Microcirculatory dysfunction in sepsis: pathophysiology, clinical monitoring, and potential therapies

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Abstract

Abnormal microvascular perfusion, including decreased functional capillary density and increased blood flow heterogeneity, is observed in early stages of the systemic inflammatory response to infection and appears to have prognostic significance in human sepsis. It is now known that improvements in systemic hemodynamics are weakly correlated with the correction of microcirculatory parameters despite an appropriate treatment of macro-hemodynamic abnormalities. Furthermore, conventional hemodynamic monitoring systems available in clinical practice fail to detect microcirculatory parameters changes and responses to treatments as they do not evaluate intrinsic events that occur in the microcirculation. Fortunately, there are some bedside diagnostic methods and therapeutic options specifically directed to the assessment and treatment of microcirculatory changes. In the present review, we discuss fundamental aspects of septic microcirculatory abnormalities, including pathophysiology, clinical monitoring, and potential therapies.

Keywords: Microcirculation; Sepsis; Microcirculatory monitoring; Microvascular resuscitation.
**Introduction**

In early stages of sepsis, proinflammatory cytokines are released in an attempt to eliminate the offending agent, generating an intense response that impairs the microcirculation (24) (69). Nearly every cellular component of the microcirculation is affected, including endothelial cells, smooth muscle cells, platelets, leukocytes, red blood cells, and adjacent parenchyma cells (59). Resulting microcirculatory dysfunction is characterized by increased number of capillaries with stopped flow and maldistribution of microvascular blood flow (15) (40) (68). These microcirculatory changes have already been observed in different models of sepsis, organs, and species (5) (31) (42) (68) (78) (95) (101) (111) and appear to have prognostic significance in human sepsis, as the severity of initial microcirculatory derangements in the early resuscitation phase of therapy and their persistence over time have been associated with lower survival rates (5) (7) (93) (105).

This paper is intended as a review of fundamental aspects of septic microcirculatory abnormalities, including pathophysiology mechanisms, available techniques for clinical monitoring, and potential therapies to rescue the microcirculation.

**The microcirculation**

The microcirculation consists of vessels with diameters less than 100 micrometers (arterioles, capillaries, venules, and microlymphatics). It is the major
site of blood oxygen release to tissues and works as an integrated system that ensures the adequacy of tissue oxygen delivery to cell oxygen demand. Main cell types found in the microvasculature are endothelial cells (which line the inside of blood vessels), smooth muscle cells (mainly present in arterioles), red blood cells, leukocytes, and platelets (59). Endothelial cells play a central role in the control of the microcirculatory function, regulating microvascular thrombosis and fibrinolysis, leukocyte adhesion and migration, vasomotor tone, trafficking of cells and nutrients, and capillary permeability and recruitment (1).

Pathophysiology of microcirculatory changes in sepsis

Local distribution of blood flow to tissues is regulated by the microcirculation. This is possible because, under physiological conditions, endothelial cells sense metabolic and physical signs and respond by regulating microvascular flow through local release of vasodilators, especially nitric oxide (NO), modulating arteriolar smooth muscle cell tone. NO is an activator of the soluble guanylate cyclase enzyme, responsible for the production of cyclic guanosine monophosphate (cGMP), the mediator of smooth muscle cell relaxation. Therefore, NO is considered a key component in the maintenance and autoregulation of the homeostasis and patency of the microcirculation (52) (104).

During sepsis, the NO system is severely affected: inducible nitric oxide synthase (iNOS) becomes heterogeneously expressed in different organic vascular beds, resulting in pathological shunt of microvascular blood flow (via arteriovenous
shunts), inappropriately deviating blood flow from suffering units. Thus, iNOS-deficient areas, which are less vasodilated, become hypoperfused (57) (104). Furthermore, increased reactive oxygen species production during sepsis interferes with NO formation by the endothelial nitric oxide synthase (eNOS) and with formed NO, further reducing its concentration (22).

In addition to NO dysregulation, the function of many cell types found in the microcirculation is impaired in sepsis syndrome (Figure 1). Endothelial cells lose their regulatory function as a consequence of changes in signal transduction pathways, loss of electrophysiological communication, and loss of control over the arteriolar smooth muscle cells (59) (70) (71) (109). Besides losing their regulation by endothelial cells, arteriolar smooth muscle cells lose their adrenergic sensitivity and tonus, contributing to perfusion abnormalities in the microcirculation (88) (107). This impaired vascular responsiveness to stimuli appears to be a result of excessive NO production by iNOS (115).

