



HAL
open science

Oxidative Stress and Endothelial Dysfunction in Sepsis and Acute Inflammation

Jérémie Joffre, Judith Hellman

► **To cite this version:**

Jérémie Joffre, Judith Hellman. Oxidative Stress and Endothelial Dysfunction in Sepsis and Acute Inflammation. *Antioxidants and Redox Signaling*, 2021, 35, pp.1291 - 1307. 10.1089/ars.2021.0027 . hal-04022309

HAL Id: hal-04022309

<https://hal.sorbonne-universite.fr/hal-04022309v1>

Submitted on 9 Mar 2023

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



FORUM REVIEW ARTICLE

Oxidative Stress and Endothelial Dysfunction in Sepsis and Acute Inflammation

Jérémie Joffre and Judith Hellman

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 (StO₂), 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 dys-

function, 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 adaptive responses to hypoxia, bacterial clearance, and

Department of Anesthesia and Perioperative Care, University of California, San Francisco School of Medicine, San Francisco, California, USA.

© Jérémie Joffre and Judith Hellman, 2021; Published by Mary Ann Liebert, Inc. This Open Access article is distributed under the terms of the Creative Commons License [CC-BY] (<http://creativecommons.org/licenses/by/4.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

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 (glutathi-

one/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 antioxidant 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)- α , interleukin (IL)-1 α , 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

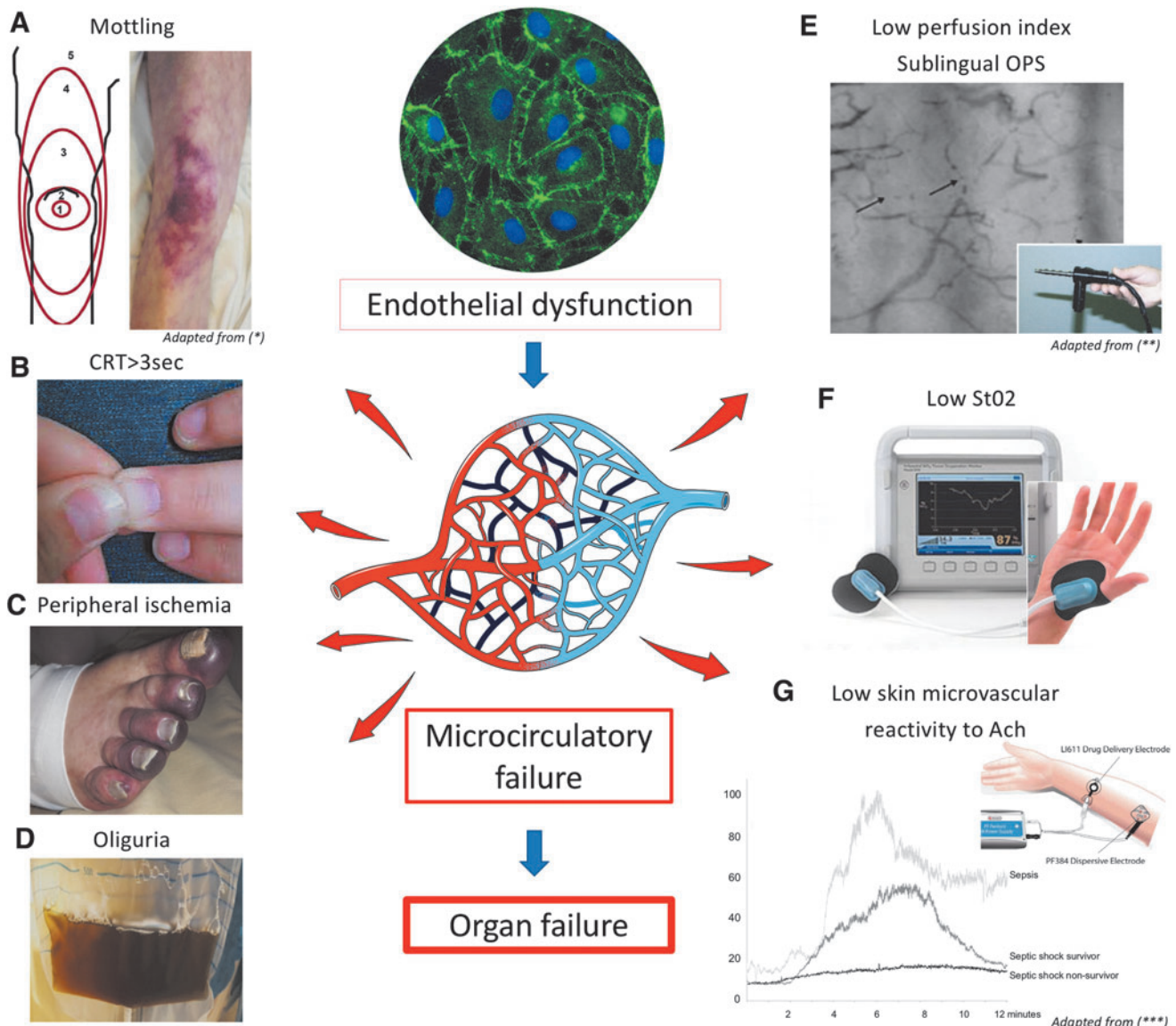


FIG. 1. Cutaneous clinical signs of microcirculatory failure and “beside” tools for microcirculation assessment. (A) Mottling are red-purple patchy marbling of the skin witnessing skin hypoperfusion. The mottling score based on the mottling extension predicts organ failure severity and mortality in patients 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: Nández-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 O₂ (StO₂). Low StO₂ 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 (inducible 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 isoproterenol (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

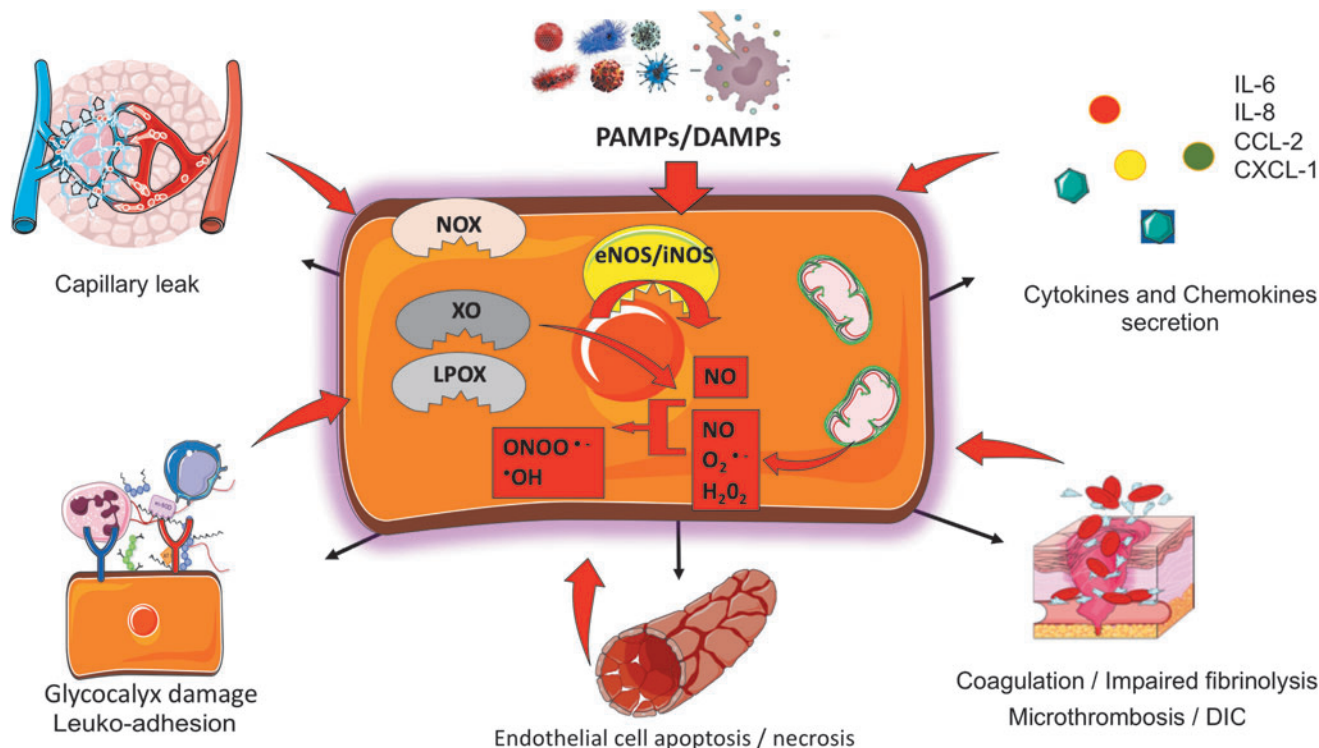


FIG. 2. The endothelium is both a source and a target for OS. During sepsis, PAMPs and DAMPs provoke accumulation of products of OS from multiple EC sources: the mitochondria, the eNOS uncoupling, and intracellular enzymes (NADPH ox, XO, and LPOX). The disruption of redox balance in EC enhances multiple facets of the endothelial dysfunction, engaging EC toward a proinflammatory, procoagulant, and proadhesive phenotype. It also favors EC apoptosis 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 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

