Endothelial and Microcirculatory Function and Dysfunction in Sepsis

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KEYWORDS
- Sepsis • Microcirculation • Glycocalyx • Intravital microscopy • Glycosaminoglycans
- Heparan sulfate

KEY POINTS
- Microcirculatory functions critical for the homeostatic control of infection can become dysregulated and harmful during sepsis.
- Microcirculation dysfunction may arise in part from septic degradation of the endothelial glycocalyx, a substantial, glycosaminoglycan (GAG)-rich layer lining the vascular lumen.
- The microcirculation can be measured at the bedside, either directly via intravital microscopy or indirectly via circulating measures of vascular damage. Such evidence of microcirculatory dysfunction is predictive of sepsis outcomes.
- Additional human studies are needed to determine if sepsis treatments, when titrated to improve- ment of microvascular function, improve patient outcomes.

ANATOMY AND FUNCTION OF THE MICROVASCULATURE
The microcirculation, comprised of less than 100 \(\mu m\)-diameter arterioles, capillary beds, and draining venules, performs essential homeostatic functions, including oxygen delivery and solute exchange.\textsuperscript{1} Although this simple construct holds true across all human tissues, there is substantial organ specificity of microcirculation structure, reflecting unique functions assigned to different vascular beds. The kidney glomerulus, tasked with plasma ultrafiltration, features afferent and efferent arterioles flanking a capillary network lined with fenestrated endothelium. In contrast, the cerebral and pulmonary vasculature are characterized by tight endothelial barriers (and supporting pericytes), reflecting organ functions that are threatened by interstitial edema. These organ-specific differences in microvascular function are paralleled by tissue-specific endothelial phenotypes, yielding varied mechanisms of endothelial-leukocyte adhesion (eg, pulmonary vs systemic circulations\textsuperscript{2}) and organ-specific endothelial glycocalyces.\textsuperscript{3}

THE NORMAL MICROVASCULAR RESPONSE TO INFECTION
To understand dysfunction of the microcirculation during sepsis, it is necessary to appreciate the appropriate microvascular response to infection. The inflammatory response to infection, as described in the first century AD, consists of calor (heat), rubor (redness), dolor (pain), and tumor (swelling).\textsuperscript{4} From a microcirculation standpoint, these responses reflect altered regional blood flow, vascular hyperpermeability, leukocyte

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recruitment, and coagulation. It is critical to recognize that these physiologic changes are appropriate and effective in the setting of acute infection. A vast majority of viral and bacterial infections are controlled quickly by the host and do not lead to disseminated infection, organ failure, and death. By allowing for the beneficial actions of calor, rubor, dolor, and tumor, and the microcirculation facilitates local quarantine of pathogens, targeted delivery of soluble anti-infectious agents (eg, complement and immunoglobulins), and chemotaxis of activated host immune cells.

**Leukocyte Adhesion**

The recruitment of leukocytes to areas of infection is a highly regulated process, consisting (in systemic venules) of active leukocyte rolling, adhesion, activation, aggregation, and transmigration, demonstrating the importance of these processes to tissue homeostasis. In the absence of infection, leukocyte-endothelial interactions are limited, occurring primarily in specialized vascular beds (eg, lymph node high endothelial venules). There is great heterogeneity across different vascular beds regarding processes of leukocyte extravasation, with rolling essential for diapedesis from systemic venules but dispensable for extravasation from the pulmonary capillaries.

**Tissue Edema**

The intense, multiprocess regulation of vascular permeability reflects its critical importance in microvascular function. The targeted extravasation of antibacterial peptides, antibodies, and complement is beneficial to the host response to infection. Barrier dysfunction, however, can become pathologic if transvascular fluid flux overwhelms lymphatic drainage or other tissuespecific safeguards against interstitial edema.

**Coagulation**

Microvascular coagulation is important to the host response to infection. Endothelial damage and inflammatory cytokines lead to a procoagulant state in the microvasculature, allowing for the development of microthrombi. This response functions to isolate infection and prevent dissemination. Murine studies have shown that anticoagulants facilitate bacterial spread after peritonitis, leading to worsened sepsis outcomes. The failures of activated protein C, antithrombin III, and tissue factor antagonists to improve sepsis outcomes perhaps reflect homoeostatic effects of microvascular coagulation.