In physiological conditions, red blood cells act as both a deliverer of oxygen and a sensor of local oxygen gradients, contributing to the auto-regulation of microvascular blood flow and oxygen delivery (14). Hemoglobin has an active role in this process, as increased local oxygen gradients induce conformational changes in the hemoglobin molecule and by altering its conformation hemoglobin facilitates the release of nitrosothiol (a NO derivative) and vasoactive ATP, signaling the microvasculature to vasodilate (61) (97). During sepsis, red blood cells lose their ability to release vasodilators in the presence of hypoxia, impairing an important physiological regulatory mechanism of microcirculatory blood flow
Moreover, red blood cells become less deformable and more easily aggregate to endothelial cells during sepsis, compromising blood flow (13) (25) (38).

The percentage of activated neutrophils, with reduced deformability and increased aggregability due to increased expression of adhesion molecules on both endothelial and immune cells, also increases during sepsis (66) (78) (107). These leukocytes generate reactive oxygen species and other inflammatory mediators that directly disrupt microcirculatory structures, such as the endothelial glycocalyx (a complex macromolecular network involved in several endothelial functions). Oxidative stress results in changes in endothelial glycocalyx structure and physiology (23) (51) (100). Continuous glycocalyx degradation and shedding exposes endothelial cells to oxidative damage, leading to loss of integrity of adherens junctions and increased paracellular permeability with subsequent impairment of endothelial barrier function (75). Increased endothelial permeability results in fluid leakage from the intravascular space and tissue edema (23) (51) (100). Accumulation of water in tissues finally leads to tissue hypoxia due to increased diffusion distances between functional capillaries and tissue cells in combination with poor oxygen solubility and transport in tissue water (60). Endothelial glycocalyx degradation during sepsis coincides with microcirculatory dysfunction and has been associated with several sepsis-associated clinical conditions, including acute lung injury and cardiovascular dysfunction (75) (84) (94). More importantly, blood levels of glycocalyx components are significantly higher in nonsurvivors than in survivors of septic shock, suggesting that glycocalyx
shedding may have prognostic significance in human sepsis (84). Glycocalyx disruption also contributes to enhanced expression of adhesion molecules with increased leukocyte trafficking and to a shift toward a pro-coagulant state (33) (62). The resulting pro-adhesive and prothrombotic effect further promotes the adhesion of red blood cells, leukocytes, and platelets to the vascular endothelium, causing vascular microthrombosis, capillary plugging, and greater compromise of capillary flow (21) (22) (26) (47) (52) (95). Additional activation of coagulation pathways results in capillary obstruction by platelet/fibrin clots secondary to disseminated intravascular coagulation, contributing to microcirculatory derangements (6) (52) (95).

Together, all aforementioned mechanisms lead to a reduction in perfused capillaries (Figure 2). Decreased functional capillary density (number of spontaneously perfused capillaries by analyzed tissue area) results in an increased distance for oxygen diffusion to surrounding parenchymal cells (40) (42) (68) (104). Thus, it is reasonable to suggest that abnormal tissue oxygen transport and tissue hypoxia may ensue when microcirculatory function is impaired, as in sepsis. Supporting this hypothesis, Ellis and colleagues (40) have shown that profound maldistribution of microvascular blood flow occurred along with changes in oxygen extraction in early stages of experimental sepsis. They suggested that impaired tissue oxygen transport is likely the result of microcirculatory dysfunction (40). In another experimental study, Bateman and colleagues have suggested that loss of microvascular autoregulation during sepsis uncouples local oxygen demand and delivery leaving some tissue regions vulnerable to hypoxia and unable to rapidly
respond to oxygen demand (15). In experimental conditions, septic microvascular abnormalities could be linked to surrogate markers of tissue hypoxia. For instance, microvascular heterogeneity has been associated with hypoxia-inducible factor gene expression (16). Furthermore, reversal of microcirculatory abnormalities has been associated with proportional improvements in lactate and NADH levels (4) (65). Finally, mathematical models of experimental sepsis predict that decreased capillary density and increased flow heterogeneity may play a role in tissue hypoxia, further corroborating the concept that microcirculatory dysfunction impairs tissue oxygenation (48) (49).

Besides microcirculatory dysfunction, other mechanisms at cellular level, such as cellular metabolic alterations, mitochondrial dysfunction, and dysregulated apoptosis, also take place during sepsis. However, these cellular abnormalities are regarded as late adaptive responses, which may be preceded or triggered by microcirculatory failure (98) (104). Indeed, evidence suggests that microcirculatory derangements are the primary event leading to cellular dysfunction, reinforcing the importance of microcirculatory assessment (10). For instance, Eipel and colleagues have shown that microvascular abnormalities are associated with cellular injury during experimental sepsis, leading to apoptosis (39). In another experimental study, Rosengarten and colleagues have demonstrated that microcirculatory dysfunction precedes sepsis-induced disturbances of neuronal cell function (91). Finally, in vitro studies have shown that NO inhibits mitochondrial respiration decreasing oxygen consumption during sepsis (20) (43) (73). This finding raises the hypothesis that sepsis-induced NO overproduction (an important
element in the pathophysiology of microcirculatory dysfunction) may contribute to both tissue hypoxia and mitochondrial inhibition (14) (20).

