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Resuscitating the Microcirculation in Sepsis: The Central Role of Nitric Oxide, Emerging Concepts for Novel Therapies, and Challenges for Clinical Trials

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Abstract

Microcirculatory dysfunction is a critical element of the pathogenesis of severe sepsis and septic shock. In this Bench-to-Bedside review, we present: (1) the central role of the microcirculation in the pathophysiology of sepsis; (2) new translational research techniques of in vivo videomicroscopy for assessment of microcirculatory flow in human subjects; (3) clinical investigations that reported associations between microcirculatory dysfunction and outcome in septic patients; (4) the potential role of novel agents to "rescue" the microcirculation in sepsis; (5) current challenges facing this emerging field of clinical investigation; and (6) a framework for the design of future clinical trials aimed to determine the impact of novel agents on microcirculatory flow and organ failure in patients with sepsis. We specifically focus this review on the central role and vital importance of the nitric oxide molecule in maintaining microcirculatory homeostasis and patency, especially when the microcirculation sustains an insult (as with sepsis), and we present the scientific rationale for clinical trials of exogenous nitric oxide administration to treat microcirculatory dysfunction and augment microcirculatory blood flow in early sepsis therapy.

Keywords

microcirculation; sepsis; severe sepsis; septic shock; resuscitation; endothelium; nitric oxide

INTRODUCTION

Sepsis is a common and devastating disease that is responsible for 215,000 deaths annually in the United States and is the leading cause of death in critically ill patients.[1, 2] This disease is

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now recognized to be a time-sensitive emergency, as patients stand the best chance for survival when effective therapeutic interventions are delivered as early as possible.[3, 4] Early protocol-driven resuscitation (e.g. early goal-directed therapy)[4] targeting optimization of global hemodynamic parameters has been associated with the largest mortality benefit to date in sepsis randomized controlled trials.[4, 5] However, severe sepsis and septic shock still carry a high mortality rate (21–28%), even after effective interventions to optimize global (i.e. macrocirculatory) hemodynamics have been applied.[6] The persistently high mortality rate despite early aggressive resuscitation suggests a need for novel therapeutic interventions to further improve survival.

After aggressive resuscitation of the septic patient, a normal or high cardiac output is typically achieved, yet tissue perfusion can remain markedly impaired. Tissue hypoxia can persist despite achievement of normal or supranormal global oxygen delivery. Clinically, this may manifest with persistent acidosis, mottled skin, or progressive multi-organ failure. Therefore, limiting goal-directed resuscitation solely to macrocirculatory perfusion indices alone (e.g. cardiac filling pressure, mean arterial pressure, cardiac output, or mixed/central venous oxygen saturation) may not be sufficient to optimize blood flow to tissues in many patients. The microcirculation (blood vessels <100μm in diameter) is the principal site of oxygen exchange between blood and underlying tissues, and there is abundant data indicating profound disruption of the microcirculation in sepsis. Future clinical trials designed to go beyond global hemodynamic optimization and test novel therapeutic strategies to augment microvessel blood flow may contribute important new information to our understanding of optimal resuscitation in patients with sepsis.

This paper is intended as a focused review of the role of microcirculatory perturbation in the pathogenesis of sepsis, with special emphasis on the importance of nitric oxide (NO) in maintaining microcirculatory homeostasis. We review the available techniques for monitoring the microcirculation in human subjects, and important considerations for designing clinical trials of therapeutic agents to rescue the microcirculation in patients with sepsis-induced tissue hypoperfusion.

SEPSIS IS A DISORDER OF THE MICROCIRCULATION

The microcirculation is an integrated functional system that ensures tissue oxygen delivery meets cellular oxygen demand throughout the body. When this system becomes unhinged, maldistribution of blood flow and tissue hypoxia may result. Although microcirculatory dysfunction may occur to some degree in most shock states (e.g. cardiogenic shock and ischemia-reperfusion injury), microcirculatory failure appears to be a hallmark of the septic state and central to sepsis pathophysiology.

The microcirculatory unit – comprised of the arteriole, capillary bed, and post-capillary venule – is the landscape where most of the pivotal events of sepsis pathogenesis take place, including loss of vasomotor reactivity, endothelial cell injury, activation of coagulation, and disordered leukocyte trafficking (Figure 1). In rat models of cecal ligation and puncture, investigators have used intravital videomicroscopy to demonstrate that sepsis is characterized by decreased microcirculatory flow velocity, an abundance of stopped-flow microvessels, increased heterogeneity of microcirculatory flow, and low density of perfused capillaries.[7–10] As these microcirculatory flow alterations can occur in the absence of global hemodynamic derangements (e.g. absence of arterial hypotension),[8, 10–12] microcirculatory dysfunction largely reflects intrinsic events occurring in the microvessels. The ensuing microcirculatory "failure" can cause marked impairment of tissue oxygen transport resulting in tissue hypoxia (Figure 2).[7]

Role of the Endothelium in Sepsis-Induced Microcirculatory Dysfunction

The endothelium is a single layer of cells lining all blood vessels, numbering ~10^{13} cells, approximately 4,000–7,000 m^2 in an average adult. The endothelium is a highly active organ that dynamically regulates microvessel thrombosis, profibrinolysis, leukocyte adhesion/migration, microvascular tone, permeability, and blood flow in both health and disease.[13] During normal function, endothelial cells (ECs) in microcirculatory networks function as an integrated system actively autoregulating vasomotor tone and upstream microvessel recruitment via cell-cell signaling based on conditions downstream in the capillary bed,[14] and recruiting blood flow primarily via local release of vasodilators, most notably NO.[15] Endotoxemia can disrupt these cell-cell signal transduction pathways resulting in maldistribution of blood flow.[14] The endothelium also protects vascular integrity in the micrcocirculation by actively maintaining tight junctions between cells.

The endothelium contributes in fundamental ways to the hemostatic balance by expressing multiple anticoagulant and procoagulant proteins. For example, ECs express thrombomodulin, which converts protein C to its activated form. Once activated, protein C cleaves and inactivates factors Va and VIIIa. In addition, the endothelium expresses tissue factor pathway inhibitor, which blocks the extrinsic coagulation pathway; synthesizes heparan, a cofactor for antithrombin III; and releases tissue-type plasminogen activator and plasminogen activator inhibitor-1. These activities all work together to maintain the anticoagulant nature of the EC surface in the healthy state.[16]

Endothelial cells are highly responsive to changes in their extracellular milieu, and sense a myriad of biomechanical and biochemical forces. They integrate these signals and respond in ways that are usually adaptive (*endothelial activation*), but are at times maladaptive and harmful to the host (*endothelial dysfunction*). Endothelial activation also describes a phenotypic response to an inflammatory stimulus that may be mediated by cytokines [interleukin (IL)-1, IL-6, or tumor necrosis factor (TNF)-alfa] or exposure to oxidative stress,[17] either directly or indirectly via activation of nuclear factor-kappa B.[18] The endothelial activation phenotype is characterized by a pro-coagulant and pro-adhesive cell surface, dysregulation of vasomotor tone, and compromised barrier function.

