Monitoring of the Sublingual Microcirculation in Cardiac Surgery Using Orthogonal Polarization Spectral Imaging

Preliminary Results

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Background: The recent introduction of orthogonal polarization spectral imaging enables the direct visualization of the microcirculation of man without imaging enhancing dyes. The authors studied the changes in microvascular perfusion of sublingual mucosa during cardiac surgery with the use of cardiopulmonary bypass (CPB) using this optical method.

Method: Orthogonal polarization spectral images were recorded in 47 patients after skin incision (T1), after the start of CPB (T2), in the late phase of CPB (T3), and 1 h after the discontinuation of CPB (T4). The images were analyzed for microvascular diameter, erythrocyte velocity, and functional capillary density using an established analysis routine for intravital microscopy studies. In a subpopulation (n = 8), the expression of the adhesion molecules CD18 on circulation leukocytes was compared with the number of visualized rolling leukocytes.

Results: Preoperatively, no significant changes of the microvascular diameter and erythrocyte velocity were seen. The functional capillary density was significantly reduced at T3 to 90% of the values observed before CPB but recovered at T4 and showed a weak but significant correlation with body temperature (r = 0.38, P < 0.01) and hemoglobin concentration (r = 0.20, P < 0.05). Expression of CD18 was significantly increased in the late phase of CPB (T3) only, whereas the numbers of rolling leukocytes increased during CPB and revealed a significant threefold increase 1 h after termination of CPB.

Conclusions: Orthogonal polarization spectral imaging revealed no major changes of microvascular perfusion during uncomplicated hypothermic CPB. The slightly reduced functional capillary density during CPB may be caused by several factors all present during CPB, including hypothermia, the artificial extracorporeal perfusion, surgical trauma, hemodilution, and inflammatory reaction. The current data do not allow differentiation between the effects of those possible causes.

The rate of complications after cardiac surgery with the use of cardiopulmonary bypass (CPB) has decreased in the past 3 decades, but some minor postoperative neurologic sequelae are common and in recent studies have been reported with an incidence of up to 70%.1 In particular, microembolism and small air bubbles are considered to cause these complications. Changes in microvascular perfusion during CPB, however, may also contribute to neurologic deficits, because they are known to play a role in other postoperative complications such as edema formation and organ failure distant to the site of injury, e.g., lungs or kidneys.3,4

Orthogonal polarization spectral (OPS) imaging enables the noninvasive visualization of microvascular perfusion in humans without the use of fluorescent dyes.5 Various clinical investigations with OPS imaging have identified microcirculatory abnormalities as a major component of the pathogenesis of sepsis6,7 and cardiogenic shock.8 Furthermore, the effect of therapeutic strategies could be studied using this technique in preterm infants9 and critically ill adults.10–12

In the current study, we aimed to determine whether OPS imaging applied to the sublingual mucosa provides relevant clinical information about the microcirculation during cardiac surgery with the use of CPB and whether signs of the systemic inflammatory reactions in response to cardiac surgery are detectable with OPS imaging. The sublingual mucosa was chosen because it is easy to access for the anesthesiologist during surgery, its nutritive blood flow is known to correlate with that of vital internal organs,13,14 and it is to date the most often used measurement site for OPS imaging in humans.6–8,12

Materials and Methods

The institutional review board of the Ludwig-Maximilians University (Munich, Germany) approved the study, and written informed consent was obtained from each patient. Forty-seven patients who underwent cardiac surgery with the use of CPB entered the study.

Anesthesia and Perioperative Management

All patients received oral premedication with a benzodiazepine the evening before and on the day of the operation. Before induction of anesthesia, an arterial line was placed into the femoral artery. Induction was performed with a continuous infusion of sufentanil (100-µg bolus followed by 100 µg/h), etomidate (0.3 mg/kg), and 8 mg pancuronium to facilitate tracheal intubation of the patient. After intubation, a two-lumen central venous line and an 8.5-French venous sheath (Arrow Deutschland, Erding, Germany) were placed in the right internal jugular vein. In addition, we inserted a pulmonary artery flotation catheter in patients with a limited cardiac reserve (ejection fraction < 50%). The perioperative

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fluid and drug management was guided by the measurement of arterial and central venous pressure and, if considered necessary, by the measurements provided by the use of the pulmonary artery flotation catheter. Usually, patients needed some catecholamine therapy after CPB, which was left to the discretion of the anesthesiologist not involved in the study.

