

## Review Article

### THE ENDOTHELIUM IN SEPSIS

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**ABSTRACT**—Sepsis affects practically all aspects of endothelial cell (EC) function and is thought to be the key factor in the progression from sepsis to organ failure. Endothelial functions affected by sepsis include vasoregulation, barrier function, inflammation, and hemostasis. These are among other mechanisms often mediated by glycocalyx shedding, such as abnormal nitric oxide metabolism, up-regulation of reactive oxygen species generation due to down-regulation of endothelial-associated antioxidant defenses, transcellular communication, proteases, exposure of adhesion molecules, and activation of tissue factor. This review covers current insight in EC-associated hemostatic responses to sepsis and the EC response to inflammation. The endothelial cell lining is highly heterogeneous between different organ systems and consequently also in its response to sepsis. In this context, we discuss the response of the endothelial cell lining to sepsis in the kidney, liver, and lung. Finally, we discuss evidence as to whether the EC response to sepsis is adaptive or maladaptive. This study is a result of an Acute Dialysis Quality Initiative XIV Sepsis Workgroup meeting held in Bogota, Columbia, between October 12 and 15, 2014.

**KEYWORDS**—Barrier function, blood, endothelium, glycocalyx, hemostasis, inflammation, microcirculation, sepsis

### INTRODUCTION

The endothelial cell lining (ECL) of the vasculature is a unique cellular system that coats the inside of blood vessels and forms the interface between the circulating blood and the parenchymal cells responsible for organ function. It is critical for the regulation of hemostasis, vasomotor control, and immunological function, by sensing and reaction through secretion of molecules, which initiate transcellular and intracellular signaling. In addition to these important functions, the endothelium forms the essential vascular barrier for solute transport and osmotic balance. Sepsis is associated with severe endothelial cell (EC) dysfunction leading to dysregulation of hemostasis and vascular reactivity, as well as tissue edema. This failure of the ECL is considered central to the progression to organ failure during sepsis.

This review discusses many of the latest insights into the physiological function of the ECL and its dysfunction in sepsis. Since many excellent reviews on the septic endothelium have preceded this study (1), we have focused our attention to studies published in the past 5 years. This endeavor arose as a part of an international Acute Dialysis Quality Initiative (ADQI) XIV Sepsis Workgroup meeting held in Bogota, Columbia, between October 12 and 15, 2014, in which the authors participated. This meeting addressed the different cellular and subcellular aspects of sepsis, and the working group of the present authors of this study focused themselves on the role of endothelium in sepsis. In this

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ADQI XIV Workgroup: A complete list is provided in Appendix 1.

Can Ince and Daniel De Backer: Denotes workgroup facilitators.

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review, we present a current update on the central role of the endothelium in sepsis.

## METHODS

Complete methods are available in the companion article to this series (2). Briefly, we assembled a group of international experts with distinct clinical and scientific backgrounds; this group included physicians; specialists in critical care, anesthesiology, nephrology, surgery, and emergency medicine; and basic scientists with expertise in biology and physiology, who were recruited on the basis of their expertise in sepsis and organ dysfunction. The group consisted of 23 international experts from 5 continents. A set of questions was generated through mutual agreement and we sought evidence to answer each question by searching the Cochrane Controlled Trials Register, the Cochrane Library, MEDLINE, and EMBASE, from 1966 to present. The search terms for questions regarding epithelial dysfunction are provided in Appendix 2. Finally, we reviewed the evidence with the group and used the Delphi method to achieve consensus.

## RESULTS

On the basis of the literature review and consensus among the workgroup members, the following key questions were considered:

1. How does sepsis affect EC function and integrity?
2. What different techniques are available to assess EC function at the bedside?
3. What is the relationship between endothelial altered function and organ function?
4. Impact of usual and microvascular targeted therapies?
5. Is EC dysfunction adaptive or maladaptive?

### The endothelial glycocalyx in sepsis

The ECL contains fenestrations and pores, which are heterogeneous between organs and the different vascular generations (3) (Fig. 1). The integrity of the ECL as a barrier and transporter of solutes is determined largely by the endothelial cytoskeleton and the glycocalyx, which are tightly regulated.

The glycocalyx is a 0.2 to 0.5- $\mu$ m thick gel-like layer lining the luminal membrane of the ECL, thought to compromise some 20% of the intravascular volume. It is a multicomponent layer consisting of proteoglycans (of which 50% to 90% is heparin sulfate) and glycoproteins, anchored to ECs by glycosaminoglycans (4). Shedding of the glycocalyx occurs in the presence of oxidants, hyperglycemia, cytokines, and bacterial endotoxins (5, 6), and is associated with many states of disease including sepsis (Fig. 2). The glycocalyx mediates several key physiological processes such as the vascular barrier function, hemostasis, leukocyte and platelet adhesion, the transmission of shear stress to the endothelium (4), and anti-inflammatory and antioxidant defenses. The main instigators for glycocalyx shedding are thought to be reactive oxygen species (ROS) such as hydrogen peroxide, hydroxyl anions, and superoxide, but other mediators include tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) and heparanase (7, 8). Their action results in an increase in shedding products of the endothelium and disruption of the barrier function—an effect that can be reversed experimentally by treatment with antioxidant enzymes such as catalase and superoxide dismutase (SOD) (9). Loss-of-barrier function induced by glycocalyx shedding is associated with the formation of edema (6) and is a key contributor to sepsis-induced organ failure. Shedding of the glycocalyx may also hamper the ability to sense and transduce blood flow-induced shear stress, resulting in the endothelial release of nitric oxide (NO) or endothelin (ET), which regulates smooth muscle cell contraction and constitutes the basis of a process referred to as myogenic control of vascular regulation (4). Increased plasma concentrations of NO and ET metabolites have been reported in endotoxic shock (10). In addition, loss of shear stress flow monitoring can alter further regional vascular control because such signals are communicated to proximal vascular structures by interendothelial communication (11) through gap junctions, resulting in upstream vasogenic control. Finally, neutrophil extracellular trap (NET)—a mechanism implicated in host defense against

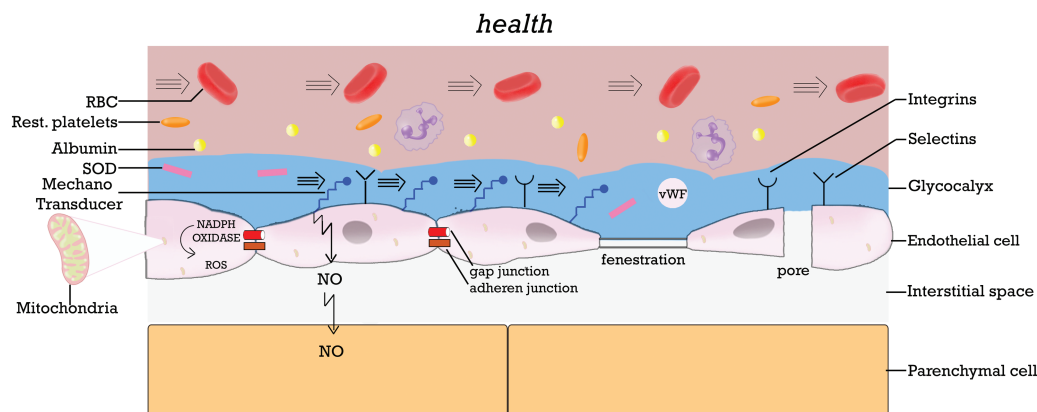


FIG. 1. **The endothelium in health.** This figure shows a number of the key elements of the endothelium in health responsible for its key role in its interface between circulating blood and the parenchymal cells. Elements relating to its function as a vascular barrier, vascular regulation, transcellular signaling, and hemostasis are shown. Highlighted are the endothelium glycocalyx housing various molecules, including mechanotransducers (and its transducer role between sensing shear stress and inducing NO affective for smooth muscle vasodilation, inactive adhesion molecules embedded in the glycocalyx, molecules essential for hemostatic, and antioxidant defense molecules such as SOD). Intra and transcellular elements of the endothelial shown include mitochondria, with its contribution to ROS generation and oxidative phosphorylation. Transcellular elements present include gap junctions for electrical communication for upstream vascular regulation and intercellular tight junctions important for maintaining vascular barrier. Morphological elements shown include transcellular fenestrations and pores. Source: Acute Dialysis Quality Initiative 14, www.ADQI.net 2014; used with permission.

