# Oxidative Stress and Endothelial Dysfunction in Sepsis and Acute Inflammation

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FORUM REVIEW ARTICLE

# Oxidative Stress and Endothelial Dysfunction in Sepsis and Acute Inflammation

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## Abstract

***Significance:*** Under homeostatic conditions, the endothelium dynamically regulates vascular barrier function, coagulation pathways, leukocyte adhesion, and vasomotor tone. During sepsis and acute inflammation, endothelial cells (ECs) undergo multiple phenotypic and functional modifications that are initially adaptive but eventually become harmful, leading to microvascular dysfunction and multiorgan failure.
***Critical Issues and Recent Advances:*** Sepsis unbalances the redox homeostasis toward a pro-oxidant state, characterized by an excess production of reactive oxygen species and reactive nitrogen species, mitochondrial dysfunction, and a breakdown of antioxidant systems. In return, oxidative stress (OS) alters multiple EC functions and promotes a proinflammatory, procoagulant, and proadhesive phenotype. The OS also induces glycocalyx deterioration, cell death, increased permeability, and impaired vasoreactivity. Thus, during sepsis, the ECs are both a significant source and one of the main targets of OS.
***Future Directions:*** This review aims at covering the current understanding of the role of OS in the endothelial adaptive or maladaptive multifaceted response to sepsis and to outline the therapeutic potential and issues of targeting OS and endothelial dysfunction during sepsis and septic shock. One of the many challenges in the management of sepsis is now based on the detection and correction of these anomalies of endothelial function. *Antioxid. Redox Signal.* 35, 1291–1307.

**Keywords:** endothelium, sepsis, oxidative stress

## Introduction

Septic shock is defined as a condition whereby the circulation cannot deliver adequate blood flow to meet the tissue's metabolic demand and/or cellular metabolism is impaired, ultimately leading to organ dysfunction (138). Microcirculatory anomalies are involved throughout the course of septic shock, and signs such as skin mottling on the knee area, prolonged capillary refill time (CRT), central-to-peripheral temperature gradient, low tissue oxygen saturation ($StO_2$), or abnormal sublingual perfusion index correlate with organ failure severity and are predictive of intensive care unit (ICU) mortality (7, 40, 47, 68). The *substratum* of microcirculatory failure is characterized by endothelial dysfunction, which is often referred to as sepsis-induced endotheliopathy. Indeed, beyond a simple malfunction, the endothelium during sepsis presents with multiple phenotypic and functional modifications that are initially adaptive but eventually become harmful.

During sepsis, the engagement of pattern-recognition receptors (PRRs), including Toll-like receptors (TLRs), drive endothelial cell (EC) reprogramming toward a proinflammatory, proapoptotic, proadhesive, and procoagulant phenotype. In addition, sepsis causes glycocalyx (GCX) damage, dysregulated microcirculatory vasoreactivity, capillary leak, and impaired tissue perfusion (83).

During sepsis, oxidative stress (OS) plays a role in promoting adative responses to hypoxia, bacterial clearance, and

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postinjury endothelial repair processes. However, sepsis can cause an imbalance between reactive oxygen species (ROS) and reactive nitrogen species (RNS), and the antioxidant system, which, in addition to sepsis-induced mitochondrial dysfunction, can, in a vicious cycle, enhance the multiple aspects of endothelial dysfunction. Indeed, OS contributes substantially to sepsis-induced endotheliopathy by playing a role in impaired vasomotricity, augmenting leukocyte and platelet adhesion to ECs and capillary permeability, and promoting cell death and a procoagulant state of the endothelium. This review aims at covering the current understanding of the role of OS in the adaptive or maladaptive multifaceted response to acute inflammation, and therapeutic avenues targeting OS and endothelial alterations during septic shock.

## Endothelial Dysfunction Is a Major Driver of Organ Failure in Sepsis

Sepsis is one of the main reasons for ICU admission and is responsible for more than 10 million deaths each year worldwide (131). Over the past two decades, clinical studies have shown that endothelial and microvascular dysfunction are primary causes of tissue hypoperfusion and organ failure. Further, the presence and intensity of microcirculatory abnormalities during septic shock have been linked, by multiple teams, to organ failure and poor outcome regardless of blood pressure or cardiac output. In 2002, De Backer *et al.* used an orthogonal polarization spectral (OPS) imaging technique to investigate the sublingual microcirculation in 10 healthy volunteers, 10 acutely ill patients without sepsis, and 50 patients with severe sepsis. They demonstrated, for the first time in humans, that sepsis causes altered microcirculatory flow, with reductions in vessel density and in the proportion of perfused vessels (47). These factors compromise tissue perfusion, and the intensity of these alterations correlates with organ failure and death. Since these pioneering observations, a large number of studies have shown that the identification of microcirculatory abnormalities using simple skin signs [mottling (7), prolonged CRT (6, 72)] or precision morphological tools [OPS (81), laser Doppler (24)] is predictive of the severity of organ failure and survival. Therefore, current resuscitation strategies target the restoration of endothelial function and preservation of the microcirculation and thereby promote adequate tissue perfusion and oxygen delivery, with the ultimate goal of reducing organ failure and improving sepsis outcomes. Figure 1 summarizes the cutaneous clinical signs and some bedside clinical tools used to assess the microcirculatory function during septic shock.

## The Endothelium Is Both a Source and a Target for OS: A Vicious Circle

The paradigm governing the relationship between OS and sepsis-induced endotheliopathy is that of pathogenic reciprocity. Under homeostatic conditions, there is a balance between the formation of reactive oxidizing/oxygen species and their removal by endogenous antioxidant scavenging of toxic compounds of the endothelium (64). The ROS are continuously produced in EC metabolism: Superoxide ($O_2^{\bullet -}$) is immediately transformed by superoxide dismutase (SOD) into hydrogen peroxide ($H_2O_2$) and then transformed by catalase and peroxidase into water ($H_2O$). In addition to SOD, mammals are equipped with various enzymatic systems (glutathione/glutathione reductase and thioredoxin/thioredoxin reductase) or nonenzymatic antioxidants (vitamins A/C/E) to counterbalance the effect of oxidants. This balance is called the ''redox homeostasis.'' Under inflammatory conditions, the endothelial cytotoxicity of OS is the combination of excessive production of ROS ($O_2^{\bullet -}$, $H_2O_2$, and hydroxyl radicals: $^{\bullet}OH$) and RNS (*e.g.,* peroxynitrite [$ONOO^-$]), and inadequate antioxidative systems including decreased SOD and catalase activity, reduced glutathione (GSH) accumulation, and potential deficiencies of vitamins (63).

Also, during sepsis, similar to conventional innate immune cells, such as neutrophils and macrophages (51), EC produce large amounts of ROS and RNS. PAMPs (pathogen-associated molecular patterns) and DAMPs (damage-associated molecular patterns) increase endothelial OS production *via* engagement of PPR, including TLRs, in addition to tissue hypoxia and reperfusion *via* several distinct mechanisms (36). Mitochondrial dysfunction (4, 35), xanthine oxidase (XO), uncoupled NO synthases (NOS), cytochrome P-450 enzymes, lipoxygenases, and NADPH oxidases all contribute to superoxide production by EC.

The mitochondrial respiratory chain is considered the primary source of $O_2^{\bullet -}$ during critical illness (112). Mitochondria generate energy by producing adenosine triphosphate (ATP) through oxidative phosphorylation (OXPHOS) and typically, OXPHOS results in up to 1%–4% of $O_2$ being incompletely reduced, leading to $O_2^{\bullet -}$ formation (77). Moreover, proinflammatory mediators such as tumor necrosis factor (TNF)-$\alpha$, interleukin (IL)-1$\alpha$, and lipopolysaccharide (LPS) may directly induce mitochondrial $O_2^{\bullet -}$ production in human umbilical vein endothelial cell (HUVEC) *via* a ceramide-dependent pathway, and in lung arterial ECs *via* upregulation of NADPH oxidase (76, 111). Corda *et al.* also reported that plasma from septic patients decreases endothelial GSH in HUVECs, increasing ROS endothelial production and ultimately endothelial injury (39).