These and other microcirculatory responses are adaptive and often successful in localizing and eliminating infectious insults. In extreme cases of overwhelming infection, however, these processes may contribute to the overall morbidity and mortality of sepsis (Fig. 1).

**EVIDENCE OF MICROVASCULAR DYSFUNCTION DURING SEPSIS**

Oxygen delivery is a function of both cardiac output and blood oxygen content. Because early sepsis is characterized by a low systemic vascular resistance/high cardiac output state, oxygen delivery is typically elevated in sepsis. The kidney, brain, and heart all experience augmented blood flow during sepsis. Despite this increased bulk delivery of oxygen, tissue hypoxia persists in sepsis and contributes to septic organ injury. This suggests that the defect of sepsis is not a loss of macrovascular blood supply but rather a loss of microvascular function. Therapeutic attempts to augment macrocirculatory oxygen delivery by increasing cardiac output or hemoglobin have failed to improve outcomes in sepsis.

This suspected microvascular defect in sepsis has been extensively investigated using animal models, identifying critical pathogenic roles of endothelial barrier dysfunction, inappropriate leukocyte adhesion, platelet activation, activation of microvascular coagulation, and aberrant control of vascular tone. These changes broadly mediate injury across numerous organ systems of relevance to sepsis outcomes, including the lung, kidney, and brain.

There is no discrete, easily apparent inflection point at which beneficial microvascular responses to infection change to pathologic contributors to sepsis. Sepsis may arise from numerous microcirculatory changes, including activation of anti-infection responses in vascular beds where no pathogens exist, or a magnitude of anti-infection response that outstrips what is necessary for microbial clearance. This complexity warrants a deeper understanding of the precise changes occurring within an individual during sepsis, potentially allowing for personalization of sepsis therapeutics.

**MEASURING SEPTIC MICROVASCULAR DYSFUNCTION IN HUMANS**

Detecting and characterizing microvascular dysfunction in humans is technically challenging, given difficulties in the direct measurement of clinically relevant vascular beds. Systemic, circulating biomarkers of tissue ischemia (eg, central venous oxygenation and lactate) are not sensitive to
microcirculatory defects, given the potential for functional shunting, in which venular $P_{O_2}$ exceeds capillary $P_{O_2}$.\textsuperscript{1} Furthermore, the value of therapeutically targeting these markers is uncertain, given recent negative studies of early goal-directed therapy.\textsuperscript{22–24} As recently reviewed elsewhere,\textsuperscript{27,34} numerous promising biomarkers for capillary endothelial dysfunction (eg, angiopoietins and glyocalyx fragments) have been identified in septic shock. These biomarkers, however, often are not easily measured point of care and have yet to be validated as clinically relevant treatment endpoints.

An alternative approach to rapidly measuring microcirculatory function is direct imaging of microvessels using intravital microscopy.\textsuperscript{35} Although nail fold or episcleral vessels can be visualized at the bedside,\textsuperscript{35} these vascular beds yield few quantitative data regarding microvascular function without the use of large microscopy systems. The development of microscopy techniques, however, such as orthogonal phase spectrometry (OPS) or sidestream dark field imaging (SDF), has led to increasing enthusiasm for the bedside imaging of the sublingual microvasculature. OPS and SDF imaging can clearly identify red blood cells (RBCs), due to the absorptive effects of hemoglobin. As such, these techniques can identify RBC-perfused vessels (Fig. 2); a lack of visualized sublingual vessels serves as evidence of absent or impaired RBC flow.\textsuperscript{36}

This visualized loss of sublingual microvascular RBC perfusion can be quantified via several techniques, either at point of care\textsuperscript{37} or during later review of recorded images. Loss of RBC flow yields a heterogeneous loss of vascular density apparent on OPS and SDF imaging, particularly involving small (<20-$\mu$m) microvessels. This microvascular dropout can be quantified by using several different validated approaches, including the De Backer score (which uses a stereological-like approach in which vessel density is calculated from intersections with overlying gridlines) or the microvascular flow index (a semiquantitative score determined from the average of qualitative assessments across 4 visual field quadrants; see Fig. 2).\textsuperscript{36,38}