The endothelial contribution to the procoagulant state in the activation phenotype is primarily related to the loss of its role in anticoagulation. There is an increase in EC tissue factor expression, decreased EC surface expression of thrombomodulin, and decreased protein C activation, the severity of which has been associated with poor outcome in sepsis.[19] Moreover, activated ECs amplify the local inflammatory response by releasing their own complement of pro-inflammatory cytokines that can propogate focal and ongoing microvascular injury in a perpetuating cycle. This injury cycle disrupts EC tight junctions, causing tissue edema that can further impair oxygen delivery to tissues.[20] Under hypoxic conditions, hypoxia inducible factor (HIF)-1 gene can be upregulated, increasing vascular endothelial growth factor (VEGF) expression, which has been associated with high severity, organ failure, and death in sepsis.[21, 22]

Activated ECs mediate leukocyte trafficking through a highly regulated multi-step adhesion cascade that involves selectin-mediated attachment and rolling (P-selectin on platelets and ECs and E-selectin on endothelium), and cell adhesion molecule-dependent firm adhesion to the endothelial surface mediated by intercellular cell adhesion molecule (ICAM)-1 and vascular cell adhesion molecule (VCAM)-1. The pro-adhesive activated endothelial phenotype is compounded by sepsis-induced changes in circulating cells, comprising not only leukocyte activation, but changes in red blood cells (RBCs) including impaired deformability causing increased viscosity,[23, 24] aggregation, and adherence.[25] The end result of EC activation/dysfunction in sepsis is a multifaceted disruption of microcirculatory homeostasis. If

uncorrected, this disruption may impair oxygen transport culminating in cellular hypoxia, acute organ dysfunction, and death.[26]

The Centrality of Nitric Oxide in Regulating Microcirculatory Homeostasis

Nitric oxide plays a pivotal and multifaceted role in the complex pathophysiology of sepsis. In the healthy state and under pathologic conditions, NO maintains microcirculatory homeostasis by regulating microvascular tone, leukocyte adhesion, platelet aggregation, microthrombi formation, and microvascular permeability.[27–34] When the microvasculature sustains an insult (e.g. sepsis) the NO molecule becomes vital to maintaining microcirculatory patency, integrity and function.

Although the sepsis pro-inflammatory response triggers a sharp increase in systemic NO production,[35] the upregulation of inducible nitric oxide synthase (iNOS) is heterogeneously expressed between and within organ systems [35, 36] and NO can be consumed by reactive oxygen species, giving the potential for localized areas of relative NO deficiency in microvascular beds despite a state of total body NO "excess".[36, 37] This can be a major factor in the heterogeneity of tissue perfusion that characterizes both experimental and human sepsis,[8, 10, 38] and may also help explain pathologic microcirculatory shunting in sepsis – the diversion of blood flow away from distressed microvascular units via opening of arteriovenous shunts within capillary beds.[39]

Clinical Investigations of the Microcirculation in Sepsis

Although clinical investigations in sepsis cardiovascular support have traditionally focused on macrocirculatory hemodynamics (i.e. heart and large arteries) that reflect the distribution of blood flow globally throughout the body, a functional microcirculation is a critical component of the cardiovascular system that is necessary for effective blood flow to tissues. This conceptual framework in the context of shock and resuscitation is shown in Figure 3. With the advent of new in vivo videomicroscopy techniques, it is now possible to visualize the microcirculation in human subjects. Although a shift of research focus from global hemodynamic parameters to indices of microvessel perfusion could potentially be viewed as a major departure for the clinical research mission, the microcirculation may actually prove to be a logical next frontier in understanding the full scope of circulatory failure in sepsis.

In septic patients, microcirculatory failure appears to be a major perturbation with prognostic significance.[11, 12, 38] Severe derangements of microcirculatory flow, including the severity of initial derangements in the early resuscitation phase of therapy as well as the persistence of microcirculatory derangements over time, have been associated with lower survival.[11, 12, 38] Impairment of microcirculatory blood flow may be an early triggering event in the development of sepsis-induced multi-organ failure,[12, 40–42] which is known to be a critical (and early) determinant of sepsis mortality.[43, 44] A lack of improvement in microcirculatory flow indices early in the ICU course has been associated with multi-organ failure, suggesting that the capacity to impact outcome via restoration of microcirculatory flow may be time-sensitive.[12] Table 1 summarizes recent published clinical investigations that used in vivo videomicroscopy to study the association between microcirculatory flow impairment and outcome in patients with sepsis.

TRANSLATIONAL VIDEOMICROSCOPY TECHNIQUES FOR ASSESSING THE MICROCIRCULATION IN HUMAN SUBJECTS

Intravital videomicroscopy in animal models has historically required a tissue dissection; however, new minimally-invasive videomicroscopy techniques permit direct visualization of the microcirculatory network beneath thin mucosal surfaces using a hand-held instrument

[Orthogonal Polarization Spectral (OPS) or Sidestream Dark Field (SDF) imaging], making microcirculatory assessment possible in human subjects (Figure 4). These techniques have been validated in experimental models and human subjects.[45–49]

The sublingual site has emerged as the preferred site for microcirculatory assessment in human subjects with overt or impending shock. Beginning with the work of Weil and coworkers, numerous investigators have demonstrated that impaired sublingual perfusion can track impairment of splanchnic perfusion and can detect early systemic perfusion failure in shock states.[50–53] Monitoring sublingual blood flow can yield important information for use in clinical studies of circulatory shock because ([1]) the sublingual mucosa shares the same embryologic (and therefore anatomic) origin as the splanchnic mucosa, ([2]) derangements in sublingual perfusion can reflect derangements in splanchnic blood flow,[50, 51, 53–56] and ([3]) the sublingual space is easily accessible. Tracking splanchnic hypoperfusion can be clinically important because it is one of the earliest indicators of systemic hypoperfusion in circulatory shock;[57–59] therefore, impaired sublingual blood flow may herald the onset of systemic hypoperfusion.[52, 53] Although some discrepancy between sublingual and gut microvascular perfusion has been reported,[60] clinical data have found sublingual flow to be independently prognostic.[11, 12, 38]

Details of our technique for sublingual image acquisition, processing, and analysis appear in an ONLINE DATA SUPPLEMENT **[E1]**. Our methodology is consistent with the recently published proceedings of a consensus conference on microcirculatory image analysis that was intended to help standardize analysis techniques among different groups of investigators.[61] The consensus recommendations advocate the calculation of multiple microcirculatory indices including a semi-quantitative flow velocity index, the proportion of perfused vessels, perfused vessel density, and the heterogeneity of flow between different sublingual sites.[61]

POTENTIAL THERAPEUTIC STRATEGIES TO AUGMENT MICROCIRCULATORY FLOW IN SEPSIS RESUSCITATION

Therapeutic approaches to counteract microcirculatory failure could represent a novel strategy to help optimize tissue perfusion in sepsis resuscitation. An ideal agent to recruit the microcirculation in sepsis would most likely be either: (a) an endothelium modulator, (b) a vasodilator to “open” low-flow microcirculatory units, or (c) both. The concept of using pharmacotherapy to augment microcirculatory flow in critically ill patients originated in the 1980s with clinical trials of agents with vasodilatory properties (prostacyclin) or combined inotropic/vasodilatory properties (dobutamine).[62, 63] These studies demonstrated increased systemic oxygen consumption (VO_2) with drug administration, suggesting that successful microcirculatory recruitment had occurred. Recently, De Backer *et al* used OPS imaging in two studies of the effects of dobutamine and recombinant human activated protein C (rhAPC) on the microcirculation in septic patients.[64, 65] Both agents were associated with increases in capillary perfusion independent of systemic hemodynamic effects, presumably via microvessel vasodilatory properties or rheologic effects in the case of dobutamine, and via modulation of leukocyte-endothelial cell interactions for rhAPC.[64–67] Additional agents that may hold promise include anticoagulants (e.g. antithrombin III), other vasodilators (e.g. pentoxifylline), and antioxidants that may attenuate oxidative stress-induced endothelial activation and/or increase NO bioavailability by reducing NO consumption by reactive oxygen species (e.g. parenteral ascorbate, N-Acetyl-L-cysteine).[68–70]