Sepsis causes alterations in mitochondrial signaling pathways controlling function and population. Inflammatory conditions, and metabolic disorders (hypoxia, hyperglycemia, reperfusion) and metabolic reprogramming toward aerobic glycolysis can reduce the activity and/or the maximum capacity of the mitochondrial respiratory chain complex I, III, and IV (55, 59), although inflammation is sometimes associated with increased mitochondrial OXPHOS, particularly in the short term. These derangements in mitochondrial function lead to elevated production of $O_2^{\bullet -}$.

Finally, sepsis causes over-expression of an inducible endothelial form of NOS that significantly increases NO production. During septic shock, eNOS (endothelial NO synthase) function is altered. In addition to NO, during sepsis, eNOS also produces large amounts of superoxide anion. This phenomenon is named NOS uncoupling, as superoxide generation mainly occurs when NOS is not coupled with its cofactor or substrate (52, 103). NO itself has a specific effect by inhibiting complexes I and IV of the respiratory chain (26, 32). Nitrosylation of cellular proteins and peroxynitrite production may indirectly lead to harm. These products induce inhibition of the respiratory chain and, therefore, direct mitochondrial toxicity, which fuels a vicious circle. Indeed, the simultaneous production of $O_2^{\bullet -}$ and NO can be 1000-fold upregulated. At these high concentrations, NO can compete with SOD and react with $O_2^{\bullet -}$ to generate $ONOO^-$ (27, 137). Moreover, NO and $ONOO^-$ can also be directly produced by the mitochondria

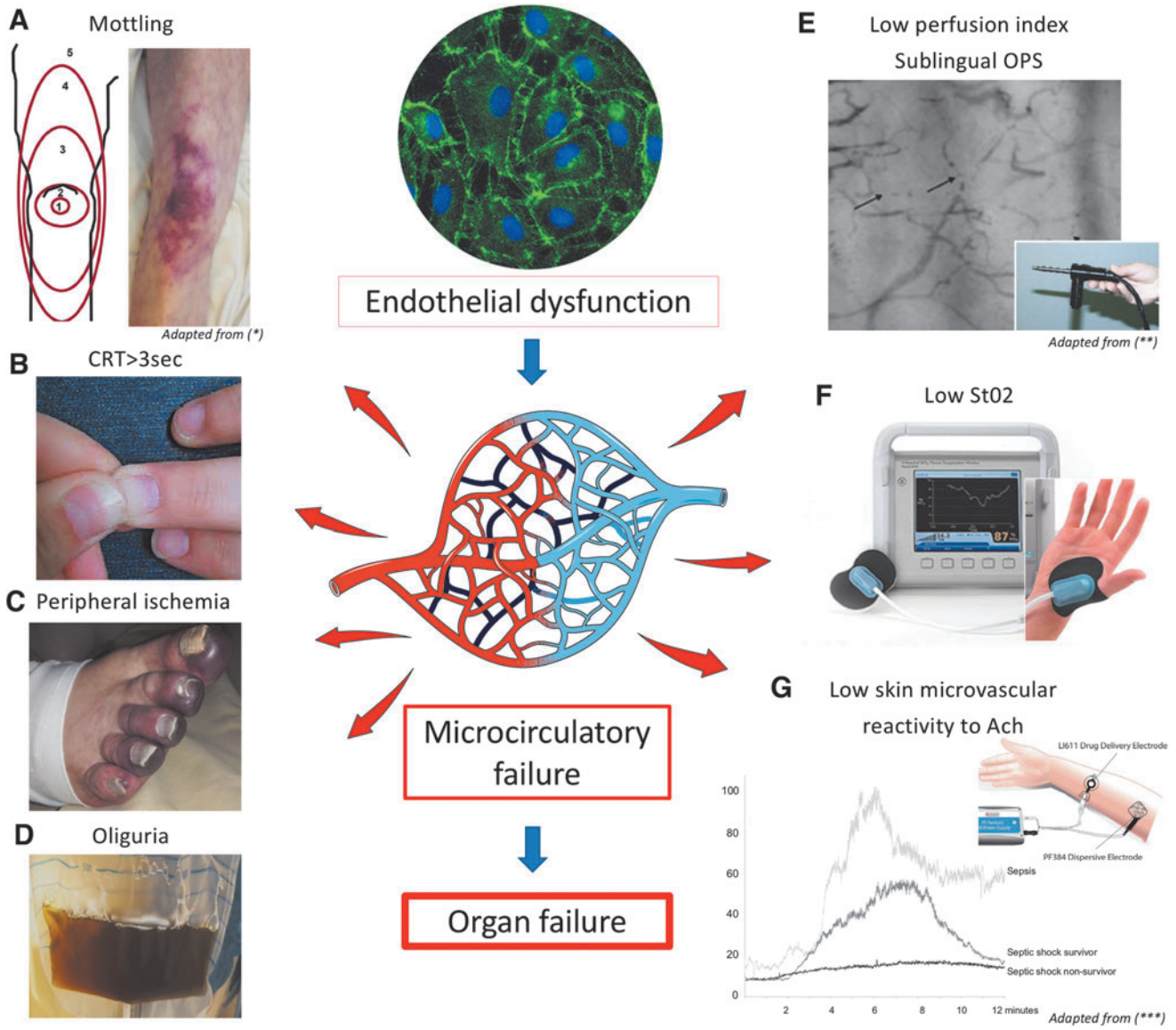


**FIG. 1. Cutaneous clinical signs of microcirculatory failure and "beside" tools for microcirculation assessment.** **(A)** Mottling are *red-purplish* patchy marbling of the skin witnessing skin hypoperfusion. The mottling score based on the mottling extension predicts organ failure severity and mortality in patients with septic shock. The mottling score, ranging from 0 to 5, is based on skin mottling area extension on legs: 0 = no mottling, 1 = "coin-sized" mottling area on the patella, 2 = mottling area not exceeding the superior edge of the knee cap, 3 = mottling area not exceeding the middle thigh, 4 = mottling area not exceeding the fold of the groin and 5 = extended beyond the groin area. The *picture* shows an example of mottling score 4. (*) Adapted from Ait-Oufella *et al.* (7). **(B)** CRT is the time taken for a distal capillary bed to regain its color after pressure that has been applied to cause blanching is released. Normal CRT is <2 s. Prolonged CRT (>3 s on the index finger) is associated with hyperlactatemia, higher SOFA score and is predictive of mortality in septic shock. **(C)** Peripheral gangrene caused by septic shock (in the absence of major vascular occlusive disease) is the ultimate sign of peripheral ischemia. It is often associated with DIC and high doses of vasopressors. **(D)** Oliguria is defined by urine output <500 mL in 24 h in an adult or <0.5 mL/kg/h in an adult or child (<1 mL/kg/h in neonates). It is an early sign of reduced renal perfusion and often precedes acute kidney injury. **(E)** Sub-lingual microcirculation imaged using orthogonal-polarization spectral imaging in a septic shock patient. The *picture* shows a reduction in total capillary density, reduced perfused vessel density, and heterogeneity of flow. (**) Adapted from: Ñáñez-Varona *et al.* (112a). **(F)** NIRS provides a continuous noninvasive measurement of oxy- and deoxyhemoglobin saturation in tissues within a few centimeters from the skin to estimate the tissue saturation in $O_2$ ($StO_2$). Low $StO_2$ at the thenar eminence and the knee area correlate with poor outcome in sepsis and septic shock. **(G)** Microcirculatory skin blood flow recorded using laser Doppler flowmetry after iontophoretic acetylcholine infusion. The increase in flow index reflects the endothelium-dependent vasodilatation reserve. Impaired skin microvascular reactivity correlates with poor outcome in septic shock. The *picture* shows the microvascular skin reactivity in the knee area at the admission of a sepsis patient, and impaired reactivity in a septic shock survivor and a nonsurvivor. (***) Adapted from: Bourcier *et al.* (24). CRT, capillary refill time; DIC, disseminated intravascular coagulation; NIRS, near-infrared spectroscopy.