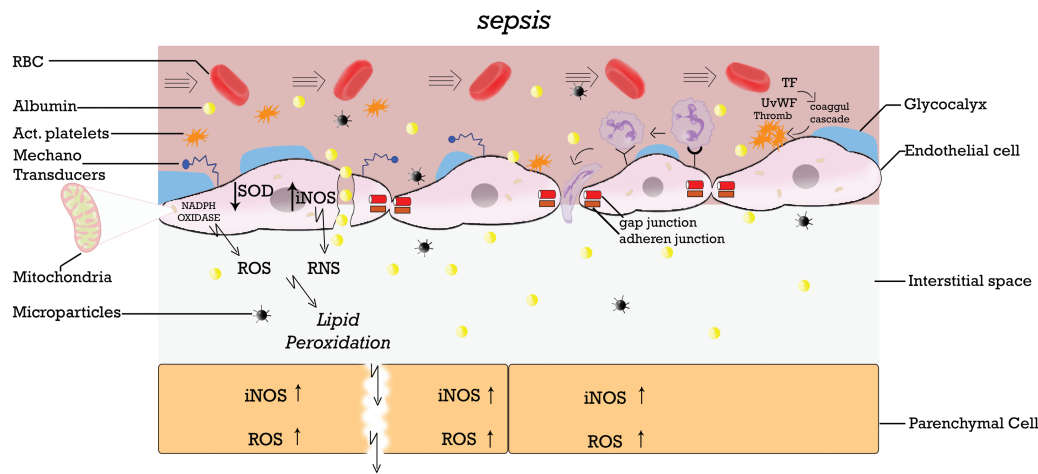


FIG. 2. **The endothelium in sepsis.** This figure shows the pathogenic effect of sepsis on the various elements of the ECL resulting in its functional impairment in terms of its function as a vascular barrier, a regulator of vasotone, and its hemostatic function. The destruction of the glycocalyx results, among many other effects, in the exposure of adhesion molecules, resulting in the trapping (selectins) and transmigration (integrins) of activated leukocytes, activation of hemostatic compounds in favor of a procoagulant state, and the loss of mechanotransducer function due to these molecules, losing their natural environment essential for the sensing of shear stress. The barrier function of the ECL is compromised by direct membrane destruction due to lipid peroxidation induced by ROS/RNS, as well as the decomposition of molecules such as tight junctions anchoring the EC together. The role of the EC as a vasomotor tone regular is lost due to the loss of function of the mechanotransducer system, the overproduction of iNOS-mediated NO, and the loss of transcellular gap junction essential for an integrative control of vasotone along the ECL. Endothelial destruction also results in the release of microparticles contributing to the pathogenic effect of EC dysfunction. Source: Acute Dialysis Quality Initiative 14, www.ADQI.net 2014; used with permission. iNOS indicates inducible nitric oxide synthase; RNS, reactive nitrogen species.

infection—can also contribute to endothelial damage and impair microvascular perfusion (12).

### Hemostasis and the endothelium

Sepsis is not only a state of systemic inflammation, it is also a state of deregulated hemostasis. Hemostasis is a complex system mediated by the endothelium, soluble plasma molecules, platelets, and leukocytes, which not only regulates the balance between pro and anticoagulant forces, but it also directs platelet and fibrin clotting to areas of focal vascular injury. The endothelium synthesizes and expresses molecules that are vital in regulating hemostasis, such as von Willebrand factor (VWF), tissue factor (TF), and plasminogen activator inhibitor type 1 (PAI-1). VWF—the largest multimeric glycoprotein in human plasma (molecular masses from 500 to 20,000 kDa)—mediates initial platelet adhesion to the damaged vessel wall by bridging platelet receptor platelet glycoprotein GPIb-IX COMPLEX (GPIb-IX) to exposed subendothelial collagen. VWF is secreted by either the constitutive pathway of lower molecular mass dimers, or the inducible pathway [inflammatory stimulation: TNF- $\alpha$ , interleukin (IL)-6, IL-8] of the larger and ultralarge multimers. The ultralarge multimers (ULVWF) are highly thrombotic and so they are rapidly cleaved to less active forms by a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13 (ADAMTS-13) (or VWF-cleaving protease) as they are released into the plasma. Thus, during normal physiology, plasma VWF binds and aggregates platelets only in the presence of modulators such as ristocetin or under conditions of high shear stress. Recently, a previously unrecognized role for GPIb-IX was reported, suggesting that platelets may actually exert an anti-inflammatory action in some models of experimental sepsis.

In normal hemostasis, coagulation and fibrinolysis are tightly regulated and kept in balance by the endothelium, such

that they allow blood to flow freely without systemic bleeding or clotting. TF is a procoagulant transmembrane glycoprotein synthesized by the endothelium and leukocytes (13), which, by creating complexes with factor VIIa activates factors IX and X, which ultimately leads to clot formation. The endothelium regulates TF by producing TF pathway inhibitor (TFPI), which limits fibrin deposition by binding to factor Xa, and inhibits TF-factor VIIa complex. In addition, the endothelium further regulates anticoagulation by activating protein C via thrombomodulin and endothelial protein C receptor, which inhibits factor V, factor VIII, and PAI-1. PAI-1—another glycoprotein synthesized by the endothelium and the liver—regulates fibrinolysis by inhibiting tissue plasminogen activator (tPA) in health, but is incrementally released during inflammation.

Sustained inflammation during severe sepsis drives hemostasis toward a prothrombotic and antifibrinolytic state, which can lead to disseminated microvascular thrombosis, organ ischemia, and multiple organ dysfunction syndrome (MODS). Clinically, this phenomenon can manifest as one of the following phenotypes—disseminated intravascular coagulation (DIC), thrombotic thrombocytopenic purpura/hemolytic uremic syndrome (TTP/HUS), or thrombocytopenia-associated multiple organ failure (TAMOF) (13, 14). Inflammatory mediators during sepsis such as IL-6, plasma-free hemoglobin, VWF proteolytic fragments, shiga toxin, and neutralizing autoantibodies can inactivate ADAMTS-13 (15, 16), the proteolytic enzyme in charge of cleaving ULVWF into smaller less thrombogenic multimers. In addition, plasmin, thrombin, products of activated coagulation, and granulocyte elastase released by activated neutrophils can proteolyze ADAMTS-13 into inactive fragments, and neutrophil-derived ROS can inhibit ADAMTS-13-mediated cleavage, which leads to an acquired ADAMTS-13 deficiency, and thus increased risk for disseminated platelet/VWF-rich microvascular thrombosis (14,



17). Furthermore, the normal anticoagulant system regulated by TFPI and protein C is defective in sepsis because TFPI is decreased due to reduced synthesis and proteolytic inactivation, and protein C activation is dysfunctional. In addition, the fibrinolytic pathway is suppressed in sepsis by the increased release of PAI-1 by the endothelium. These imbalances can ultimately lead to the dissemination of fibrin-rich microvascular thrombi as observed in DIC, which occurs in 25% to 50% of septic patients.

Drugs have been tested in attempts to address the hemostatic imbalances associated with sepsis. For example, recombinant human (rh) TFPI, rh-activated protein C, rh-soluble thrombomodulin, protein C concentrate, heparin, antithrombin III, and platelet-activating factor antagonist have all been unsuccessful in large randomized control trials as monotherapy for sepsis (35). Because the hemostatic system is so complex with many molecules being altered during sepsis, blood purification offers a chance to achieve hemostatic balance by removing molecules that are causing harm and replenishing deficient soluble plasma factors. Therapeutic plasma exchange has been tried with various successes in patients with sepsis-induced disseminated microvascular thromboses (DIC, TTP/HUS, and TAMOF) (18). A future approach of employing combination therapy would more likely achieve success once clinicians have appropriate tools/biomarkers to fully assess the underlying pathogenic mechanism of disseminated microvascular thrombosis.

### The inflamed endothelium

Neutrophil/monocyte-EC interaction plays an important role in the pathogenesis of sepsis, leading to organ failure. This interaction is mediated by adhesion molecules to which leukocytes anchor themselves, allowing them to eventually extravagate into the tissues cells—a process referred to as diapedesis. There, they can release inflammatory mediators and reactive molecules to destroy pathogens, but at the same potentially causing tissue damage. The integrity of the glycocalyx is of prime importance in this process. The adhesion molecules responsible for leukocyte adhesion leading to extravasation in conditions of health are embedded in the glycocalyx, shielding them from adherence to the leukocytes (Fig. 1). In conditions of inflammation and sepsis, cytokines and reactive species induce glycocalyx shedding, exposing the adhesion molecules initiating leukocyte adhesion, leading to transmigration to the tissues (Fig. 2).

Of the inflammatory mediators, oxidative and nitrosative stress (ROS, reactive nitrogen species [RNS]) resulting from oxidant release by mitochondria, xanthine oxidase, nicotinamide adenine dinucleotide phosphate hydrogen (NADPH) oxidase, and by uncoupled endothelial NO synthase (eNOS) damages the ECL glycocalyx and alters endothelial function. Shedding of the glycocalyx exposes the otherwise hidden adhesion molecules to circulating leukocytes, which in turn facilitates adhesion and ultimately transmigration through the ECL and into the parenchyma, in addition to altering NO and ET production and contributing to the loss of vascular reactivity. Selectins (E, L, and P) mediate sticking and rolling, whereas integrins such as intercellular adhesion molecule 1 (ICAM-1) and vascular cell adhesion molecule 1 (VCAM-1)

mediate firm adhesion and transcellular trafficking of the leukocytes to the parenchymal cells. Depletion of essential cofactors necessary for eNOS activity, such as tetrahydrobiopterin, uncouples the enzyme and results in the generation of superoxide anion and reduced NO production, a process referred to as eNOS uncoupling. Because NO metabolism also plays a key role in the regulatory function of the ECL, reduced activity of eNOS exacerbates organ injury. Mitochondria are regarded as the main source of ROS in the endothelium, which is supported by the observation that scavenging mitochondrial oxidants can reduce oxidative and nitrosative stress even in the parenchyma (19). Finally, endothelial damage results in the release of endothelium derived microparticles (20), which are composed of membrane lipids and proteins, and express several endothelium-derived surface antigens such as adhesion molecules and ULVWF. Their action can cause vascular hyporeactivity, amplify the hemostatic response, and induce increases in ROS and RNS, further fueling endothelial dysfunction (20).

