Microvascular Blood Flow Is Altered in Patients with Sepsis

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Microvascular blood flow alterations are frequent in animal models of sepsis and may impair tissue oxygenation. We hypothesized that alterations of the microcirculation are present in patients with sepsis. We used an orthogonal polarization spectral imaging technique to investigate the sublingual microcirculation in 10 healthy volunteers, 16 patients before cardiac surgery, 10 acutely ill patients without sepsis (intensive care unit control subjects), and 50 patients with severe sepsis. The effects of topical application of acetylcholine (10^{-2} M) were tested in 11 patients with sepsis. In each subject, five to seven sublingual areas were recorded and analyzed semiquantitatively. Data were analyzed with nonparametric tests and are presented as medians (25th–75th percentiles). No significant difference in microvascular blood flow was observed between healthy volunteers and patients before cardiac surgery or intensive care unit control subjects. The density of all vessels was significantly reduced in patients with severe sepsis (4.5 [4.2–5.2] versus 5.4 [5.4–6.3]/mm in volunteers, $p < 0.01$). The proportion of perfused small (< 20 μm) vessels was reduced in patients with sepsis (48 [33–61] versus 90 [89–92]% in volunteers, $p < 0.001$). These alterations were more severe in nonsurvivors. The topical application of acetylcholine totally reversed these alterations. In conclusion, microvascular blood flow alterations are frequent in patients with sepsis and are more severe in patients with a worse outcome.

Keywords: blood flow heterogeneity; healthy volunteers; microcirculation; septic shock; tissue oxygenation

Multiple organ failure occurs commonly in patients with sepsis, even after restoration of a stable hemodynamic status, and can be related to direct impairment of cellular functions or cytopathic hypoxia (1) and/or redistribution of blood flow between and within the organs at the microcirculatory level. The relative contribution of each of these factors is difficult to delineate.

Various experimental studies have reported a direct impairment of cellular functions after endotoxin administration, including uncoupling of cytochrome oxidative processes. Endotoxin can decrease the rate of oxygen consumption in hepatocyte (2) or enterocyte (1) cultures, although oxygen was adequately provided in the culture media. Evidence that this phenomenon may occur in humans is more limited (3). However, evidence reporting that early optimization of blood flow can improve survival in patients with sepsis (4) suggests that blood flow alterations remain important.

Alterations of microvascular blood flow have been described in various experimental models of sepsis (5–8). Endotoxin has been reported to induce marked microvascular alterations. In rats, the diameter of arterioles decreased in large arterioles but increased in small arterioles, whether cardiac output was increased or not (5). In dogs, capillary density is decreased (9). Similar observations have been reported in models of peritonitis. In a normodynamic sepsis model obtained by cecal ligation and perforation in rats, the perfused capillary density was reduced and the number of stopped-flow capillaries increased in striated muscles (6) and in the small bowel mucosa (8). In addition, the spatial distribution of perfused capillaries was much more heterogeneous. However, these alterations may not be similar in all organs, especially in models using an extraperitoneal source of infection. In an animal model of sepsis induced by lung instillation of live bacteria, microvascular blood flow alterations were observed in the muscularis layer, whereas blood flow to the mucosa was preserved (10).

Evidence that these microvascular alterations also occur in humans is tenuous; the lack of adequate techniques to investigate the microcirculation has been a major limitation. Indeed, the study of the microcirculation has long required the use of large microscopes, limiting the investigation to the nailfold area, where capillary blood cell velocity is decreased in normotensive febrile patients (11). Studies using laser Doppler techniques (12–14) or plethysmography (14, 15) in patients with severe sepsis have reported impaired microvascular blood flow and blunted hyperemic response after transient ischemia obtained by cuff inflation. However, these techniques provide only a global measurement of microvascular blood flow. The microvascular blood flow measurements obtained with laser Doppler techniques represent the average of the blood flow measured in all the vessels comprised in the sampling volume, whatever their direction and diameter. In addition, laser Doppler techniques do not assess the heterogeneity of the microcirculation, a major feature reported in experimental data.

Orthogonal polarization spectral (OPS) imaging has been developed as a noninvasive technique to investigate the human microvasculature (16). The technique has been described in detail elsewhere (16). Briefly, polarized light illuminates the area of interest, is reflected by the background, and is absorbed by hemoglobin. Specific optical filtration allows the elimination of the light reflected at the surface of the tissue to produce high-contrast reflected light images of the microcirculation. Hence, red blood cells will appear dark and white blood cells and platelets may be visible, sometimes, as refringent bodies. However, the vessel walls are not visible (Figure 1) and, therefore, vessels will be visible only if they contain red blood cells. The OPS imaging device is particularly convenient for studying tissues protected by a thin epithelial layer, such as mucosal surfaces. This novel technique has been validated in various experimental models (17–19).