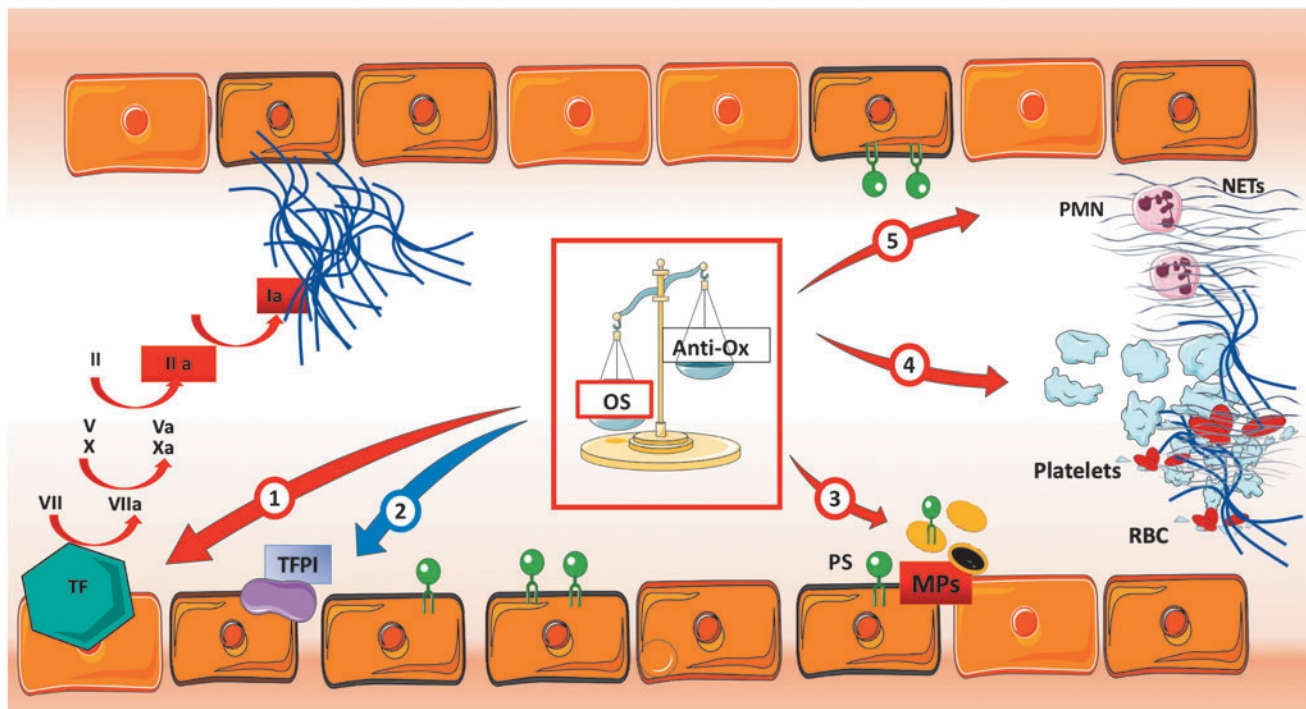


FIG. 3. OS drives EC toward a procoagulant phenotype. During sepsis, redox homeostasis is disrupted, leading to OS and accumulation of mediators that engage multiple cellular pathways that promote the development of microvascular thrombosis and DIC: OS upregulates TF expression at the EC membrane and 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, H₂O₂ 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 H₂O₂ 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- κ B (NF- κ 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- κ B and AP-1, which are two critical transcription factors involved in the proinflammatory switch of ECs (136, 164). In general, reducing agents decrease NF- κ B activities, but it conversely enhances AP-1 activity (104). Oxidants, on the other hand, broadly activate NF- κ B activity (109, 136), whereas they can also activate AP-1 by activating the JNK

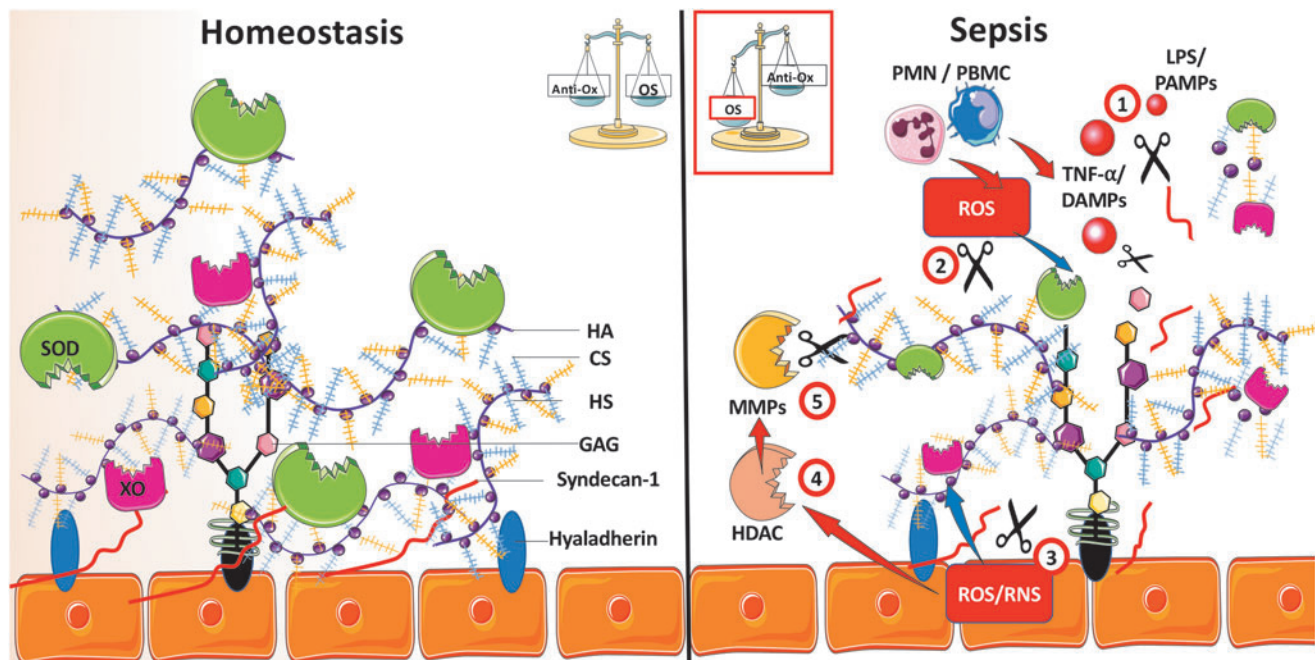


FIG. 4. OS damages the endothelial GCX. Under homeostatic conditions, the GCX is a thick and dense heparan sulfate-rich layer of glycosaminoglycans and proteoglycans that coats the vascular endothelium. The GCX contributes to maintenance of the endothelial barrier, and it helps to reduce leukocyte adhesion, 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- κ B regulation sites in their promoters, including IL-6, IL-8, TNF- α , vascular endothelial growth factor, RANTES, CCL-2, intercellular 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). H₂O₂, •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 H₂O₂ 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* (HYPER2S) study, the groups receiving mechanical ventilation with FiO₂ = 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