**Cardiopulmonary Bypass**

All patients received heparin (400 U/kg), which resulted in an activated clotting time of greater than 450 s. The priming solution contained 900 ml NaCl, 0.9%; 500 ml hydroxyethyl starch, 6%; and 30 ml/kg mannitol, 20%. During CPB, patients were cooled to a core temperature of 28°–32°C. CPB was performed with a closed circuit (HMT, Fürstenfeldbruck, Germany) using a membrane oxygenator (Medtronic, Düsseldorf, Germany), with a mean flow of 2.2–2.4 l/m² body surface area and with a maintenance of mean blood pressure between 60 and 70 mmHg. If mean arterial blood pressure decreased below 50 mmHg, bolus injections of 10 μg norepinephrine were given into the CPB circuit. After declamping of the aorta, 6 mmol magnesium was given intravenously. Gradual rewarming was undertaken during the reperfusion of the heart and lung, which was conducted for at least 30% of the clamping time of the aorta. The mean duration of CPB was 126 ± 40 min, with a mean time of aortic clamping of 84 ± 31 min.

Weaning from CPB was performed with the administration of dopamine, epinephrine, norepinephrine, nitroglycerine, or milrinone or a combination of those drugs as considered necessary by the anesthesiologist in charge of the case. Generally, we aimed for a hemoglobin concentration of greater than 9 g/dl after CPB, which necessitated transfusion of erythrocytes in 20 of 47 patients. Heparin was fully antagonized with intravenous protamine after decannulation of the aorta. If nonsurgical bleeding was diagnosed, fresh frozen plasma and platelets were given depending on the duration of CPB and the judgment of the overall clotting situation.

**OPS Imaging**

The technique has been described in detail previously³,⁵,¹⁵,¹⁶; hence we will only outline its use in this study. We observed the microcirculation using the Cytoscan A/R (Cytometrics Inc., Philadelphia, PA) with a 10x objective giving a magnification of approximately 450-fold. To visualize erythrocytes, polarized light is passed through a green filter and the images produced are captured using a mini charge-coupled device camera (Costar CV-M536 CCIR; JAI, Yokohama, Japan). All images were recorded on S-VHS tape and analyzed off-line.

Orthogonal polarization spectral images (fig. 1) were obtained from the sublingual mucosa where the probe was put in direct contact with the tissue. Once blood vessels were discernible, it was manually focused, and by stabilizing the probe on the teeth, it was possible to obtain good images with minimal movement artifacts. Because the probe had to be in direct contact with the mucosa, the exerted pressure could impair the sublingual tissue blood flow. We took great care in placing the probe, and the contact pressure was minimized to a point where the venules and capillaries remained in focus and showed maximum flow, judged by the on-line image depicted on the monitor.

Orthogonal polarization spectral images were recorded after skin incision (T1), after the start of CPB (T2), in the late phase of CPB and always well after the release of the aortic cross clamp (T3), and 1 h after the discontinuation of CPB (T4).

Five sequences of 20 s duration were recorded at each time point. All visible microvessels in each field were analyzed, resulting in 15–30 observed vessels per patient and time point. We measured microvascular diameter and erythrocyte velocity using CAP-image software (Dr. Zeintl GmbH, Heidelberg, Germany).¹⁷ Functional capillary density (FCD) is known to show a good correlation with nutritive blood flow and represents the length of capillaries (cm) revealing erythrocyte flow for a given area (cm²).¹⁸ This measurement can also be obtained with CAP-image.

**Interaction of Leukocyte and Microvascular Endothelium**

Particularly during the late reperfusion phase, we could identify many rolling leukocytes in postcapillary venules. They appeared as a sparing in the erythrocyte column and revealed a slow pattern of movement along the vessel wall. We made a quantitative assessment of the numbers of rolling leukocytes by subdividing the screen of a high-
resolution 20-inch monitor (Trinitron Monitor PVM-20; Sony Inc., Tokyo, Japan) into nine rectangles (13 × 10 cm) corresponding to approximately 0.26 × 0.20 mm in the image (fig. 2). In eight patients (one female; mean age, 62.5 yr), we counted the number of postcapillary venules and capillaries in each square and the number of rolling leukocytes that could be identified in each postcapillary venule, expressed in rolling leukocytes per 20 s.