The history of investigating NO modulation in sepsis has been largely driven by the hypothesis that NO is deleterious. The thought that NO is harmful in sepsis comes from its consequences on the macrocirculation (arterial hypotension), disregarding its possible beneficial effects on the microcirculation. Indeed, iNOS upregulation and the concomitant NO-induced relaxation

of microvascular tone can produce arterial hypotension.[71–74] However, although NOS inhibition is clearly effective at raising arterial pressure in sepsis,[75–79] it can simultaneously worsen the impairment of microcirculatory perfusion and oxygen transport to tissues.[28, 32, 80–88] Blocking NO production in sepsis worsens leukocyte adhesion,[89, 90] platelet aggregation and microthrombosis,[28, 32] and microvascular permeability,[34, 82] causing decreased splanchnic[28, 83–88] and myocardial[81] blood flow and defects in tissue oxygenation that do not recover with fluid resuscitation alone.[80] Although iNOS-deficient septic animals have less severe arteriolar hyporesponsiveness to adrenergic agents,[73] they have increased endothelial adhesion molecule expression and leukocyte-endothelial interactions.[91–93] Due to the dichotomy of macrocirculatory and microcirculatory effects, the concept of NOS inhibition in sepsis has been considered a "double-edged sword".[94] In human subjects, a phase III randomized controlled trial of nonspecific NOS inhibition was stopped early because of increased mortality in the NOS inhibition group.[95]

As NO preserves microcirculatory patency and function, upregulation of NO may be adaptive (rather than maladaptive) and in fact *protective* in sepsis. Taking a contrarian approach to studying NO modulation in septic patients, administration of exogenous NO could potentially improve tissue perfusion indices. Nitric oxide is especially attractive as a candidate therapy to treat microcirculatory dysfunction in sepsis because it could in theory recruit microcirculatory flow by two potential mechanisms of action – modulation of leukocyte-endothelial interactions and microvessel vasodilation – simultaneously. In experimental models, administration of a NO-donor decreased endothelial adhesion molecule expression and leukocyte adhesion,[96] augmented splanchnic microcirculatory blood flow,[97, 98] and optimized tissue oxygen transport.[99, 100] In two clinical studies of sepsis patients utilizing OPS imaging, the sepsis-induced impairment of sublingual microcirculatory blood flow was reversed with ([1]) topical administration of acetylcholine (suggesting that the endothelium was still NO-responsive)[11], and ([2]) intravenous nitroglycerin (an NO donor).[101] Because administration of intravenous nitroglycerin could cause or exacerbate a drop in arterial pressure in septic patients,[102] a clinical trial of exogenous NO administration to human subjects with sepsis would require an agent that would not be expected to induce or exacerbate arterial hypotension – e.g. inhaled nitric oxide (INO).[103, 104]

Although the classical view of NO metabolism assumed that the bioavailability of INO was limited to the lung due to rapid binding to heme iron, it is now recognized that the inhaled route can deliver NO to the systemic circulation and exert extrapulmonary effects via two mechanism: formation of nitrite and/or S-nitrosothiol (SNO).[105] Under hypoxic conditions, RBCs can convert circulating nitrite to NO and release SNO bioavailability. As such, RBCs are capable of dilating microvessels and regulating of blood flow.[106, 107] In human subjects, administration of INO effectively delivered SNO to extrapulmonary vascular beds and dilated the peripheral microvasculature.[108–111] In studies of microcirculatory impairment induced by NOS inhibition, INO attenuated mesenteric vasoconstriction and leukocyte adhesion in experimental models and reversed distal extremity vasoconstriction in human subjects,[112–115] indicating that INO administration can generate circulating molecules with NO-carrying capacity, exert distant (i.e. extrapulmonary) effects, and help maintain microvascular homeostasis. These data support the concept that INO could be a novel treatment for a disease characterized by systemic endothelial dysfunction. In previous studies (Table 2) INO improved microcirculatory homeostasis by multiple separate and distinct effects.

It is notable that, although three randomized controlled trials of INO failed to improve outcome in patients with acute respiratory distress syndrome (ARDS),[104, 131] 132 only 4% of 742 total subjects had sepsis-associated ARDS. Therefore, the efficacy of INO in treating patients with sepsis has not yet been adequately tested.

CHALLENGES

There are important challenges (and limitations) to studying the microcirculation in critically ill patients. The main challenges to overcome in development of analysis techniques are the need for a fully quantitative (rather than semi-quantitative) measurement of microcirculatory blood flow velocity, and automated image analysis that can provide real-time readout at the bedside. Ideally, one software-based image analysis solution would satisfy both of these needs, and this represents a bioengineering opportunity. As OPS/SDF microcirculatory imaging requires focused training to become proficient in the technique, a broader challenge is that it remains unclear whether the technique can effectively translate to widespread use in critically ill patients, outside the confines of dedicated laboratories, or if its use will be limited to use only by experienced operators and investigators. If efficacy of microcirculation-directed therapies is demonstrated by dedicated labs in clinical trials, this question will be of paramount importance in subsequent trials testing effectiveness of these therapies when applied broadly.

The most important limitation of studying microcirculatory dysfunction in general may be that circulatory failure only represents one of multiple complex mechanisms leading to cellular dysfunction in shock. Mechanisms at a cellular level such as mitochondrial failure (i.e. “cytopathic hypoxia”) and apoptosis are pivotal factors in the development of sepsis-associated cell death and organ failure.[133–137] Although evaluation of the microcirculation might provide information on oxygen delivery to tissues that is not available from macrocirculatory parameters, imaging alone does not provide information on oxygen utilization by the cells. However, some authors have suggested that abnormalities at a cellular level are a late adaptive response that may be preceded (or perhaps triggered) by circulatory failure.[138]

FUTURE DIRECTIONS: IMPORTANT CONSIDERATIONS FOR CLINICAL TRIAL DESIGN

Going forward, it will be imperative to ascertain whether or not (or to what extent) the effects of microcirculation-directed therapies are clinically meaningful. This will be best addressed in a randomized controlled trial (RCT) design employing both microcirculatory and patient-oriented outcome measures. Choosing the optimal patient-oriented outcome measure in this context is vital.[139] Although 28-day mortality is the typical outcome measure selected for sepsis randomized controlled trials, it captures no information on the biologic or physiologic activity of an intervention, or its capacity to modulate disease processes,[139] and potentially could be confounded by a number of factors that are non-physiologic, not least of which, for example, may be family preferences for limitations of support later in the hospital course.