The ECL is highly heterogeneous in morphology and function, not only between the wide diversity of vessels (i.e., arteries, arterioles, capillaries, venules, and the veins) (3), but also between the organs. Such diversity is reflected in the heterogeneity of the response of the various organs to the septic insult, and thus, we will discuss the features of endothelial dysfunction in three organ systems at special risk during sepsis: the kidney, the lung, and the liver.

**The kidney**—The microcirculation of the kidney is unique in that it is comprised of two specialized capillary beds—the glomerular and the peritubular beds—connected in series by the efferent arteriole. Specialized ECs line each of these capillary beds and are the first defense against a blood-borne systemic microbial infection. Accordingly, experimental sepsis [by lipopolysaccharide (LPS) or cecal ligation and puncture (CLP)] results in early (within hours) endothelial activation, increased neutrophil activation, adhesion and migration (21), increased microvascular permeability in both the glomerular (21, 22) and peritubular (23) capillary beds, impaired control of local perfusion with heterogeneous blood flow distribution and areas of hypoperfusion and hypoxemia (23–29), and increased microvascular coagulation, all of which can participate in the rapid development of acute kidney injury (AKI).

Although the mechanisms responsible for renal microcirculatory failure are not fully understood, disruption of the ECL is considered an important mechanism for enhanced microvascular permeability during sepsis (30), which in turn has been associated with progression of decreased flow and vascular congestion (19, 23, 27). The unique anatomical arrangement of capillaries and tubules within the kidney means that changes in the microcirculation will have a profound effect on renal function and that signaling cross-talk between microvascular ECs and the tubular epithelium must be considered when exploring new therapeutic options (31). In addition, changes in the interstitial space due, for example, to edema, and/or tubular dilation and vacuolization, which occur during sepsis (28), can compress the microcirculation, further reducing the nutritive flow. Also, ROS/RNS, cytokines, and other stress-signaling molecules released from injured tubular epithelial cells (21, 28) may directly damage the ECs, contributing to a cycle of injury.



The glomerular capillary EC glycocalyx appears to be especially vulnerable during sepsis. In rats subjected to CLP, increased urinary albumin is associated with ultrastructural changes in the glomerular filtration barrier and decreased expression of syndecan-1, hyaluronic acid, and sialic acid (22), which are the key components of the glycocalyx in the glomerular capillary. Alterations to the glomerular glycocalyx also occur in mice following LPS administration (21). The key question is why does the endothelial glycocalyx and permeability barrier become damaged during sepsis? To begin to address this, Xu et al. (21) found that administration of TNF- $\alpha$  produced similar changes to the glomerular ultrastructure and glycocalyx, as did LPS. Moreover, they found that mice lacking the TNF receptor 1 (TNFR1<sup>-/-</sup>) were resistant to LPS-induced changes in glomerular permeability and were resistant to LPS-induced expression of heparanase, an enzyme that degrades heparan sulfate and weakens the permeability barrier. This pathway of glycocalyx degradation is also activated in the lung (7) and likely represents a common mode of injury to the endothelium during sepsis throughout the microcirculation.

Targeting the endothelial permeability barrier to restore stability is a rapidly expanding area of research. It is becoming clear that maintaining the endothelial permeability barrier improves outcomes. Sphingosine-1-phosphate (S1P)—a phospholipid generated from ceramide—enhances the endothelial permeability barrier through stimulation of the endothelial Rac1 GTPase-coupled sphingosine-1-phosphate receptor 1 (S1P1), leading to the assembly of adherens junctions, cytoskeletal reorganization, and focal adhesion formation. Importantly, S1P has shown to protect the endothelial glycocalyx through S1P1 by inhibiting matrix metalloproteinase-dependent shedding of glycosaminoglycans (32). Interestingly, actinonin—an inhibitor of the tubule brush-border metalloproteinase meprin A—protects the renal microcirculation during sepsis (33), supporting the notion of signaling cross-talk. Recently, Wang et al. (19) showed that the S1P1 agonist SEW2871 was able to reduce renal microvascular permeability and restore peritubular capillary perfusion in a murine CLP model. These findings link stimulation of S1P1 to repair of the microcirculation and preservation of renal function.

The endothelial permeability barrier can also be stabilized by agents that increase endothelial cyclic adenosine monophosphate (cAMP) levels, which promote Rac1 activation (34). Rolipram is a phosphodiesterase 4 inhibitor shown to protect the mesenteric microvascular permeability barrier from LPS in the rat, and restore the renal microvascular permeability barrier and peritubular capillary perfusion in the murine CLP model (35). Phosphodiesterase inhibitors may offer an additional benefit during sepsis by reducing renal vascular resistance to also improve the renal blood flow (35).

**The liver**—The liver receives about 25% of the cardiac output via the portal vein and the hepatic artery. The liver microcirculatory bed is comprised of hepatic sinusoids, which are lined by a thin discontinuous endothelium (open pores or fenestrae) with underlying basal lamina that is absent over large areas. Anatomically, the fenestrae diameter decreases slightly from the periportal to the centrilobular zone, and importantly, the diameter and number of fenestrae can dynamically change

because they are influenced by diverse stimuli (36). Structural integrity of the sinusoidal endothelial fenestrae is believed to be indispensable for the preservation of normal exchanges of fluids, solutes, particles, and metabolites between parenchymal and sinusoidal blood. Liver sinusoidal ECs (LSECs) and Kupffer cells (KCs) represent the predominant nonparenchymal cell types of the hepatic sinusoid, in addition to other cells with immunological activity, such as dendritic cells (DCs). Nonparenchymal cells such as LSECs, resident DCs (comprising both myeloid and plasmacytoid DCs), hepatic stellate cells (HSCs), and KCs have demonstrated capabilities to recognize endotoxin, express TLR-4, and play a key role in liver immune tolerance. Along with the KCs, the LSECs exert a key role in host defense mechanism and blood flow regulation, and represent a major target for injury during the early phases of inflammation. The LSECs have the capacity to produce immunoregulatory and proinflammatory cytokines, such as IL-1, IL-6, and interferon. Furthermore, these cells produce eicosanoids such as thromboxane A2 (TxA2) and prostaglandin E2 (PGE2), as well as other mediators that contribute to the regulation of the vascular tone such as NO and ET. Importantly, intact microvascular function requires that the diverse population of liver sinusoidal cells act in concert.

The failure of sinusoidal perfusion has been shown in experimental models of sepsis to be a key factor in the pathogenesis of organ failure. Microcirculatory failure of the liver during sepsis is largely characterized by heterogeneity of the microvascular blood flow, resulting in an oxygen supply-demand mismatch, with consequent depletion of high-energy phosphates, which ultimately leads to hepatocellular injury and dysfunction. Several mechanisms have been implicated. LPS injection induces a massive loss of the sieve-plate architecture of the sinusoidal endothelium, with gap formation (37) and up-regulation of ICAM-1 on LSEC that promotes leukocyte adhesion and sequestration, which facilitates leukocyte–hepatocyte interactions (37), decreases flow velocities, and increases heterogeneity and flow perfusion deficits. Endotoxin also decreases protein S mRNA levels in LSECs and thrombomodulin activity (38), which contributes to a procoagulant state. The balance between vasodilators and vasoconstrictors, critical for hepatic blood flow regulation, is altered in sepsis. Although sepsis induces mRNA encoding for vasoconstrictor (ET-1) and vasodilator (NO) mediators, NO overproduction is generated by inducible nitric oxide synthase (iNOS), whereas eNOS appears to be inactivated, which may actually contribute to microvascular dysfunction (39). Finally, direct cytotoxic effects of LPS and TNF- $\alpha$  can happen without coexistence of such sinusoidal perfusion failure.

A number of specific strategies have been used to prevent the liver microvascular failure. The use of radical scavengers (SOD, tocopherol, and allopurinol) has been associated with some beneficial effects in the setting of injury/reperfusion models by inhibition of leukocyte accumulation and by improvement of microvascular perfusion failure and lipid peroxidation. NADPH inhibitors can also prevent the generation of free radicals via NADPH oxidase, thereby inhibiting nuclear factor-kappa beta (NF- $\kappa$ B) and TNF- $\alpha$  mRNA expression. Both endogenous and exogenous NO can protect

hepatocytes and LSECs against hepatic ischemia/reperfusion injury and LPS-mediated liver damage (40). In addition, NO can reduce the cytokine-mediated interaction between leukocyte and endothelium by inhibition of adhesion molecule expression. However, RNS may also contribute to sepsis-induced hepatic injury through generation of peroxynitrite (41). Hence, despite promising experimental results demonstrating that preventing or reversing microvascular dysfunction can attenuate injury, little of this knowledge has been translated to the clinical setting.