If not corrected, tissue hypoxia, metabolite accumulation, and mitochondrial dysfunction of parenchymal cells trigger a cascade of pathogenic mechanisms that eventually leads to organ failure and death (15) (59). The underlying process that culminates in organ dysfunction in sepsis is not fully understood but microvascular alterations have been implicated (113). Indeed, Doerschung and colleagues have shown that impaired microvascular reactivity is related to tissue dysoxia and organ dysfunction in humans sepsis (32). Sakr and colleagues (93) have also shown an association between microcirculatory alterations and organ dysfunction in human subjects. More importantly, capillary perfusion was related to the severity of organ failure when shock resolved (93). Later trials have corroborated these findings, demonstrating an association between the severity of microvascular dysfunction and the development of organ failure (96) (105) (106). All these studies have suggested the existence of a potential connection between microcirculatory alterations and multiple organ failure in sepsis. However, statistical association between outcome and microcirculatory derangements does not imply a mechanistic relation. To date, a cause-and-effect relationship could not be demonstrated (52).

Septic microcirculatory impairment is characterized by heterogeneity of blood flow, with some capillaries being hypoperfused while others exhibit normal or even abnormally high blood flow (15) (37) (40) (110). Some vulnerable microcirculatory units become hypoxic, determining the oxygen extraction deficit
associated with sepsis. Due to microcirculatory perfusion heterogeneity, areas of hypoxic tissue may be found in close vicinity to well-oxygenated ones. An impaired ability to regulate local oxygen delivery becomes evident and may further contribute to impaired oxygen extraction (32) (34) (40) (57).

Although microcirculatory dysfunction may occur to varying degrees in most clinical conditions that result in shock, autoregulatory mechanisms of microvascular function are most severely impaired during sepsis, indicating the microcirculatory dysfunction as a pathophysiological mark of sepsis syndrome (83) (104).

**Clinical monitoring**

Hemodynamic coherence between macro and microcirculation exists when therapeutic interventions aimed at the correction of systemic hemodynamics variables are effective in correcting regional and microcirculatory parameters (60). Loss of such coherence is most frequently found during sepsis, when microcirculatory recruitment is hardly observed despite an appropriate treatment of macro-hemodynamic abnormalities (60) (93) (106). The disparity between macro and micro-hemodynamic is explained by the presence of functional shunts in the microcirculation that "hide" the microcirculatory dysfunction from systemic circulation. Sepsis is associated with dysregulation of the opening of these shunts, pushing the microcirculatory partial pressure of oxygen (pO$_2$) to a level below the venous one, constituting the "pO$_2$ gap" and determining tissue distress despite adequate systemic hemodynamic parameters (57). This distributive defect
associated with sepsis is characterized by stagnation of capillary blood flow, reducing functional capillary density, in the presence of practically normal flow in larger blood vessels of the microcirculation (59). Microvascular blood flow depression occurs more intensely in smaller vessels, affecting fewer larger vessels, which partly explains why systemic hemodynamic variables do not reflect the hemodynamic properties of the microcirculation in a reliable manner. Hypoxic tissues with severe oxygen extraction deficit may be found even when total blood flow to the respective organ is preserved (58).

Besides blood flow heterogeneity, other microcirculatory alterations underlying the loss of hemodynamic coherence have also been identified, such as hemodilutional microvascular anemia, stasis of microcirculatory blood flow, and increased capillary leak (60). These alterations cannot be detected by conventional hemodynamic monitoring systems available in clinical practice. By simply monitoring the macrocirculation, these systems fail to detect changes in microcirculatory parameters and responses to treatments as they do not evaluate intrinsic events that occur in the microcirculation (58) (60).

A reliable diagnostic tool capable of assessing the microcirculation is urged in the diagnosis and treatment of sepsis. The ideal monitoring method for critically ill patients should allow assessment at bedside and be able to assess local transport of oxygen and changes in vascular permeability, inflammatory response, and coagulation. Finally, the method should allow direct noninvasive visualization of microvascular changes, such as decreases in functional capillary density, vascular diameters, and flow rates (11). Unfortunately, there is no single method
capable of monitoring all these functions related to the microcirculation. Moreover, whatever the monitoring method, it should be known that only the microvascular bed on which the method is being used is actually being assessed at that time. This is particularly important in sepsis, a systemic syndrome characterized by great heterogeneity of the microcirculation.

