y)	65 [53–75]	62 [39–73]	65 [58–75]
ICU LOS (d)	5 [3–9]	4.5 [2–7]	5 [3–10]
APACHE II score	18 [12–24]	12 [8–19]	20 [15–25]**
Basal SOFA score	8 [5–10]	6 [3–8]	9 [6–11]**
ICU mortality (%)	13.7	5.2	17.4*
Hospital mortality (%)	17.6	7.9	20.9*
Patients in MV (%)	79	71	82*
Length of MV (d)	2 [1–5]	1 [0–3.7]	3 [1–7]*
Renal replacement therapy	19	3	16*
Sepsis source (%)			
Pulmonary	27	26	28
Abdominal	45	45	44
Other	28	29	28
Adequate initial AB empiric coverage (%)			
Yes	81	71	85
No	13	16	12
Unknown	6	13	3
Comorbidities (%)			
Diabetes	20	19	21
Hypertension	26	23	27
Chronic kidney disease	7	6	8
Stroke	24	0	3
Atrial fibrillation	11	0	15

* $P < 0.05$ for the comparison between subgroups.
** $P < 0.01$ for the comparison between subgroups.
Data are shown as median [interquartile range] or percentage. ICU: intensive care unit; LOS: length of stay; APACHE: acute physiology and chronic health evaluation; SOFA: sequential organ failure assessment; MV: mechanical ventilation; AB: antibiotic.

Table 2: Baseline and peak laboratory parameters of organ dysfunction.

	Lactate < 2.5 mmol/l	Lactate ≥ 2.5 mmol/l
Baseline PaO_2/FiO_2	260 [185–388]	275 [160–339]
Lowest PaO_2/FiO_2	257 [184–340]	218 [150–286]
Baseline D-dimer levels (ng/mL)	3070 [2031–4198]	3788 [2096–5480]
Peak D-dimer levels (ng/mL)	3447 [2182–4771]	5298 [2885–7392]
Baseline platelet count ($\times 10^3/mm^3$)	192 [157–332]	145 [101–255]*
Lowest platelet count ($\times 10^3/mm^3$)	171 [116–261]	83.5 [43.3–162.5]**
Baseline bilirubin levels (mg/dL)	0.7 [0.5–1.3]	1 [0.6–1.9]
Peak bilirubin levels (mg/dl)	0.7 [0.6–1.7]	1.1 [0.7–3]
Baseline C-reactive protein levels (mg/dL)	15.9 [8.5–25.9]	14.7 [5.7–27.6]
Peak C-reactive protein levels (mg/dL)	24.4 [15.2–33.9]	28 [19.7–36]
Baseline serum creatinine levels (mg/dL)	0.8 [0.6–1.6]	1.7 [1–3]**
Peak serum creatinine levels (mg/dL)	1 [0.6–1.7]	1.7 [1.1–2.9]**

* $P < 0.05$.
** $P < 0.01$.
Data are shown as median [interquartile range].

Table 3: Hemodynamic and perfusion parameters in subgroups of patients.

	Lactate < 2.5 mmol/L	Lactate ≥ 2.5 mmol/L
Peak lactate level (mmol/l)	1.7 [1.3–2]	4.5 [3.4–7.4]**
Baseline lactate levels (mmol/l)	1.2 [1–1.8]	4 [3–5.8]**
Baseline PAOP (mmHg)	18 [13–26.5]	19.5 [15.3–23.8]
Baseline CI (l/min/m^2)	3.2 [1.9–3.5]	3 [2.4 –3.7]
Lowest CI (l/min/m^2)	2 [1.9–3.2]	2.4 [2 –2.7]
Lowest $ScvO_2$ (%)	67 [59–71]	66 [58 –72]
Lowest SvO_2 (%)	69 [65 –74]	68 [61 –75]
Peak NE dose (ug/kg/min)	0.08 [0.04 – 0.17]	0.2 [0.07–0.53]**
NE use (h)	22 [11–41]	35 [17–69]*
24 h fluid balance (mL)	1903 [845–2835]	4000 [1973–5509]**
Cumulative 72 h fluid balance (mL)	2857 [1130–5264]	5978 [3674–9551]**
Dobutamine use (% of patients)	18	46**
Basal P(cv-a)CO_2(mmHg)	5.5 [3–8]	6.1 [4.7–8]
Peak intra-abdominal pressure (mmHg)	19 [12.5–24]	17 [15–19]

* $P < 0.05$.
** $P < 0.01$.
Data are shown as median [interquartile range] or percentage. PAOP: pulmonary artery occlusion pressure; CI: cardiac index; $ScvO_2$: central venous oxygen saturation; SvO_2: mixed venous oxygen saturation; NE: norepinephrine; P(cv-a)CO_2: central venous-to-arterial PCO_2 difference.

Table 4: Hemodynamic, perfusion and microcirculatory parameters in 45 patients evaluated with sublingual SDF videomicroscopy.

	Lactate < 2.5 mmol/L	Lactate ≥ 2.5 mmol/L
n (%)	14 (31%)	31 (69%)
NE (ug/kg/min)	0.2 [0.09–0.39]	0.48 [0.22–0.93]*
Lactate (mmol/l)	1.4 [1.2–2.1]	5.8 [3.9–8.4]*
$ScvO_2$ (%)	73 [67–77]	71 [66–78]
TVD (n/mm)	12.9 [10.7–13.9]	12.9 [11.1–14.9]
PVD (n/mm)	10.5 [9.5–12]	10.1 [6.6–12.4]
PPV (%)	87.3 [81.6–90.6]	75.5 [60.9–86.4]*
MFI	2.44 [2.25–2.61]	2.11 [1.7–2.32]*
Het MFI	0.33 [0.18–0.49]	0.42 [0.27–0.72]

* $P < 0.01$
Data are shown as median [interquartile range] or percentage. NS: nonsignificant ($P > 0.05$). NE: norepinephrine; $ScvO_2$: central venous oxygen saturation; P(cv-a)CO_2: central venous-to-arterial PCO_2 difference; TVD: total vascular density; PVD: perfused vascular density; PPV: percentage of perfused vessels; MFI: microvascular flow index; Het MFI: MFI heterogeneity.