**The lung**—Pulmonary endothelial dysfunction plays a major role in septic-induced lung injury. Several pathogenic factors induce a variety of changes in ECs, resulting in secretion of inflammatory and chemotactic substances, expression of adhesion molecules, enhanced procoagulant pathways, and alteration of the alveolar-capillary barrier, with subsequent increased permeability, pulmonary edema, and impairment of epithelial alveolar fluid clearance mechanisms (42).

Pulmonary endothelial dysfunction may be a deleterious consequence of excessive cytokine production. Mediators such as TNF-α activate signaling events that culminate in cytoskeletal contraction and increased microvascular permeability (42). In addition, activated neutrophils release NETs, macromolecular structures formed of extruded nuclear chromatin, and bactericidal proteins, which have been shown to exert cytotoxic effects on ECs (43). Blockade of cytokine signaling by inhibiting NF-κB activation in an endotoxic model results in decreased lung inflammation and endothelial permeability, as well as in improved lung function.

Alterations in tight junction proteins, key to maintaining the integrity of the alveolar-capillary barrier, may increase paracellular permeability of the alveolar epithelium (30). Similarly, vascular endothelial (VE)-cadherin is the major component of endothelial adherens junctions—tightly regulated protein complexes that join adjacent ECs and prevent leukocyte emigration and vascular leak. Endocytosis of VE-cadherin is sufficient to induce gaps between ECs, leading to increased permeability (30). Tight junction proteins are also targets of oxidative stress and, thus, ROS overproduction may alter alveolar–capillary barrier function. Alveolar endothelial barrier function is also regulated by members of the connexin (Cx) family, which form functional gap junctional channels in the endothelium and allow cell–cell flux of small molecules and solutes (44). Cx43 has been shown to mediate interendothelial Ca<sup>2+</sup> movement that upregulates the leukocyte adhesion receptor P-selectin, thereby

contributing to the propagation of inflammation. Inhibition of this Cx prevents the increase in endothelial permeability observed in an experimental model of lung injury (44). Finally, high-mobility group protein B1—a late mediator of sepsis—has been shown to induce the formation of endothelial paracellular gaps, perhaps providing a potential therapeutic target in acute respiratory distress syndrome (ARDS) secondary to sepsis (45).

The endothelial glycocalyx has also been recognized to be a critical regulator of barrier integrity in the alveolar endothelium. The glycocalyx actively regulates barrier function via mechanotransduction, and its alteration may lead to augmentation in EC hydraulic conductivity and subsequent formation of pulmonary edema (42). During sepsis in the lung, leukocytes interact with the endothelial glycocalyx and promote its degradation (7). In summary, pulmonary edema from increased endothelial permeability is the hallmark of acute lung injury during sepsis, and as summarized above, several pathogenic factors may be involved in this process.

Assessment of endothelial function

Monitoring the function of the ECL at the bedside can be regarded as the single main obstacle in the evaluation of the pathogenesis of endothelial dysfunction and in its therapeutic management in states of critical illness such as sepsis. Several methods, mostly experimental, have been introduced at the bedside to monitor EC function (Table 1), although, accurate validation of these as surrogates for ECL function is incomplete. Reactive hyperemic response using, for example, laser Doppler (45), near-infrared spectroscopy (46), or fingertip tonometry (47), has been used to identify endothelial dysfunction in sepsis. The ability to directly visualize the microcirculation at the bedside using hand-held microscopes has greatly increased the appreciation for the need to monitor the microcirculation in critically ill patients (48). Several recent studies in septic patients have shown that microcirculatory alterations are closely associated with organ failure and mortality (49, 50), independent of variations in systemic hemodynamics. Such alterations are directly related to EC dysfunction, although other factors such as red blood cell deformability or increased aggregation can also cause microcirculatory alterations. Several studies, however, attempted to use hand-held microscopy to specifically identify dysfunction of the ECL. These have included the identification of leukocyte rolling (51), the response to acetylcholine administration (52), and the measurement of capillary boundary changes as a means to observe

TABLE 1. Functional monitoring of the septic endothelium

Function of normal endothelial cells	Phenotype of septic endothelial cell	Techniques for bedside monitoring
Barrier function	Capillary leak	Dye exclusion, glycocalyx shedding, vital microscopy, biomarkers, EVLW
Signal transduction	Tissue hypoperfusion	Ach challenge vital microscopy
Vasomotor regulation	Vasoplegia	Reactive hyperemia (NIRS, capillary refill, vital microscopy)
Control of coagulation	DIC, purpura, HUS, TTP TAMOF	Biomarkers, ex vivo functional coagulation Monitor
Oxidative defense		Biomarkers
Immunological		Leukocyte adhesion/rolling
		Vital microscopy

DIC indicates disseminated intravascular coagulation; EVLW, extra vascular lung water; HUS, hemolytic uremic syndrome; NIRS, near infra red spectroscopy; TAMOF, thrombocytopenia-associated multiple organ failure; TTP, thrombotic thrombocytopenic purpura.

alterations in the glycocalyx (53). There is also growing interest in using the presence of soluble adhesion molecules in the circulation as biomarkers of sepsis-related EC activation.

Many **biomarkers** have been identified, which may serve as surrogates for **endothelial dysfunction**, and excellent reviews have appeared on the subject (54). These include proteases, soluble VCAMs, **glycocalyx components**, and coagulation factors such as TF and PAI-1. Selectins and integrins that are released during states of inflammation have been linked in several studies as indicators of EC activation (54). Such soluble biomarkers can indeed be sensitive indicators of subsequent development of sepsis (55). Recently, a large prospective multicenter observational study (55) confirmed that biomarkers of EC activation strongly correlate with outcome. In addition, it has been shown that the presence of ECs in the circulation correlates with damage to the ECL in lung injury (56). The **use of biomarkers** is, however, **limited** due to their **uncertain origin**, the **cost and time** to measure, the difficulty in monitoring their presence over time, and importantly, the lack of standardization and validation.

**Glycocalyx shedding** is a **sensitive** indicator of injury to the ECL. Heparin sulfate, sialic acid, **hyaluronic acid**, and **syndecan-1** can be detected in plasma from patients with sepsis (57). However, as yet, there is **no “gold standard”** for *in vivo* assessment of **glycocalyx shedding**, and the validation of surrogate biomarkers is still underway. The use of vital dyes of different sizes in combination with dyes measuring red blood cell volume, although very cumbersome, has also been applied in clinical scenarios for measuring the presence of the ECL glycocalyx (53). A different more experimental approach to evaluate the glycocalyx has been to calculate its width by analysis of hand-held video images of sublingual microcirculation and calculating the change in capillary perfused boundary width. A key limitation that must be addressed during the validation of biomarkers is the heterogeneity of the endothelium between organs and the heterogeneity of vessels within each organ. Still, as technologies improve, advances in hand-held bedside imaging (58) coupled with biomarker assays could reveal dynamic changes in the function of the ECL that should help direct therapy.

### The impact of therapy

**Conventional therapies** used in the treatment of sepsis **can actually cause injury to the ECL**. For example, **fluid** therapy may be beneficial when applied early in the course of sepsis (60), but may be ineffective in correcting sepsis-induced microcirculatory alterations when applied later (59, 60), and may even have **deleterious** effects on endothelial function when applied in **excessive** amounts by **altering shear stress** and inducing **glycocalyx degradation**, leading to loss-of-barrier function (6, 61, 62). **Catecholamines**, which are often administered during septic shock, have been **suggested** to contribute to **endothelial dysfunction** and promote **increases in glucose levels**, which can potentially **adversely** affect the ECL. Also, **antibiotics** such as **vancomycin** can have **deleterious** effects on **endothelial** cells (63) and **inducing** the **release** of **proinflammatory cytokines** such as **IL-6** (64), which can contribute to organ failure (64).

The **loss of antioxidant defenses** in the **glycocalyx** of the ECL makes the **antioxidant therapy** a logical candidate for protecting the ECL. Indeed, addition of catalase and SOD to EC cultures can block  $H_2O_2$ -shedding of the glycocalyx and loss-of-barrier function (9). Still, randomized clinical **trials** have **failed** to show conclusively that generalized **antioxidants** **improve outcomes** in septic patients. A possible reason for the mixed results of antioxidant studies may be ineffective targeting of antioxidant therapy. In this regard, several animal studies have tested targeted delivery, and have demonstrated, for instance, that conjugated SOD with antibodies targeted to endothelial endosomes had beneficial effects in endotoxin-challenged mice (65). Patil et al. (66) tested the mitochondria-targeted antioxidant, MitoTEMPO, in mice made septic by CLP, and found improved organ function and increased survival. Given the potential deleterious effects of RNS generated by sepsis-induced iNOS, the use of selective iNOS inhibitors has been extensively investigated in animal models of sepsis and has shown to be protective (28). Similarly, administration of tetrahydrobiopterin analogs such as sepiapterin, targeting the uncoupling of eNOS to reduce superoxide generation, can restore NO generation and protect the endothelium and organ function (67, 68).