*via* mtNOS (an isoform of nNOS that is expressed in the mitochondria) (95, 127). $ONOO^-$ causes rapid oxidation of sulfhydryl and thioether groups, as well as nitration and hydroxylation of aromatic compounds such as tyrosine, tryptophan, and guanine. These modifications are reported to induce numerous cellular function alterations, such as decreased protein activity, alteration of the mitochondrial respiratory chain, peroxidation of membrane lipids, and dysfunction of $Na^+/K^+$ membrane pumps ATPase, and even direct damage to deoxyribonucleic acid (DNA) (144).

Collectively these mechanisms result in the disbalance of the endothelial redox homeostasis. The resultant OS disrupts multiple facets of microvascular endothelial function such as vasomotor tone, coagulation, GCX integrity, barrier function, leukocytes, and platelet adhesion, and it ultimately promotes cell death. In return, some of these alterations increase OS, which further amplifies endothelial dysfunction and injury and ultimately causes organ dysfunction and failure (Fig. 2). Consistent with this paradigm, some clinical studies have reported a positive correlation between OS, mitochondrial dysfunction, and severity in septic shock (25, 43, 76, 78).

### Vascular Tone and OS

The OS directly alters vascular tone by modulating NO bioavailability or signaling. Animal models of sepsis revealed significantly increased levels of NO in the first hours of sepsis secondary to increased tissue iNOS (inductible NO synthase) expression (149). However, eNOS uncoupling limits the bioavailability of NO because of immediate transformation in $ONOO^-$, which is believed to participate in microcirculatory failure by limiting the capillary vasodilation that is necessary to maintain organ perfusion. Consistent with this concept, experimental studies on rat coronary arteries revealed that application of $ONOO^-$ prevented vasodilatation induced by acetylcholine or isoprotenol (156). Concomitantly, the production of NO by iNOS increases levels of two potent vasoconstrictive factors, endothelin-1 and thromboxane A2 (50, 70). Several clinical studies reported increased NO levels in early sepsis and altered bioavailability (45, 46, 162). However, difficulties with measuring NO coupled with the dynamic regulation in its production and bioavailability during the different phases of sepsis add complexity to the understanding of the role of NO in sepsis. These factors may partly explain the negative results of clinical studies attempting to inhibit or increase its production during sepsis (described below).

### OS and Coagulation

Under homeostatic conditions, the GCX layer, as well as anticoagulant molecules produced by EC, such as tissue factor pathway inhibitor (TFPI), EC protein C receptor, and thrombomodulin, prevent microvascular thrombosis and maintain optimal blood fluidity (20). The EC-anticoagulant

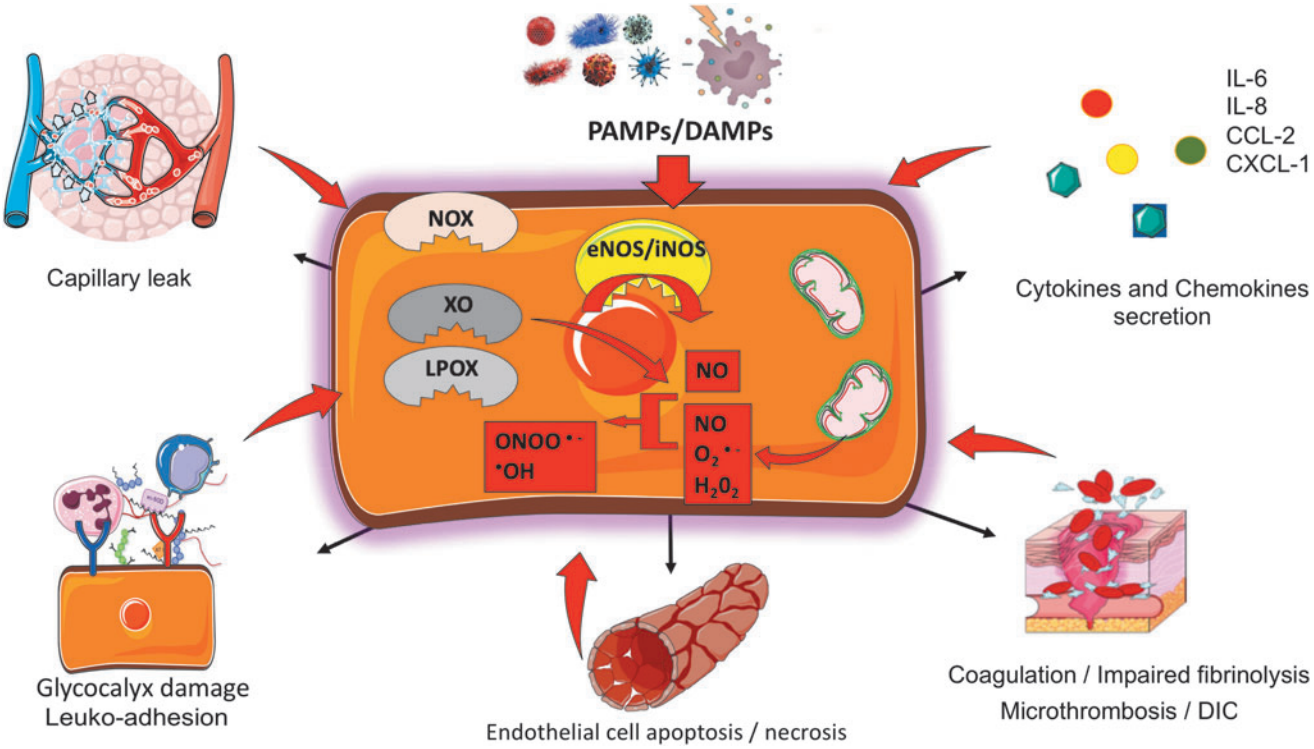


**FIG. 2. The endothelium is both a source and a target for OS.** During sepsis, PAMPs and DAMPs provoke accumulation of products of OS from multiple EC sources: the mitochondria, the eNOS uncoupling, and intracellular enzymes (NADPH ox, XO, and LPOX). The disruption of redox balance in EC enhances multiple facets of the endothelial dysfunction, engaging EC toward a proinflammatory, procoagulant, and proadhesive phenotype. It also favors EC apoptonecrosis and GCX damage, leading to the loss of endothelium barrier function and capillary leak. All of the alterations in the endothelial homeostasis will, in return, amplify OS and fuel this vicious circle. DAMP, damage-associated molecular pattern molecules; EC, endothelial cells; eNOS, endothelial NO synthase; GCX, glycocalyx; LPOX, lipo-peroxidase; NOX, NADPH oxidase; OS, oxidative stress; PAMP, pathogen-associated molecular pattern molecules; XO, xanthine oxidase.

functions are impaired during acute inflammation and sepsis, which leads to platelet adhesion and imbalances in the formation and breakdown of thrombin, and ultimately to the development of micro thrombosis (34). Experimental studies provide insight into the crucial role of redox regulation of the endothelium in triggering or promoting dysregulation of the coagulation pathways. The OS has been reported to directly inactivate TFPI on EC (37). Treatment of EC with ROS-generating systems (xanthine/XO or $H_2O_2$) for 24 h resulted in reduced activity of TFPI, without significant changes at the mRNA level, suggesting a direct action on TFPI structure (28, 105). Tissue factor (TF) is a primary initiator of the extrinsic coagulation cascade. During homeostasis, the active form of TF is absent or poorly expressed on the surface of EC, and it is maintained in an inactive ''crypted'' form. During acute inflammation, post-transcriptional modifications and membrane phosphatidylserine (PS) exposure convert crypted TF to fully active TF (5, 97). Active TF has a high affinity to FVII/FVIIa and FX/FXa, which creates a procoagulant environment resulting in the generation of thrombin, fibrin formation, and platelet activation (33, 152). In 2018, Ebert *et al.* demonstrated that deficiency of antioxidative protein paraoxonase-2 (PON2) causes vascular inflammation and a procoagulant endothelial phenotype *via* deregulated redox homeostasis. They observed that *PON2*$^{-/-}$ mice display superoxide accumulation in EC and a proinflammatory phenotype (higher IL-6 and CCL-2 production) at baseline. They hypothesized that the antioxidant role played by PON2 controlled the decryption of TF (48). Also, both PON2 deficiency and OS decrypt TF procoagulant activity and trigger coagulation (9, 65). Finally, EC and platelet-derived ROS enhance platelet activation and adhesion and promote coagulation during inflammation (38, 93, 150).