Currently available methods in clinical use for microvascular perfusion monitoring can be divided into biomarkers, videomicroscopy techniques, tissue oxygenation evaluation techniques, and partial pressure of carbon dioxide (pCO₂) based evaluation techniques.

Biomarkers

Blood lactate measurement is widely used in the evaluation of critically ill patients. When present, hyperlactatemia may indicate tissue hypoperfusion with increased lactate production by anaerobic metabolism, but other mechanisms not related to cellular hypoxia and anaerobic metabolism may explain the elevation of lactate concentration in critically ill patients. When absent, inadequate tissue perfusion is not excluded, being a diagnostic test with low sensitivity and specificity. Despite its limitations, lactate is a good surrogate marker of tissue hypoperfusion in shock states. A blood lactate level ≥ 4 mmol.l⁻¹ increases the probability of finding clinically relevant microcirculatory abnormalities, which may help to explain the high mortality risk associated with hyperlactatemia. Accordingly, a decrease of at least 10 percent in serial measurements of blood lactate level is
part of the goals to be achieved in the treatment of severe sepsis/septic shock,
existing evidence showing a correlation between lactate level fall and improved
microvascular perfusion (2) (52) (63) (85).

Direct biomarkers of endothelial damage could be of great interest during
sepsis. For instance, adhesion molecules (intercellular adhesion molecule-1
[ICAM-1], vascular cell adhesion molecule-1 [VCAM-1], E-selectin, and P-selectin)
can be assayed in the serum of septic patients and reflect endothelial cell
activation (or endothelium disruption) and its interaction with leukocytes. Although
not commonly used in clinical practice, serum increase of these molecules was
correlated with severe microcirculatory changes in pediatric patients with
meningococcemia, pending validation (86).

Direct microcirculatory assessment (videomicroscopy techniques)

Historically, intravital microscopy needed anatomical dissections to be
performed in animal models. The development of new techniques and portable
instruments for minimally invasive microscopy allowed direct visualization of the
microcirculation in tissues using videocapillaroscopy, orthogonal polarization
spectral imaging (OPS), sidestream dark field (SDF), or incident dark field (IDF)
illumination, making it possible to assess the microcirculation in humans. The main
limitation of all these methods is related to the difficulty of their continued use,
limiting the information to that acquired at the time of the examination.
Nailfold videocapillaroscopy was the first method used at bedside and evaluates capillaries located at the region between the cuticle and the nail using a microscope (114). It allows the detection of morphological, capillary density, and microvascular flow abnormalities at rest and after vascular occlusion tests (arterial and venous). This method has not become popular for the monitoring of septic patients because the nailfold vascular bed is very sensitive to peripheral vasoconstriction, vasopressor agents, and changes in temperature, making its use very restricted in this population.

OPS and SDF techniques are based on a common principle: the use of polarized light with a wavelength of 530 nm, absorbed by the hemoglobin of red blood cells regardless of its state of oxygenation, generating grayscale images. Thus, only blood vessels filled with red blood cells are displayed. In OPS, depolarized light provides the contrast when it is reflected by deeper layers of the evaluated tissue, generating a clear background. In SDF, image contrast is given by a pulsating green light. Unfortunately, for technical reasons, neither method allows direct visualization of blood vessel walls. Captured image is optically magnified five times, and digitally increased 340 times in OPS or 380 times in SDF. Functional capillary density, predominant type of blood flow, and degree of flow heterogeneity are generally analyzed (9). Microcirculatory blood flow parameters are calculated as surrogates of the convective capacity of the microcirculation, while functional capillary density represents the diffusive capacity (52). Among these variables, functional capillary density is the most validated parameter to assess microcirculatory function. Unfortunately, specific cut-off values that
correlate to relevant clinical outcomes, such as disease severity or mortality, have not been identified yet (52). Main limitations for the use of these technologies include the need of special equipment and operator training (to avoid pressure and motion artifacts) and the possibility of bleeding of the site under analysis. OPS technique is more prone to artifacts and is no longer routinely used (19).

Orthogonal polarization techniques reach a depth of five millimeters, only allowing microcirculatory assessment in tissues covered by a thin epithelial layer, being the sublingual mucosa the most studied and used (Figure 3). This region is preferred due to its embryological origin, which is the same as the gastrointestinal tract. Consequently, microcirculatory changes found in the sublingual mucosa could reflect splanchnic microcirculatory abnormalities (5). However, a correlation between sublingual and intestinal microcirculatory parameters is still in debate (52).