Although specific therapies directed at reducing adhesion of leukocytes such as antibodies raised against adhesion molecules like CD11a have been shown to be effective in sepsis-induced lung injury (69), **no clinical therapies are available for limiting adhesion of leukocytes to the endothelium**. Of course, protecting the ECL in other ways, perhaps through NO donors, could result, indirectly, in modulating leukocyte adhesion. It could be argued that vasodilators may have favorable effects on rescuing endothelial function. For example, **phosphodiesterase 4 inhibition** has been shown to **improve microvascular flow**, as well **endothelial barrier function**, in **animal** models of sepsis (70, 71).

A number of different therapeutic strategies have been proposed, which target endothelial tight junctions and leakage. The reader is directed to an excellent recent review on this topic (72). Another emerging area of interest is the possibility of targeting endothelial repair mechanisms with endothelial progenitor cells, but although promising, there is still much to learn (47, 73).

Endothelial NF- $\kappa$ B activation plays a key role in the cascade of events leading to EC dysfunction in sepsis. Blocking NF- $\kappa$ B activation by anti-inflammatory drugs results in reduced iNOS expression, reduced nitrosative stress, and attenuated eNOS downregulation (74), all of which have a beneficial protective role for the ECS. Inhibiting NF- $\kappa$ B activation in an endotoxic model results in decreased lung inflammation and endothelial permeability, as well as in improved lung function. Although controversial in sepsis, there are various lines of evidence to suggest the protective effects of synthetic steroids to the endothelium (75). **Corticosteroids** have been shown to **protect** the **glycocalyx**, and **dexamethasone** was shown to be **beneficial** in **protecting the renal microcirculation** (76). The protective action of synthetic steroids to EC function, however, can be neutralized by the **excessive** presence of **NO**, which can **block** the **glucocorticoid receptor** (77). Although clinically no longer



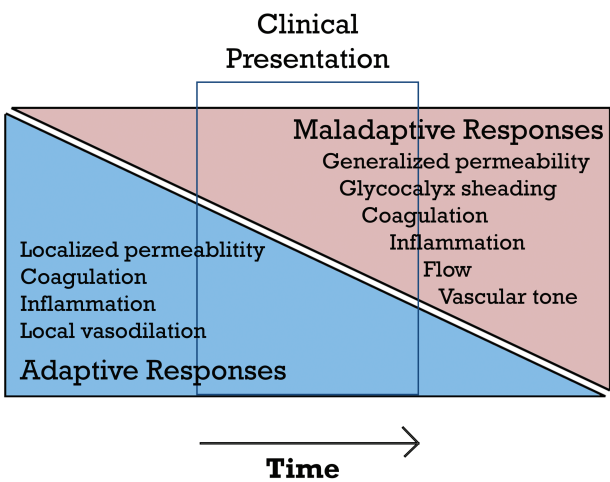


FIG. 3. Adaptive versus maladaptive response to sepsis. Source: Acute Dialysis Quality Initiative 14, www.ADQI.net 2014; used with permission.

available, activated protein C inhibits NF-κβ and has been shown in a number of studies to have a protective effect on the endothelial function in conditions of sepsis and improved organ function (57, 78). An alternative innovative method of protecting the endothelium at risk may be the use hemoperfusion with polymyxin B-immobilized fibers, which have already been shown to have a beneficial effect in PaO<sub>2</sub>/FiO<sub>2</sub> ratio in intensive care patients. In a recent experimental study, it was shown to also reduce leukocyte and platelet adhesion to ECs (79).

Alterations to the ECL: an adaptive or a maladaptive response?

An important question to address prior to considering therapeutic maneuvers is whether microvascular alterations are adaptive or maladaptive, and in extension of this idea, an initiator, a promoter, or just a bystander. The categorization as adaptive versus maladaptive is context and time-dependent (Fig. 3, Table 2). Under conditions of focal infection (e.g., pneumonia or soft tissue infection), local vasodilation independent of metabolic needs and increased permeability is required to allow leukocytes to reach the infection site and in particular the interstitial tissue where microorganisms are populating. In addition, activation of coagulation and downstream vasoconstriction helps to prevent dissemination of the

infection. In a model of focal pneumonia, early administration of activated protein C before development of sepsis decreased local formation of fibrin, and favored dissemination of infection and development of systemic sepsis (80). At more advanced stages, however, alterations in the ECL contributed to the septic phenotype, with marked decrease in vascular tone, diffuse alterations in microvascular perfusion, generalized increase in permeability, and DIC. At these stages, it was difficult to imagine any adaptive benefit in the diffuse increase in permeability, contributing to lung edema and compartmental syndrome (including in the kidney) or in DIC.

With regard to alterations in microvascular perfusion, the benefit/detriment may be more debatable. Because alterations in cellular metabolism occur in sepsis and microvascular perfusion adapts to meet metabolic needs, one must consider whether alterations in microvascular perfusion observed just after initial resuscitation is adaptive or maladaptive. Several factors suggest that the alterations in microvascular perfusion at sites of ECL dysfunction are primary events leading to organ injury. However, this notion is complicated because of the heterogeneity of microvascular perfusion during sepsis. For example, perfused capillaries are in close proximity to non-perfused capillaries, leading to alterations in oxygen extraction, hypoxic zones, and functional shunting, even when total blood flow to the organ is preserved (81, 82). It is of significance to note that even in the presence of severe microcirculatory alterations associated with sepsis, ECs can still be functional in terms of their responsiveness to acetylcholine-induced vasodilation (52, 83). Heterogeneity of perfusion is associated with heterogeneity in oxygenation (24), and also with altered oxygen extraction capabilities (84). Under normal conditions, the heterogeneity of perfusion is minimal and it further decreases under stress (i.e., hemorrhage). In sepsis, heterogeneity of perfusion is already increased at baseline and further increases when stressed (84). These heterogeneous alterations in microvascular perfusion are colocalized with low PO<sub>2</sub>, production of hypoxia-inducible factor (85), altered redox potential, or even cell death (86) in experimental models. Microvascular alterations can lead to cellular injury. In addition, several trials have demonstrated an association between the severity of microvascular dysfunction and the development of organ dysfunction (46, 87, 88) and mortality (49, 83, 89). Second, oxygen

TABLE 2. Adaptive and maladaptive responses of endothelial cell function in sepsis

Organ	Location	Adaptive response in sepsis	Maladaptive response in sepsis
Kidney	Glomerular capillary		Shedding of glycocalyx; increased permeability; activation of coagulation
	Peritubular capillary	Localized leukocyte adhesion/transmigration; generation of NO and endothelin; blood flow regulation	Shedding of glycocalyx; widespread and sustained increase in permeability; generation of ROS/RNS; propagation of the inflammatory response
Lung	Alveoli	Production of tight junction proteins such as VE-cadherin; maintenance of endothelial glycocalyx	Secretion of inflammatory and chemotactic substances; expression of adhesion molecules; enhanced procoagulant pathways; increased permeability with pulmonary edema
Liver	Liver sinusoids	Localized leukocyte adhesion/transmigration; generation of NO and endothelin; blood flow regulation; antigen presentation; liver immune tolerance	Loss of sieve-plate architecture of the sinusoidal endothelium; inflammation-induced sinusoidal endothelial gap formation; contribution to procoagulant state; changes in the patterns of ET receptor expression; heterogeneous hepatic sinusoidal perfusion, hypoxia

ET indicates endothelin; NO, nitric oxide; RNS, reactive nitrogen species; ROS, reactive oxygen species; VE, vascular endothelial.

saturation at the capillary end of well-perfused capillaries is low, not elevated, suggesting that the tissues are using the delivered oxygen (90), although in tissue oxygenation measurements, venous  $pO_2$  values higher than microcirculatory  $pO_2$  values have been reported, indicating the presence of functional shunting (82). Third, tissue-to-arterial  $PCO_2$  gradient—the  $PCO_2$  gap—is increased in sepsis (52, 91–93). In addition, there is an inverse relationship between sublingual microvascular perfusion and the  $PCO_2$  gap (52). A similar inverse relationship is found between ileal mucosal perfusion and ileal-to-arterial  $PCO_2$  gap (93). If flow alterations were just matching metabolism,  $CO_2$  production would be low because the primary alteration is the decrease in metabolism, and  $PCO_2$  gap would be normal, even at low flows. Fourth, perfusion abnormalities precede alterations in organ function (94). Finally, animal models of sepsis have shown that the improvement in microvascular perfusion is associated with an improvement in redox potential (28) and decrease in the number of dead cells (95). In patients with septic shock, the improvement in the sublingual microcirculation in response to initial resuscitation procedures was associated with an improvement of organ function 24 h later (96). The decrease in lactate levels was also proportional to the improvement of the microcirculation during dobutamine administration (52).