Blood Samples
Arterial blood samples were drawn from each patient at each time point for analysis of hemoglobin concentration, lactate concentration, and leukocyte count. In those eight patients where the number of rolling leukocytes was determined, we also measured the expression of the adhesion molecule CD18 on polymorphonuclear leukocytes by flow cytometry as previously described.19

Statistical Analysis
We calculated a mean value for FCD, microvascular diameter, and erythrocyte velocity for each patient and time point. Because not all parameters were normally distributed tested by Kolmogorov-Smirnov test, data are expressed as median [25th, 75th percentiles] and non-parametric tests were used. Data were analyzed for statistical difference using the Friedman repeated-measures analysis of variance on ranks followed by the Dunn method post hoc test. Correlations were tested with Spearman rank order correlation and multiple linear regressions. Significance was assumed at $P < 0.05$. The statistical analysis was processed using Sigma Stat (Jandel Scientific, Erkrath, Germany).

Results
Demographic data of the study population are given in table 1. OPS images could be obtained in all patients. At T1 and T4, no adverse events were noted. However, after full heparinization for CPB, some patients revealed microbleeding in the sublingual mucosa, which was most likely due to the contact of the probe with the tissue. After suction of the mouth and sometimes flushing the sublingual mucosa with normal saline, good-quality images could still be obtained even when bleeding had occurred. OPS sequences with evidence of perivascular erythrocytes were excluded from analysis. Postoperative intraoral evaluation revealed neither bleeding nor discomfort of any patient included in the study.

The hemodilution, which occurred in response to CPB, resulted in a marked decrease of median hemoglobin concentration (table 2). This was always discernible in the OPS images leading to a marked reduction in the numbers of erythrocytes particularly at T2 after the start of CPB, when the priming solution entered the patients’ circulation. The changes in hemoglobin concentration values are given in table 2, and examples of the resulting OPS images are shown in figures 1 and 2, which correspond to hemoglobin concentrations of 10.3 g/dl (T1) and 9.0 g/dl (T4), respectively.

Mean arterial pressure was reduced in the late phase of CPB (T3) under hypothermic conditions. Heart rate was significantly higher at T4; however, epicardial stimulation with pacemakers was used in this phase of surgery to guarantee a heart rate greater than 90 beats/min (table 2).

The microvascular diameters and erythrocyte velocities in postcapillary venules are given in table 2 where no significant change in erythrocyte velocity and in diameter was observed.

Functional capillary density, however, showed a moderate but significant reduction during late CPB at T3, which returned to the preoperative value 1 h after termination of CPB (table 2).
Correlation analysis of OPS imaging (FCD, microvascular diameter, and erythrocyte velocity) and hemodynamic parameters (mean arterial pressure and heart rate) as well as esophageal temperature of pooled values from T1 to T4 revealed a significant but weak correlation of FCD with esophageal temperature (fig. 3; \( r = 0.38, P < 0.01 \)) and hemoglobin concentration (\( r = 0.20; P = 0.05 \)). Neither mean arterial pressure nor heart rate showed a significant correlation with the microcirculatory parameters. Low-dose epinephrine (mean dosage, 0.06 mg kg\(^{-1}\) min\(^{-1}\)), norepinephrine (0.02 mg kg\(^{-1}\) min\(^{-1}\)), and milrinone (0.05 mg kg\(^{-1}\) min\(^{-1}\)) were used in all patients after weaning from CPB, whereas no catecholamine therapy was used during T2 and T3. We could not find a correlation between the administration of inotropic and/or vasoactive substances and microvascular parameters.