We used this new technique to investigate the microcirculation of the sublingual area of patients with severe sepsis or septic shock and compared it with data obtained from healthy human volunteers. We hypothesized that microvascular blood flow alterations are present in patients with sepsis and that they are proportional to the severity of the disease.

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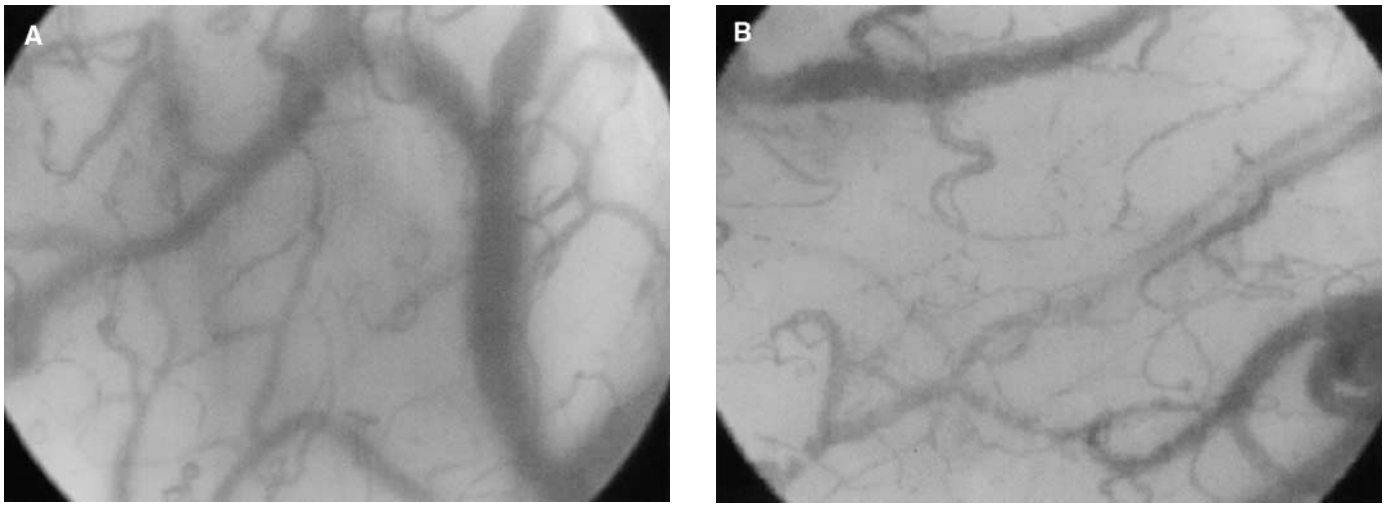

Figure 1. Representative examples of the sublingual microvasculature in a healthy volunteer (*A*) and in a patient with septic shock (*B*). Note the rich density in large and small vessels in the volunteer and the decrease in the density of small vessels in sepsis. Physiologic data of the volunteer: temperature, 36.8° C; heart rate, 65 bpm; mean arterial pressure, 82 mm Hg. Patient's hemodynamic data: temperature, 38° C; heart rate, 120 bpm; mean arterial pressure, 60 mm Hg; mean pulmonary artery pressure, 30 mm Hg; pulmonary artery occluded pressure, 16 mm Hg; right atrial pressure, 13 mm Hg; cardiac index, 3.5 L/min · m^2; pH 7.32; Pa_{CO_2}, 38 mm Hg; Pa_{O_2}, 65 mm Hg; Sa_{O_2}, 93%; mixed-venous oxygen saturation, 68%; hemoglobin, 8.1 g/dl; lactate, 2.9 mEq/L; dopamine, 20 μg/kg · min; norepinephrine, 0.4 μg/kg · min.

METHODS

This study included 50 patients with severe sepsis, defined on the basis of American College of Chest Physicians/Society of Critical Care Medicine consensus conference criteria (20). In each patient, the presence of infection was established using the Centers for Disease Control and Prevention criteria. Circulatory shock was defined as hypotension with a mean arterial pressure of less than 65 mm Hg requiring the administration of pressor agents (dopamine at dosages up to 20 μg/kg · min and/or norepinephrine at any dose) after the correction of hypovolemia.