Admittedly, cellular metabolic alterations may also contribute to organ dysfunction, and this will be covered in other part of this series. Importantly, there is an interplay between hypoxia and inflammation, and mitochondrial dysfunction (97). Limiting perfusion abnormalities in a timely fashion is associated with a lower expression of inflammation molecules, caspases, and mitochondrial abnormalities (98). The second part of the question is probably the easiest to address. In sepsis, infection always begins in a single spot and transformation from focal infection to sepsis includes alterations in endothelial function. Propagation of the inflammatory response and of endothelial dysfunction systemically results in the clinical pattern of sepsis. Thus, in summary, the clinical challenge in being able to assess whether ECL and by extension the microcirculatory response is adaptive or maladaptive lies in being able to distinguish whether the ECL is able to respond to stimuli and then mediate an appropriate change in microcirculatory perfusion. Assessment of such functional changes at the bedside, although feasible, remains a challenge (52). If this limitation could be overcome and EC function be readily assessed at the level of the microcirculation at the bedside, it could dramatically improve care of the septic patient.

## CONCLUSIONS

It is clear from the recent literature that the ECL plays a central role in the pathogenesis of sepsis leading to multiorgan failure syndrome. The implication of this conclusion offers a major challenge to the clinical management of the septic patient. Hemodynamic management in terms of fluids and vasopressors has little contribution to either protecting or resuscitating EC function. The antioxidant and anti-inflammatory therapies, although used, have not proven themselves in large randomized controlled trial (RCT) trials. One can draw a conclusion that

these compounds have limited effect, but one may also consider that large-scale randomized controlled trials may not be adequate to evaluate therapies in complex and heterogeneous populations. More mechanistically oriented trial designs are required, focusing on phenotypes of organ and cellular function, to demonstrate potential benefits of protective strategies for the ECS in the clinical management of sepsis. To this end, diagnostic methods to assess EC function at the bedside will have to be further developed, synchronous to therapeutic interventions to fortify and repair the endothelium system at risk during sepsis.

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## APPENDIX 2. SEARCH TERMS

Endothelium, sepsis, inflammation, barrier function, hemostasis, blood, glycocalyx, vectorial transport, microcirculation, capillary, blood flow, acute respiratory distress syndrome, ARDS, acute lung injury, ALI, adherens junction, gap junction, biomarkers, plasma, blood, intestine, liver, translocation, bacteria, bacterial translocation, ions, transport, microbiota, mucosa.

## REFERENCES

1. Ait-Oufella H, Maury E, Lehoux S, Guidet B, Offenstadt G: The endothelium: physiological functions and role in microcirculatory failure during severe sepsis. *Intensive Care Med* 36:1286–1298, 2010.

2. Kellum JA, Gómez H, Gómez A, Murray P, Ronco C: Acute Dialysis Quality Initiative (ADQI) XIV sepsis phenotypes and targets for blood purification in sepsis: The Bogota Consensus. *Shock* 45:242–248, 2016.
3. Aird WC: Endothelial cell heterogeneity. *Cold Spring Harb Perspect Med* 2:a006429, 2012.
4. Weinbaum S, Tarbell JM, Damiano ER: The structure and function of the endothelial glycocalyx layer. *Annu Rev Biomed Eng* 9:121–167, 2007.
5. Zuurbier C, Vink H, Koeman A, Demirci C, Ince C: Short-term hyperglycemia increases endothelial glycocalyx permeability and decreases lineal density of capillaries with flowing RBC's. *J Appl Physiol* 99:1471–1476, 2005.
6. Rubio-Gayosso I, Platts SH, Duling BR: Reactive oxygen species mediate modification of glycocalyx during ischemia-reperfusion injury. *Am J Physiol Heart Circ Physiol* 290:H2247–H2256, 2006.
7. Schmidt EP, Yang Y, Janssen WJ, Gandjeva A, Perez MJ, Barthel L, Zemans RL, Bowman JC, Koyanagi DE, Yunt ZX, et al.: The pulmonary endothelial glycocalyx regulates neutrophil adhesion and lung injury during experimental sepsis. *Nat Med* 18:1217–1223, 2012.
8. Nieuwdorp M, Meuwese MC, van Lieshout MHP, Levi M, Meijers JCM, Ince C, Vink H, Kastelein JJP, Erik SG, Stroes ESG: TN- $\alpha$  inhibition dampens endotoxin-induced endothelial glycocalyx perturbation and inflammatory effects in vivo. *Atherosclerosis* 202:296–303, 2009.
9. Singh A, Ramnath RD, Foster RR, Wylie EC, Fridén V, Dasgupta I, Haraldsson B, Welsh GI, Mathieson PW, Satchell SC: Reactive oxygen species modulate the barrier function of the human glomerular endothelial glycocalyx. *PLoS One* 8:e55852, 2013.
10. Forni M, Mazzola S, Ribeiro LA, Pirrone F, Zannoni A, Bernardini C, Bacci ML, Albertini M: Expression of endothelin-1 system in a pig model of endotoxic shock. *Regul Pept* 131:89–96, 2005.
11. Bolon ML, Peng T, Kidder GM, Tymi K: Lipopolysaccharide plus hypoxia and reoxygenation synergistically reduce electrical coupling between microvascular endothelial cells by dephosphorylating connexin40. *J Cell Physiol* 217:350–359, 2008.
12. Camici G, Pozner R, de Larrañaga G: Neutrophil extracellular traps in sepsis. *Shock* 42:286–294, 2014.
13. Levi M, van der Poll T: Disseminated intravascular coagulation: a review for the internist. *Internal Med* 8:23–32, 2013.
14. Nguyen TC, Han YY, Kiss JE, Hall MW, Hassett AC, Jaffe R, Orr RA, Janosky J, Carcillo JA: Intensive plasma exchange increases a disintegrin and metalloprotease with thrombospondin motifs-13 activity and reverses organ dysfunction in children with thrombocytopenia-associated multiple organ failure\*. *Crit Care Med* 36:2878–2887, 2008.
15. Studt JD, Hovinga JA, Antoine G, Hermann M, Rieger M, Scheiflinger F, Lammle B: Fatal congenital thrombotic thrombocytopenic purpura with apparent ADAMTS13 inhibitor: in vitro inhibition of ADAMTS13 activity by hemoglobin. *Blood* 105(2):542–544, 2005.
16. Nolasco LH, Turner NA, Bernardo A, Tao Z, Cleary TG, Dong JF, Moake JL: Hemolytic uremic syndrome-associated Shiga toxins promote endothelial-cell secretion and impair ADAMTS13 cleavage of unusually large von Willebrand factor multimers. *Blood* 106:4199–4209, 2005.
17. Nguyen TC, Liu A, Liu L, Ball C, Choi H, May WS, Aboulfatova K, Bergeron AL, Dong JF: Acquired ADAMTS-13 deficiency in pediatric patients with severe sepsis. *Haematologica* 92:121–124, 2007.
18. Zhou F, Peng Z, Murugan R, Kellum JA: Blood purification and mortality in sepsis: a meta-analysis of randomized trials. *Crit Care Med* 41:2209–2220, 2013.
19. Wang Z, Sims CR, Patil NK, Gokden N, Mayeux PR: Pharmacological targeting of sphingosine phosphate receptor 1 improves the renal microcirculation during sepsis in the mouse. *J Pharmacol Exp Ther* 352:61–66, 2015.
20. Reid VL, Webster NR: Role of microparticles in sepsis. *Brit J Anaesth* 109:503–513, 2012.
21. Xu C, Chang A, Hack BK, Eadon MT, Alper SL, Cunningham PN: TNF-mediated damage to glomerular endothelium is an important determinant of acute kidney injury in sepsis. *Kidney Int* 85:72–81, 2014.
22. Adembri C, Sgambati E, Vitali L, Selmi V, Margheri M, Tani A, Bonaccini L, Nosi D, Calzavara AL, Formigli L, et al.: Sepsis induces albuminuria and alterations in the glomerular filtration barrier: a morphofunctional study in the rat. *Crit Care* 15:R277, 2011.
23. Wang Z, Holthoff JH, Seely KA, Pathak E, Spencer HJ 3rd, Gokden N, Mayeux PR: Development of oxidative stress in the peritubular capillary microenvironment mediates sepsis-induced renal microcirculatory failure and acute kidney injury. *Am J Pathol* 180:505–516, 2012.
24. Legrand M, Bezemer R, Payen D, Ince C: The role of renal hypoperfusion in the development of renal microcirculatory dysfunction in endotoxemic rats. *Intensive Care Med* 37:1534–1542, 2011.
25. Johannes T, Mik EG, Ince C: Nonresuscitated endotoxemia induces microcirculatory hypoxic areas in the renal cortex in the rat. *Shock* 31:97–103, 2009.
26. Seely KA, Holthoff JH, Burns ST, Wang Z, Thakali KM, Gokden N, Rhee SW, Mayeux PR: Hemodynamic changes in the kidney in a pediatric rat model of sepsis-induced acute kidney injury. *Am J Physiol Renal Physiol* 301:F209–F217, 2011.
27. Wu L, Gokden N, Mayeux PR: Evidence for the role of reactive nitrogen species in polymicrobial sepsis-induced renal peritubular capillary dysfunction and tubular injury. *J Am Soc Nephrol* 18:1807–1815, 2007.
28. Wu L, Mayeux PR: Effects of the inducible nitric oxide synthase inhibitor L-N<sup>G</sup>-(1-iminoethyl)-lysine on microcirculation and reactive nitrogen species generation in the kidney following lipopolysaccharide administration in mice. *J Pharmacol Exp Ther* 320:1061–1067, 2007.
29. Wu L, Tiwari MM, Messer KJ, Holthoff JH, Gokden N, Brock RW, Mayeux PR: Peritubular capillary dysfunction and renal tubular epithelial cell stress following lipopolysaccharide administration in mice. *Am J Physiol Renal Physiol* 292:F261–F268, 2007.
30. Lee WL, Slutsky AS: Sepsis and endothelial permeability. *N Engl J Med* 7:689–691, 2010.
31. Mayeux PR1, MacMillan-Crow LA: Pharmacological targets in the renal peritubular microenvironment: implications for therapy for sepsis-induced acute kidney injury. *Pharmacol Ther* 134:139–155, 2012.
32. Zeng Y, Adamson RH, Curry FR, Tarbell JM: Sphingosine-1-phosphate protects endothelial glycocalyx by inhibiting syndecan-1 shedding. *Am J Physiol Heart Circ Physiol* 306:H363–H372, 2014.
33. Wang Z, Herzog C, Kaushal GP, Gokden N, Mayeux PR: Actinonin, a meprin A inhibitor, protects the renal microcirculation during sepsis. *Shock* 35:141–147, 2011.
34. Schlegel N, Waschke J: cAMP with other signaling cues converges on Rac1 to stabilize the endothelial barrier- a signaling pathway compromised in inflammation. *Cell Tissue Res* 355(3):587–596, 2014.
35. Holthoff JH, Wang Z, Patil NK, Gokden N, Mayeux PR: Rolipram improves renal perfusion and function during sepsis in the mouse. *J Pharmacol Exp Ther* 347:357–364, 2013.
36. Braet F: How molecular microscopy revealed new insights in the dynamics of hepatic endothelial fenestrae in the past decade. *Liver Int* 24:532–539, 2004.
37. Ito Y, Abril ER, Bethea NW, McCuskey MK, Cover C, Jae-schke H, McCuskey RS: Mechanisms and pathophysiological implications of sinusoidal endothelial cell gap formation following treatment with galactosamine/endotoxin in mice. *Am J Physiol Gastrointest Liver Physiol* 291:G211–G218, 2006.
38. Kume M, Hayashi T, Yuasa H, Tanaka H, Nishioka J, Ido M, Gabazza EC, Kawarada Y, Suzuki K: Bacterial lipopolysaccharide decreases thrombomodulin expression in the sinusoidal endothelial cells of rats: a possible mechanism of intrasinusoidal microthrombus formation and liver dysfunction. *J Hepatol* 38:9–17, 2003.
39. Matejovic M, Krouzecky A, Martinkova V, Rokyta R Jr, Kralova H, Treska V, Radermacher P, Novak I: Selective inducible nitric oxide synthase inhibition during long-term hyperdynamic porcine bacteremia. *Shock* 21:458–465, 2004.
40. Mojena M, Hortelano S, Castrillo A, et al.: Protection by nitric oxide against liver inflammatory injury in animals carrying a nitric oxide synthase-2 transgene. *FASEB J* 15:583–585, 2001.
41. La Mura V, Pasarín M, Rodríguez-Vilarrupla A, García-Pagán JC, Bosch J, Abalde JG: Liver sinusoidal endothelial dysfunction after LPS administration: a role for inducible-nitric oxidesynthase. *J Hepatol* 61:1321–1327, 2014.
42. Maniatis NA, Orfanos SE: The endothelium in acute lung injury/acute respiratory distress syndrome. *Curr Opin Crit Care* 14:22–30, 2008.
43. Saffarzadeh M, Juenemann C, Queisser MA, Lochnit G, Barreto G, Galuska SP, Lohmeyer J, Preissner KT: Neutrophil extracellular traps directly induce endothelial and endothelial cell death: a predominant role of histones. *PLoS One* 7:e32366, 2012.
44. Parthasarathi K: Endothelial connexin43 mediates acid-induced increases in pulmonary microvascular permeability. *Am J Physiol Lung Cell Mol Physiol* 303:L33–L42, 2012.
45. Neviere D, Mathieu D, Chagnon J, Lebleu N, Millien JP, Watiel N: Skeletal muscle microvascular blood flow and oxygen transport in patients with severe sepsis. *Am J Resp Crit Care Med* 153:191–195, 1996.
46. Doerschug KC, Delsing AS, Schmidt GA, Haynes WG: Impairments in microvascular reactivity are related to organ failure in human sepsis. *Am J Physiol Heart Circ Physiol* 293:H1065–H1071, 2007.
47. Schmidt-Lucke C, Fichtlscherer S, Aicher A, Tschöpe C, Schultheiss HP, Zeiher AM, Dimmeler S: Quantification of circulating endothelial progenitor cells using the modified ISHAGE protocol. *PLoS One* 5:e13790, 2010.
48. Hernandez G, Bruhn A, Ince C: Microcirculation in sepsis: new perspectives. *Curr Vasc Pharmacol* 11:161–169, 2013.