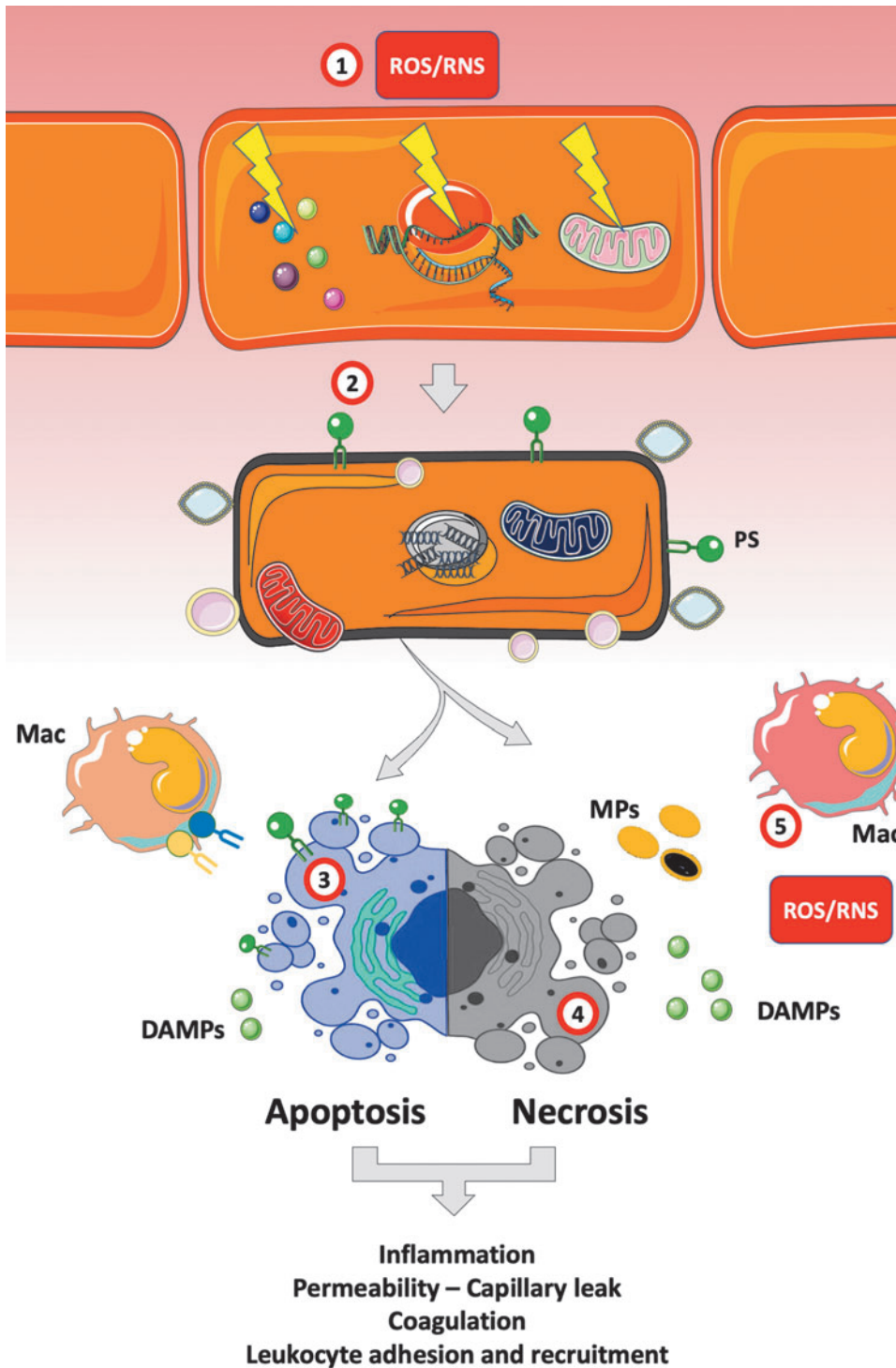


FIG. 5. EC OS accumulation initiates cell death programs. ROS and RNS damage cell integrity by oxidizing lipids, membranes, proteins, DNA, and organelles (mitochondria and ER), and they trigger a cell death program (1). OS initiates the apoptosis program *via* both mitochondrial and extrinsic pathways, 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- α , NF- κ 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- κ B activation in peripheral blood mononuclear cell (PBMC) and reduced plasma IL-8 (121). Another study 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 $N = 797$ 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 $N = 49$ Inhaled nitric oxide 40 ppm*6h vs. Sham	Inhaled NO did not improve microcirculatory flow, lactate clearance, or organ dysfunction.
	The effect of nitroglycerin on microcirculatory abnormalities during sepsis (NISMIS) (163)	Sepsis $N = 70$ 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 $N = 111$ Methylene blue iv 2 mg/kg bolus then 0.5 mg/kg/h*6 h vs. standard care	Completed Awaiting results
Methylene blue as a third-line vasopressor in septic shock (NCT04089072)	Refractory septic shock $N = 250$ ProvatBlue® 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 $N = 30$ 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 ($p < 0.01$). 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 $N = 20$ 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- κ B activation in patients' mononuclear leukocytes, and IL-8 in patients receiving NAC at 72h
	Randomized trial of NAC in septic shock (136)	Septic shock $N = 22$ Placebo vs. NAC iv 150 mg/kg bolus, then infusion of 50 mg/kg over 4h	Survivors who received NAC had shorter ventilation (7 ± 2 days vs. 20 ± 7 days; $p < 0.05$) and shorter ICU stays (13 ± 2 days vs. 32 ± 9 days; $p < 0.05$) Recruiting
	Randomized controlled clinical trial of antioxidant therapy in critically ill patients with septic shock (NCT03557229)	Septic shock $N = 125$ 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) Lactated Ringer <i>versus</i> albumin in early sepsis therapy (RASP) (164) ALBumIn Italian Outcome Septic Shock-BALANCED trial (ALBIOS-BAL) (NCT03654001) Albumin replacement therapy in septic shock (ARISS) (NCT03869385)	Severe sepsis or septic shock <i>N</i> = 1818 20% albumin and crystalloid solution <i>vs.</i> crystalloid solution alone Cancer and septic shock <i>N</i> = 110 Albumin 4% <i>vs.</i> ringer lactate in the first 12 h Septic shock <i>N</i> = 1252 4 groups: albumin + BAL, albumin + saline, BAL, saline Septic shock <i>N</i> = 1662 60 g loading dose of albumin 20%, then 40–80 g/day until day 28 <i>vs.</i> crystalloids	Albumin 20% + crystalloids compared with crystalloids alone did not improve survival at 28 and 90 days Albumin 4% as compared with lactated Ringer did not improve survival at 30 days Recruiting Recruiting
Vitamin C	The vitamin C, hydrocortisone, and thiamine in patients with septic shock trial (VITAMINS) (NCT0333278) Hydrocortisone, vitamin C, and thiamine for the treatment of sepsis and septic shock (HYVCTSSS) (NCT03258684) Metabolic resuscitation using vitamin C, thiamine, and glucocorticoids in sepsis (ORANGES) (NCT03422159) Vitamin C and thiamine in sepsis (NCT03592277) Vitamin C, hydrocortisone, and thiamine for septic shock (CORVICTES) (NCT03592693) Vitamin C, corticosteroids, and thiamine in sepsis (ACTS) trial (NCT03389555) Vitamin C and thiamine effect in septic shock (ATESS) (NCT03756220) Evaluation of hydrocortisone, vitamin C, and thiamine for the treatment of septic shock (HYVITS) (NCT03380507)	Septic shock <i>N</i> = 211 High-dose vitamin C (6 g/day), thiamine (400 mg/day), and hydrocortisone (200 mg/day) <i>vs.</i> hydrocortisone Septic shock <i>N</i> = 140 Vitamin C (4 g/day), hydrocortisone (200 mg/day), and thiamine (400 mg/day) <i>vs.</i> saline Severe sepsis or septic shock <i>N</i> = 140 Vitamin C (4 g/day), hydrocortisone (200 mg/day), and thiamine (400 mg/day) <i>vs.</i> placebo Severe sepsis or septic shock <i>N</i> = 120 Vitamin C (4 g/day) and thiamine (400 mg/day) <i>vs.</i> placebo Septic shock <i>N</i> = 400 Vitamin C (6 g/day) and hydrocortisone (250 mg/dL then 200 mg/day) <i>vs.</i> placebo Septic shock <i>N</i> = 200 Vitamin C (6 g/day), hydrocortisone (200 mg/day), and thiamine (400 mg/day) <i>vs.</i> placebo Septic shock <i>N</i> = 116 Vitamin C (100 mg/day) and thiamine (400 mg/day) <i>vs.</i> saline Septic shock <i>N</i> = 212 Vitamin C (6 g/day), hydrocortisone (200 mg/day), and thiamine (400 mg/day) <i>vs.</i> standard care	Combination of intravenous vitamin C, hydrocortisone, and thiamine does not lead to a more rapid resolution of septic shock compared with intravenous hydrocortisone alone Completed Awaiting results Completed Awaiting results Completed Awaiting results Recruiting Recruiting Recruiting Recruiting

BAL, low-chloride balanced crystalloid solutions; GSH, reduced glutathione; ICU, intensive care unit; IL, interleukin; MDA, malondialdehyde; NAC, *N*-acetylcysteine; NF- κ B, nuclear factor- κ 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 H₂O₂ 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 (β -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 pro-coagulant, 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.

Author Disclosure Statement

No competing financial interests exist.

Funding Information

No funding was received for this work.

References

1. Ackermann M, Verleden SE, Kuehnel M, Haverich A, Welte T, Laenger F, Vanstapel A, Werlein C, Stark H, Tzankov A, Li WW, Li VW, Mentzer SJ, and Jonigk D.