Data obtained in these 50 patients with sepsis were compared with those obtained in 10 healthy volunteers, 16 patients before cardiac surgery, and 5 acutely ill noninfected patients (intensive care unit control subjects).

This study was approved by the ethics committee of Erasme University Hospital (Brussels, Belgium), and informed consent was obtained from the patients or their relatives.

General Management of Patients with Sepsis

All patients with sepsis had in place an arterial catheter and a central venous catheter; 43 patients were also monitored with a pulmonary artery catheter (Swan-Ganz catheter; Edwards, Irvine, CA). Fluid challenges with crystalloids or artificial colloids were repeated as needed to maximize stroke volume.

All patients were mechanically ventilated. If needed, light sedation (with midazolam up to 4 mg/hour) and analgesia (with morphine up to 3 mg/hour) were provided.

Measurements

Temperature, heart rate, arterial pressure, and central venous pressure were obtained in all patients. In patients monitored with a pulmonary artery catheter, complete hemodynamic measurements were obtained. After obtaining these measurements, arterial blood samples were drawn for the determination of blood gases, hemoglobin saturation, and hemoglobin and lactate concentrations (ABL700; Radiometer, Copenhagen, Denmark). Simultaneously, mixed venous blood samples were drawn for measurement of mixed-venous oxygen saturation. Oxygen delivery, oxygen consumption, and oxygen extraction were calculated according to standard formulas. The Acute Physiology and Chronic Health Evaluation (APACHE) II score (21) and the sepsis-related organ failure assessment (SOFA) score (22) were calculated.

Microvideoscopic Measurements and Analysis

The microvascular network of the sublingual mucosa was studied with a Cytoscan ARII (Cytometrics, Philadelphia, PA) with a ×5 objective providing ×167 magnification. The device was applied without pressure on the lateral side of the tongue, in an area roughly 1.5 to 4 cm from the tip of the tongue. Saliva and other secretions were gently removed with gauze. Five to seven areas were recorded on disk, using a computer and a video card (MiroVideo; Pinnacle Systems, Mountain View, CA). The five best sequences (minimum duration of 20 seconds each) were selected for technical reasons (absence or limited movement artifacts, absence of saliva film) and stored by random number designation for further analysis. An investigator blinded to the clinical data later analyzed these sequences semiquantitatively. Three equidistant horizontal and three equidistant vertical lines were drawn. The vascular density was calculated as the number of vessels crossing these lines divided by the total length of the lines. The type of flow was defined as continuous, intermittent, or absent. The vessels were separated into large and small vessels, using a diameter cutoff value of 20 μm (23, 24). In each patient, the data of the five areas were averaged. To assess the heterogeneity of microvascular blood flow between the five areas, the coefficient of variation of blood flow was calculated as the standard deviation of the five values of blood flow divided by their mean value.

The intra and interobserver variability were determined with three sequences that were analyzed five times at one week intervals by two observers (D.D. and M.J.D.). The coefficient of variability of the determination of one sequence ranged from 2.5 to 4.7% (intraobserver) and from 3.0 to 6.2% (interobserver) for the total number of vessels, and from 0.9 to 4.5% (intraobserver) and from 4.1 to 10.0% (interobserver) for the proportion of perfused vessels (all sizes).

Administration of Acetylcholine

In the last 11 patients with septic shock, we also tested whether local acetylcholine application could affect the sublingual microvasculature. After baseline measurements, gauze imbibed with acetylcholine at a concentration of 10^{-2} M was gently applied to the sublingual area for 1 minute. After removal of the gauze, measurements were repeated.

Statistical Analysis

After exclusion of a normal distribution of the data by Kolmogorov–Smirnov test, nonparametric tests were used. Differences between

groups were estimated by Kruskal–Wallis with *post hoc* Mann–Whitney analysis with adjustment for multiple comparisons. The effects of acetylcholine were assessed with a Wilcoxon test. To assess the possible influence of systemic factors, the relationship between the proportion of perfusion of the vessels (dependent variable) and some hemodynamic and biologic parameters was assessed by Spearman rank correlation. To investigate the possible consequence of the microcirculatory alterations, the correlation between the proportion of perfusion of the vessels (independent variable) and pH and lactate was similarly assessed. A p value lower than 0.05 was considered statistically significant. All analyses were performed with the StatView program (StatView for Windows, version 5; SAS Institute, Cary, NC). Data are presented as medians (25th–75th percentiles) in text and tables and as box plots in Figures 2–4.