Fig. 1. Homeostatic versus pathologic (septic) pulmonary and renal microvascular responses to infection.
Although consensus statements have detailed standardized approaches to the quantification of intravital measures of microvascular function,
there remain several practical challenges to the widespread implementation of these approaches. A major concern is the risk of visual artifacts (eg, capillary dropout) produced from undue pressure of the microscope objective on the sublingual microvessels.\textsuperscript{36,39} Even when excluding video clips that have such artifacts, only 30.8\% of SDF recordings were found of excellent technical quality.\textsuperscript{39} Despite these concerns, a recently published international study (Microcirculatory Shock Occurrence in Acutely Ill Patients [microSOAP]) performed across 36 ICUs performed SDF sublingual microvascular measurements in 501 patients, with low variation in microvascular flow index (MFI) (2\%) and De Backer scoring (7\%).\textsuperscript{40}

An additional concern regarding the sublingual microcirculation is the relevance of this vascular bed during sepsis, particularly given divergent responses of the sublingual microvasculature from vascular beds more proximal to the site of a sepsis-inducing infection (eg, the submucosa of an intestinal ostomy during abdominal sepsis).\textsuperscript{41} Convergent findings from multiple groups, however, have linked sublingual microvascular alterations with clinical outcomes in sepsis, providing reassurance for the relevance of these measurements. Using OPS imaging of the sublingual microvasculature, De Backer and colleagues\textsuperscript{42} compared 10 healthy volunteers, 16 patients prior to cardiac surgery, 5 nonseptic ICU patients, and 50 patients with sepsis/septic shock. Patients with sepsis had significant loss (or intermittent interruption) of RBC perfusion in small (<20-\mu m) sublingual microvessels. Perfusion was highly variable in patients with sepsis, and vessel perfusion was lower in nonsurvivors. These changes were independent of measures of macrovascular function, including mean arterial pressure and need for vasopressor medications. Further studies demonstrated that septic shock survivors tended to have rapid (albeit incomplete) correction of early microvascular dysfunction, as opposed to persistent abnormalities in patients who ultimately died.\textsuperscript{43} An increase in small vessel perfusion of greater than 7.8\% in the first 24 hours of sepsis was 82\% specific for survival.\textsuperscript{43} In the microSOAP study, 17\% of mixed ICU patients (septic and nonseptic) demonstrated abnormal sublingual microvascular function; in the subgroup of patients with tachycardia, this dysfunction predicted hospital mortality.\textsuperscript{40} These studies and others\textsuperscript{44--46} support the feasibility (and reproducibility) of bedside measures of sublingual microvascular function and their relevance to sepsis outcomes.

As with any observational human approach, it is difficult to prove that observed changes in microvascular dysfunction during sepsis are causal to, as opposed to a consequence of, organ dysfunction. For example, it is possible that loss of microvascular flow is an appropriate response to decreased tissue metabolic demand. Sepsis-induced suppression of mitochondrial oxidative phosphorylation is expected to decrease cellular oxygen demand, triggering a reactive decrease in microvascular flow and vascular density. This phenomenon, however, is not supported by available experimental data. During sepsis, extravascular tissue CO\textsubscript{2} partial pressures (a measure of cellular respiration quantifiable by sublingual capnometry) increase as microvascular flow decreases, suggesting that tissue metabolic activity outstrips microvascular blood supply.\textsuperscript{47}

### PATHOGENESIS OF MICROVASCULAR DYSFUNCTION DURING SEPSIS

Given the potential causal importance of microvascular dysfunction during sepsis, the
pathogenic mechanisms underlying these changes are attractive therapeutic targets. Likely contributors to these changes include pathophysiologic events typically implicated in septic organ injury, including aberrant vascular tone, inappropriate barrier dysfunction (and consequent tissue edema), inappropriate leukocyte adhesion (and inflammation), and activation of microvascular coagulation. These pathophysiologic events can yield a signature appearance on intravital microscopy, with extraluminal (tissue edema and vasoconstriction) and intraluminal (coagulation and leukocyte adhesion) events conspiring to produce a loss of visualized RBC flow. Loss of RBC flow has physiologic consequence, leading to tissue hypoxia in the setting of tissue injury-amplified metabolic demands. Although loss of microvascular flow can be compensated by increased flow through other vessels, this compensation can produce a functional shunt, in which the high velocity of flow through patent collateral microvessels decreases the capillary dwell time of RBCs, diminishing oxygen diffusion and potentially leading to additional hypoxia surrounding perfused microvessels. 