- Pulmonary vascular endothelialitis, thrombosis, and angiogenesis in Covid-19. *N Engl J Med* 383: 120–128, 2020.
2. Adachi T, Fukushima T, Usami Y, and Hirano K. Binding of human xanthine oxidase to sulphated glycosaminoglycans on the endothelial-cell surface. *Biochem J* 289(Pt 2): 523–527, 1993.
 3. Adamson RH, Clark JF, Radeva M, Kheirulomoom A, Ferrara KW, and Curry FE. Albumin modulates SIP delivery from red blood cells in perfused microvessels: mechanism of the protein effect. *Am J Physiol Heart Circ Physiol* 306: H1011–H1017, 2014.
 4. Addabbo F, Montagnani M, and Goligorsky MS. Mitochondria and reactive oxygen species. *Hypertension* 53: 885–892, 2009.
 5. Ahamed J, Belting M, and Ruf W. Regulation of tissue factor-induced signaling by endogenous and recombinant tissue factor pathway inhibitor 1. *Blood* 105: 2384–2391, 2005.
 6. Ait-Oufella H, Bige N, Boelle PY, Pichereau C, Alves M, Bertinchamp R, Baudel JL, Galbois A, Maury E, and Guidet B. Capillary refill time exploration during septic shock. *Intensive Care Med* 40: 958–964, 2014.
 7. Ait-Oufella H, Lemoine S, Boelle PY, Galbois A, Baudel JL, Lemant J, Joffre J, Margetis D, Guidet B, Maury E, and Offenstadt G. Mottling score predicts survival in septic shock. *Intensive Care Med* 37: 801–807, 2011.
 8. Ali MM, Mahmoud AM, Le Master E, Levitan I, and Phillips SA. Role of matrix metalloproteinases and histone deacetylase in oxidative stress-induced degradation of the endothelial glycocalyx. *Am J Physiol Heart Circ Physiol* 316: H647–H663, 2019.
 9. Altenhofer S, Witte I, Teiber JF, Wilgenbus P, Pautz A, Li H, Daiber A, Witan H, Clement AM, Forstermann U, and Horke S. One enzyme, two functions: PON2 prevents mitochondrial superoxide formation and apoptosis independent from its lactonase activity. *J Biol Chem* 285: 24398–24403, 2010.
 10. Anand D, Ray S, Srivastava LM, and Bhargava S. Evolution of serum hyaluronan and syndecan levels in prognosis of sepsis patients. *Clin Biochem* 49: 768–776, 2016.
 11. Armour J, Tyml K, Lidington D, and Wilson JX. Ascorbate prevents microvascular dysfunction in the skeletal muscle of the septic rat. *J Appl Physiol* 90: 795–803, 2001.
 12. Arouma OI, Halliwell B, Hoey BM, and Butler J. The antioxidant action of N-acetylcysteine: its reaction with hydrogen peroxide, hydroxyl radical, superoxide, and hypochlorous acid. *Free Radic Biol Med* 6: 593–597, 1989.
 13. Asfar P, Schortgen F, Boisrame-Helms J, Charpentier J, Guerot E, Megarbane B, Grimaldi D, Grelon F, Anguel N, Lasocki S, Henry-Lagarrigue M, Gonzalez F, Legay F, Guitton C, Schenck M, Doise JM, Devaquet J, Van Der Linden T, Chatellier D, Rigaud JP, Dellamonica J, Tamion F, Meziani F, Mercat A, Dreyfuss D, Seegers V, Radermacher P; HYPER2S Investigators; REVA research network. Hyperoxia and hypertonic saline in patients with septic shock (HYPER2S): a two-by-two factorial, multicentre, randomised, clinical trial. *Lancet Respir Med* 5: 180–190, 2017.
 14. Bakker J, Grover R, McLuckie A, Holzapfel L, Andersson J, Lodato R, Watson D, Grossman S, Donaldson J, Takala J, and Glaxo Wellcome International Septic Shock Study G. Administration of the nitric oxide synthase inhibitor NG-methyl-L-arginine hydrochloride (546C88) by intravenous infusion for up to 72hours can promote the resolution of shock in patients with severe sepsis: results of a randomized, double-blind, placebo-controlled multicenter study (study no. 144-002). *Crit Care Med* 32: 1–12, 2004.
 15. Baxter AA. Stoking the fire: how dying cells propagate inflammatory signalling through extracellular vesicle trafficking. *Int J Mol Sci* 21: 7256, 2020.
 16. Becker M, Menger MD, and Lehr HA. Heparin-released superoxide dismutase inhibits postischemic leukocyte adhesion to venular endothelium. *Am J Physiol* 267: H925–H930, 1994.
 17. Blackwell TS, Blackwell TR, Holden EP, Christman BW, and Christman JW. In vivo antioxidant treatment suppresses nuclear factor-kappa B activation and neutrophilic lung inflammation. *J Immunol* 157: 1630–1637, 1996.
 18. Boerma EC, Koopmans M, Konijn A, Kaiferova K, Bakker AJ, van Roon EN, Buter H, Bruins N, Egbers PH, Gerritsen RT, Koetsier PM, Kingma WP, Kuiper MA, and Ince C. Effects of nitroglycerin on sublingual microcirculatory blood flow in patients with severe sepsis/septic shock after a strict resuscitation protocol: a double-blind randomized placebo controlled trial. *Crit Care Med* 38: 93–100, 2010.
 19. Bombeli T, Karsan A, Tait JF, and Harlan JM. Apoptotic vascular endothelial cells become procoagulant. *Blood* 89: 2429–2442, 1997.
 20. Bombeli T, Mueller M, and Haerberli A. Anticoagulant properties of the vascular endothelium. *Thromb Haemost* 77: 408–423, 1997.
 21. Bombeli T, Schwartz BR, and Harlan JM. Endothelial cells undergoing apoptosis become proadhesive for non-activated platelets. *Blood* 93: 3831–3838, 1999.
 22. Borrelli E, Roux-Lombard P, Grau GE, Girardin E, Ricou B, Dayer J, and Suter PM. Plasma concentrations of cytokines, their soluble receptors, and antioxidant vitamins can predict the development of multiple organ failure in patients at risk. *Crit Care Med* 24: 392–397, 1996.
 23. Borutaite V, Morkuniene R, and Brown GC. Release of cytochrome c from heart mitochondria is induced by high Ca²⁺ and peroxynitrite and is responsible for Ca²⁺-induced inhibition of substrate oxidation. *Biochim Biophys Acta* 1453: 41–48, 1999.
 24. Bourcier S, Joffre J, Dubee V, Preda G, Baudel JL, Bige N, Leblanc G, Levy BI, Guidet B, Maury E, and Ait-Oufella H. Marked regional endothelial dysfunction in mottled skin area in patients with severe infections. *Crit Care* 21: 155, 2017.
 25. Brealey D, Brand M, Hargreaves I, Heales S, Land J, Smolenski R, Davies NA, Cooper CE, and Singer M. Association between mitochondrial dysfunction and severity and outcome of septic shock. *Lancet* 360: 219–223, 2002.
 26. Brown GC and Borutaite V. Inhibition of mitochondrial respiratory complex I by nitric oxide, peroxynitrite and S-nitrosothiols. *Biochim Biophys Acta* 1658: 44–49, 2004.
 27. Brown GC and Borutaite V. Nitric oxide, cytochrome c and mitochondria. *Biochem Soc Symp* 66: 17–25, 1999.
 28. Broze GJ, Jr. and Girard TJ. Tissue factor pathway inhibitor: structure-function. *Front Biosci* 17: 262–280, 2012.
 29. Caironi P, Tognoni G, Masson S, Fumagalli R, Pesenti A, Romero M, Fanizza C, Caspani L, Faenza S, Grasselli G, Iapichino G, Antonelli M, Parrini V, Fiore G, Latini R, Gattinoni L; ALBIOS Study Investigators. Albumin replacement in patients with severe sepsis or septic shock. *N Engl J Med* 370: 1412–1421, 2014.
 30. Calzia E, Asfar P, Hauser B, Matejovic M, Ballestra C, Radermacher P, and Georgieff M. Hyperoxia may be beneficial. *Crit Care Med* 38: S559–S568, 2010.