This highlights OS's direct role in triggering thrombotic complications and potentially disseminated intravascular coagulation (99). Figure 3 summarizes the direct effect of ROS in sepsis-associated coagulopathy and microvascular thrombosis.

### Sepsis- and OS-Induced GCX Damage

The GCX is a heparan sulfate-rich layer of glycosaminoglycans and proteoglycans that coats the healthy vascular endothelium. The GCX is critically involved in microvascular barrier function maintenance, leukocyte trafficking, local anticoagulation, and capillary rheology (60, 100, 129). Inflammatory conditions cause an early and durable disruption of GCX structure and function (92). Wiesinger *et al.* observed reduced GCX thickness after challenges with LPS or TNF-α (161) on mice aorta freshly isolated and human cultivated lung microvascular EC. Electron microscopy has recently enabled 3D ultrastructure visualization of capillary GCX at baseline and after LPS challenge in the heart, kidney, liver (114), and lung microvasculature (80) in mice. Kataoka

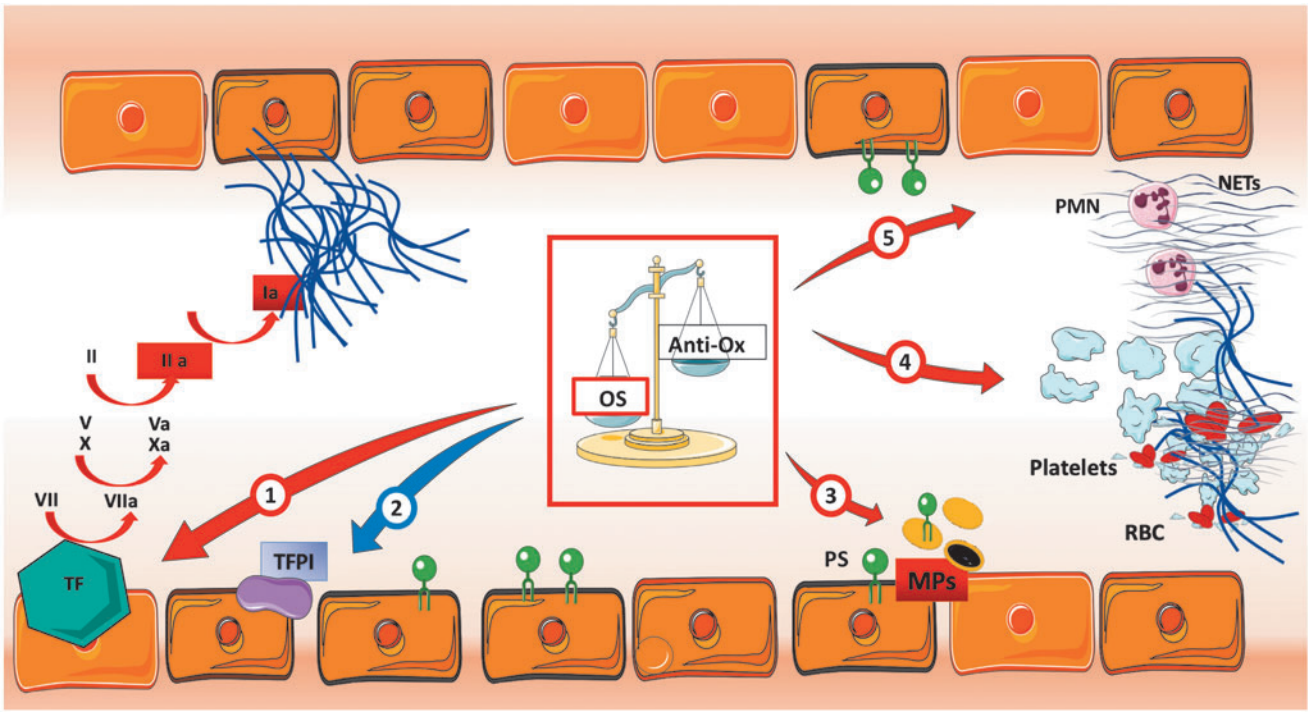


**FIG. 3. OS drives EC toward a procoagulant phenotype.** During sepsis, redox homeostasis is disrupted, leading to OS and accumulation of mediators that engage multiple cellular pathways that promote the development of microvascular thrombosis and DIC: OS upregulates TF expression at the EC membrane and its bioavailability, which triggers the coagulation cascade (1). OS reduces TFPI activity (2). OS provokes cell apoptosis and necrosis, leading to a large amount of procoagulant PS+ microparticles release (3). OS promotes the recruitment, adhesion, and activation of platelets (4) and PMN (5). These factors promote microvascular immuno-thrombosis, leading to microvascular shunt and tissue hypoxia. The local redox imbalance is further amplified by ROS produced by activated platelets and PMN. Ia, fibrin; IIa, Thrombin; MP, microparticles; NETs, neutrophils extracellular traps; PMN, polymorphonuclear; PS, phosphatidylserine; RBC, red blood cells; ROS, radical oxygen species; TF, tissue factor; TFPI, tissue factor pathway inhibitor.

*et al.* showed that GCX destruction increased macromolecule permeability and leukocyte adhesion in microcirculation (86).

Human studies have reported evidence that shedding of the GCX component occurs in many critical illnesses where OS play a significant role, including in patients with septic shock or postcardiac-arrest syndrome (62) or ischemia–reperfusion syndrome (153). For example, shed GCX components, such as Syndecan-1 and Hyaluronan, are higher in septic patients than healthy control (116, 141) and positively correlate with severity of illness, capillary leak (125, 140), and mortality (10, 74, 79, 113).

Under homeostatic conditions, the intact GCX is a sanctuary of two major antioxidant enzymes. The GCX heparan sulfate has a high affinity for the SOD (16, 84, 85), and sulfated proteoglycans dock active XO (2). Therefore, a healthy GCX can quench and neutralize a high amount of systemic and self-generated free radicals and maintain NO bioavailability, thereby preserving redox homeostasis (91). Conversely, when GCX is damaged, the redox homeostasis is broken. In addition, ROS can directly alter the endothelial GCX density by decreasing the EC surface content of glycosaminoglycans and heparan sulfate proteoglycans, and which amplifies endothelial dysfunction (130). For example, $H_2O_2$ of human glomerular EC has been reported to directly increase the cleavage of sulfated glycosaminoglycan, to reduce Trans-endothelial Electrical Resistance, and to alter the selective barrier function to proteins (139). Moreover, oxidative mechanisms, including OS-induced proteolysis, can also lead to shedding of syndecan-1 (90). Another study demonstrated that OS induced by exposure to $H_2O_2$ upregulates the activity of endothelial histone deacetylase and metalloproteinases and, ultimately, amplifies syndecan-1 shedding (8).

Finally, sepsis-induced injury to the GCX, reduced antioxidant defense of the endothelium, and increasing ROS production by the activated EC and leukocytes amplify the phenomenon in a vicious circle and aggravate multiple facets of the endothelial dysfunction (Fig. 4).