Because many pathophysiologic events contribute to microvascular dysfunction during sepsis, it is unlikely that targeting a discrete contributor to vascular injury would have broad beneficial effects on patient outcomes in sepsis. As such, there has been great effort invested in identifying, and subsequently targeting, unifying mechanisms upstream of endothelial barrier dysfunction, inflammation, and microthrombosis. Particularly intense attention has been dedicated to the immunopathogenesis of sepsis, a broad topic ranging from the initial infection-associated release of pathogen-associated molecular patterns, consequent induction of pattern receptor (eg, toll-like) signaling, downstream induction of inflammatory cytokine production (cytokine storm), leukocyte recruitment, and tissue damage with release of immune-amplifying damage-associated molecular patterns. These events coincide with induction/augmentation of coagulation pathway signaling. These proinflammatory pathways, however, have largely failed to identify clinically effective immunotherapies for sepsis. Although these failures may be largely the consequence of practical challenges in therapeutically interrupting hyperacute events driving sepsis onset, it may also reflect an incomplete understanding of the complex immunologic events surrounding sepsis. Recent efforts have highlighted the pathologic significance of anti-inflammatory signaling in severe sepsis and septic shock. 

These limitations of the classic cytokine storm theory as a unifying mechanism of microcirculatory dysfunction have raised the need to identify novel pathophysiologic pathways of organ dysfunction (and microcirculatory failure) in sepsis. De Backer and colleagues demonstrated that septic sublingual microcirculatory heterogeneity can be completely corrected by the topical administration of vasodilators (eg, acetylcholine). This rapid reversibility suggests that septic microvascular failure may arise largely from pathologic involvement of processes associated with the dynamic regulation of vascular tone. Accordingly, nitric oxide (NO) has been the intense focus of research as a mediator of sepsis and septic organ injury. Unfortunately, NO signaling is highly complex, with context-specific functions that can be both homeostatic and pathologic. Human studies of NO-targeted microvascular therapeutics have accordingly been disappointing, potentially reflecting broad, nonspecific effects of NO manipulation. These challenges (and opportunities) of NO-based therapies are reviewed in detail elsewhere. The limitations of systemic, NO-targeted therapeutic approaches in sepsis have raised interest in other, more specific, manipulations of vascular tone. Although many pathways are currently the focus of intense investigation, this review focuses on 1 particularly promising therapeutic target—the endothelial glycocalyx.

ENDOTHelial GLYCOcalyx AND THE Septic Microcirculation

The endothelial glycocalyx is a layer of GAGs and associated proteoglycans lining the vascular lumen (Fig. 3). First described as a 20 nm–thick “endocapillary layer” in 1966, the glycocalyx was long thought to be a structure of trifling significance. This underappreciation of glycocalyx structure/significance likely reflected glycocalyx aberrance in vitro as well as its frequent degradation during tissue fixation. With the advent and optimization of intravital microscopy, it is now apparent that in vivo, negatively charged glycocalyx GAGs sequester water, forming a massive (0.5 μm–11 μm) endothelial surface layer (ESL) with measurable rigidity. The ESL has several homeostatic functions, including maintenance of the endothelial barrier to fluid and protein as well as regulation of leukocyte-endothelial adhesion. The ESL also serves as a mechanotransducer of shear stress: in the presence of sufficient shear, the ESL-replete endothelium activates endothelial NO synthase, leading to vasodilation and accommodation of increased flow. Experimental ESL degradation induces edema, inappropriate leukocyte adhesion,
and loss of microvascular autoregulation. Accordingly, degradation of the ESL in animal models increased microvascular heterogeneity, with some vessels becoming occluded to RBC flow and others becoming hyperemic.