31. Carlos TM, Clark RS, Francicola-Higgins D, Schiding JK, and Kochanek PM. Expression of endothelial adhesion molecules and recruitment of neutrophils after traumatic brain injury in rats. *J Leukoc Biol* 61: 279–285, 1997.
32. Cassina A and Radi R. Differential inhibitory action of nitric oxide and peroxynitrite on mitochondrial electron transport. *Arch Biochem Biophys* 328: 309–316, 1996.
33. Chantrathammachart P, Mackman N, Sparkenbaugh E, Wang JG, Parise LV, Kirchofer D, Key NS, and Pawlinski R. Tissue factor promotes activation of coagulation and inflammation in a mouse model of sickle cell disease. *Blood* 120: 636–646, 2012.
34. Chen XL, Zhang Q, Zhao R, Ding X, Tummala PE, and Medford RM. Rac1 and superoxide are required for the expression of cell adhesion molecules induced by tumor necrosis factor-alpha in endothelial cells. *J Pharmacol Exp Ther* 305: 573–580, 2003.
35. Chen Y, Zhou Z, and Min W. Mitochondria, oxidative stress and innate immunity. *Front Physiol* 9: 1487, 2018.
36. Chung HY, Yokozawa T, Kim MS, Lee KH, Kim KW, Yang R, and Choi JH. The mechanism of nitric oxide and/or superoxide cytotoxicity in endothelial cells. *Exp Toxicol Pathol* 52: 227–233, 2000.
37. Cimmino G, Cirillo P, Ragni M, Conte S, Uccello G, and Golino P. Reactive oxygen species induce a procoagulant state in endothelial cells by inhibiting tissue factor pathway inhibitor. *J Thromb Thrombolysis* 40: 186–192, 2015.
38. Cooper D, Stokes KY, Tailor A, and Granger DN. Oxidative stress promotes blood cell-endothelial cell interactions in the microcirculation. *Cardiovasc Toxicol* 2: 165–180, 2002.
39. Corda S, Laplace C, Vicaut E, and Duranteau J. Rapid reactive oxygen species production by mitochondria in endothelial cells exposed to tumor necrosis factor-alpha is mediated by ceramide. *Am J Respir Cell Mol Biol* 24: 762–768, 2001.
40. Creteur J, Carollo T, Soldati G, Buchele G, De Backer D, and Vincent JL. The prognostic value of muscle StO2 in septic patients. *Intensive Care Med* 33: 1549–1556, 2007.
41. Crimi E, Liguori A, Condorelli M, Cioffi M, Astuto M, Bontempo P, Pignalosa O, Vietri MT, Molinari AM, Sica V, Della Corte F, and Napoli C. The beneficial effects of antioxidant supplementation in enteral feeding in critically ill patients: a prospective, randomized, double-blind, placebo-controlled trial. *Anesth Analg* 99: 857–863, table of contents, 2004.
42. Cuzzocrea S, Mazzon E, Dugo L, Serraino I, Ciccolo A, Centorrino T, De Sarro A, and Caputi AP. Protective effects of N-acetylcysteine on lung injury and red blood cell modification induced by carrageenan in the rat. *FASEB J* 15: 1187–1200, 2001.
43. Daga MK, Khan NA, Singh H, Chhoda A, Mattoo S, and Gupta BK. Markers of oxidative stress and clinical outcome in critically ill septic patients: a preliminary study from North India. *J Clin Diagn Res* 10: OC35–OC38, 2016.
44. Daiber A and Chlopicki S. Revisiting pharmacology of oxidative stress and endothelial dysfunction in cardiovascular disease: evidence for redox-based therapies. *Free Radic Biol Med* 157: 15–37, 2020.
45. Davis JS, Darcy CJ, Yeo TW, Jones C, McNeil YR, Stephens DP, Celermajer DS, and Anstey NM. Asymmetric dimethylarginine, endothelial nitric oxide bioavailability and mortality in sepsis. *PLoS One* 6: e17260, 2011.
46. Davis JS, Yeo TW, Thomas JH, McMillan M, Darcy CJ, McNeil YR, Cheng AC, Celermajer DS, Stephens DP, and Anstey NM. Sepsis-associated microvascular dysfunction measured by peripheral arterial tonometry: an observational study. *Crit Care* 13: R155, 2009.
47. De Backer D, Creteur J, Preiser JC, Dubois MJ, and Vincent JL. Microvascular blood flow is altered in patients with sepsis. *Am J Respir Crit Care Med* 166: 98–104, 2002.
48. Ebert J, Wilgenbus P, Teiber JF, Jurk K, Schwierczek K, Dohrmann M, Xia N, Li H, Spiecker L, Ruf W, and Horke S. Paraoxonase-2 regulates coagulation activation through endothelial tissue factor. *Blood* 131: 2161–2172, 2018.
49. Elmore S. Apoptosis: a review of programmed cell death. *Toxicol Pathol* 35: 495–516, 2007.
50. Eum HA, Park SW, and Lee SM. Role of nitric oxide in the expression of hepatic vascular stress genes in response to sepsis. *Nitric Oxide* 17: 126–133, 2007.
51. Fialkow L, Wang Y, and Downey GP. Reactive oxygen and nitrogen species as signaling molecules regulating neutrophil function. *Free Radic Biol Med* 42: 153–164, 2007.
52. Forstermann U and Munzel T. Endothelial nitric oxide synthase in vascular disease: from marvel to menace. *Circulation* 113: 1708–1714, 2006.
53. Fowler AA, 3rd, Truitt JD, Hite RD, Morris PE, DeWilde C, Priday A, Fisher B, Thacker LR, 2nd, Natarajan R, Brophy DF, Sculthorpe R, Nanchal R, Syed A, Sturgill J, Martin GS, Sevransky J, Kashiouris M, Hamman S, Egan KF, Hastings A, Spencer W, Tench S, Mehkri O, Bindas J, Duggal A, Graf J, Zellner S, Yanny L, McPolin C, Hollrith T, Kramer D, Ojielo C, Damm T, Cassidy E, Wieliczko A, and Halquist M. Effect of vitamin C infusion on organ failure and biomarkers of inflammation and vascular injury in patients with sepsis and severe acute respiratory failure: the CITRIS-ALI randomized clinical trial. *JAMA* 322: 1261–1270, 2019.
54. Fox ES, Brower JS, Bellezzo JM, and Leingang KA. N-acetylcysteine and alpha-tocopherol reverse the inflammatory response in activated rat Kupffer cells. *J Immunol* 158: 5418–5423, 1997.
55. Fredriksson K, Tjader I, Keller P, Petrovic N, Ahlman B, Scheele C, Wernerman J, Timmons JA, and Rooyackers O. Dysregulation of mitochondrial dynamics and the muscle transcriptome in ICU patients suffering from sepsis induced multiple organ failure. *PLoS One* 3: e3686, 2008.
56. Fujii T, Luethi N, Young PJ, Frei DR, Eastwood GM, French CJ, Deane AM, Shehabi Y, Hajjar LA, Oliveira G, Udy AA, Orford N, Edney SJ, Hunt AL, Judd HL, Bitker L, Cioccarelli L, Naorungroj T, Yanase F, Bates S, McGain F, Hudson EP, Al-Bassam W, Dwivedi DB, Peppin C, McCracken P, Orosz J, Bailey M, Bellomo R, and Investigators VT. Effect of vitamin C, hydrocortisone, and thiamine vs hydrocortisone alone on time alive and free of vasopressor support among patients with septic shock: the VITAMINS randomized clinical trial. *JAMA* 323: 423–431, 2020.
57. Galley HF, Davies MJ, and Webster NR. Ascorbyl radical formation in patients with sepsis: effect of ascorbate loading. *Free Radic Biol Med* 20: 139–143, 1996.
58. Galley HF, Howdle PD, Walker BE, and Webster NR. The effects of intravenous antioxidants in patients with septic shock. *Free Radic Biol Med* 23: 768–774, 1997.
59. Gasparetto A, Corbucci GG, Candiani A, Gohil K, and Edwards RH. Effect of tissue hypoxia and septic shock on human skeletal muscle mitochondria. *Lancet* 2: 1486, 1983.
60. Gaudette S, Hughes D, and Boller M. The endothelial glycocalyx: structure and function in health and critical illness. *J Vet Emerg Crit Care (San Antonio)* 30: 117–134, 2020.