### Endothelial Inflammation and OS

During sepsis, microbial products and endogenous proinflammatory mediators can directly stimulate EC by binding to PRRs (87, 134), which leads to the activation of intracellular inflammatory pathways mediated by nuclear factor-$\kappa$B (NF-$\kappa$B) and the mitogen-activated protein kinases (134, 159). Activation of these signaling pathways drives EC toward a proinflammatory phenotype characterized by secretion of a tremendous amount of IL-6, IL-8, CCl-2, and CXCL-1 (87). The OS has been reported to modulate NF-$\kappa$B and AP-1, which are two critical transcription factors involved in the proinflammatory switch of ECs (136, 164). In general, reducing agents decreases NF-$\kappa$B activities, but it conversely enhances AP-1 activity (104). Oxidants, on the other hand, broadly activate NF-$\kappa$B activity (109, 136), whereas they can also activate AP-1 by activating the JNK

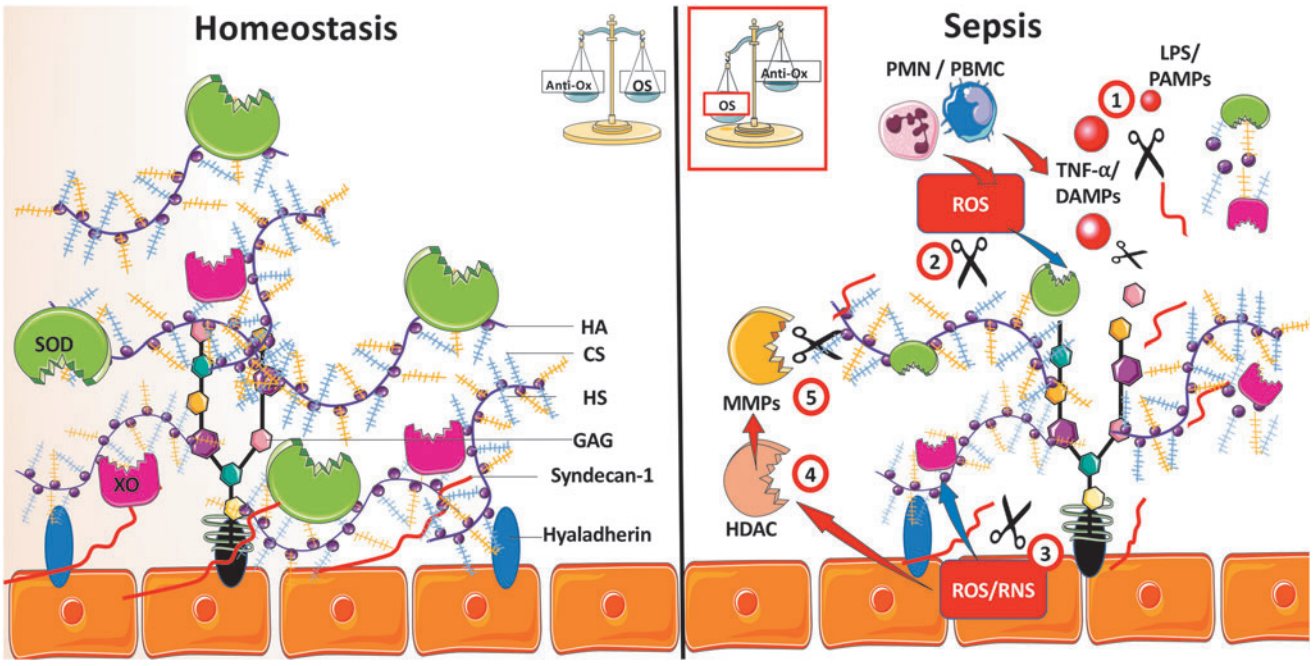


**FIG. 4. OS damages the endothelial GCX.** Under homeostasic conditions, the GCX is a thick and dense heparan sulfate-rich layer of glycosaminoglycans and proteoglycans that coats the vascular endothelium. The GCX contributes to maintenance of the endothelial barrier, and it helps to reduce leukocyte adhesion, maintain local anticoagulant conditions, and regulate capillary rheology. The GCX is also a harbor for antioxidant enzymes such as SOD and XO. During sepsis, PAMPs, DAMPS (1), and ROS derived from multiples cellular sources (recruited PBMC and PMNs, and EC) provoke shedding of GCX components (*e.g.,* HS, CS, GAG, syndecan-1) (2), which reduces its quality, density, and antioxidant properties. Moreover, EC-derived ROS and RNS (3) upregulate HDAC activity (4), leading to activation of MMPs, which amplifies cleavage of the GCX components (5). CS, chondroitin sulfate; GAG, glycosaminoglycans; HA, hyaluronic acid; HDAC, histone deacetylase; HS, heparan sulfate; MMP, metalloproteinase; PBMC, peripheral blood mononuclear cells; RNS, radical nitrogen species; SOD, superoxide dismutase.

pathway *via* oxidative thiol modification of the apoptosis signal-regulating kinase 1 (124, 133). In microvascular EC, numerous genes that play critical roles in endothelial dysfunction have NF-κB regulation sites in their promoters, including IL-6, IL-8, TNF-α, vascular endothelial growth factor, RANTES, CCL-2, intercellular cell adhesion molecule 1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), TF, and the nitric oxide synthases. Moreover, ROS and RNS fuel the endothelial inflammation indirectly by promoting the release of DAMPs, which perpetuates the inflammatory stimuli.

### OS and Endothelium-Leukocyte Adhesion

The EC regulates leukocyte trafficking through their production of chemokines and expression of adhesion molecules, primarily in the venous part of the microcirculatory network. Integrin family glycoproteins, including CD 62E/P, VCAM-1, PECAM (platelet endothelial cell adhesion molecule-1), and ICAM-1/2, orchestrate leukocyte adhesion, rolling, and crawling, the necessary steps before trans- or paracellular diapedesis (108, 154). In addition, the activation of circulating polymorphonuclear leukocytes facilitates host defense against circulating microorganisms. Luminal expression of these receptors by EC is rapidly upregulated during sepsis or acute inflammation, which enhances the recruitment of phagocytic cells to infectious sites (31). Under pathologic conditions, such as septic shock, tissue infiltration by activated leukocytes contributes to tissue injury and downstream dysfunction and failure of multiple organs. Many experimental data indicate that ROS play an essential role in regulating the production of leukocyte adhesion molecules. For example, overexpression of SOD expression in EC has been reported to limit TNF-α, and to abrogate the upregulation of VCAM-1, E-selectin, and ICAM-1-induced OS (34). Takano *et al.*, based on HUVECs experiments, also reported that EC P-selectin upregulation induced by thrombin or TNF-α is dependent on ROS generated by the EC mainly *via* the NADPH oxidase (145). These phenotypic modifications of the EC luminal surface, triggered by the endothelial OS and the systemic inflammatory mediators, accelerate the recruitment and the activation of polymorphonuclear leukocytes and macrophages, which are, themselves, powerful generators of ROS. The tissue accumulation of OS from multiple sources can ultimately precipitate organ failure.

### OS-Induced EC Death

A large body of evidence supports a key role for ROS and RNS in triggering cell death programs (128, 132). $H_2O_2$, $^{\bullet}OH$, and $ONOO^-$ cause cell injuries by oxidizing biological molecules (*e.g.,* lipids) membranes, proteins, DNA, and organelles such as the mitochondria or the endoplasmic reticulum (ER) (23, 66, 127). Therefore, OS can trigger the apoptosis program *via* the mitochondrial and extrinsic (death receptor and ER) pathways (49, 106, 122). For example, $ONOO^-$ can directly permeabilize the mitochondrial outer membrane, triggering the mitochondrial permeability transition, an irreversible process that leads to a cessation of electron transfer and ATP production (23), ultimately provoking cell death (77). $ONOO^-$ and $H_2O_2$ also cause rapid activation (1–3 h) of the Fas death receptor pathway and caspases-2/7/9 (118). Notably, low doses of ROS have been found to activate cell survival signaling pathways, whereas moderate ROS/RNS concentrations result in apoptosis, and higher concentrations or long exposure have been associated with necrosis. Therefore, the intensity of the redox disbalance will engage EC toward apoptosis or necroptosis, both of which can dramatically enhance endothelial dysfunction. First, apoptotic (and *a fortiori* necrotic) EC become highly procoagulant. In staurosporine-induced apoptosis experiments, Bombeli *et al.* reported a rapid exposure of PS and active TF. EC anticoagulant features are impaired in a few hours, and they include reduced production of thrombomodulin, TFPI, and heparan sulfate (19, 21). Necrotic cells release a large amount of DAMPs and PS-positive microparticles that amplify local and disseminated coagulation and inflammation (Fig. 5) (15, 101). Finally, EC death involves loss of structural and membrane properties, which aggravates the sepsis- and ROS-induced permeability, the capillary protein and fluids leakage, and therefore organ injury (61).