Recently, a third generation handheld video-microscope, based on the principle of IDF illumination, has been introduced for clinical use. This imaging device was developed in an attempt to overcome technical limitations of its predecessors (OPS and SDF) which resulted in poor quality of image acquisition, such as use of analogue video cameras, heavy weight of the devices leading to pressure-induced microcirculatory alterations, and the requirement for hand operated focusing. A pen-like lightweight probe, highly illuminating light emitting diodes (LEDs), high-resolution lenses, computer-controlled high-resolution image sensor, and improved focusing mechanism are some of the technical improvements that have been implemented in the IDF video-microscope. Additionally, a very short LED pulse time is utilized to avoid motion induced blurring
caused by fast moving red blood cells. In recent clinical studies, IDF imaging has provided better image quality than SDF, allowing detection of more capillaries and more accurate determination of vessels’ perfusion (3) (41) (46). Finally, the IDF video-microscope allows direct analysis of microcirculatory parameters by a specialized software that automatically detects and quantitatively assesses vessels’ diameters and flow velocity of red blood cells (46).

Besides videomicroscopy techniques, other methods, such as laser Doppler flowmetry and tissue reflectance spectrophotometry, are also used at bedside for the assessment of microcirculatory blood flow. However, it is unclear to what extent these techniques reflect actual microcirculatory abnormalities or just changes in regional blood flow (52).

Although direct evaluation of the microcirculation provides information on tissue perfusion that is not available from macro-circulatory parameters, an image per se cannot provide information about the actual supply and demand of oxygen by cells. As a way to complement the information provided by videomicroscopy techniques, oxygen supply and consumption abnormalities at cellular level can be indirectly evaluated by gastric tonometry, blood or tissue dosage of lactate, pH and base excess, central or mixed venous oxygen saturation monitoring, or by venoarterial pCO₂ gradient (delta CO₂) determination (55).

Assessment of tissue oxygenation
One of the main goals of sepsis treatment is to adjust oxygen transport and tissue delivery to its pathological condition of consumption. Unfortunately, direct monitoring of many components involved in the oxygen supply/consumption balance is not possible in clinical practice. However, some indirect measurement methods are available for routine use.

Oxygen consumption can be indirectly assessed by Near-infrared spectroscopy (NIRS). NIRS technique uses electromagnetic waves in the near-infrared spectral region to measure the chromophores oxy and deoxyhemoglobin, myoglobin, and oxidized cytochrome aa3. Oxy and deoxyhemoglobin measurements are used to calculate the tissue oxygen saturation (StO₂) while oxidized cytochrome aa3 measurement allows the assessment of mitochondrial oxygen tension. As the wavelength used (ranging approximately between 650 and 900 nm) goes through the skin, subcutaneous tissue, skeletal muscle, and bone tissue, NIRS is able to penetrate the body up to a few centimeters, allowing organ-specific StO₂ monitoring. Brain and muscles are the most commonly monitored organs. Tissue oxygen saturation can be evaluated at rest and after venous and arterial occlusion tests. Rest StO₂ mainly reflects local venous compartment oxygen saturation while post-occlusion tests allow the calculation of muscle oxygen consumption (mVO₂), peripheral/regional blood flow, and deoxygenation and reoxygenation velocities. The dynamic analysis of StO₂ changes produced by transient ischemic maneuvering (vascular occlusion test) allowed the assessment of microcirculatory/endothelial dysfunction and has added clinical value to this technology (29). Main limitations of the method include the presence of thick
adipose tissue and/or swelling at the site of sensor application, low temperatures, and the use of vasoactive drugs (72).

Real-time monitoring of oxygen saturation in venous blood obtained by a central venous catheter (central venous oxygen saturation) or a pulmonary artery catheter (mixed venous oxygen saturation) is often used in the management of severe sepsis and septic shock. Venous oxygen saturation value is assumed to reflect the balance between oxygen transport and its global consumption provided arterial blood oxygen saturation is normal (102). However, it does not show direct correlation with microvascular dysfunction and fails to predict microcirculatory abnormalities in septic patients because venous oxygen saturation monitoring is unable to assess local perfusion deficits (5). Consequently, it may present normal or increased values even in situations where regional microcirculation is greatly impaired, with shunt, heterogeneous flow, and intense tissue hypoxia (76). Difficulties and risks related to the insertion of catheters should also be taken into account when choosing this monitoring method. Despite these drawbacks, central venous oxygen saturation is still widely used in clinical practice to guide resuscitation of septic patients.