61. Gill SE, Rohan M, and Mehta S. Role of pulmonary microvascular endothelial cell apoptosis in murine sepsis-induced lung injury in vivo. *Respir Res* 16: 109, 2015.
62. Grundmann S, Fink K, Rabadzheva L, Bourgeois N, Schwab T, Moser M, Bode C, and Busch HJ. Perturbation of the endothelial glycocalyx in post cardiac arrest syndrome. *Resuscitation* 83: 715–720, 2012.
63. Gutteridge JM. Lipid peroxidation and antioxidants as biomarkers of tissue damage. *Clin Chem* 41: 1819–1828, 1995.
64. Gutteridge JM and Mitchell J. Redox imbalance in the critically ill. *Br Med Bull* 55: 49–75, 1999.
65. Hagmann H, Kuczkowski A, Ruehl M, Lamkemeyer T, Brodessaer S, Horke S, Dryer S, Schermer B, Benzing T, and Brinkkoetter PT. Breaking the chain at the membrane: paraoxonase 2 counteracts lipid peroxidation at the plasma membrane. *FASEB J* 28: 1769–1779, 2014.
66. Halliwell B. Free radicals and antioxidants—quo vadis? *Trends Pharmacol Sci* 32: 125–130, 2011.
67. Hariri G, Joffre J, Deryckere S, Bige N, Dumas G, Baudel JL, Maury E, Guidet B, and Ait-Oufella H. Albumin infusion improves endothelial function in septic shock patients: a pilot study. *Intensive Care Med* 44: 669–671, 2018.
68. Hariri G, Joffre J, Leblanc G, Bonsey M, Lavillegrand JR, Urbina T, Guidet B, Maury E, Bakker J, and Ait-Oufella H. Narrative review: clinical assessment of peripheral tissue perfusion in septic shock. *Ann Intensive Care* 9: 37, 2019.
69. Hasan SS, Radford S, Kow CS, and Zaidi STR. Venous thromboembolism in critically ill COVID-19 patients receiving prophylactic or therapeutic anticoagulation: a systematic review and meta-analysis. *J Thromb Thrombolysis* 50: 814–821, 2020.
70. Hellyer PW, Johnson LW, and Olson NC. Effect of NG-nitro-L-arginine-methyl-ester on cardiopulmonary function and biosynthesis of cyclooxygenase products during porcine endotoxemia. *Crit Care Med* 25: 1051–1058, 1997.
71. Helmerhorst HJ, Schultz MJ, van der Voort PH, de Jonge E, and van Westerloo DJ. Bench-to bedside review: the effects of hyperoxia during critical illness. *Crit Care* 19: 284, 2015.
72. Hernandez G, Pedreros C, Veas E, Bruhn A, Romero C, Rovegno M, Neira R, Bravo S, Castro R, Kattan E, and Ince C. Evolution of peripheral vs metabolic perfusion parameters during septic shock resuscitation. A clinical-physiologic study. *J Crit Care* 27: 283–288, 2012.
73. Heyland D, Muscedere J, Wischmeyer PE, Cook D, Jones G, Albert M, Elke G, Berger MM, Day AG; Canadian Critical Care Trials Group. A randomized trial of glutamine and antioxidants in critically ill patients. *N Engl J Med* 368: 1489–1497, 2013.
74. Holzmann MS, Winkler MS, Strunden MS, Izbicki JR, Schoen G, Greiwe G, Pinnschmidt HO, Poppe A, Saugel B, Daum G, Goetz AE, and Heckel K. Syndecan-1 as a biomarker for sepsis survival after major abdominal surgery. *Biomark Med* 12: 119–127, 2018.
75. Hovaguimian F, Lysakowski C, Elia N, and Tramer MR. Effect of intraoperative high inspired oxygen fraction on surgical site infection, postoperative nausea and vomiting, and pulmonary function: systematic review and meta-analysis of randomized controlled trials. *Anesthesiology* 119: 303–316, 2013.
76. Huet O, Cherreau C, Nicco C, Dupic L, Conti M, Borderie D, Pene F, Vicaut E, Benhamou D, Mira JP, Duranteau J, and Batteux F. Pivotal role of glutathione depletion in plasma-induced endothelial oxidative stress during sepsis. *Crit Care Med* 36: 2328–2334, 2008.
77. Huet O, Dupic L, Harrois A, and Duranteau J. Oxidative stress and endothelial dysfunction during sepsis. *Front Biosci* 16: 1986–1995, 2011.
78. Huet O, Obata R, Aubron C, Spraul-Davit A, Charpentier J, Laplace C, Nguyen-Khoa T, Conti M, Vicaut E, Mira JP, and Duranteau J. Plasma-induced endothelial oxidative stress is related to the severity of septic shock. *Crit Care Med* 35: 821–826, 2007.
79. Ikeda M, Matsumoto H, Ogura H, Hirose T, Shimizu K, Yamamoto K, Maruyama I, and Shimazu T. Circulating syndecan-1 predicts the development of disseminated intravascular coagulation in patients with sepsis. *J Crit Care* 43: 48–53, 2018.
80. Inagawa R, Okada H, Takemura G, Suzuki K, Takada C, Yano H, Ando Y, Usui T, Hotta Y, Miyazaki N, Tsujimoto A, Zaikokuji R, Matsumoto A, Kawaguchi T, Doi T, Yoshida T, Yoshida S, Kumada K, Ushikoshi H, Toyoda I, and Ogura S. Ultrastructural alteration of pulmonary capillary endothelial glycocalyx during endotoxemia. *Chest* 154: 317–325, 2018.
81. Ince C, Boerma EC, Cecconi M, De Backer D, Shapiro NI, Duranteau J, Pinsky MR, Artigas A, Teboul JL, Reiss IKM, Aldecoa C, Hutchings SD, Donati A, Maggiorini M, Taccone FS, Hernandez G, Payen D, Tibboel D, Martin DS, Zarbock A, Monnet X, Dubin A, Bakker J, Vincent JL, Scheeren TWL; Cardiovascular Dynamics Section of the ESICM. Second consensus on the assessment of sublingual microcirculation in critically ill patients: results from a task force of the European Society of Intensive Care Medicine. *Intensive Care Med* 44: 281–299, 2018.
82. Investigators SS, Finfer S, Bellomo R, McEvoy S, Lo SK, Myburgh J, Neal B, and Norton R. Effect of baseline serum albumin concentration on outcome of resuscitation with albumin or saline in patients in intensive care units: analysis of data from the saline versus albumin fluid evaluation (SAFE) study. *BMJ* 333: 1044, 2006.
83. Joffre J, Hellman J, Ince C, and Ait-Oufella H. Endothelial responses in sepsis. *Am J Respir Crit Care Med* 202: 361–370, 2020.
84. Karlsson K, Lindahl U, and Marklund SL. Binding of human extracellular superoxide dismutase C to sulphated glycosaminoglycans. *Biochem J* 256: 29–33, 1988.
85. Karlsson K and Marklund SL. Extracellular superoxide dismutase in the vascular system of mammals. *Biochem J* 255: 223–228, 1988.
86. Kataoka H, Ushiyama A, Akimoto Y, Matsubara S, Kawakami H, and Iijima T. Structural behavior of the endothelial glycocalyx is associated with pathophysiologic status in septic mice: an integrated approach to analyzing the behavior and function of the glycocalyx using both electron and fluorescence intravital microscopy. *Anesth Analg* 125: 874–883, 2017.
87. Khakpour S, Wilhelmssen K, and Hellman J. Vascular endothelial cell Toll-like receptor pathways in sepsis. *Innate Immun* 21: 827–846, 2015.
88. Kheir-Eldin AA, Motawi TK, Gad MZ, and Abd-ElGawad HM. Protective effect of vitamin E, beta-carotene and N-acetylcysteine from the brain oxidative stress induced in rats by lipopolysaccharide. *Int J Biochem Cell Biol* 33: 475–482, 2001.
89. Kilgannon JH, Jones AE, Shapiro NI, Angelos MG, Milcarek B, Hunter K, Parrillo JE, Trzeciak S; Emergency Medicine Shock Research Network (EMShockNet) Investigators. Association between arterial hyperoxia fol-