Alternatively, indices of acute organ system dysfunction (e.g. Sequential Organ Failure Assessment [SOFA] scores[140, 141]) are measures of morbidity that provide important serial assessments of physiology and response to treatment. Acute multi-organ dysfunction is a critical event in sepsis pathogenesis that is closely linked with survival.[1, 44, 142] Early evidence of organ failure and early changes in organ function are especially strong mortality predictors,[43, 44, 140, 141] whereas later changes in organ function have little predictive value.[43] Serial SOFA scores, therefore, can be a dynamic index of disease progression and response to a novel therapy.[139, 143]

Table 3 is a framework that could be employed in designing clinical trials of new interventions to augment microcirculatory flow and reduce organ failure in sepsis. Using this framework, all of the possible study outcomes could yield important new information about the pathogenesis and treatment of sepsis.

Overcoming heterogeneity among subjects in sepsis clinical trials

Historically, a plethora of clinical trials of novel agents for sepsis failed to demonstrate a benefit; this may be due (in part) to enrollment of highly heterogeneous populations of patients with nonspecific sepsis syndromes.[144] As sepsis is characterized by activation of a multitude of different pathophysiologic pathways that are heterogeneously expressed, the capacity to respond to a novel agent may be a function of the degree of abnormal expression of a specific pathophysiologic mechanism at which the novel therapy is aimed (or some other subclinical phenotype that is a determinant of response to therapy). Failing to screen for expression of these factors prior to the decision to randomize may accrue a large volume of subjects in the sample with little or no capacity to respond to the new therapy, causing the clinical trial to be underpowered to show a treatment effect. For example, for a randomized clinical trial of a novel microcirculation-directed therapy in sepsis, having even a small percentage of subjects in the sample with no (or minimal) microcirculatory flow impairment could cause the trial to be underpowered. Therefore, screening sepsis subjects at the bedside for the presence or absence of microcirculatory dysfunction and limiting randomization only to those who manifest significant microcirculatory impairment despite aggressive conventional resuscitation should yield a more homogeneous sample and maximize the number of potential "responders" in the trial (Figure 5). This type of tailored clinical trial design could be considered analogous to a "personalized medicine" approach (i.e. driven by the phenotype of an individual patient). Another important consideration for clinical trials is the potential for genetic heterogeneity in response to microcirculation-directed agents.

Defining the control group interventions

Subjects in both control and treatment arms of RCTs of microcirculation-directed therapies should receive early protocol-directed hemodynamic optimization with standard interventions (i.e. intravenous fluids, vasopressors, etc.) targeting pre-defined quantitative resuscitation goals, in order to help ensure homogeneity in the adequacy of conventional resuscitation and normalization of global hemodynamic parameters. This homogeneity in macrocirculatory indices would be necessary in order for investigators to isolate (and better test hypotheses about) the impact of novel therapies on the microcirculation and determine the microcirculation-specific treatment effects (Figure 5).

Timing of microcirculation-directed therapies

The concept of using the resuscitation phase of therapy to investigate microcirculation-directed therapies in sepsis is based on the understanding that timing of interventions for circulatory optimization is a critical determinant of the capacity to impact outcome. On the continuum of sepsis treatment, early phase and late phase sepsis appear to be physiologically different because earlier interventions to optimize hemodynamics have been shown to be beneficial,[4, 5, 145–147] whereas later interventions have not.[146, 148, 149] Furthermore, organ failure in early sepsis is thought to be perfusion-mediated to a greater extent than the organ failure associated with late-phase sepsis, which may relate more closely to mitochondrial failure.[134, 135, 138] Therefore, for future clinical trials of microcirculation-directed interventions, the resuscitation phase of therapy appears to be the greatest window of opportunity for demonstrating a treatment effect.

SUMMARY

Microcirculatory dysfunction is a pivotal event in the development of sepsis, and a critical component of sepsis-induced circulatory failure. Although there are still important challenges to overcome for translation of microcirculation imaging techniques to practice, obtaining microcirculatory perfusion indices may yield physiologic information that macrocirculatory indices cannot. Novel agents to "rescue" the microcirculation may prove to be a cutting-edge

strategy to optimize tissue perfusion in sepsis resuscitation. As the NO molecule is vital to microcirculatory homeostasis, it appears to protect microcirculatory patency when the microcirculation sustains the septic insult. Exogenous NO administration may improve microcirculatory perfusion in sepsis, and we submit that there is sufficient scientific rationale and safety data for a clinical trial of exogenous INO administration in sepsis. In designing clinical trials to find novel microcirculation-directed therapies, the resuscitation phase of therapy appears to be the best window of opportunity for impact. Ultimately, the aim for this line of clinical investigation would be to give clinicians a novel intervention in their armamentarium to optimize tissue perfusion in the acute-phase management of sepsis.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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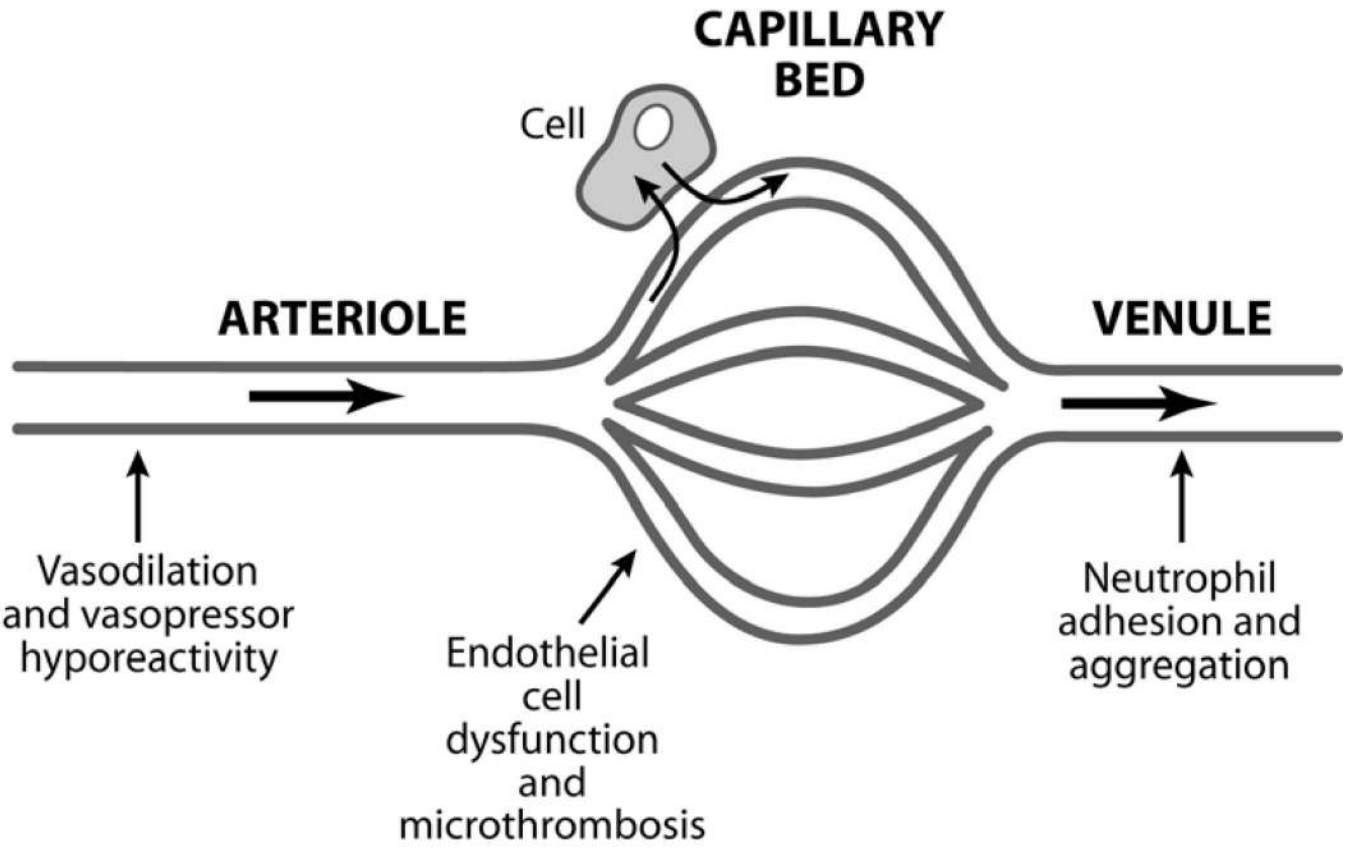