### OS as a Goal for an Endothelial-Targeted Therapy in Sepsis

There is currently a focus in septic shock on preserving endothelial function and optimizing the microcirculation (98). Several approaches targeting OS have been explored based on the important role of OS in EC dysregulation during sepsis and acute inflammation (Table 1) (44, 123).

#### *Avoiding hyperoxia*

Based on the assumption of improving the delivery of oxygen to the tissue and promoting bacterial defenses *via* oxidative burst (117, 160), some authors have proposed hyperoxia as a therapy for sepsis (71). Although small studies reported encouraging results in reducing wound infection postsurgery (30, 75), in the *hyperoxia and hypertonic saline in patients with septic shock* (HYPERS2S) study, the groups receiving mechanical ventilation with $FiO_2 = 1$ had a higher risk of mortality, which resulted in discontinuation of the study before completing full enrollment (13). This deleterious effect of hyperoxia may be partly due to the increase in ROS generation, which has been observed in several critical illnesses such as resuscitated cardiac arrest (89) or acute myocardial infarction (143).

#### *Limiting NO production*

Several clinical trials investigated the NOS isoform inhibitors in the years after the recognition of NO as a critical mediator in septic shock-associated vasoplegia (96). Although phase II trials showed improved systemic vascular resistance and better macro hemodynamics, in the phase III trial there was significant excess mortality in the treatment group and the trial was stopped early (14, 102). The complexity of the role of NO between macro/microvessels, the dynamic regulation of iNOS and eNOS, and issues of NO bioavailability during sepsis and septic shock may explain the failure of a basic approach aiming at inhibiting NO production (96). Currently, two clinical trials are underway evaluating methylene blue, as the third line of vasopressor in septic shock (NCT04089072) or in cirrhotic patients with refractory vasoplegia (NCT03120637).

#### *Enhancing antioxidant defenses*

*N*-acetylcysteine. *N*-acetylcysteine (NAC) has been safely used to treat acetaminophen overdose for decades (151). Its direct antioxidant properties, which increase glutathione synthesis, and its lack of severe side effects make it an excellent

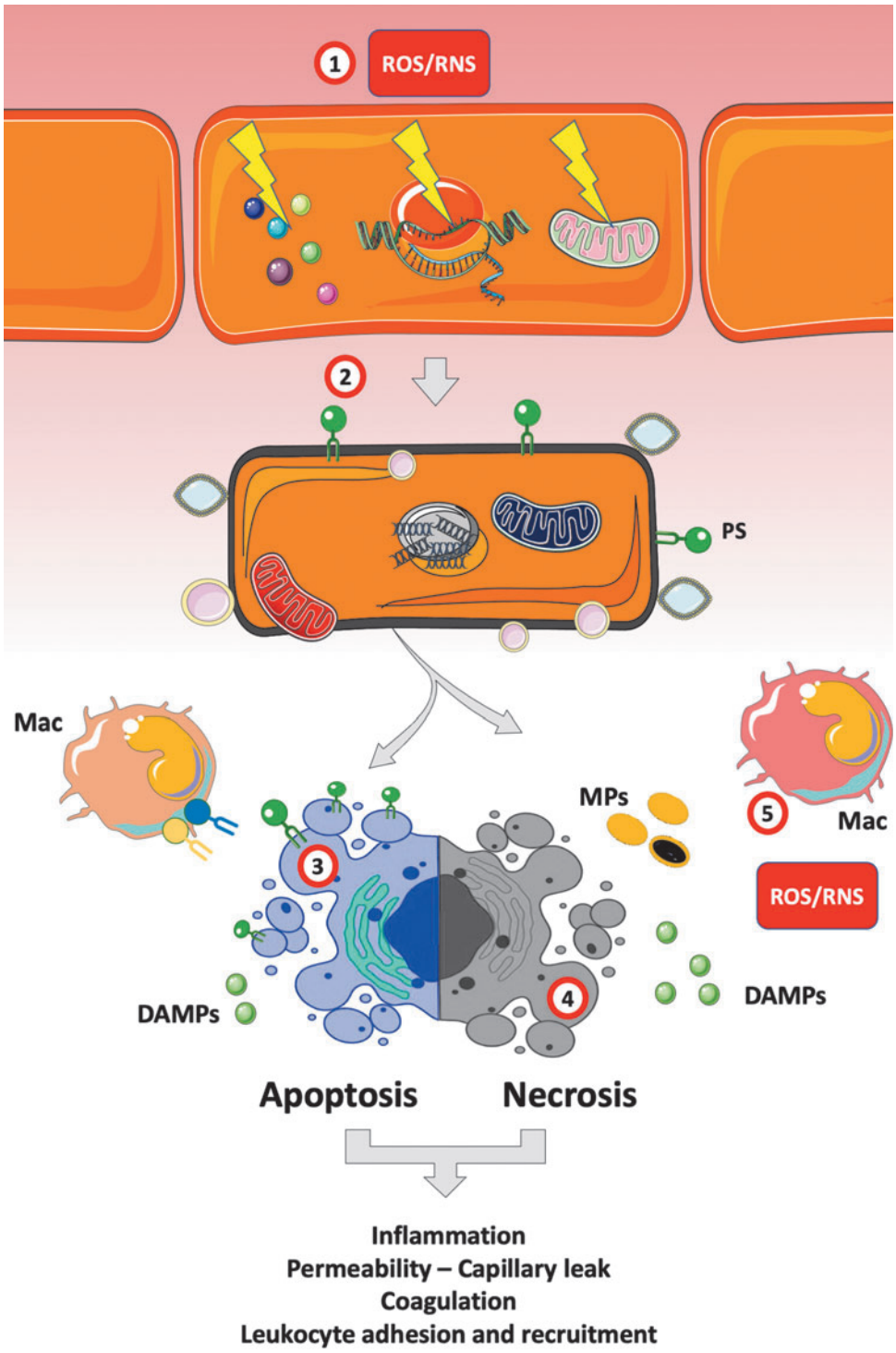


**FIG. 5. EC OS accumulation initiates cell death programs.** ROS and RNS damage cell integrity by oxidizing lipids membranes, proteins, DNA, and organelles (mitochondria and ER), and they trigger a cell death program (1). OS initiates the apoptosis program *via* both mitochondrial and extrinsic pathways, leading to cell shrinkage, membrane PS exposure and blebbing chromatin condensation, and nuclear fragmentation (2). Apoptotic cells and apoptotic bodies promote the efferocytosis by neighboring cells or macrophages with minimal damage to surrounding cells and tissues (3). However, EC necrosis can result if the OS is too intense or prolonged. The EC necrosis generates a large amount of proinflammatory DAMPs, MPs, and oxidant-free radicals (4), which amplifies local and systemic inflammation (5). Ultimately, EC apoptosis and necrosis disrupt the endothelial monolayer, worsen the sepsis-induced permeability, and enhance EC inflammation, coagulation, and leukocyte recruitment and adhesion. DNA, deoxyribonucleic acid; ER, endoplasmic reticulum; Mac, macrophages.