**Table 2. Orthogonal Polarization Spectral Imaging Parameters, Temperature, and Hemodynamic and Laboratory Data during Cardiac Surgery (n = 47)**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>T1</th>
<th>T2</th>
<th>T3</th>
<th>T4</th>
</tr>
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<tbody>
<tr>
<td><strong>Orthogonal polarization</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Erythrocyte velocity, ( \mu \text{m/s} )</td>
<td>606 [530, 685]</td>
<td>576 [529, 648]</td>
<td>606 [528, 719]</td>
<td>564 [503, 716]</td>
</tr>
<tr>
<td>Microvascular diameter, ( \mu \text{m} )</td>
<td>33 [26, 39]</td>
<td>32 [25, 45]</td>
<td>31 [25, 37]</td>
<td>34 [26, 38]</td>
</tr>
<tr>
<td>FCD, ( \text{cm}^2 )</td>
<td>147 [135, 157]</td>
<td>136 [128, 155]</td>
<td>133 [117, 140]; §</td>
<td>142 [132, 162]</td>
</tr>
<tr>
<td><strong>Hemodynamic data and temperature</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean arterial pressure, mmHg</td>
<td>75 [66, 78]</td>
<td>60 [57, 69]</td>
<td>55 [52, 66]; §</td>
<td>67 [58, 79]</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>67 [60, 75]</td>
<td>NA</td>
<td>NA</td>
<td>100 [96, 106];</td>
</tr>
<tr>
<td>Central venous pressure, mmHg</td>
<td>7 [4, 8]</td>
<td>6 [4, 8]</td>
<td>4 [2, 8]</td>
<td>10 [8, 12]; §†</td>
</tr>
<tr>
<td>Temperature, °C</td>
<td>36.0 [35.2, 36.9]</td>
<td>34.7 [34.0, 35.2]; §</td>
<td>30.6 [28.9, 33.6]; §</td>
<td>36.9 [36.1, 37.0]</td>
</tr>
<tr>
<td><strong>Laboratory data</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hemoglobin concentration, g/dl</td>
<td>12.3 [10.4, 14.7]</td>
<td>8.3 [7.0, 9.9]; *</td>
<td>8.8 [7.7, 9.1]</td>
<td>9.4 [8.9, 10.1]</td>
</tr>
<tr>
<td>Lactate, mM</td>
<td>0.8 [0.7, 1.1]</td>
<td>0.7 [0.6, 0.8]</td>
<td>1.0 [0.8, 1.5]</td>
<td>2.3 [1.7, 3.2]; †‡</td>
</tr>
</tbody>
</table>

Data are presented as median [25th, 75th percentiles]. Statistical analysis by Friedman repeated-measures analysis of variance with post hoc Dunn method: \( \ast P < 0.05 \) vs. T1, \( \dagger P < 0.05 \) vs. T2, \( \ddagger P < 0.05 \) vs. T3, \( \ddagger P < 0.05 \) vs. T4; Wilcoxon test for heart rate: \( \ddagger P < 0.001 \).

FCD = functional capillary density; NA = not applicable.

**Leukocyte Endothelium Interaction and Expression of Adhesion Molecules**

We measured a significant increase in systemic leukocyte count at T3 and T4 (table 3). CD18 expression on polymorphonuclear leukocytes was significantly increased at T3 and returned to the values before CPB at T4. Using OPS imaging, we always saw rolling and sticking leukocytes in the images obtained during CPB. A typical example is shown in figure 2, where the rolling leukocytes are marked; because in OPS imaging the leukocytes are not labeled, they appear as a single white space in the column of moving erythrocytes.

The quantitative analysis of the OPS images (table 3) showed an increase in the number of rolling leukocytes at T3 (not significant) and particularly T4 (\( P < 0.05 \)). We found weak but significant correlations between the numbers of rolling leukocytes and CD18 expression (\( r = 0.47, P = 0.04 \)) as well as leukocyte count (\( r = 0.45, P = 0.04 \)).

**Discussion**

Using OPS imaging, we obtained the first images of microvascular perfusion during cardiac surgery with the use of CPB in the human sublingual mucosa. We could show that microvascular flow seemed well maintained during uncomplicated CPB. FCD, an index of nutritive blood flow, was only moderately reduced during CPB and recovered to its initial value 1 h after reperfusion. However, we found evidence for a systemic inflammatory response after reperfusion because the number of rolling leukocytes in the postcapillary venules observed with OPS imaging and the expression of adhesion molecules were significantly increased.