Transcutaneous pO$_2$ (PtcO$_2$) measurement is a commonly used method for the indirect estimation of oxygen transport. Initially created to be correlated with arterial pO$_2$, avoiding serial collection of arterial blood samples for blood gas analysis, this method uses a Stow-Severinghaus type sensor coupled to the skin. As in septic patients, especially those in circulatory shock, there is no correlation between blood oxygenation and skin oxygenation, PtcO$_2$ can be interpreted as a
surrogate marker of skin perfusion (or oxygen transport): the PtcO$_2$ index (transcutaneous pO$_2$/arterial pO$_2$) estimates the adequacy of cardiac output and peripheral blood flow. Besides raw data, trend analysis is used for the diagnosis of shock and therapy optimization. The main limitations of the method include the presence of skin lesions that prevent electrode installation, swelling or thick adipose tissue, and the use of vasoactive drugs.

Microcirculatory assessment based on pCO$_2$

Tissue pCO$_2$ represents the balance between local blood flow and carbon dioxide production. Tissue hypercarbia ensues when carbon dioxide clearance is reduced due to decreased vascular flow and/or when its generation is increased. Transcutaneous pCO$_2$ (PtcCO$_2$) is measured by the same monitor and sensor used to measure PtcO$_2$ and correlates inversely with cardiac index during low-flow shock. The rise in PtcCO$_2$ has been proposed to be an early and better index of tissue hypoxia than traditional markers (27). Raw data and trends should be evaluated. Besides arterial hypercapnia, the main limitations of the method are similar to those discussed for PtcO$_2$ monitoring (90).

Gastric tonometry is an organ-specific minimally invasive technique that measures pCO$_2$ in the gastric mucosa, assessing the adequacy of the balance between oxygen supply and consumption in this mucosa. As anatomical characteristics of the intestinal mucosa make it particularly vulnerable to hypoxia, gastric tonometry attracted special interest in the past. Unfortunately, no correlation
between gastric pCO₂ and overall splanchnic perfusion has been found in septic patients (28). The main limitation of the technique, which restricts its use, is the interference caused by enteral nutrition or duodenogastric reflux.

pCO₂ can also be measured in the sublingual mucosa in a noninvasive manner, using a microelectrode sensor. An inverse correlation between mucosal pCO₂ values and functional capillary density in the sublingual mucosa has already been shown in septic patients (27). As tissue carbon dioxide is greatly influenced by arterial pCO₂, a better way to assess mucosal pCO₂ is to calculate the gradient between mucosal and arterial pCO₂ (the tissue to arterial pCO₂ gradient or pCO₂-gap) (76). This gradient is increased in sepsis.

Potential therapies

Persistent microcirculatory abnormalities are more commonly found among non-surviving septic patients than in those who survive (93) (106). Thus, drugs that assist in the reversal of microcirculatory changes could be decisive in sepsis treatment.

It has already been demonstrated that the dysfunctional endothelium is still able to respond to stimulation and that sepsis-associated microcirculatory derangements are functional and liable to complete reversal with adequate treatment (10). As multiple mechanisms are involved in the pathogenesis of microvascular dysfunction, a single pathway intervention is unlikely to be effective in the recruitment of the microcirculation. In fact, drugs or treatments with
pleiotropic effects have greater therapeutic potential than compounds directed
against a single target. To date, the best approach seems to be a multimodal
therapy targeting different mechanisms involved in microcirculatory distress (19).

Currently available treatments to correct microcirculatory abnormalities in
septic patients aim at favorably modulating systemic response to infection and/or
increasing the driving pressure of blood flow at microcirculation (Figure 4). Further
research should focus on whether these therapeutic approaches are successful in
improving the outcome of septic patients.

Fluid resuscitation

Fluid resuscitation is an essential therapy for sepsis-induced hypoperfusion
and may improve microcirculatory blood flow through several mechanisms (Figure
4). Beneficial microvascular effects were seen in early but not in the late phase of
sepsis, supporting current recommendations of timely aggressive fluid loading in
sepsis treatment (64) (85). Interestingly, microcirculatory effects of fluids are
independent of global hemodynamic effects.

In respect to the type of fluid, the use of hydroxyethyl starch 130/0.4 allowed
better resuscitation of the microcirculation than crystalloid solutions (35) (64).
However, several randomized clinical trials have provided evidence that starches
impair kidney function and haemostasis, and may increase mortality in critically ill
septic patients. Human serum albumin solutions have also proved to be effective in
microcirculatory resuscitation and may have beneficial effects on endothelial
dysfunction, by decreasing inflammatory response and oxidative stress (19) (67) (77).