candidate to alleviate systemic and endothelial OS in sepsis (12). *In vitro*, NAC can reduce by about 20% the EC apoptosis induced by plasma from patients with septic shock (78). In mice with endotoxemia or with sepsis induced by cecal ligation and puncture (CLP), NAC administration reduced systemic inflammation (TNF-$\alpha$, NF-$\kappa$B) (42) and organ injury (54, 88), and increased survival (17, 157). The administration of high doses of NAC added to glutathione (GSH) has been reported to significantly decrease the peroxidative stress of patients with septic shock as measured by expired ethane, plasma malondialdehyde, erythrocyte deformability, and complement activation (115). Despite a small sample size, the mortality at day 10 in the NAC-treated patients was half that of the untreated group (115). In a randomized controlled pilot trial, NAC infusion reduced NF-$\kappa$B activation in peripheral blood mononuclear cell (PBMC) and reduced plasma IL-8 (121). Another study reported reported reduced IL-8 levels and better lung oxygenation and compliance in early septic shock

Table 1. Clinical Trials Targeting Systemic Oxidative Stress or Endothelial-Related Oxidative Stress in Sepsis and Septic Shock

| *Target* | *Human clinical trial* | *Population and intervention* | *Results/current status* |
|---|---|---|---|
| NO pathway | Randomized trial of nitric oxide synthase inhibitor 546C88 in patients with septic shock (126) | Septic shock $N=797$<br>546C88 or placebo (5% dextrose) for up to 7 or 14 days | NOS inhibitor 546C88 increased mortality |
| | Randomized trial of inhaled nitric oxide to augment tissue perfusion in sepsis (162) | Severe sepsis and septic shock $N=49$<br>Inhaled nitric oxide 40 ppm*6 h *vs.* Sham | Inhaled NO did not improve microcirculatory flow, lactate clearance, or organ dysfunction |
| | The effect of nitroglycerine on microcirculatory abnormalities during sepsis (NISMIS) (163) | Sepsis $N=70$<br>Nitroglycerin iv loading dose of 4 mg/h iv in the first 30 mn, 2 mg/h iv in the next 23 h and 30 mn *vs.* placebo | Intravenous nitroglycerin does not promote sublingual microcirculatory blood flow |
| | Evaluating in cirrhotics with refractory vasoplegia the effect of methylene blue (CRuMBS) (NCT03120637) | Septic shock in cirrhotic patient $N=111$<br>Methylene blue iv 2 mg/kg bolus then 0.5 mg/kg/h*6 h *vs.* standard care | Completed<br>Awaiting results |
| | Methylene blue as a third-line vasopressor in septic shock (NCT04089072) | Refractory septic shock $N=250$<br>ProvayBlue® bolus then 2 mg/kg/h*24 h *vs.* phenylephrine as third-line vasopressor | Not yet recruiting |
| NAC | Randomized trial of NAC and GSH in early septic shock (134) | Septic shock $N=30$<br>Placebo (A) *vs.* GSH iv 70 mg/kg/day (B) or GSH+NAC iv 75 mg/kg/day (C) | Reduced mortality in groups B and C ($p<0.01$).<br>Peroxidative indexes (ethane in the expirate and plasma MDA) were significantly reduced in groups B and C |
| | Randomized trial of NAC in sepsis (135) | Septic $N=20$<br>Saline *vs.* NAC 50 mg/kg iv over 15 min, then 50 mg/kg over 4 h, and finally infusion of 50 mg/kg/day up to 72 h | Decreased NF-$\kappa$B activation in patients' mononuclear leukocytes, and IL-8 in patients receiving NAC at 72 h |
| | Randomized trial of NAC in septic shock (136) | Septic shock $N=22$<br>Placebo *vs.* NAC iv 150 mg/kg bolus, then infusion of 50 mg/kg over 4 h | Survivors who received NAC had shorter ventilation ($7\pm2$ days *vs.* $20\pm7$ days; $p<0.05$) and shorter ICU stays ($13\pm2$ days *vs.* $32\pm9$ days; $p<0.05$) |
| | Randomized controlled clinical trial of antioxidant therapy in critically ill patients with septic shock (NCT03557229) | Septic shock $N=125$<br>Placebo *vs.* NAC (1200 mg/12 h iv) + vitamin C (4 g/day) + vitamin E (400 unit/8 h) + melatonin (50 mg/day) for up to 5 days | Recruiting |

(*continued*)

Table 1. (Continued)

| *Target* | *Human clinical trial* | *Population and intervention* | *Results/current status* |
|---|---|---|---|
| Albumin | Volume replacement with albumin in severe sepsis (ALBIOS) (146) | Severe sepsis or septic shock $N=1818$<br>20% albumin and crystalloid solution *vs.* crystalloid solution alone | Albumin 20% + crystalloids compared with crystalloids alone did not improve survival at 28 and 90 days |
| | Lactated Ringer *versus* albumin in early sepsis therapy (RASP) (164) | Cancer and septic shock $N=110$<br>Albumin 4% *vs.* ringer lactate in the first 12 h | Albumin 4% as compared with lactated Ringer did not improve survival at 30 days |
| | ALBumin Italian Outcome Septic Shock-BALANCED trial (ALBIOSS-BAL) (NCT03654001) | Septic shock $N=1252$<br>4 groups: albumin + BAL, albumin + saline, BAL, saline | Recruiting |
| | Albumin replacement therapy in septic shock (ARISS) (NCT03869385) | Septic shock $N=1662$<br>60 g loading dose of albumin 20%, then 40–80 g/day until day 28 *vs.* crystalloids | Recruiting |
| Vitamin C | The vitamin C, hydrocortisone, and thiamine in patients with septic shock trial (VITAMINS) (NCT03333278) | Septic shock $N=211$<br>High-dose vitamin C (6 g/day), thiamine (400 mg/day), and hydrocortisone (200 mg/day) *vs.* hydrocortisone | Combination of intravenous vitamin C, hydrocortisone, and thiamine does not lead to a more rapid resolution of septic shock compared with intravenous hydrocortisone alone |
| | Hydrocortisone, vitamin C, and thiamine for the treatment of sepsis and septic shock (HYVCTTSSS) (NCT03258684) | Septic shock $N=140$<br>Vitamin C (4 g/day), hydrocortisone (200 mg/day), and thiamine (400 mg/day) *vs.* saline | Completed<br>Awaiting results |
| | Metabolic resuscitation using vitamin C, thiamine, and glucocorticoids in sepsis (ORANGES) (NCT03422159) | Severe sepsis or septic shock $N=140$<br>Vitamin C (4 g/day), hydrocortisone (200 mg/day), and thiamine (400 mg/day) *vs.* placebos | Completed<br>Awaiting results |
| | Vitamin C and thiamine in sepsis (NCT03592277) | Severe sepsis or septic shock $N=120$<br>Vitamin C (4 g/day) and thiamine (400 mg/day) *vs.* placebos | Completed<br>Awaiting results |
| | Vitamin C, hydrocortisone, and thiamine for septic shock (CORVICTES) (NCT03592693) | Septic shock $N=400$<br>Vitamin C (6 g/day) and hydrocortisone (250 mg/dL then 200 mg/day) *vs.* placebos | Recruiting |
| | Vitamin C, corticosteroids, and thiamine in sepsis (ACTS) trial (NCT03389555) | Septic shock $N=200$<br>Vitamin C (6 g/day), hydrocortisone (200 mg/day), and thiamine (400 mg/day) *vs.* placebos | Recruiting |
| | Vitamin C and thiamine effect in septic shock (ATESS) (NCT03756220) | Septic shock $N=116$<br>Vitamin C (100 mg/day) and thiamine (400 mg/day) *vs.* saline | Recruiting |
| | Evaluation of hydrocortisone, vitamin C, and thiamine for the treatment of septic shock (HYVITS) (NCT03380507) | Septic shock $N=212$<br>Vitamin C (6 g/day), hydrocortisone (200 mg/day), and thiamine (400 mg/day) *vs.* standard care | Recruiting |

BAL, low-chloride balanced crystalloid solutions; GSH, reduced glutathione; ICU, intensive care unit; IL, interleukin; MDA, malondialdehyde; NAC, *N*-acetylcysteine; NF-$\kappa$B, nuclear factor-$\kappa$B; NOS, NO synthase.

in NAC-treated patients. These data suggest a beneficial role of NAC in blunting the inflammatory response to sepsis (142). However, none of these trials focused on endothelial dysfunction, nor did they show a substantial reduction in sepsis mortality with NAC treatment.