RESULTS

We investigated 10 healthy volunteers (median age, 30 [26–36] years), 16 patients before cardiac surgery (median age, 66 [56–74] years), 5 intensive care unit control subjects (median age, 64 [52–66] years), and 50 patients with sepsis (median age, 61 [50–72] years), including 42 patients in septic shock. The intensive care unit control subjects included four males admitted after scheduled operation for aortic aneurysm and one female admitted for subarachnoid hemorrhage. All these patients were free of infection, and were intubated and mechanically ventilated. The patient with subarachnoid hemorrhage was treated with norepinephrine at a dosage of 0.2 μg/kg · min to maintain an adequate perfusion pressure. The characteristics of the patients with sepsis are listed in Table 1 and the main hemodynamic and biologic variables are listed in Table 2. The most common sources of infection were the lungs (42%) and the abdomen (36%). Most patients were treated with vasoactive agents, including an inotropic agent (38% dobutamine) and/or pressor agents (82% dopamine and/or 34% norepinephrine and 2% epinephrine).

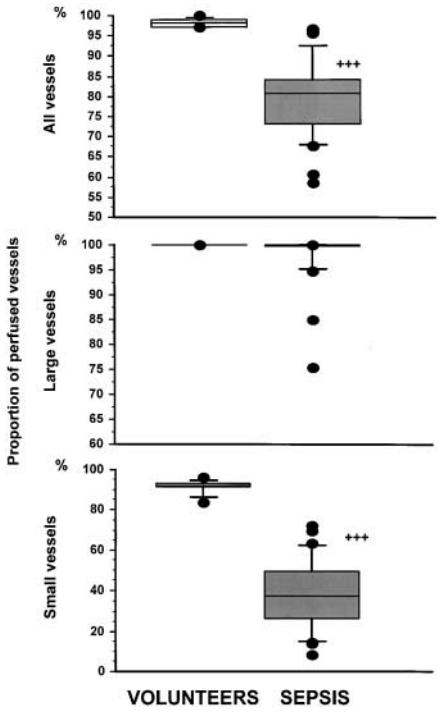

Figure 2. Proportion of perfused vessels. *Top*: All vessels. *Middle*: Large vessels (diameter larger than 20 μm). *Bottom*: Small vessels (diameter smaller or equal to 20 μm). Volunteers are represented by *open rectangles,* patients with sepsis by *gray rectangles.* $^{+++}p < 0.001$ versus volunteers. The physiologic data of the volunteers and of the patients are presented in Table 2.

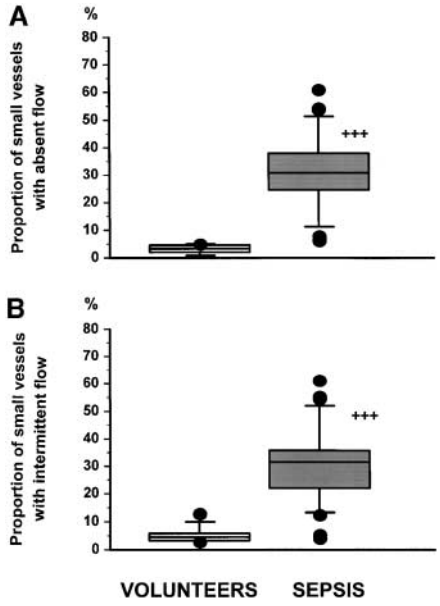

Figure 3. Proportion of small vessels with absent (*A*) or intermittent (*B*) perfusion. Volunteers are represented by *open rectangles,* patients with sepsis by *gray rectangles.* $^{+++}p < 0.001$ versus volunteers. The physiologic data of the volunteers and of the patients are presented in Table 2.

Characteristics of the Sublingual Microvascular Network

A typical example of sublingual vascularization is provided in Figure 1 and video recordings are available in the online data supplement (Figures E1 and E2). Healthy volunteers had a dense network of tortuous vessels, with very few small vessels not continuously perfused. In patients with sepsis, the proportion of small vessels typically decreased, and the proportion of nonperfused or intermittently perfused vessels increased. On a few occasions, we observed adherence or rolling of white blood cells to the walls of venules.

Vascular Density

No significant difference was observed between healthy volunteers and patients before cardiac surgery or the intensive care unit control subjects. The density of all vessels decreased significantly in sepsis (Table 3), suggesting that some vessels did not contain red blood cells. The density of perfused vessels also significantly decreased, especially for the small vessels (Table 3). The proportion of perfused vessels in patients with sepsis decreased in small vessels but not in large vessels (Figure 2). The decreased proportion of perfused vessels in small vessels was related to an increased proportion of both nonperfused and intermittently perfused vessels (Figure 3).