49. De Backer D, Donadello K, Sakr Y, Ospina-Tascon G, Salgado D, Scolletta S, Vincent JL: Microcirculatory alterations in patients with severe sepsis: impact of time of assessment and relationship with outcome. *Crit Care Med* 41:791–799, 2013.
50. Edul VS, Enrico C, Laviolle B, Vazquez AR, Ince C, Dubin A: Quantitative assessment of the microcirculation in healthy volunteers and in patients with septic shock. *Crit Care Med* 40:1443–1448, 2012.
51. Bauer A, Kofler S, Thiel M, Eifert S, Christ F: Monitoring of the sublingual microcirculation in cardiac surgery using orthogonal polarization spectral imaging: preliminary results. *Anesthesiology* 107:939–945, 2007.
52. De Backer D, Creteur J, Dubois MJ, Sakr Y, Koch M, Verdant C, Vincent JL: The effects of dobutamine on microcirculatory alterations in patients with septic shock are independent of its systemic effects. *Crit Care Med* 34:403–408, 2006.
53. Nieuwdorp M, Meuwese MC, Mooij HL, Ince C, Broekhuizen LN, Kastelein JP, Stroes ESG, Vink H: Measuring endothelial glycocalyx dimensions in humans: a novel tool to monitor vascular vulnerability. *J Applied Physiol* 104:845–852, 2008.
54. Xing K, Murthy S, Liles WC, Singh JM: Clinical utility of biomarkers of endothelial activation in sepsis: a systematic review. *Crit Care* 16(1):R7, 2012.
55. Skibsted S, Jones AE, Puskarich MA, Arnold R, Sherwin R, Trzeciak S, Schuetz P, Aird WC, Shapiro NI: Biomarkers of endothelial cell activation in early sepsis. *Shock* 39:427–432, 2013.
56. Moussa MD, Santonocito C, Fagnoul D, Donadello K, Pradier O, Gaussem P, De Backer D, Vincent JL: Evaluation of endothelial damage in sepsis-related ARDS using circulating endothelial cells. *Intensive Care Med* 41(2):231–238, 2015.
57. Donati A, Damiani E, Botticelli L, Adrario E, Lombrano MR, Domizi R, Marini B, Van Teeffelen JW, Carletti P, Girardis M, Pelaia P, Ince C: The aPC treatment improves microcirculation in severe sepsis/septic shock syndrome. *BMC Anesthesiol* 13(1):25, 2013.
58. Aykut G, Veenstra G, Scorcea, Ince C, Boerma C: Cytocam-IDF (incident dark field illumination) imaging for bed-side monitoring of the microcirculation. *Inten Care Med Exp* 3:4, 2015.
59. Ospina-Tascon G, Neves AP, Occhipinti G, Donadello K, Büchele G, Simion D, Chierego ML, Silva TO, Fonseca A, Vincent JL, De Backer D: Effects of fluids on microvascular perfusion in patients with severe sepsis. *Inten Care Med* 36:949–955, 2010.
60. Ince C: The rationale for microcirculatory-guided fluid therapy. *Curr Opin in Crit Care* 20:301–308, 2014.
61. Aksu U, Bezemer R, Yavuz B, Kandil A, Demirci C, Ince C: Balanced vs. unbalanced crystalloid resuscitation in a near-fatal model of hemorrhagic shock and the effects on renal oxygenation, oxidative stress, and inflammation. *Resuscitation* 83:767–773, 2012.
62. Johannes T, Mik EG, Nohé B, Raat NJH, Unertl KE, Ince C: Influence of fluid resuscitation on renal microvascular PO<sub>2</sub> in a normotensive rat model of endotoxemia. *Crit Care* 10(R88):1–13, 2006.
63. Drouet M, Chai F, Barthélémy C, Lebuffe G, Debaene B, Décaudin B, Odou P: Influence of vancomycin infusion methods on endothelial cell toxicity. *Antimicrob Agents Chemother*; 2014:3694–3714, 2014.
64. Cianferoni S, Devigili A, Ocampos-Martinez E, Penaccini L, Scolletta S, Abdelhadi A, De Backer D, Beumier M, Jacobs F, Vincent JL, Taccone FS: Development of acute kidney injury during continuous infusion of vancomycin in septic patients. *Infection* 41:811–820, 2013.
65. Shuvaev VV, Han J, Tliba S, Arguiri E, Christofidou-Solomidou M, Ramirez SH, Dykstra H, Persidsky Y, Atochin DN, Huang PL, Muzykantor VR: Anti-inflammatory effect of targeted delivery of SOD to endothelium: mechanism, synergism with NO donors and protective effects in vitro and in vivo. *PLoS One* 8(10):e77002, 2013.
66. Patil NK, Parajuli N, MacMillan-Crow LA, Mayeux PR: Inactivation of renal mitochondrial respiratory complexes and manganese superoxide dismutase during sepsis: mitochondria-targeted antioxidant mitigates injury. *Am J Physiol Renal Physiol* 306(7):F734–F743, 2014.
67. Legrand M, Kandil A, Payen D, Ince C: Effects of sepiapterin infusion on renal oxygenation and early acute renal injury following supra-renal aortic clamping in rats. *J Cardiovasc Pharmacol* 58:192–198, 2011.
68. He X, Su F, Velissaris D, Salgado DR, de Souza Barros D, Lorent S, Taccone FS, Vincent JL, De Backer D: Administration of tetrahydrobiopterin improves the microcirculation and outcome in an ovine model of septic shock. *Crit Care Med* 40:2833–2840, 2012.
69. Wang Y, Roller J, Menger MD, Thorlacius H: Sepsis-induced leukocyte adhesion in the pulmonary microvasculature in vivo is mediated by CD11a and CD11b. *Eur J Pharmacol* 702:135–141, 2013.
70. Flemming S, Schlegel N, Wunder C, Meir M, Baar W, Wollborn J, Roewer N, Germer CT, Schick MA: Phosphodiesterase 4 inhibition dose dependently stabilizes microvascular barrier functions and microcirculation in a rodent model of polymicrobial sepsis. *Shock* 41:537–545, 2014.
71. Holthoff JH, Wang Z, Seely KA, Gokden N, Mayeux P: Resveratrol improves renal microcirculation, protects the tubular epithelium, and prolongs survival in a mouse model of sepsis-induced acute kidney injury. *Kidney Int* 81:370–378, 2012.
72. Darwish I, Liles WC: Emerging therapeutic strategies to prevent infection-related microvascular endothelial activation and dysfunction. *Virulence* 4(6):572–582, 2013.
73. van Ierssel SH, Van Craenenbroeck EM, Hoymans VY, Vrints CJ, Conraads VM, Jorens PG: Endothelium dependent vasomotion and in vitro markers of endothelial repair in patients with severe sepsis: an observational study. *PLoS One* 8(8):e69499, 2013.
74. Ding J, Song D, Ye X, Liu SF: A pivotal role of endothelial-specific NF-kappaB signaling in the pathogenesis of septic shock and septic vascular dysfunction. *J Immunol* 183:4031–4038, 2009.
75. Chappell D, Jacob M, Hofmann-Kiefer K, Bruegger D, Rehm M, Conzen P, Welsch U, Becker BF: Hydrocortisone preserves the vascular barrier by protecting the endothelial glycocalyx. *Anesthesiology* 107:776–784, 2007.
76. Johannes T, Mik EG, Klingel K, Dieterich HJ, Unertl KE, Ince C: Low-dose dexamethasone supplemented fluid resuscitation reverses Endotoxin-induced acute renal failure and prevents cortical microvascular hypoxia. *Shock* 31:521–528, 2009.
77. Duma D, Silva-Santos J, Assreuy J: Inhibition of glucocorticoid receptor binding by nitric oxide in endotoxemic rats. *Crit Care Med* 32:2304–2310, 2004.
78. Marechal X, Favory R, Joulin O, Montaigne D, Hassoun S, Decoster B, Zerimech F, Neviere R: Endothelial glycocalyx damage during endotoxemia coincides with microcirculatory dysfunction and vascular oxidative stress. *Shock* 29:572–576, 2008.
79. Iba T, Nagaoka I, Yamada A, Nagayama M, Miki T: Effect of hemoperfusion using polymyxin B-immobilized fibers on acute lung injury in a rat sepsis model. *Int J Med Sci* 11:255–256, 2014.
80. Robriquet L, Collet F, Tournays A, Prangère T, Nevière R, Fourrier F, Guery BP: Intravenous administration of activated protein C in Pseudomonas-induced lung injury: impact on lung fluid balance and the inflammatory response. *Respir Res* 7:41, 2006.
81. Walley KR: Heterogeneity of oxygen delivery impairs oxygen extraction by peripheral tissues: theory. *J Appl Physiol* 81:885–894, 1996.
82. Ince C, Sinaasappel M: Microcirculatory oxygenation and shunting in sepsis and shock. *Crit Care Med* 27:1369–1377, 1999.
83. De Backer D, Creteur J, Preiser JC, Dubois MJ, Vincent JL: Microvascular blood flow is altered in patients with sepsis. *Am J Respir Crit Care Med* 166:98–104, 2002.
84. Goldman D, Bateman RM, Ellis CG: Effect of decreased O<sub>2</sub> supply on skeletal muscle oxygenation and O<sub>2</sub> consumption during sepsis: role of heterogeneous capillary spacing and blood flow. *Am J Physiol Heart Circ Physiol* 290:H2277–H2285, 2006.
85. Bateman RM, Tokunaga C, Kareco T, Dorscheid DR, Walley KR: Myocardial hypoxia-inducible HIF-1 $\alpha$ , VEGF, and GLUT1 gene expression is associated with microvascular and ICAM-1 heterogeneity during endotoxemia. *Am J Physiol Heart Circ Physiol* 293:H448–H456, 2007.
86. Eipel C, Borden R, Nickels RM, Menger MD, Vollmar B: Impact of leukocytes and platelets in mediating hepatocyte apoptosis in a rat model of systemic endotoxemia. *Am J Physiol Gastrointest Liver Physiol* 286:G769–G776, 2004.
87. Shapiro NI, Arnold R, Sherwin R, O'Connor J, Najjarro G, Singh S, Lundy D, Nelson T, Trzeciak SW, Jones AE: The association of near infrared spectroscopy-derived tissue oxygenation measurements with sepsis syndromes, organ dysfunction and mortality in emergency department patients with sepsis. *Crit Care* 15:R223, 2011.
88. Trzeciak S, McCoy JV, Phillip Dellinger R, Arnold RC, Rizzuto M, Abate NL, Shapiro NI, Parrillo JE, Hollenberg SM: Early increases in microcirculatory perfusion during protocol-directed resuscitation are associated with reduced multi-organ failure at 24 h in patients with sepsis. *Inten Care Med* 34:2210–2217, 2008.
89. Top AP, Ince C, de Meij N, van Dijk M, Tibboel D: Persistent low microcirculatory vessel density in nonsurvivors of sepsis in pediatric intensive care. *Crit Care Med* 39:8–13, 2011.
90. Ellis CG, Bateman RM, Sharpe MD, Sibbald WJ, Gill R: Effect of a maldistribution of microvascular blood flow on capillary O<sub>2</sub> extraction in sepsis. *Am J Physiol Heart Circ Physiol* 282:H156–H164, 2002.
91. Marik PE: Sublingual capnography: a clinical validation study. *Chest* 120:923–927, 2001.
92. Vallée F, Mateo J, Dubreuil G, Poussant T, Tachon G, Ouanounou I, Payen D: Cutaneous ear lobe Pco<sub>2</sub> at 37°C to evaluate microperfusion in patients with septic shock. *Chest* 138:1062–1070, 2010.