Albumin. Albumin is the most abundant endogenous plasma protein and the central extracellular molecule responsible for maintaining the plasma redox balance. The composition of albumin explains its tremendous antioxidant properties, including a free cysteine located in position 34, which has a thiol group that represents 80% of the antioxidant capacity of the plasma, the ''binding site 1'' attach homocysteine and bilirubin, and its N-terminal portion, which contains DAHK amino acids and is a binding site for pro-oxidant metals (Cu, Fe, Co, and Ni) (146). Moreover, albumin is a carrier of sphingosine-1 phosphate that protects GCX by inhibiting syndecan-1 shedding (3, 163). Several observational studies and meta-analyses have revealed that hypoalbuminemia is an independent risk factor for septic shock mortality (158). Indeed, each decrease of albumin by 10 g/L is associated with significant worse morbidity and a higher risk of death (OR = 2.37). In endotoxemic mice, resuscitation with albumin decreased ROS and RNS production, and it improved vascular reactivity in mesenteric arteries (110).

A recent pilot proof-of-concept study in stabilized septic shock patients showed that infusion of 20% human serum albumin (HSA) improved skin microvascular endothelial-mediated vasoreactivity as compared with saline (67). HSA infusion has been assessed in large clinical trials in septic patients (120). Although it showed the capability to restore a sustained increase in plasma thiols (126), so far it has not improved 28 and 90 day survival rates (29, 82).

Vitamin C. Vitamin C (L-ascorbic acid) is a potent electron donor that reacts with both superoxide and hydroxyl radicals. *In vitro* and *ex vivo* studies support a critical role for vitamin C in cellular mechanisms that are relevant to the pathophysiology of sepsis in PBMC, macrophages, and ECs (94, 155). For example, vitamin C supplementation inhibits bacterial replication and prevents $H_2O_2$ injury to cultured microvascular EC. Vitamin C was also reported to improve microvascular perfusion *in vivo* in rats with sepsis induced by CLP (11).

In humans, circulating vitamin C concentrations are markedly depleted in patients with sepsis, and they are potentially associated with worse outcomes (22, 57, 58, 135). Therefore, in 2013 the REDOXS study evaluated glutamine and antioxidant vitamins ($\beta$-carotene, vitamins C and E) on mortality in a general ICU population. The combination of glutamine or antioxidants did not improve clinical outcomes, and glutamine alone was associated with an increase in mortality among critically ill patients with multiorgan failure (73). Another study investigated the supplemental administration of vitamins C and E in critically ill patients requiring 10 or more days of enteral nutrition. They observed a decreased mortality (45.7% in the antioxidant group and 67.5% in the regular-feeding group; $p < 0.05$) (41). However, the control group received lower doses of vitamins C and E than usually recommended (50 mg and 10 IU, respectively), and this study highlights the potential harm of vitamin deficiencies. A small retrospective before/after study in patients with severe sepsis and septic shock found that early use of the combination of intravenous vitamin C, corticosteroids, and thiamine reduced progressive organ dysfunction (acute kidney injury) and mortality (107). However, recent large randomized prospective clinical trials failed to confirm these results (53, 56). The results of several clinical trials investigating the potential benefit of vitamin C in sepsis and septic shock are still expected. Table 1 summarizes previous and ongoing clinical trials that investigate the effect of reducing systemic OS or endothelial-related OS in sepsis and septic shock.

## Current Challenges and Futures Directions

The EC dysfunction is the primary *substratum* of microcirculatory failure during sepsis, leading to multiorgan failure and death. Sepsis-induced endotheliopathy is a multifaceted syndrome combining impaired coagulation and fibrinolysis, increased vascular permeability and leukocyte recruitment, and dysregulated vascular tone. Moreover, sepsis is a major inducer of OS in EC. The rupture of the redox balance toward OS in EC enhances all aspects of the sepsis-induced dysfunction, and it can fuel a vicious circle of pro-oxidant condition.

The COVID-19 pandemic has recently increased interest in endothelial dysfunction in severe infections. Although to date there are minimal direct experimental data published that directly show microvascular involvement specific to the betacoronavirus SARS Cov-2 infection, emerging evidence from patients with COVID-19 suggests that EC may critically contribute to the initiation of acute lung injury and propagation of organ failure in severe COVID-19. Pulmonary EC are a potential sanctuary for viral replication, and EC infection could lead to a sepsis-like endotheliopathy characterized by vascular inflammation, capillary leakage, OS, and a procoagulant, proadhesive, and proangiogenesis state (1, 69, 147). The hypothesis that pulmonary EC play an important role in driving COVID-19-induced acute respiratory distress syndrome requires further investigation.

The re-establishment of tissue perfusion is a cornerstone of the early management of septic shock. Therefore, targeting one or more of these pro-oxidant mechanisms might improve microcirculatory function and reduce the development of sepsis-induced organ dysfunction. Although results of many preclinical studies and pilot clinical trials of antioxidant treatment for sepsis have been encouraging, large controlled randomized trials have not yet convincingly shown that antioxidant strategies improve sepsis outcomes. Better identification of septic patients suffering from latent EC dysfunction may help to define those individuals who are more likely to benefit from antioxidant therapies. Finally, a deeper understanding of OS's complex role in sepsis-induced EC dysfunction and downstream shock and organ failure may reveal additional therapeutic targets and strategies.

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**Abbreviations Used**

ATP = adenosine triphosphate
BAL = low-chloride balanced crystalloid solutions
CLP = cecal ligation and puncture
CRT = capillary refill time
CS = chondroitin sulfate
DAMPs = damage-associated molecular patterns
DIC = disseminated intravascular coagulation
DNA = deoxyribonucleic acid
EC = endothelial cell(s)
eNOS = endothelial NO synthase
ER = endoplasmic reticulum
GAG = glycosaminoglycans
GCX = glycocalyx
GSH = reduced glutathione
$H_2O$ = water
$H_2O_2$ = hydrogen peroxide
HA = hyaluronic acid
HDAC = histone deacetylase
HS = heparan sulfate
HSA = human serum albumin
HUVEC = human umbilical vein endothelial cell
HYPERS2S = *hyperoxia and hypertonic saline in patients with septic shock*
ICAM-1/2 = intercellular cell adhesion molecule-1/2
ICU = intensive care unit
IL = interleukin
iNOS = inductible NO synthase
JNK = c-Jun N-terminal kinases
LPOX = lipo-peroxidase
LPS = lipopolysaccharide
Mac = macrophages
MDA = malondialdehyde
MMP = metalloproteinase
MP = microparticles
mtNOS = mitochondria NO synthase
NAC = *N*-acetylcysteine
NADPH = reduced nicotinamide adenine dinucleotide phosphate
NCT = national clinical trial
NETs = neutrophils extracellular traps
NF-$\kappa$B = nuclear factor-$\kappa$B
NIRS = near-infrared spectroscopy
NOS = NO synthase
NOX = NADPH oxidase
$O_2^{\bullet-}$ = superoxide
$^{\bullet}OH$ = hydroxyl radicals
$ONOO^-$ = peroxynitrite
OPS = orthogonal polarization spectral
OS = oxidative stress
OXPHOS = oxidative phosphorylation
PAMPs = pathogen-associated molecular patterns
PBMC = peripheral blood mononuclear cell
PECAM = platelet endothelial cell adhesion molecule-1
PMN = polymorphonuclear
PON2 = paraoxonase-2
PRR = pattern-recognition receptor
PS = phosphatidylserine
PS = phosphatidylserine
RBC = red blood cells
RNS = reactive nitrogen species
ROS = reactive oxygen species
SOD = superoxide dismutase
$StO_2$ = tissue oxygen saturation
TF = tissue factor
TFPI = tissue factor pathway inhibitor
TLRs = toll-like receptors
TNF = tumor necrosis factor
VCAM-1 = vascular cell adhesion molecule-1
XO = xanthine oxidase