Endothelial glycocalyx/ESL integrity is, therefore, highly relevant to septic organ injury and microvascular dysfunction. In experimental models of polymicrobial sepsis, GAG degradation occurred within the pulmonary and renal vascular beds, contributing to both lung edema/inflammation as well as loss of glomerular filtration.

In a rat model of endotoxemia, loss of intestinal capillary density occurred in association with mesenteric ESL degradation. In humans, several techniques exist for the detection and quantification of glycocalyx degradation in critically ill patients (Table 1). Loss of glycocalyx/ESL integrity was apparent within the sublingual microcirculation after endotoxin administration to healthy volunteers, coincident with loss of capillary density. Patients with sepsis demonstrate elevated circulating ESL degradation products, including proteoglycans as well as GAGs heparan sulfate, hyaluronic acid, and chondroitin sulfate. Accordingly, glycocalyx/ESL degradation is predictive of clinical outcomes in critical illness.

The development of rapid, point-of-care assays for glycocalyx breakdown products (see Table 1) may allow for microvascular personalization of sepsis treatment, identifying patients who may benefit the most from vascular-protective therapies.

**THERAPEUTIC TARGETING OF THE MICROCIRCULATION IN SEPSIS**

The ability to directly visualize the sublingual microcirculation in human subjects has allowed for hypothesis-generating human studies identifying treatments that, by virtue of rescuing the dysfunctional microvasculature, could serve as clinically effective treatments for sepsis. Such microcirculation-protective therapies, however, have largely failed to improve patient outcomes when broadly applied across large, multicenter trials (Table 2).

The failure of activated protein C as a treatment of sepsis is particularly disappointing, due to not only the promising microcirculation-protective effects observed in animal models and preliminary human studies but also the initial success of drotrecogin alfa as reported in the seminal Recombinant Human Activated Protein C Worldwide Evaluation in Severe Sepsis (PROWESS) study. Ultimately, the futility of drotrecogin alfa was demonstrated in the Administration of Drotrecogin Alpha [Activated] in Early Stage Severe Sepsis (ADDRESS) and PROWESS-Shock studies, paralleling negative studies of other anticoagulants such as antithrombin III and tissue factor antagonists. A recent meta-analysis suggested, however, a mortality decrease with heparin treatment during sepsis (odds ratio 0.88), although this analysis is derived largely from a single study of low-dose heparin for venous thromboembolism prophylaxis (a dosing regimen of uncertain relevance to the septic microcirculation).
Table 1
Measurement of endothelial glycocalyx/endothelial surface layer degradation in humans