- lowing resuscitation from cardiac arrest and in-hospital mortality. *JAMA* 303: 2165–2171, 2010.
90. Kliment CR, Englert JM, Gochuico BR, Yu G, Kaminski N, Rosas I, and Oury TD. Oxidative stress alters syndecan-1 distribution in lungs with pulmonary fibrosis. *J Biol Chem* 284: 3537–3545, 2009.
 91. Kliment CR, Tobolewski JM, Manni ML, Tan RJ, Enghild J, and Oury TD. Extracellular superoxide dismutase protects against matrix degradation of heparan sulfate in the lung. *Antioxid Redox Signal* 10: 261–268, 2008.
 92. Kolarova H, Ambruzova B, Svihaalkova Sindlerova L, Klinke A, and Kubala L. Modulation of endothelial glycocalyx structure under inflammatory conditions. *Mediators Inflamm* 2014: 694312, 2014.
 93. Krotz F, Sohn HY, and Pohl U. Reactive oxygen species: players in the platelet game. *Arterioscler Thromb Vasc Biol* 24: 1988–1996, 2004.
 94. Kuhn SO, Meissner K, Mayes LM, and Bartels K. Vitamin C in sepsis. *Curr Opin Anaesthesiol* 31: 55–60, 2018.
 95. Lacza Z, Pankotai E, and Busija DW. Mitochondrial nitric oxide synthase: current concepts and controversies. *Front Biosci* 14: 4436–4443, 2009.
 96. Lambden S. Bench to bedside review: therapeutic modulation of nitric oxide in sepsis—an update. *Intensive Care Med Exp* 7: 64, 2019.
 97. Langer F and Ruf W. Synergies of phosphatidylserine and protein disulfide isomerase in tissue factor activation. *Thromb Haemostasis* 111: 590–597, 2014.
 98. Legrand M, De Backer D, Depret F, and Ait-Oufella H. Recruiting the microcirculation in septic shock. *Ann Intensive Care* 9: 102, 2019.
 99. Levi M and Ten Cate H. Disseminated intravascular coagulation. *N Engl J Med* 341: 586–592, 1999.
 100. Lipowsky HH, Gao L, and Lescanic A. Shedding of the endothelial glycocalyx in arterioles, capillaries, and venules and its effect on capillary hemodynamics during inflammation. *Am J Physiol Heart Circ Physiol* 301: H2235–H2245, 2011.
 101. Liu Y, Zhang R, Qu H, Wu J, Li L, and Tang Y. Endothelial microparticles activate endothelial cells to facilitate the inflammatory response. *Mol Med Rep* 15: 1291–1296, 2017.
 102. Lopez A, Lorente JA, Steingrub J, Bakker J, McLuckie A, Willatts S, Brockway M, Anzueto A, Holzapfel L, Breen D, Silverman MS, Takala J, Donaldson J, Arneson C, Grove G, Grossman S, and Grover R. Multiple-center, randomized, placebo-controlled, double-blind study of the nitric oxide synthase inhibitor 546C88: effect on survival in patients with septic shock. *Crit Care Med* 32: 21–30, 2004.
 103. Luo S, Lei H, Qin H, and Xia Y. Molecular mechanisms of endothelial NO synthase uncoupling. *Curr Pharm Des* 20: 3548–3553, 2014.
 104. Macdonald J, Galley HF, and Webster NR. Oxidative stress and gene expression in sepsis. *Br J Anaesth* 90: 221–232, 2003.
 105. Mackman N and Antoniak S. Tissue factor and oxidative stress. *Blood* 131: 2094–2095, 2018.
 106. Mahmood Z and Shukla Y. Death receptors: targets for cancer therapy. *Exp Cell Res* 316: 887–899, 2010.
 107. Marik PE, Khangoora V, Rivera R, Hooper MH, and Catravas J. Hydrocortisone, vitamin C, and thiamine for the treatment of severe sepsis and septic shock: a retrospective before-after study. *Chest* 151: 1229–1238, 2017.
 108. McEver RP, Moore KL, and Cummings RD. Leukocyte trafficking mediated by selectin-carbohydrate interactions. *J Biol Chem* 270: 11025–11028, 1995.
 109. Meyer M, Schreck R, and Baeuerle PA. H₂O₂ and antioxidants have opposite effects on activation of NF- κ B and AP-1 in intact cells: AP-1 as secondary antioxidant-responsive factor. *EMBO J* 12: 2005–2015, 1993.
 110. Meziani F, Kremer H, Tesse A, Baron-Menguy C, Mathien C, Mostefai HA, Carusio N, Schneider F, Asfar P, and Andriantsitohaina R. Human serum albumin improves arterial dysfunction during early resuscitation in mouse endotoxic model via reduced oxidative and nitrosative stresses. *Am J Pathol* 171: 1753–1761, 2007.
 111. Muzaffar S, Jeremy JY, Angelini GD, Stuart-Smith K, and Shukla N. Role of the endothelium and nitric oxide synthases in modulating superoxide formation induced by endotoxin and cytokines in porcine pulmonary arteries. *Thorax* 58: 598–604, 2003.
 112. Nagar H, Piao S, and Kim CS. Role of mitochondrial oxidative stress in sepsis. *Acute Crit Care* 33: 65–72, 2018.
 - 112a. Nãñez-Varona DP, Tróchez-Zuleta AL, and Vargas-Garzón WA. Restoring microcirculation in anesthesia: Impact, usefulness and controversies. *Colomb J Anesthesiol* 44: 140–145, 2016.
 113. Nelson A, Berkestedt I, Schmidtchen A, Ljunggren L, and Bodelsson M. Increased levels of glycosaminoglycans during septic shock: relation to mortality and the antibacterial actions of plasma. *Shock* 30: 623–627, 2008.
 114. Okada H, Takemura G, Suzuki K, Oda K, Takada C, Hotta Y, Miyazaki N, Tsujimoto A, Muraki I, Ando Y, Zaikokuji R, Matsumoto A, Kitagaki H, Tamaoki Y, Usui T, Doi T, Yoshida T, Yoshida S, Ushikoshi H, Toyoda I, and Ogura S. Three-dimensional ultrastructure of capillary endothelial glycocalyx under normal and experimental endotoxemic conditions. *Crit Care* 21: 261, 2017.
 115. Ortolani O, Conti A, De Gaudio AR, Moraldi E, Cantini Q, and Novelli G. The effect of glutathione and N-acetylcysteine on lipoperoxidative damage in patients with early septic shock. *Am J Respir Crit Care Med* 161: 1907–1911, 2000.
 116. Ostrowski SR, Haase N, Muller RB, Moller MH, Pott FC, Perner A, and Johansson PI. Association between biomarkers of endothelial injury and hypocoagulability in patients with severe sepsis: a prospective study. *Crit Care* 19: 191, 2015.
 117. Paiva CN and Bozza MT. Are reactive oxygen species always detrimental to pathogens? *Antioxid Redox Signal* 20: 1000–1037, 2014.
 118. Pallepati P and Averill-Bates DA. Mild thermotolerance induced at 40 degrees C protects HeLa cells against activation of death receptor-mediated apoptosis by hydrogen peroxide. *Free Radic Biol Med* 50: 667–679, 2011.
 119. Park CHL, de Almeida JP, de Oliveira GQ, Rizk SI, Fukushima JT, Nakamura RE, Mourao MM, Galas F, Abdala E, Pinheiro Freire M, Kalil Filho R, Costa Auler JO, Jr., Nardelli P, Martin GS, Landoni G, and Hajjar LA. Lactated Ringer's versus 4% albumin on lactated Ringer's in early sepsis therapy in cancer patients: a pilot single-center randomized trial. *Crit Care Med* 47: e798–e805, 2019.
 120. Patel A, Laffan MA, Waheed U, and Brett SJ. Randomised trials of human albumin for adults with sepsis: systematic review and meta-analysis with trial sequential analysis of all-cause mortality. *BMJ* 349: g4561, 2014.
 121. Paterson RL, Galley HF, and Webster NR. The effect of N-acetylcysteine on nuclear factor- κ B activation, interleukin-6, interleukin-8, and intercellular adhesion