**Handling of OPS Probe and Adverse Events**

The OPS probe was easy to use, and staff did not require specific training to obtain good images. Only the applica-
tion of minimum pressure required some skill, to minimize this potential source of error. OPS could be used in all patients without any severe side effects. In some patients, microbleeding of the sublingual mucosa was noted due to the contact of the probe. It was only seen after full heparinization and is a common feature during CPB, usually as a result of the insertion of gastric tubes or transesophageal echocardiography probe. OPS recordings with any evidence of bleeding were excluded from microcirculatory analysis because of the potential effect of extravascular hemostasis and is a common feature during CPB, usually as a result of the insertion of gastric tubes or transesophageal echocardiography probe. OPS recordings with any evidence of bleeding were excluded from microcirculatory analysis because of the potential effect of extravascular hemorrhage and is a common feature during CPB, usually as a result of the insertion of gastric tubes or transesophageal echocardiography probe. OPS recordings with any evidence of bleeding were excluded from microcirculatory analysis because of the potential effect of extravascular hemorrhage.

Data are presented as median [25th, 75th percentiles]. Statistical analysis by Friedman repeated-measures analysis of variance with post hoc Dunn method: * P < 0.05 vs. T1. rFlU = relative fluorescence units.

### Table 3. Numbers and Activation of Leukocytes in a Subgroup of Eight Patients

<table>
<thead>
<tr>
<th></th>
<th>T1</th>
<th>T2</th>
<th>T3</th>
<th>T4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leukocytes, 10³/µl</td>
<td>5.7 [3.4, 9.3]</td>
<td>3.4 [2.5, 4.6]</td>
<td>6.7 [3.1, 6.8]*</td>
<td>11.2 [5.9, 18.0]*</td>
</tr>
<tr>
<td>CD18, rFlU</td>
<td>45 [42, 53]</td>
<td>57 [52, 63]</td>
<td>70 [60, 77]*</td>
<td>59 [56, 67]</td>
</tr>
</tbody>
</table>

Measurement of Diameter, Velocity, and Functional Capillary Density

In an animal model of the hamster skinfold chamber, Harris et al.²⁰ showed that measurement of vessel diameter and erythrocyte velocity obtained with OPS imaging are comparable to intravital microscopy images obtained from the same vessel with fluorescent dye. Because the image quality of the sublingual mucosa in humans is comparable to that in the animal model, we believe that OPS imaging can be used to provide reliable data on the changes in blood vessel diameter and velocity in humans.²¹ Therefore, microvascular diameter as well as changes in microvascular erythrocyte velocity were measured off-line using Capim- age.¹⁷ In the image, the diameter is typically calculated from four lines manually drawn across the blood vessel of interest. Measurement error is introduced, because this does not represent the whole area of the vessel under investigation and the individual judgment of the vessel border may vary between examiners. In OPS imaging, the diameter of the vessel is determined by the width of the column of erythrocytes, whereas in fluorescent intravital microscopy, the dye containing plasma provides the contrast; therefore, the true vessel diameter should be underestimated with OPS imaging. This was confirmed by Harris et al.,²⁰ who compared identical microvessels with OPS imaging and fluorescent intravital microscopy and measured a 4- to 5-µm larger diameter with the latter technique. This difference may partially be due to the known fluid layer of 0.4–0.6 µm between flowing erythrocytes and the vessel wall.²² Moreover, the interfascial scattering, an out-of-focus effect known to occur during intravital microscopy, may have contributed to larger diameter measurements when fluorescent dyes were used.²³ The values of erythrocyte velocity are obtained by determining the spatial change of an individual pixel over time, which must be chosen by the examiner. Although this technique is sufficiently validated, it introduces potential bias for better quality images. Moreover, velocity values of more than 1 mm/s cannot be measured with a high degree of accuracy because of the limits given by frame rate of the S-VHS recording.²⁴ This technical limitation thus only enabled velocity measurement in venules and capillaries, excluding arteries and arterioles of human tissues.