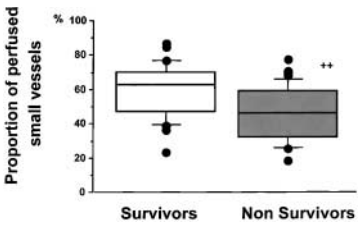

Figure 4. Proportion of perfused small vessels in survivors (n = 22) and nonsurvivors (n = 28). Survivors are represented by the *open rectangle,* nonsurvivors by the *gray rectangle.* $^{++}p < 0.01$ versus survivors. The physiologic data of these patients are presented in Table E1.

TABLE 1. CHARACTERISTICS OF PATIENTS WITH SEPSIS

Characteristic	Value
Source of infection	
Lung, n	21
Abdominal, n	18
Urinary tract, n	4
Soft tissues, n	4
Miscellaneous, n	3
Vasoactive agents*	
Dobutamine, μg/kg · min	19; 10 (5–20)
Dopamine, μg/kg · min	41; 20 (12–20)
Norepinephrine, μg/kg · min	17; 0.22 (0.12–0.86)
Epinephrine, μg/kg · min	1; 0.1
Analgo-sedation*	
Morphine, mg/min	32; 2 (1–2)
Midazolam, mg/min	29; 2 (1–2)
APACHE II score†	21 (17–25)
SOFA score‡	13 (10–15)
No. of survivors, n (%)	22 (44%)

Definition of abbreviations: APACHE = acute physiology and chronic health evaluation; SOFA = sepsis-related organ failure assessment.

* For adrenergic agents and analgo-sedation, data are presented as number of patients; treated with dose (25th–75th percentiles).

† Knauss and coworkers (21).

‡ Vincent and coworkers (22).

Variability between Areas

The coefficient of variability for the perfused vessels between the five sublingual sites was significantly greater in patients with sepsis than in volunteers (22 [15–26] versus 13 [11–17]%, $p < 0.05$).

Relationship with Severity of the Disease and Influence of Systemic Factors

The proportion of perfused vessels was higher in the survivors than in the nonsurvivors when considering all vessels (90.4 [80.7–95.3] versus 85.4 [79.4–88.0]%, $p < 0.05$), as well as small vessels (Figure 4). The proportion of perfused vessels was similar in patients treated with or without adrenergic agents (in small vessels 47.1 [32.9–59.8] versus 56.9 [32.0–61.5]%, respectively, p = NS).

For all vessels and for large vessels, there was no significant relationship between the proportion of perfused vessels and temperature, mean arterial pressure, cardiac index, pH, mixed-venous oxygen saturation, hemoglobin concentration, and lactate level. In small vessels, the proportion of perfused vessels was weakly but significantly related to pH ($0.02 \times pH + 7.23$, $r^2 = 0.26$; $p < 0.001$) and inversely related to lactate level ($-0.02 \times$ lactate $+ 3.96$, $r^2 = 0.10$; $p < 0.05$), whereas the other relationships were not significant.

Effects of Acetylcholine

Acetylcholine was administered locally in 11 patients with septic shock treated with high doses of vasoactive agents (dopamine, n = 11, 20 [15–20] μg/kg · min; norepinephrine, n = 4, 0.9 [0.4–1.2] μg/kg · min; epinephrine, n = 1, 8 μg/kg · min; dobutamine, n = 7, 20 [5–20] μg/kg · min) and with a poor outcome (survivors, n = 4; 36%). In these patients, topical application of acetylcholine significantly increased the number of vessels and the proportion of perfused vessels up to values similar to those seen in the volunteers (Table 4).

DISCUSSION

This is the first study reporting direct visualization of microvascular blood flow in critically ill patients. OPS imaging techniques document significant microvascular blood flow alterations in patients with sepsis. These include decreased vascular density, especially in the small vessels, a large number of nonperfused and intermittently perfused vessels in small vessels, and a marked heterogeneity between the areas. These alterations were more severe in nonsurvivors, were not affected by the global hemodynamic state or the use of adrenergic agents, and were totally reversible with the topical application of acetylcholine.