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<th>Assay</th>
<th>Human Studies</th>
<th>Advantages</th>
<th>Disadvantages</th>
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<td>Detection of circulating glycocalyx fragments</td>
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| Dimethylmethylene blue/alcian blue    | • Plasma GAGs (via alcian blue binding) increased in septic shock, correlated with mortality\(^{75}\)  
  • Urinary, plasma GAGs increased in pediatric meningococccemia\(^{83}\)  
  • Modified dimethylmethylene blue assay feasible in trauma patients\(^{84}\) | Rapid, inexpensive colorimetric assay                      | Only detects sulfated GAGs (cannot detect hyaluronic acid)   |
| ELISA/latex agglutination assay       | • Plasma hyaluronan increased in endotoxin-treated volunteers,\(^{74}\) patients with septic shock\(^{75,82}\)  
  • Plasma heparan sulfate increased in septic shock\(^{79}\)  
  • Plasma syndecan-1 shedding increased in sepsis patients\(^{75,81}\)      | Quantitative measure of proteoglycans, GAGs                | Insufficient rapidity to date for bedside use; uncertain specificity of antibody binding to GAGs |
| Mass spectrometry                    | • Elevated heparan sulfate in patients with severe sepsis\(^{78}\)  
  • Chondroitin sulfate unchanged in severe sepsis\(^{78,79}\)             | High sensitivity; allows detection of sulfation signatures, potentially identifying tissue source | Expensive, impractical for rapid bedside use                   |
| Thromboelastography                  | Trauma patients with thromboelastography evidence of circulating sulfated heparan sulfate fragments had higher plasma syndecan-1, injury severity\(^{85}\) | Rapid, inexpensive measurement of circulating heparan sulfate fragments with anticoagulant ability | Detection limited to highly sulfated heparan pentasaccharides (or larger) |
| Measurement of whole-body glycocalyx volume |                                                                                |              |                                                 |
| Tracer dilution technique\(^{86}\)   | In healthy volunteers, total body glycocalyx volume correlated with sublingual intravital imaging\(^{86}\) | Can measure whole-body ESL volume based on differences in tracer volumes of distribution | Technical assumptions controversial\(^{87}\)                   |
| Intravital microscopy                |                                                                                |              |                                                 |
| Sublingual SDF imaging               | Loss of sublingual glycocalyx thickness in endotoxin-treated volunteers,\(^{74}\) ICU patients\(^{88}\) | Rapid, point-of-care assay; allows for simultaneous measurements of microvascular function | Concerns regarding relevance of imaged vascular bed and interobserver variability; need for specialized training |
in patients receiving activated protein C. Low-dose heparin administration had been previously implicated as detrimental in sepsis studies of antithrombin III and tissue factor antagonists. This complexity may reflect the varied biologic effects of heparin, including the ability of this highly sulfated GAG to inhibit selectins, influence growth factor signaling, and inhibit enzymes implicated in endothelial glycocalyx degradation (ie, heparanase). Many anticoagulants (including activated protein C and antithrombin) have multiple biologic effects; the failure of these agents to improve sepsis outcomes, therefore, cannot be viewed as a direct repudiation of the pathophysiological importance of tissue thrombosis to organ injury.

**NOVEL MICROCIRCULATION-PROTECTIVE THERAPIES**

The general failures of microcirculation-targeted therapies to improve patient outcomes highlights a need to identify new therapeutic targets in sepsis. Given the known benefit of early antibiotics...
in sepsis, studies of the impact of antibiotic administration on microcirculatory function would be instructive as to potential new therapeutic targets. Shedases implicated in septic glycocalyx degradation may be targeted, including the use of doxycycline or sphingosine-1-phosphate to inhibit matrix metalloproteinases responsible for proteoglycan shedding. Alternatively, coagulant or nonanticoagulant variants of heparin can be used to block heparanase, a heparan sulfate-degrading endoglucuronidase responsible for septic endothelial glycocalyx degradation and lung and kidney injury. Furthermore, interventions aimed at promoting glycocalyx reconstitution may hasten a return of microvascular homeostasis. Rosuvastatin improved glycocalyx reconstitution in patients with familial hyperlipidemia; however, a randomized trial of statins failed to show benefit as a sepsis therapeutic.

Although the general failure of microcirculation-protective interventions to improve clinical outcomes may reflect a lack of novel therapeutic targets, a more compelling explanation might lie in the indiscriminant administration of microcirculation-protective therapies in multicenter trials. Microvascular-protective treatments might only benefit patients who demonstrate baseline abnormalities of microvascular function. Ideally, future studies will pursue such microvasculature-targeted, personalized approaches to sepsis resuscitation. This assessment of baseline microvascular status could be based on bedside intravital microscopy (with its accompanying technical limitations) or systemic markers of endothelial damage (with their accompanying logistic concerns as point-of-care tests). The promise of such personalized approaches to infection treatment has been demonstrated in recent studies of pneumonia, in which a benefit of adjunctive corticosteroids existed largely in patients with baseline evidence of systemic inflammation.

**SUMMARY**

The microcirculation is a promising therapeutic target in sepsis. Although several techniques allow for the detection of microcirculation dysfunction in humans (including intravital imaging and measures of glycocalyx degradation), these approaches have yet to guide sepsis therapeutics in a manner that demonstrably (in phase III studies) improves patient outcomes. Validating, multicenter, patient outcome-focused studies of interventions titrated to improving microcirculation function are needed to create new treatment paradigms in sepsis.

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