Perioperative Microvascular Perfusion

We measured erythrocyte velocity in postcapillary venules and capillaries of the sublingual tissue and could not observe a significant change during and after CPB. FCD was significantly reduced by 10% compared with the initial value after induction of anesthesia despite normal flow (2.2–2.4 l/m² body surface area) of the CPB. Our data indicate that also minor changes of microvascular perfusion can be detected with OPS imaging, thus adding to the validity of the current study.

Several factors during CPB may contribute to changes in microvascular perfusion, e.g., artificial nonpulsatile blood flow, hemodilution,²⁵,²⁶ hypothermia,²⁸,²⁹ or inflammatory response.²⁸,²⁹ We found weak but significant correlations of FCD with hemoglobin concentration and body temperature in our study.

Cardiopulmonary bypass with a blood-free priming of the extracorporeal circuit results in a rapid isovolemic hemodilution. In an animal model of isovolemic hemodilution, it could be shown that only a marked (> 50%) reduction of hematocrit alters microvascular perfusion monitored with OPS imaging.²⁶ No data from human studies on the influence of normovolemic hemodilution on FCD are available so far. The lack of a control group without hemodilution in our study does not allow for a clear evaluation of the role of hematocrit on microvascular perfusion.

Hypothermia decreases FCD during an extracorporeal circulation model in the hamster skinfold chamber.²⁷ However, only deep hypothermia has been shown to be associated with changes in FCD so far.²⁵,²⁷

Hypothermia and hemodilution coincide with other factors that may contribute to the observed changes during CPB (i.e., artificial nonpulsatile blood flow, activation of blood by artificial surfaces). The lack of a
control group with similar surgical conditions, however, without CPB does not allow for a clear differentiation and evaluation of the role of these different factors. The current study presents, to our knowledge, the first measurements of FCD in humans during cardiac surgery. The fact that we could only demonstrate a moderate decrease in FCD during CPB supports previous data suggesting that neither the nonpulsatile flow of CPB nor mild hypothermia causes severe microvascular impairment.²⁵,³⁰

For the evaluation of FCD, we selected approximately five sites per measuring point. The measurements were performed where at least some capillaries with flow could be identified. However, this introduces a potential bias because only areas with at least some capillaries revealing erythrocytes where analyzed. In the case of severe alteration of microcirculatory perfusion (e.g., ischemia-reperfusion injuries), large areas without any flow may exist³¹ and are thus not quantified by our form of analysis. An automated analyses routine that reviews all available images of the tissue would therefore greatly enhance the clinical value of OPS imaging in this respect.

**Evaluation of the Leukocyte Interaction with the Microvascular Endothelium Using OPS Imaging**

Leukocytes are of great importance for inflammatory reactions as well as for the development of ischemia-reperfusion injuries after CPB.²⁹ Sawa et al.²⁹ demonstrated that the removal of leukocytes during the reperfusion phase after CPB results in less inflammatory injury and less damage to both myocytes and endothelial cells. Intravital microscopy of the cerebral microcirculation via a cranial window in a piglet model of CPB and deep hypothermic cardiac arrest revealed evidence of increased leukocyte-endothelial cell interaction after CPB.²⁵ Leukocytes must be activated before they can adhere to the wall of microvessels and migrate into the surrounding tissue. This process requires inflammatory cytokines and the expression of adhesion molecules on leukocytes and endothelial cells, which result in an increased rolling and sticking of leukocytes to the postcapillary venules.³¹-³⁵ These phenomena could be detected with OPS imaging and correlate with the combination of increased numbers in circulating leukocytes and expression of CD18. However, the design of this study does not allow for a clear conclusion about whether the observed activation of leukocytes was caused by the extracorporeal circulation via CPB or by surgical trauma.