Red blood cell transfusion

Red blood cell transfusion is another commonly used therapy to restore oxygen carrying capacity in critically ill patients. Microvascular response to transfusions is quite variable due to considerable interindividual variation. Conflicting data regarding the effects of red blood cell transfusion over microcirculatory parameters suggest that microvascular perfusion and reactivity improve in patients with abnormal microcirculatory parameters at baseline, but deteriorate in patients with normal baseline parameters (30) (92). Apparently, patients with severe microvascular dysfunction at baseline may benefit from transfusion therapy, highlighting the importance of microvascular monitoring in septic patients to target therapies on an individual basis (52).

Other variables may influence the net effect of blood transfusion over tissue oxygenation and microcirculation. For instance, the storage process is related to reduction in red blood cells deformability and 2,3-diphosphoglycerate levels, while the capacity of stored red blood cells to capture NO is enhanced. These alterations may affect the efficacy of transfusions leading to the worsening of tissue oxygen delivery and further microcirculatory dysfunction (52).
Vasopressors

In septic shock, vasopressors are used to counteract the intense vasoplegia and could improve tissue perfusion when mean arterial pressure (MAP) falls below the autoregulatory threshold (60-65 mmHg), as below this level of MAP organ perfusion becomes pressure dependent (45) (103). However, vasopressors may increase MAP at the expense of microcirculatory flow (17) (52). Despite considerable variation in individual responses, microvascular response to vasopressors appears to be dependent on the basal condition of the microcirculation: capillary perfusion improves in patients with an altered perfusion at baseline, but decreases in those with close to normal baseline perfusion (36). This suggests that vasopressors should be titrated under microcirculatory monitoring on an individual basis (64).

Inotropes

Dobutamine, milrinone, and levosimendan have been shown to improve microvascular perfusion during sepsis in experimental, small, or uncontrolled studies (4) (79) (81). Although both inotropic and vasodilatory effects are common to these drugs, microvascular effects were independent of changes in systemic hemodynamic variables. Unfortunately, the role of inotropes in microcirculatory recruitment is still not defined due to conflicting results in recent controlled experimental and clinical studies (52) (53).
Vasodilators

The use of vasodilators could compensate for the heterogeneity of septic microcirculatory blood flow, promoting the perfusion of ischemic/hypoxic areas (called “microcirculatory weak units”) (52). Indeed, it has already been shown that NO donors, such as nitroglycerin, are capable to improve microcirculatory oxygenation in experimental and human sepsis when used in combination with fluids (99). However, this result has been challenged by recent experimental and clinical studies (18). Negative results may be explained by the use of non-selective agents, which dilate both perfused and non-perfused microvascular units, leading to hyperperfusion of some areas and flow diversion from others. As for inotropes, the role of vasodilators in microcirculatory recruitment is still not defined (10) (52) (64).

Sedatives

Studies addressing the effects of propofol and midazolam on microcirculation agree that they exert negative effects, even in non-septic states, leading to microcirculatory derangements. Conversely, recent data suggest that dexmedetomidine yields beneficial effects on microcirculatory function during experimental sepsis, attenuating capillary perfusion deficits (78).

Anticoagulants
Activated protein C, antithrombin, unfractionated heparin, and low molecular weight heparin have shown beneficial microcirculatory effects during sepsis (12) (54) (56) (80). Interestingly, microcirculatory recruitment appeared to be independent of anticoagulant effects of these drugs (6) (10) (54) (64) (80). Despite microvascular improvement, none of these agents have consistently improved the outcome of septic patients in numerous clinical trials, but all significantly increased the risk of bleeding (44) (80). Activated protein C was withdrawn from markets worldwide in the wake of a clinical trial showing that this agent did not significantly reduce mortality, as compared with placebo, in patients with septic shock (89). Increasing uncertainty about efficacy and concerns about bleeding risk challenged the role of anticoagulants in microcirculatory recruitment.

NO synthase modulation

Non-selective NO synthase inhibition aggravates microvascular perfusion abnormalities, affecting tissue oxygenation and increasing mortality (74) (104). On the other hand, eNOS modulation by BH4 (tetrahydrobiopterin), leads to increased microvascular perfusion, improved organ function, and greater survival during experimental sepsis due to stimulation of NO production, suggesting that upregulation of NO formation in some microvascular beds may be an adaptive and protective mechanism during sepsis (10) (19) (50) (108).

Conclusions
Multiple experimental and clinical trials have shown that microcirculatory dysfunction occurs in sepsis. Several mechanisms have been involved in the development of microvascular abnormalities, such as NO dysregulation and functional impairment of many cell types found in the microcirculation, particularly the endothelial cell. Despite recent advances in the understanding of pathophysiological mechanisms involved in septic microcirculatory dysfunction, the diagnosis of microcirculatory changes and the adoption of therapeutic measures aimed at the treatment of these changes are not yet part of routine management of the majority of septic patients, due to the lack of easy-to-use and widely available bedside technology, validated microcirculatory endpoints for resuscitation, or efficient treatment (that actually improves patient outcomes).