The use of OPS imaging techniques to visualize the microcirculation has been validated in animals and in humans. When

TABLE 2. PRINCIPAL PHYSIOLOGIC VARIABLES OF HEALTHY VOLUNTEERS, PATIENTS BEFORE CARDIAC SURGERY, INTENSIVE CARE UNIT CONTROL SUBJECTS, AND PATIENTS WITH SEPSIS

	Healthy Volunteers	Patients before Cardiac Surgery	ICU Control Subjects	Patients with Sepsis
Age, yr	29 (25–35)	66 (56–74)*	64 (56–69)*	61 (50–72)*
Temperature, ° C	37.0 (36.8–37.1)	36.7 (36.0–37.1)	36.8 (36.4–37.5)	37.0 (36.4–38.0)
Heart rate, bpm	69 (64–72)	68 (65–74)	69 (63–106)	105 (91–110)
Mean arterial pressure, mm Hg	82 (80–87)†	91 (79–99)‡	88 (75–94)†	71 (63–79)
Cardiac index, L/min · m^2	N/A	N/A	3.03 (2.4–3.60)	3.63 (2.62–4.69)
$\dot{D}_{O_2}$, ml/min · m^2	N/A	N/A	440 (380–499)	421 (333–525)
$\dot{V}_{O_2}$, ml/min · m^2	N/A	N/A	119 (115–134)	122 (96–152)
E_{O_2}, %	N/A	N/A	29 (27–30)	29 (24–33)
pH	N/A	N/A	7.44 (7.35–7.48)†	7.35 (7.27–7.39)
Pa_{CO_2}, mm Hg	N/A	N/A	33 (28–34)	37 (32–43)
Pa_{O_2}, mm Hg	N/A	N/A	142 (107–169)†	94 (73–108)
Sa_{O_2}, %	N/A	N/A	99 (99–99)†	97 (94–98)
$S\bar{v}_{O_2}$, %	N/A	N/A	70 (69–72.5)	68 (62–73)
Hemoglobin, g/dl	N/A	12.6 (11.5–14.5)‡	11.1 (9.2–11.8)‡	8.3 (7.4–9.9)
Lactate, mEq/L	N/A	N/A	1.4 (1.3–1.7)	2.2 (1.5–3.4)
APACHE II score§	N/A	5 (3–5)‡	10 (7–20)†	21 (17–25)
SOFA score‖	N/A	0 (0–0)‡	3 (1–8)‡	13 (10–15)

Definition of abbreviations: $\dot{D}_{O_2}$ = oxygen delivery; E_{O_2} = oxygen extraction; ICU = intensive care unit; N/A = not available; $S\bar{v}_{O_2}$ = mixed venous oxygen saturation; $_{O_2}$ = oxygen consumption.

* $p < 0.01$ versus volunteers.

† $p < 0.05$ versus sepsis.

‡ $p < 0.01$ versus sepsis.

§ Knauss and coworkers (21).

‖ Vincent and coworkers (22).

TABLE 3. VESSEL DENSITY

	Healthy Volunteers	Patients before Cardiac Surgery	ICU Control Subjects	Patients with Sepsis
Total number of vessels	5.4 (5.4–6.3)	5.8 (5.4–6.7)	5.7 (5.4–6.0)	4.5 (4.2–5.2)*
Perfused vessels	5.4 (5.2–6.1)	5.8 (5.3–6.6)	5.7 (5.3–5.9)	3.6 (3.1–4.3)†
Perfused large vessels	2.3 (2.2–2.6)	1.9 (1.4–2.3)	2.4 (2.1–2.6)	1.7 (1.4–1.9)†
Perfused small vessels	3.1 (2.8–3.2)	3.8 (3.2–4.5)	3.1 (2.9–3.3)	1.1 (0.7–1.5)†

Vessel density is expressed as number per millimeter. Data are presented as medians (25th–75th percentiles).
* $p < 0.01$ versus volunteers.
† $p < 0.001$ versus volunteers.

OPS imaging and standard intravital fluorescence video microscopy were used to observe a hamster dorsal skinfold chamber, vessel diameters and functional capillary density were similar with both techniques (16) and the agreement in red blood cell velocity between the two techniques was good, both under control conditions and during ischemia (17). In addition, measurements of microvascular blood flow with OPS imaging were not influenced by the hemoglobin concentration (18). The OPS technique has also been validated for the observation of nailfold microcirculation in healthy humans (19): Capillary blood flow and vessel diameters were similar when measured by OPS imaging and by conventional capillaroscopy. However, our observations of the human sublingual microcirculation are limited by the semiquantitative analysis of the data. Direct quantitative measurement of blood flow in each vessel was not feasible for two reasons. First, the image studied corresponds to the projection onto a plane of the vessels located in a small volume. In this volume, the vessels are tortuous and oriented in multiple directions, so that the identification of each vessel is impossible. We had to estimate capillary density by an indirect count corresponding to the intersection with three equidistant horizontal and vertical lines. Second, small movement artifacts made measurements of blood flow in each capillary impossible, as it was not possible to eliminate movements of the background. To minimize observer-related bias, and despite the limited interobserver variability, all measurements were performed in a blind fashion by the same investigator, and the intraobserver variability was less than 5%.