When comparing and quantifying the interaction between leukocytes and the postcapillary endothelium seen with conventional fluorescence intravital microscopy and OPS imaging, it must be stated that in OPS imaging, it is frequently impossible to distinguish between plasma gaps and leukocytes because the latter are not marked. Plasma gaps may be falsely identified as leukocytes, or they may contain leukocytes which are not detected because no erythrocytes are present to provide the necessary contrast. Moreover, the visualization of leukocytes is dependent on the microvascular hematocrit and blood flow, which changes during CPB. Therefore, we are certain that we did not identify all rolling leukocytes. However, because this limitation was present at all time points, we believe that the profound increase in the number of rolling leukocytes supports the contention of a systemic inflammatory reaction after CPB. This is corroborated by the fact that an increased expression of adhesion molecules was also found in the same patients during CPB. As far as the timing of these observations are concerned, we suspect that the increased expression of CD18 on circulating leukocytes precedes the adherence of leukocytes to the microvascular endothelial cells. Activated, CD18-positive leukocytes start rolling on, firmly adhere to, and later transmigrate through the microvascular endothelial cells and thus are removed from the circulating population of leukocytes. This could explain the discrepancy between the normalization of the CD18 expression 1 h after termination of CPB and the simultaneous further increase in the numbers of visualized leukocyte-endothelial cell interactions. Our data are in good agreement with other studies which also have reported an increased expression of adhesion molecules after CPB²⁸ and post-CPB leukocyte-endothelial cell adhesion.²⁵ However, this study was not designed to clarify the effect of CPB, because it lacks a control group without CPB (e.g., off-pump coronary artery bypass grafting).

**Current Limitations of OPS Imaging**

A major problem with OPS imaging for microvascular monitoring is the great variability of the vessels measured. Identical vessels cannot be examined over time, in contrast to intravital microscopy in animal experiments (e.g., hamster skin fold chamber) or capillaroscopy of the nail fold. Hence, many more sites of interest must be studied to enable reliable statistical analysis. In sublingual tissue, mainly capillaries and postcapillary venules can be visualized. Because of the specific anatomical geometry, arterioles are rarely seen, and in those the velocity can not be evaluated. The introduction of a stroboscope as a light source may, however, overcome this problem in the near future.²⁴ Another problem stems from movement artifacts, which make it difficult to obtain a series of stable images, necessary to measure velocity with the Cap-image software. Moreover, application pressure is uncontrolled, which may lead to pressure artifacts and result in an underestimation of the microvascular erythrocyte velocity values, diameter, and FCD. However, this potential error would be random and present in all patients at all time points. We assume that changes throughout a procedure or differences between patients should still be detectable.

We used the well-established Cap-image for analysis of the OPS images. Although this software has been vali-
BEFORE Microcirculatory monitoring during the intraoperative phase of uncomplicated surgery in 47 patients. Different stimuli that are known to alter microvascular perfusion exist in the current study, i.e., artificial nonpulsatile perfusion by CPB, contact of the blood with artificial surfaces, hemodilution, hypothermia, and surgical trauma. Our study lacks a control group, e.g., off-pump coronary artery bypass grafting where patients are exposed to similar surgical trauma without the use of CPB. However, off-pump cardiac surgery would not yield the same degree of hemodilution or hypothermia. From this study, we are not able to identify a specific factor that is responsible for the moderate microcirculatory changes during and after CPB or to evaluate the role of the different factors on the microvascular perfusion. We believe that the reported changes are the result of a complex pathophysiology during CPB. Furthermore, the observed changes in microvascular perfusion during uncomplicated cardiac surgery were only moderate and may not be of major clinical relevance. However, microcirculatory monitoring during complicated cases in high-risk patients may add further insight to the knowledge of perioperative complications.

In summary, we found that the sublingual application of OPS imaging gave new insights into changes in microvascular perfusion during CPB. The quality and contrast of the images were sufficient to allow quantitative assessment of microvascular diameter and erythrocyte velocity. We could show that microvascular perfusion seems slightly reduced during uncomplicated hypothermic CPB but is recovering to its initial value 1 h after reperfusion. Nevertheless, we found early evidence of an inflammatory reaction to the CPB and could visualize increased rolling of leukocytes in the postcapillary venules that correlated with the activation of leukocytes.

**Limitations of This Study**

This is the first study using OPS imaging during cardiac surgery with the use of CPB. We present preliminary results of the microcirculatory monitoring during the intraoperative phase of uncomplicated surgery in 47 patients. Persistent microcirculatory alterations are associated with organ failure and death in patients with septic shock. Crit Care Med 2004; 32:1825–31