- molecule-1 expression in patients with sepsis. *Crit Care Med* 31: 2574–2578, 2003.
122. Peter ME. Programmed cell death: apoptosis meets necrosis. *Nature* 471: 310–312, 2011.
 123. Prauchner CA. Oxidative stress in sepsis: pathophysiological implications justifying antioxidant co-therapy. *Burns* 43: 471–485, 2017.
 124. Psenakova K, Hexnerova R, Srb P, Obsilova V, Veverka V, and Obsil T. The redox-active site of thioredoxin is directly involved in apoptosis signal-regulating kinase 1 binding that is modulated by oxidative stress. *FEBS J* 287: 1626–1644, 2020.
 125. Puskarich MA, Cornelius DC, Tharp J, Nandi U, and Jones AE. Plasma syndecan-1 levels identify a cohort of patients with severe sepsis at high risk for intubation after large-volume intravenous fluid resuscitation. *J Crit Care* 36: 125–129, 2016.
 126. Quinlan GJ, Margaron MP, Mumby S, Evans TW, and Gutteridge JM. Administration of albumin to patients with sepsis syndrome: a possible beneficial role in plasma thiol repletion. *Clin Sci* 95: 459–465, 1998.
 127. Radi R, Cassina A, and Hodara R. Nitric oxide and peroxynitrite interactions with mitochondria. *Biol Chem* 383: 401–409, 2002.
 128. Redza-Dutordoir M and Averill-Bates DA. Activation of apoptosis signalling pathways by reactive oxygen species. *Biochim Biophys Acta* 1863: 2977–2992, 2016.
 129. Reitsma S, Slaaf DW, Vink H, van Zandvoort MA, and oude Egbrink MG. The endothelial glycocalyx: composition, functions, and visualization. *Pflugers Arch* 454: 345–359, 2007.
 130. Rubio-Gayosso I, Platts SH, and Duling BR. Reactive oxygen species mediate modification of glycocalyx during ischemia-reperfusion injury. *Am J Physiol Heart Circ Physiol* 290: H2247–H2256, 2006.
 131. Rudd KE, Johnson SC, Agesa KM, Shackelford KA, Tsoi D, Kievlan DR, Colombara DV, Ikuta KS, Kisssoon N, Finfer S, Fleischmann-Struzek C, Machado FR, Reinhart KK, Rowan K, Seymour CW, Watson RS, West TE, Marinho F, Hay SI, Lozano R, Lopez AD, Angus DC, Murray CJL, and Naghavi M. Global, regional, and national sepsis incidence and mortality, 1990–2017: analysis for the Global Burden of Disease Study. *Lancet* 395: 200–211, 2020.
 132. Ryter SW, Kim HP, Hoetzel A, Park JW, Nakahira K, Wang X, and Choi AM. Mechanisms of cell death in oxidative stress. *Antioxid Redox Signal* 9: 49–89, 2007.
 133. Saito J, Toriumi S, Awano K, Ichijo H, Sasaki K, Kobayashi T, and Tamura S. Regulation of apoptosis signal-regulating kinase 1 by protein phosphatase 2Cepsilon. *Biochem J* 405: 591–596, 2007.
 134. Salvador B, Arranz A, Francisco S, Cordoba L, Punzon C, Llamas MA, and Fresno M. Modulation of endothelial function by Toll like receptors. *Pharmacol Res* 108: 46–56, 2016.
 135. Schorah CJ, Downing C, Piripitsi A, Gallivan L, Al-Hazaa AH, Sanderson MJ, and Bodenham A. Total vitamin C, ascorbic acid, and dehydroascorbic acid concentrations in plasma of critically ill patients. *Am J Clin Nutr* 63: 760–765, 1996.
 136. Sen CK and Packer L. Antioxidant and redox regulation of gene transcription. *FASEB J* 10: 709–720, 1996.
 137. Sharpe MA and Cooper CE. Reactions of nitric oxide with mitochondrial cytochrome c: a novel mechanism for the formation of nitroxyl anion and peroxynitrite. *Biochem J* 332(Pt 1): 9–19, 1998.
 138. Singer M, Deutschman CS, Seymour CW, Shankar-Hari M, Annane D, Bauer M, Bellomo R, Bernard GR, Chiche JD, Coopersmith CM, Hotchkiss RS, Levy MM, Marshall JC, Martin GS, Opal SM, Rubenfeld GD, van der Poll T, Vincent JL, and Angus DC. The third international consensus definitions for sepsis and septic shock (Sepsis-3). *JAMA* 315: 801–810, 2016.
 139. Singh A, Ramnath RD, Foster RR, Wylie EC, Friden V, Dasgupta I, Haraldsson B, Welsh GI, Mathieson PW, and Satchell SC. Reactive oxygen species modulate the barrier function of the human glomerular endothelial glycocalyx. *PLoS One* 8: e55852, 2013.
 140. Smart L, Bosio E, Macdonald SPJ, Dull R, Fatovich DM, Neil C, and Arendts G. Glycocalyx biomarker syndecan-1 is a stronger predictor of respiratory failure in patients with sepsis due to pneumonia, compared to endocan. *J Crit Care* 47: 93–98, 2018.
 141. Smart L, Macdonald SPJ, Burrows S, Bosio E, Arendts G, and Fatovich DM. Endothelial glycocalyx biomarkers increase in patients with infection during Emergency Department treatment. *J Crit Care* 42: 304–309, 2017.
 142. Spapen H, Zhang H, Demanet C, Vleminckx W, Vincent JL, and Huyghens L. Does N-acetyl-L-cysteine influence cytokine response during early human septic shock? *Chest* 113: 1616–1624, 1998.
 143. Stub D, Smith K, Bernard S, Nehme Z, Stephenson M, Bray JE, Cameron P, Barger B, Ellims AH, Taylor AJ, Meredith IT, Kaye DM; AVOID Investigators. Air versus oxygen in ST-segment-elevation myocardial infarction. *Circulation* 131: 2143–2150, 2015.
 144. Szabo C and Modis K. Pathophysiological roles of peroxynitrite in circulatory shock. *Shock* 34(Suppl 1): 4–14, 2010.
 145. Takano M, Meneshian A, Sheikh E, Yamakawa Y, Wilkins KB, Hopkins EA, and Bulkley GB. Rapid upregulation of endothelial P-selectin expression via reactive oxygen species generation. *Am J Physiol Heart Circ Physiol* 283: H2054–H2061, 2002.
 146. Taverna M, Marie AL, Mira JP, and Guidet B. Specific antioxidant properties of human serum albumin. *Ann Intensive Care* 3: 4, 2013.
 147. Teuwen LA, Geldhof V, Pasut A, and Carmeliet P. COVID-19: the vasculature unleashed. *Nat Rev Immunol* 20: 389–391, 2020.
 148. Trzeciak S, Glaspey LJ, Dellinger RP, Durlinger P, Anderson K, Dezfulian C, Roberts BW, Chansky ME, Parrillo JE, and Hollenberg SM. Randomized controlled trial of inhaled nitric oxide for the treatment of microcirculatory dysfunction in patients with sepsis*. *Crit Care Med* 42: 2482–2492, 2014.
 149. Tsao CM, Li KY, Chen SJ, Ka SM, Liaw WJ, Huang HC, and Wu CC. Levosimendan attenuates multiple organ injury and improves survival in peritonitis-induced septic shock: studies in a rat model. *Crit Care* 18: 652, 2014.
 150. Tynl K. Critical role for oxidative stress, platelets, and coagulation in capillary blood flow impairment in sepsis. *Microcirculation* 18: 152–162, 2011.
 151. Vale JA and Proudfoot AT. Paracetamol (acetaminophen) poisoning. *Lancet* 346: 547–552, 1995.
 152. van der Poll T. Tissue factor as an initiator of coagulation and inflammation in the lung. *Crit Care* 12(Suppl 6): S3, 2008.
 153. van Golen RF, van Gulik TM, and Heger M. Mechanistic overview of reactive species-induced degradation of the endothelial glycocalyx during hepatic ischemia/reperfusion injury. *Free Radic Biol Med* 52: 1382–1402, 2012.
 154. Vestweber D. How leukocytes cross the vascular endothelium. *Nat Rev Immunol* 15: 692–704, 2015.

155. Victor VV, Guayerbas N, Puerto M, Medina S, and De la Fuente M. Ascorbic acid modulates in vitro the function of macrophages from mice with endotoxic shock. *Immunopharmacology* 46: 89–101, 2000.
156. Villa LM, Salas E, Darley-Usmar VM, Radomski MW, and Moncada S. Peroxynitrite induces both vasodilatation and impaired vascular relaxation in the isolated perfused rat heart. *Proc Natl Acad Sci U S A* 91: 12383–12387, 1994.
157. Villa P and Ghezzi P. Effect of N-acetyl-L-cysteine on sepsis in mice. *Eur J Pharmacol* 292: 341–344, 1995.
158. Vincent JL, Dubois MJ, Navickis RJ, and Wilkes MM. Hypoalbuminemia in acute illness: is there a rationale for intervention? A meta-analysis of cohort studies and controlled trials. *Ann Surg* 237: 319–334, 2003.
159. Wang W, Deng M, Liu X, Ai W, Tang Q, and Hu J. TLR4 activation induces nontolerant inflammatory response in endothelial cells. *Inflammation* 34: 509–518, 2011.
160. West AP, Brodsky IE, Rahner C, Woo DK, Erdjument-Bromage H, Tempst P, Walsh MC, Choi Y, Shadel GS, and Ghosh S. TLR signalling augments macrophage bactericidal activity through mitochondrial ROS. *Nature* 472: 476–480, 2011.
161. Wiesinger A, Peters W, Chappell D, Kentrup D, Reuter S, Pavenstadt H, Oberleithner H, and Kumpers P. Nanomechanics of the endothelial glycocalyx in experimental sepsis. *PLoS One* 8: e80905, 2013.
162. Winkler MS, Kluge S, Holzmann M, Moritz E, Robbe L, Bauer A, Zahrte C, Priefer M, Schwedhelm E, Boger RH, Goetz AE, Nierhaus A, and Zoellner C. Markers of nitric oxide are associated with sepsis severity: an observational study. *Crit Care* 21: 189, 2017.
163. Zeng Y, Adamson RH, Curry FR, and Tarbell JM. Sphingosine-1-phosphate protects endothelial glycocalyx by inhibiting syndecan-1 shedding. *Am J Physiol Heart Circ Physiol* 306: H363–H372, 2014.
164. Zhong W, Qian K, Xiong J, Ma K, Wang A, and Zou Y. Curcumin alleviates lipopolysaccharide induced sepsis and liver failure by suppression of oxidative stress-related inflammation via PI3K/AKT and NF-kappaB related signaling. *Biomed Pharmacother* 83: 302–313, 2016.

Address correspondence to:

Dr. Jérémie Joffre

Department of Anesthesia and Perioperative Care

University of California,

San Francisco School of Medicine

500 Parnassus Avenue Box 0648

San Francisco, CA 94143

USA

E-mail: jeremie.joffre@ucsf.edu

Date of first submission to ARS Central, February 11, 2021;
date of acceptance, February 23, 2021.

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
H₂O = water
H₂O₂ = 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 = inducible 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-κB = nuclear factor-κB
NIRS = near-infrared spectroscopy
NOS = NO synthase
NOX = NADPH oxidase
O₂^{•-} = superoxide
•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
StO₂ = 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