Figure 1.
Sepsis is a disorder of the microcirculation. Much of the pathophysiology of sepsis can be explained within the microcirculatory unit – the terminal arteriole, capillary bed, and the post-capillary venule. The arteriole is where the characteristic vasodilation and vasopressor hyporesponsiveness of sepsis occurs. The capillary bed is where the effects of endothelial cell activation/dysfunction are most pronounced and microvascular thromboses are formed. The post-capillary venule is where leukocyte trafficking is most disordered – leukocytes adhere to the vessel wall, aggregate, and further impair flow through the microcirculation.

Figure 2a

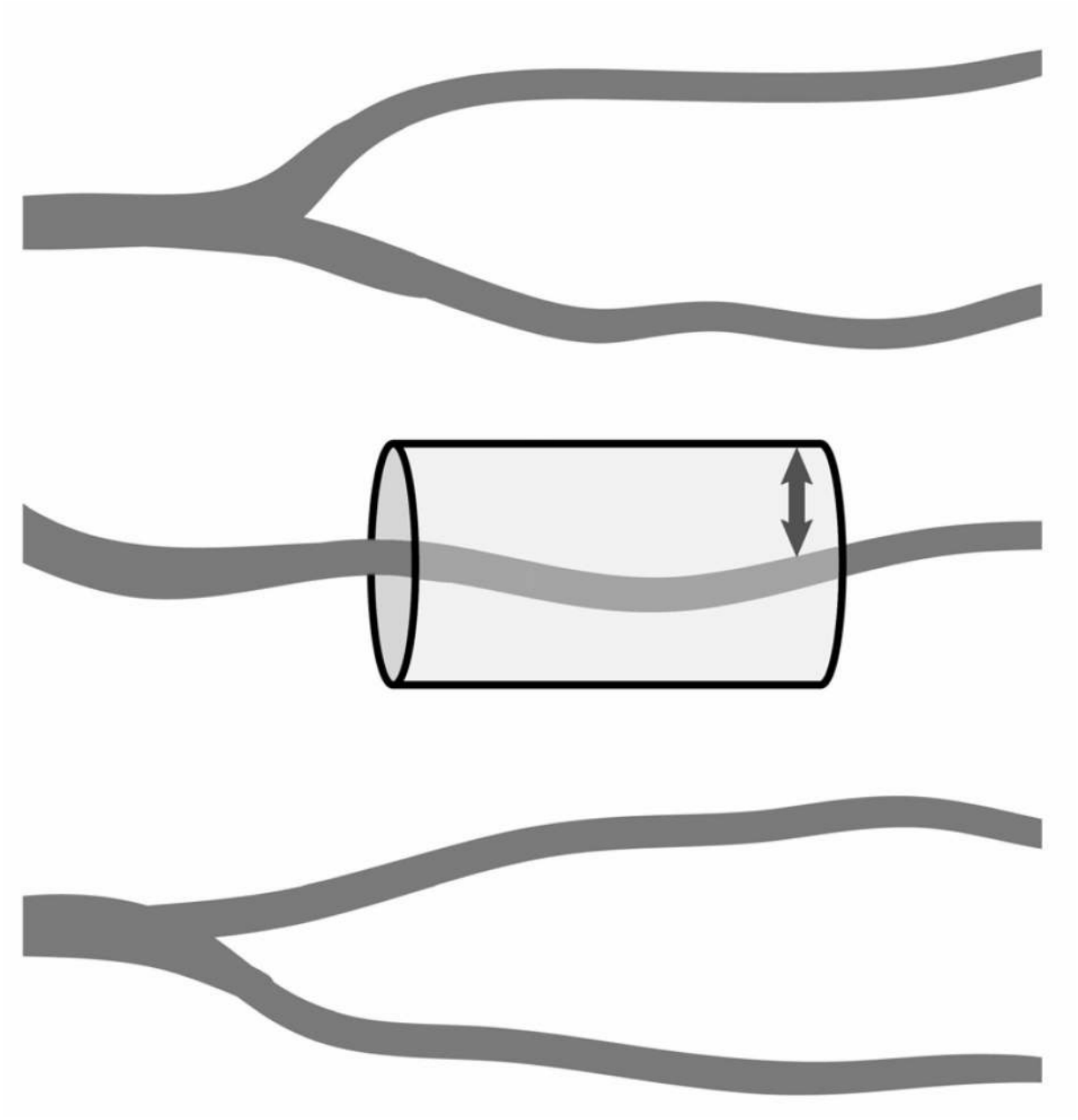

Figure 2b

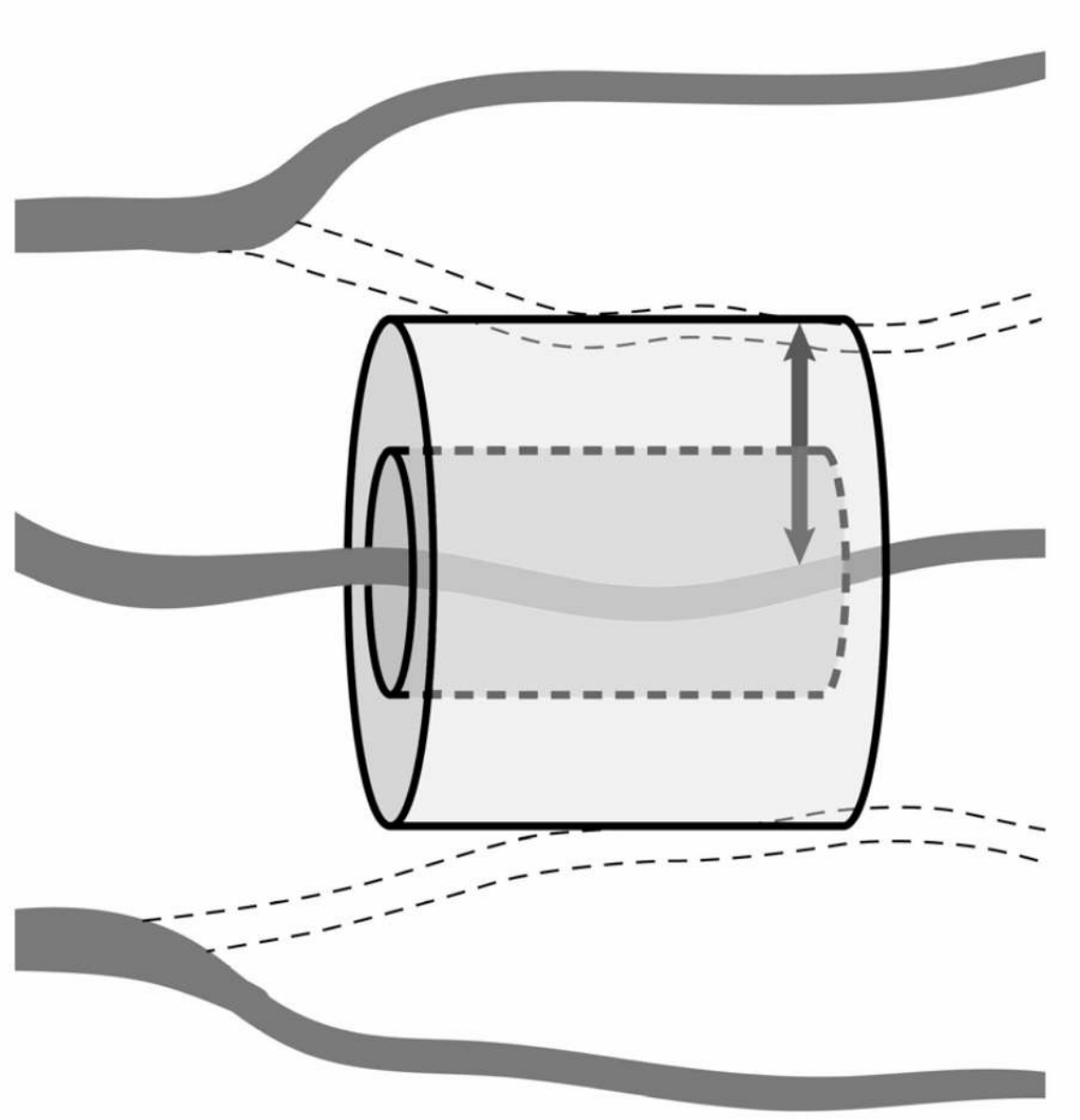