93. Dubin A, Kanoore Edul VS, Pozo M, Murias G, Canullán C, Martins E, Ferrara G, Canales H, Laporte M, Estenssoro E, Ince C: Persistent villi hypoperfusion explains intramucosal acidosis in sheep endotoxemia. *Crit Care Med* 36:535–542, 2008.
94. Rosengarten B, Hecht M, Auch D, Ghofrani HA, Schermuly RT, Grimminger F, Kaps M: Microcirculatory dysfunction in the brain precedes changes in evoked potentials in endotoxin-induced sepsis syndrome in rats. *Cerebrovasc Dis* 23:140–147, 2007.
95. Kao R, Xenocostas A, Rui T, Yu P, Huang W, Rose J, Martin CM: Erythropoietin improves skeletal muscle microcirculation and tissue bioenergetics in a mouse sepsis model. *Crit Care* 11:R58, 2007.
96. Trzeciak S, McCoy JV, Phillip Dellinger R, Arnold RC, Rizzuto M, Abate NL, Shapiro NI, Parrillo JE, Hollenberg SM: Early increases in microcirculatory perfusion during protocol-directed resuscitation are associated with reduced multi-organ failure at 24 h in patients with sepsis. *Intensive Care Med* 34:2210–2217, 2008.
97. Eltzschig HK, Carmeliet P: Hypoxia and inflammation. *N Engl J Med* 364:656–665, 2011.
98. Rivers EP, Kruse JA, Jacobsen G, Shah K, Loomba M, Otero R, Childs EW: The influence of early hemodynamic optimization on biomarker patterns of severe sepsis and septic shock. *Crit Care Med* 35:2016–2024, 2007.