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**Figure Captions**

**Figure 1. Pathophysiology of microcirculatory changes in sepsis.** Several mechanisms have been involved in the development of microvascular abnormalities, such as nitric oxide (NO) dysregulation and functional impairment of many cell types found in the microcirculation. eNOS: endothelial nitric oxide synthase; iNOS: inducible nitric oxide synthase.

**Figure 2. Septic microcirculatory dysfunction.** Microcirculatory changes include impairment of nitric oxide (NO)-induced arteriolar vasodilation (A and B) and increased venular leukocyte-endothelial interactions (E and F). Together, these abnormalities contribute to a decreased functional capillary density during sepsis (C and D). A and B: intravital microscopy of skinfold chamber preparation in a Golden Syrian hamster showing arteriolar diameter before (A) and after (B) cecal
ligation and puncture (CLP) procedure – arteriolar diameter has fallen by almost half after CLP; C and D: image of the sublingual microcirculation obtained by sidestream dark field technique from healthy (C - control) and septic (D) subjects – number of perfused capillaries is remarkably decreased in the septic patient; E and F: intravital microscopy of skinfold chamber preparation in a Golden Syrian hamster showing a single adhered leukocyte (E - white arrow) before lipopolysaccharide (LPS) administration and massive leukocyte adhesion and aggregation after endotoxemia induction.

**Figure 3. The sublingual microcirculation.** Image of the sublingual microcirculation from a healthy volunteer (obtained by sidestream dark field technique - SDF).

**Figure 4. Recruiting the microcirculation.** Currently available therapies to correct microcirculatory abnormalities in septic patients may improve microvascular blood flow and recruit the microcirculation through several mechanisms (8) (10) (19) (52) (53) (78) (79) (80) (82) (87) (112). MAP: mean arterial pressure; NO: nitric oxide.
Infection/initial hit

Inflammation

Tissue hypoxia and cellular distress

Nitric oxide
Endothelial cells
Smooth muscle cells
Red blood cells
Platelets
Leukocytes

Microcirculatory dysfunction

Nitric oxide
iNOS heterogeneous expression (with deficient areas)
Decreased production by eNOS

Endothelial cells
Loss of regulatory function over microcirculation
Disruption of glycocalyx and adherens junctions

Smooth muscle cells
Loss of regulation by endothelial cells
Loss of adrenergic sensitivity
Loss of tonus

Red blood cells
Decreased ability to release vasodilators during hypoxia
Decreased deformability
Increased aggregation to endothelial cells

Platelets and leukocytes
Increased platelet aggregation
Increased leukocyte-endothelial interactions
Arterioles
Capillary bed
Venules

66 μm
38 μm

Before CLP
After CLP

A B

Control
Septic

NO deficient area / Vasoconstriction

Impairment of blood flow / Microthrombosis

Neutrophil adhesion and aggregation

E F

Before LPS
After LPS
| **Fluid resuscitation** | Increases perfusion pressure at microcirculatory level;  
| | Triggers NO-induced vasodilation at microcirculation;  
| | Decreases blood viscosity;  
| | Decreases leukocyte-endothelial interactions;  
| | Decreases platelet aggregation.  

| **Red blood cell transfusion** | Increases functional capillary density by filling red blood cells-depleted capillaries.  

| **Vasopressors** | Norepinephrine restores perfusion pressure at microcirculatory level when MAP falls below the autoregulatory threshold;  
| | Conversely, phenylephrine and vasopressin analogues may have detrimental microcirculatory effects.  

| **Inotropes** | Dobutamine may decrease leukocytes adhesion;  
| | Milrinone reduces platelet aggregation and exerts protective effects on endothelial barrier function;  
| | Levosimendan may exert anti-inflammatory effects;  
| | All three drugs induce vasodilation at microcirculatory level.  

| **Vasodilators** | Increase the driving pressure at microcirculatory level, perfusing hypoxic areas;  
| | May aggravate flow heterogeneity leading to hyperperfusion of some areas and flow diversion from others.  

| **Sedatives** | Dexmedetomidine increases capillary perfusion by decreasing venular leukocyte-endothelial interactions.  

| **Anticoagulants** | Decrease leukocyte and platelet rolling and adhesion;  
| | Favor glycocalyx integrity;  
| | Improve endothelial function;  
| | Trigger vasodilation at microcirculatory level.  

| **NO synthase modulation** | Stimulates NO production decreasing leukocyte and platelet rolling and adhesion;  
| | Triggers NO-induced vasodilation at microcirculatory level.  