Figure 2A and 2B.

A conceptual model of oxygen diffusion from capillaries. These figures illustrate how sepsis-induced microcirculatory dysfunction can play a key role in the impairment of tissue oxygen transport and contribute to tissue hypoxia. **(2A)***Healthy state*: A cylinder represents the area of tissue that is supplied with oxygen by an individual capillary. The diffusion distance for oxygen in the tissues is shown (small arrow). **(2B)***Sepsis*: Intrinsic microcirculatory dysfunction results in non-perfused capillaries (dotted line vessels). This decreases the density of perfused vessels, increasing the diffusion distance for oxygen in the tissues (large arrow).

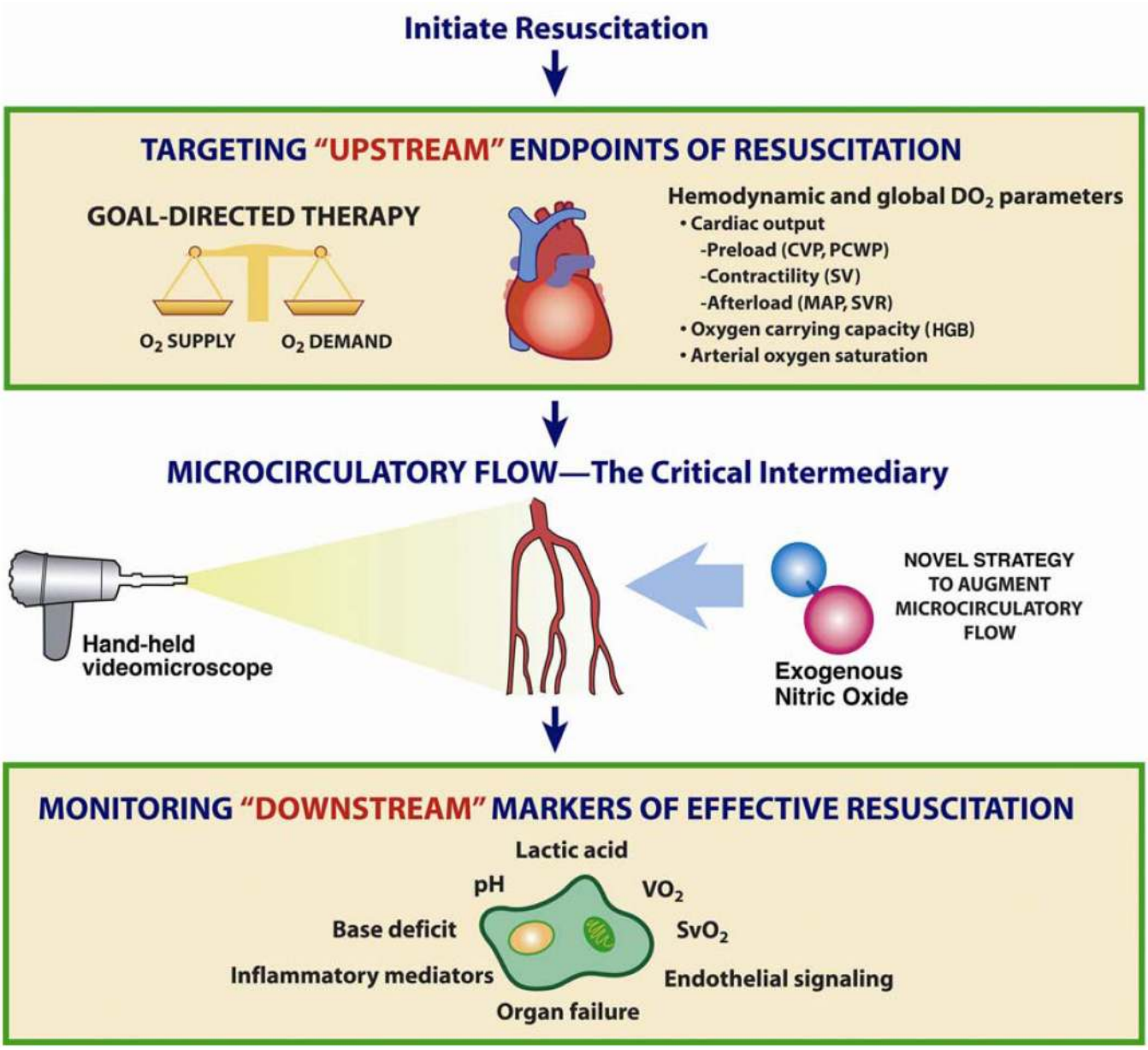

Figure 3.