Our results are in accordance with experimental data reporting decreased capillary density and increased blood flow heterogeneity in various experimental models of sepsis (6, 8, 9). Previous human studies using laser Doppler techniques (12–14) or plethysmography (14, 15) have suggested that microvascular blood flow is impaired in sepsis. Skin blood flow was increased (13, 15), whereas resting skeletal muscle blood flow was decreased (12), even when whole body oxygen delivery was elevated. More importantly, the hyperemic response after transient ischemia obtained by cuff inflation was blunted in patients with sepsis (12, 13, 15, 25). Using an indirect method to investigate microvascular blood flow, Christ and coworkers (26) observed in a mixed group of critically ill patients that microvascular blood flow and vasomotion were more severely altered in nonsurvivors than in survivors. Our observations extended these data, demonstrating the increased heterogeneity in blood flow. Our observations also precisely located these alterations to vessels smaller than 20 μm in diameter.

Multiple causes can be suggested to explain these microvascular alterations, but the immediate restoration of a normal microvascular pattern after acetylcholine suggests that vasoconstriction played a central role. Thus, the microvascular endothelial response to vasodilating stimuli was preserved (27) even though some endothelial dysfunction cannot be excluded in the absence of a dose–response study. Adrenergic agents may be responsible for vasoconstriction in the microvasculature (28, 29). However, we observed that the microvascular alterations were similar in patients with sepsis, regardless of the administration of adrenergic agents, so that these could not have played a major role. Various inflammatory mediators may be involved. In rats, the administration of tumor necrosis factor, a central mediator of sepsis, elicited a decrease in microvascular blood flow (30). Also, endothelin, a potent vasoconstrictor often found to be elevated in patients with sepsis (31), can cause microvascular vasoconstriction. Several other mechanisms may have coexisted and would further impair microvascular blood flow. First, microthrombi can form under septic conditions (32, 33). This mechanism is strongly supported by the results of a study demonstrating that the administration of activated protein C significantly improved survival of patients with severe sepsis (34). Second, sepsis impairs leukocyte (35) and erythrocyte (15, 25) deformability and promotes adhesion to endothelial cells (15, 36). In some patients, we observed leukocytes adhering to or rolling on the walls of venules, but the technique does not allow this phenomenon to

TABLE 4. EFFECT OF TOPICAL ACETYLCHOLINE ADMINISTRATION IN 11 PATIENTS WITH SEPSIS

	Patients with Sepsis*,† (*n = 11*)		Volunteers (*n = 10*)
	Baseline	Acetylcholine (10^{-2} *M*)	
Total number of vessels, n/mm	4.9 (4.1–5.7)	6.0 (4.7–6.4)‡	5.4 (5.4–6.3)‡
Proportion of vessels perfused, %	83 (77–96)	99 (98–100)‡	98 (97–99)‡
Proportion of venules perfused, %	100 (100–100)	100 (100–100)	100 (100–100)
Proportion of capillaries perfused, %	44 (24–60)	94 (77–96)‡	94 (92–95)‡
Absent flow (capillaries), %	29 (8–44)	1 (0–3)‡	3 (2–5)‡
Intermittent flow (capillaries), %	24 (19–38)	8 (3–19)‡	5 (3–6)‡

Data are presented as medians (25th–75th percentiles).
* All the patients with sepsis were treated with vasoactive agents (dopamine, n = 11, 20 [15–20] μg/kg · min; norepinephrine, n = 4, 1.23 [0.59–2.10] μg/kg · min; dobutamine, n = 7, 20 [5–20] μg/kg · min; epinephrine, n = 1, 0.1 μg/kg · min).
† The principal physiologic variables of the septic patients are reported in Table E2 in the online data supplement.
‡ $p < 0.01$ versus sepsis.

be visualized reliably. Also, microvascular blood flow heterogeneity can occur independently of leukocyte rolling and adhesion (37). Third, interstitial edema may compress small vessels. However, the increase in erythrocyte flow heterogeneity after cecal ligation and perforation in rats was not related to tissue edema as measured by wet-to-dry ratio and albumin flux (7). Hence, it is likely that many of these mechanisms contributed to the microvascular alterations, with reversible vasoconstriction in some capillaries playing a predominant role.