($P = 0.08$), and a relatively preserved microcirculatory flow (PPV and MFI). Taken together, these data suggest that the absence of hyperlactatemia could be related, at least in part, to less severe endothelial and microcirculatory dysfunctions. As a matter of fact, macrohemodynamic variables, oxygen-derived parameters such as SvO_2, and venous-arterial pCO_2 gradients were not different between subgroups, thus suggesting that systemic flow disturbances are not major determinants of the genesis of hyperlactatemia in this setting.

The relationship between hyperlactatemia and microcirculatory abnormalities in septic patients is somehow controversial. Three studies reported a poor correlation between MFI and hyperlactatemia after single assessments [23–25]. In contrast, De Backer et al., testing the effect of dobutamine on microcirculatory abnormalities, found that an improvement in PPV was significantly associated with a decrease in lactate levels [26]. The same group confirmed these findings in another study addressing the effects of fluids on microvascular flow [27]. These discrepancies could be better explained by different study designs, concerning timing and number of microcirculatory assessments and therapeutic interventions. As a matter of fact, the latter group [26, 27] performed 2 sequential microcirculatory evaluations, thus comparing the time course of microvascular flow recovery and lactate decrease. In our case, although we performed a single microcirculatory assessment per patient, the main difference with the studies cited above [23–25] is that we compared microcirculatory derangements between two mutually exclusive subgroups and found a significant correlation between several microcirculatory flow-related parameters and lactate. Although methodological differences preclude a direct comparison between studies, in our opinion they ultimately suggest that there is an effective association

between hyperlactatemia and microcirculatory abnormalities, at least during the early stages of septic shock. However, no definite cause-effect relationship can be established at this point.

Another interesting finding is the relatively moderate degree of microcirculatory derangements found in our study, as shown by a mean MFI of 2.1 and a PPV of 75.5% in patients with hyperlactatemia. However, while our observation is consistent with recent studies that found similar mean basal MFI values [28–30], it is in sharp contrast with another trial reporting MFI values of less than 1.5 early after emergency room admission [31]. Moreover, Boerma et al. [32] reported that MFI improved over time (from 1.4 to 2.2) during resuscitation in the placebo arm of their nitroglycerin trial. These data considered together suggest that MFI values are very low in nonresuscitated patients but may improve rapidly after initial aggressive resuscitative maneuvers, resembling what happens with $ScvO_2$. Nevertheless, this fact does not invalidate our results, since both subgroups, with and without hyperlactatemia, presented similar pre-ICU management and time from diagnosis to ICU admission (data not shown). Therefore, we believe that the observed differences in microcirculatory flow indexes are relevant and provide interesting potential clinical and physiological implications.

Our study suggests that persistent sepsis-induced hypotension without hyperlactatemia, traditionally included under septic shock definitions, constitutes a different subgroup in terms of prognosis and endothelial/microcirculatory dysfunction. Remarkably, more than 90% of these patients had this condition resolved and were discharged from ICU without further complications. Moreover, they required less intensive critical care treatment. The 2001 Sepsis Definition Conference proposed vasopressor requirements as a mandatory criterion for septic shock diagnosis, irrespective of lactate levels [7]. In this sense, besides confirming our previous retrospective findings [9], the present study provides more clinical and physiological data for a potential reappraisal of current septic shock definitions. The question whether persistent sepsis-induced hypotension without hyperlactemia constitutes a different pathophysiological entity, or simply a mild form of septic shock, should be addressed in future studies.

Our study has several limitations. This was a single-centre study, thus limiting the extrapolation of our results. Microcirculatory assessments were performed at different time points during early resuscitation, were limited to a subset of patients, and did not include serial measurements. We did not evaluate other potential mechanisms involved in the genesis of hyperlactatemia, such as hyperadrenergia with accelerated glycolysis, hepatosplanchnic flow, or mitochondrial dysfunction. No sample size calculation was performed, and our cohort was relatively small. We cannot rule out the possibility of having missed some high lactate values between sampling, although this is unlikely considering the frequent sampling. Finally, it was beyond our scope to comprehensively address all the potential causes of persistent hyperlactatemia. As stated in the introduction, this has been matter of extensive recent research, but briefly many potential nonhypoxic causes could contribute including hepatosplanchnic hypoperfusion, liver dysfunction, adrenergic-driven aerobic glycolysis, hyperinflammation, among others [1–3]. Nevertheless, we think that these results provide valuable information concerning the clinical and physiological significance of the absence of hyperlactatemia during sepsis-related circulatory dysfunction.

7. Conclusions

Persistent sepsis-induced hypotension without hyperlactatemia is associated with less severe organ dysfunctions and a very low mortality risk. Systemic flow parameters are not related to the presence or absence of hyperlactatemia. Our data suggest a relationship between coagulation, microcirculatory derangements, and lactate levels. This study tends to support the notion that patients with persistent sepsis-induced hypotension without hyperlactatemia exhibit a distinctive clinical and physiological profile within the spectrum of septic shock. This subject should be addressed in future studies.

Disclosure

Dr. C. Ince has been a consultant in the past to MicroVision Medical, maker of the sidestream dark field technology, but as a result of irreconcilable differences, has broken all contact with this company for more than 2 years.

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