Conceptual framework of the importance of the microcirculation in septic shock and resuscitation. Conventional resuscitation targets optimization of "upstream" (i.e. macrocirculatory) hemodynamic parameters (e.g. mean arterial pressure, cardiac output), with monitoring of "downstream" markers of tissue perfusion (e.g. acidosis, organ function) to determine the effectiveness of resuscitation efforts. The microcirculation represents a critical intermediary. Although the macrocirculation circulates blood throughout the body, an intact and functional microcirculation is necessary for effective blood flow to tissues. Therefore, intrinsic microcirculatory failure may contribute to sepsis-associated tissue hypoperfusion. Sublingual microcirculatory blood flow can now be visualized directly in sepsis clinical research using a hand-held videomicroscope (shown on left). In this paper, we present a scientific rationale for a clinical trial of a novel agent (e.g. exogenous nitric oxide administration, shown on right) to reduce microcirculatory dysfunction and augment

microcirculatory blood flow in sepsis resuscitation. [CVP = central venous pressure; PCWP = pulmonary capillary wedge pressure; SV = stroke volume; MAP = mean arterial pressure; SVR = systemic vascular resistance; HGB = hemoglobin; VO_2 = oxygen consumption; SvO_2 = mixed venous oxygen saturation] Adapted from: Trzeciak S, Dellinger RP, Parrillo JE, Septic Shock, In: Parrillo JE and Dellinger RP (3rd Edition) Critical Care Medicine: Principles of Diagnosis and Management in the Adult. (2008) Philadelphia, PA: Mosby Elsevier.

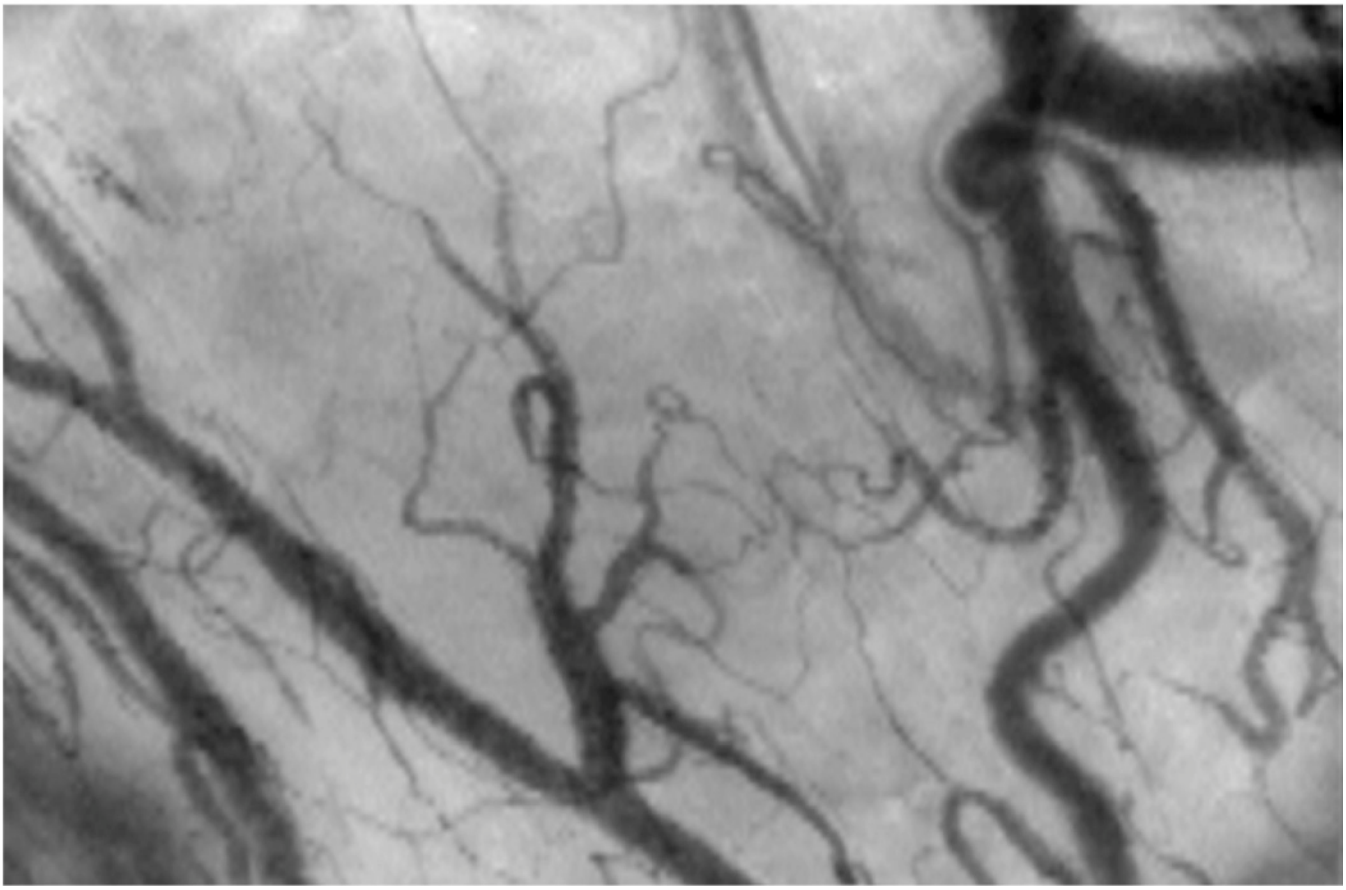

Figure 4.
A still image of the human sublingual microcirculation as visualized with Orthogonal Polarization Spectral (OPS) videomicroscopy. The videomicroscope uses a 5X objective (167X magnification) giving a 940 × 1259 μm field of view. Real-time video of healthy and dysfunctional microcirculation is available for viewing or download at: http://www.cooperhealth.org/content/gme_fellowship_shock.htm