An important finding is that the topical application of acetylcholine was able to fully reverse the sepsis-induced microvascular blood flow alterations. The relaxation in response to acetylcholine is decreased in large arteries as well as in proximal arterioles in sepsis (38). The effects of acetylcholine on the microcirculation are more controversial. Some authors have observed that acetylcholine-induced relaxation in the smallest arterioles was preserved or even increased under inflammatory conditions (39, 40). On the other hand, Tyml and coworkers (29) observed a decreased dilatory response to acetylcholine after cecal ligation in rats, with a smaller increase in red blood cell velocity in capillaries. In patients with septic shock, Kubli and coworkers (41) observed that topical acetylcholine application increased skin microvascular blood flow, estimated by laser Doppler techniques. Of note, an increase in laser Doppler blood flow does not necessarily imply an improvement in the microcirculation, because heterogeneity of blood flow may be unaffected. We observed that topical acetylcholine not only increased microvascular blood flow but also decreased the blood flow heterogeneity, indicating that the endothelium in the microcirculation is still able to respond to vasodilatory stimuli in humans.

Because OPS imaging techniques use light absorbance by hemoglobin, vessels can be visualized only when these are filled by red blood cells. Hence, the decrease in the absolute number of vessels per field, whatever the type of flow, implies that some vessels did not contain any red blood cells. This was not artifactual because it was restored after topical application of acetylcholine.

Several factors may have influenced the sublingual microcirculation of the patients with sepsis, including age, oral intubation, analgesia and sedation, arterial pressure, hemoglobin concentrations, and blood temperature. We eliminated the role of these confounding factors by investigating the sublingual microcirculation of three different control groups, including an intensive care unit control group in which patients were also intubated and had a similar type and level of analgesia and sedation. Blood temperature was similar in all groups and the median blood temperature of the patients with sepsis was within the normal range. Although mean arterial pressure was lower in patients with sepsis, this factor alone is unlikely to explain the differences we observed. Animal studies suggest that the microcirculatory alterations are independent of arterial hypotension. The perfusion of diaphragmatic capillaries was markedly decreased in septic rats compared with hypovolemic control subjects with a similar degree of hypotension (42). In addition, the microcirculatory alterations were not correlated with blood pressure in the patients with sepsis we studied. Although hemoglobin concentration was slightly lower in patients with sepsis than in control subjects, these differences are unlikely to explain the development of microcirculatory alterations, and if anything, should improve the microcirculation (43, 44). Hence, the microvascular alterations observed can be attributed to sepsis.

Alterations observed in the sublingual area may not be representative of other areas. It is commonly accepted that the splanchnic circulation may be altered earlier, and recover later, than other parts of the body. However, the sublingual mucosa, which shares a similar embryologic origin with the digestive mucosa, may also be of interest. Weil and coworkers (45, 46) reported that sublingual capnometry similarly reflected the severity of shock states and outcome. Sublingual capnometry and gastric tonometry revealed parallel alterations, suggesting that both areas can be similarly and simultaneously affected. These results were confirmed by another group of investigators (47). Hence, the sublingual region may be more readily accessible than, and as useful as, the splanchnic area for monitoring.

Our observations may have important implications, especially regarding the controversy between the cellular and vascular origin of tissue oxygenation impairment in sepsis. Experimental studies suggest that alterations in microvascular blood flow may be responsible for tissue hypoxia. Using phosphorescence quenching, Ince and Sinaasappel (48) reported that microvascular P_{O_2} was lower than venular P_{O_2}, suggesting microvascular shunting. The heterogeneity of microvascular blood flow may account for the alterations in oxygen extraction capabilities that can occur in sepsis. In a mathematical model of the determinants of oxygen delivery and consumption (49), an increase in blood flow heterogeneity was associated with an increase in critical oxygen delivery. These results were supported by experimental data reporting that gut (23) and muscle (50) blood flow heterogeneity increased together with the impaired oxygen extraction after endotoxin administration or fecal peritonitis in pigs. In our study, microvascular alterations were more severe in patients with a worse outcome. Finally, the complete reversal of the sepsis-induced microvascular blood flow alterations with topical application of acetylcholine suggests that further research should focus on agents able to dilate the microcirculation under septic conditions.

In conclusion, microvascular alterations are frequent in patients with sepsis and can be easily visualized with OPS imaging. This technique has the advantage that it can be used easily at the bedside. Further studies are required to characterize the time course of these alterations as well as the influence of various therapeutic interventions.

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