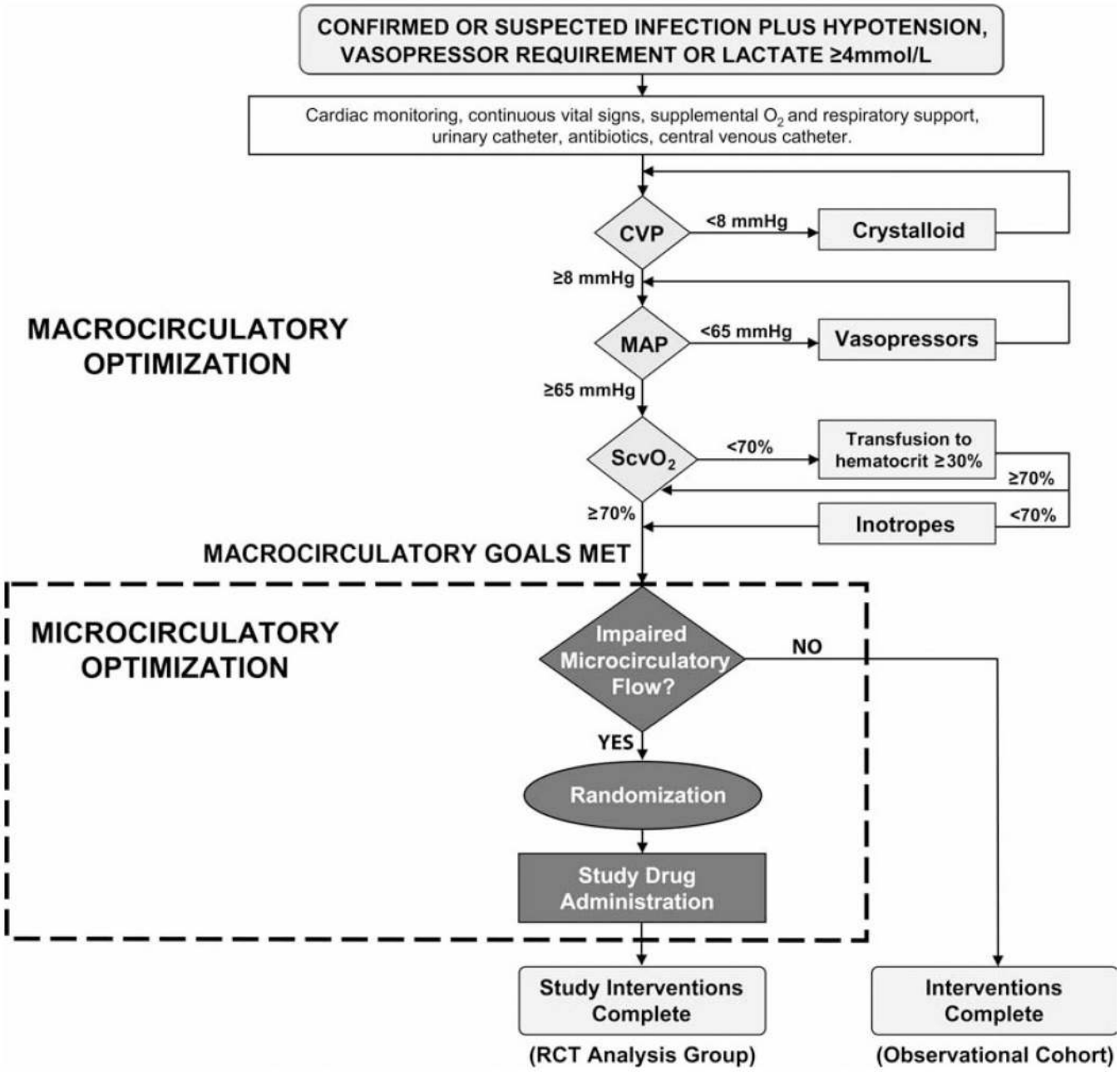

Figure 5.

A template for designing a randomized clinical trial of a novel agent to augment microcirculatory flow and improve outcome in sepsis resuscitation. Microcirculatory flow would be assessed with in vivo videomicroscopy at the bedside. Although clinically speaking, an agent that improves microcirculatory flow might optimally be initiated immediately at the time of severe sepsis identification, a requisite for this type of clinical trial would be early achievement of homogeneity in macrocirculatory hemodynamic optimization (e.g. early goal-directed therapy as per Rivers *et al* [4] or a similar resuscitation algorithm) in both the control and treatment subjects, in order to permit precise determination of the treatment effect of microcirculatory optimization on outcome. Because enrolling patients who do not manifest the microcirculatory dysfunction phenotype could cause the clinical trial to be underpowered to

show a treatment effect, we advocate a "personalized" trial design employing a real-time assessment of microcirculatory flow prior to the decision to randomize (as shown). [CVP = central venous pressure; MAP = mean arterial pressure; $ScvO_2$ = central venous oxygen saturation; RCT = randomized controlled trial]

Table 1

Published manuscripts that used in vivo videomicroscopy to examine the association between microcirculatory perfusion indices and clinical outcome in patients with severe sepsis and/or septic shock. [ED = Emergency Department; ICU = Intensive Care Unit]

AUTHORS	COUNTRY	YEAR	n	SUBJECTS	SETTING	PRIMARY OUTCOME MEASURE(S)	MAIN FINDINGS
De Backer *et al* [11]	Belgium	2002	50	Severe sepsis	ICU	Mortality	Microcirculatory perfusion impairment was more severe in sepsis non-survivors compared to survivors, independent of global hemodynamics.
Sakr *et al* [12]	Belgium	2004	46	Septic shock	ICU	Multi-organ failure and mortality	With longitudinal (daily) measurements of microcirculatory perfusion in sepsis patients, failure to improve microcirculatory perfusion was strongly associated with the development of multi-organ failure and death.
Trzeciak *et al* [38]	USA	2007	26	Severe sepsis and septic shock	ED and ICU	Mortality	During the early resuscitation phase of sepsis therapy, microcirculatory flow velocity was more severely impaired and more heterogeneous in patients that ultimately did not survive, compared to survivors.

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Table 2

Mechanisms by which inhaled nitric oxide (INO) could potentially attenuate microcirculatory dysfunction and improve microcirculatory homeostasis in shock states.

MECHANISMS	SUPPORTING EVIDENCE
Modulation of microvascular tone	In experimental models of ischemia/reperfusion injury, INO restored flow to ischemic myocardium[116,117] and raised renal blood flow and glomerular filtration.[118]
Preservation of microvascular integrity	In sepsis and endotoxemia models, INO attenuated neutrophil transmigration across the local (pulmonary) endothelial barrier[119,120] as well as the influx of inflammatory cells into systemic (extrapulmonary) organs.[121] In ischemia/reperfusion models, INO decreased microvascular injury and neutrophil transmigration, decreasing the tissue inflammatory reaction.[112,122,123]
Endothelial-dependent effects	In models of endotoxemia, ischemia/reperfusion, and oxidative endothelial activation, INO demonstrated an anti-adhesive effect on distant (mesenteric) inflamed microvasculature by attenuating endothelial dysfunction and leukocyte adhesion with an increase in microcirculatory flow.[112–114,121124] In patients with acute respiratory distress syndrome, INO decreased endothelial adhesion molecule expression, platelet aggregation, and fibrinogen binding.[125]
Leukocyte-dependent effects	INO may exert an indirect effect on the microcirculation by "pacifying" leukocytes in transit through the pulmonary circulation.[126] In experimental models, INO attenuated the oxidative burst from activated neutrophils,[119,120] prevented neutrophil-mediated, oxygen-radical dependent endothelial damage,[127] and reduced neutrophil adhesion and sequestration into tissues by inhibiting integrin-mediated firm adhesion to the endothelium.[126,128]
Direct anti-inflammatory effect	INO can decrease the amount of NF-kappa B available for binding to the regulatory region of genes that produce pro-inflammatory cytokines.[129130]

Table 3

A framework of possible outcomes for randomized controlled trials of novel interventions to augment microcirculatory flow and attenuate organ failure in sepsis. Using this framework for trial design would not only test the ability of a new intervention to (A) augment microcirculatory flow and (B) reduce sepsis-associated organ failure, but also could help test the hypothesis that microcirculatory perfusion is a key determinant of organ failure in patients with sepsis. Because it is also necessary to ascertain whether or not short-term administration of a therapy has sustainable benefit over the long-term, serial organ failure assessments well beyond 24 hours should also be incorporated.

HYPOTHESIS A: **Intervention *X* improves microcirculatory blood flow in sepsis resuscitation**	**HYPOTHESIS B:** **Intervention *X* during sepsis resuscitation reduces organ failure at 24 hours.**	**RESULTS INTERPRETATION**
YES	YES	Intervention *X* during sepsis resuscitation improves microcirculatory perfusion and reduces organ failure. This supports the concept that organ failure in sepsis can be a perfusion-mediated phenomenon.
YES	NO	Intervention *X* improves microcirculatory perfusion in sepsis resuscitation; however, organ failure in early sepsis may not be a perfusion-mediated phenomenon.
NO	YES	Intervention *X* improves organ failure is sepsis but not through a perfusion-mediated mechanism. Possible explanations for the beneficial effects of intervention *X* on organ failure could be modulation of mitochondrial respiration or cellular apoptosis.
NO	NO	Intervention *X* does not improve microcirculatory perfusion and is not beneficial in sepsis. Other methods of resuscitating the microcirculation should be investigated.