Microcirculatory dysfunction and tissue oxygenation in critical illness

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The authors declare that they have no conflicts of interest.

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Abstract
Severe sepsis is defined by organ failure, often of the kidneys, heart, and brain. It has been proposed that inadequate delivery of oxygen, or insufficient extraction of oxygen in tissue, may explain organ failure. Despite adequate maintenance of systemic oxygen delivery in septic patients, their morbidity and mortality remain high.

The assumption that tissue oxygenation can be preserved by maintaining its blood supply follows from physiological models that only apply to tissue with uniformly perfused capillaries. In sepsis, the microcirculation is profoundly disturbed, and the blood supply of individual organs may therefore no longer reflect their access to oxygen.

We review how capillary flow patterns affect oxygen extraction efficacy in tissue, and how the regulation of tissue blood flow must be adjusted to meet the metabolic needs of the tissue as capillary flows become disturbed as observed in critical illness. Using the brain, heart, and kidney as examples, we discuss whether disturbed capillary flow patterns might explain the apparent mismatch between organ blood flow and organ function in sepsis. Finally, we discuss diagnostic means of detecting capillary flow disturbance in animal models and in critically ill patients, and address therapeutic strategies that might improve tissue oxygenation by modifying capillary flow patterns.

Editorial comment: what this article tells us
In this narrative review article, the relations of microcirculatory blood flow and oxygen delivery in vital organs during severe sepsis are explored. Disturbances in oxygen extraction efficiency are examined, together with diagnostic and therapeutic aspects in critically ill patients.
Septic patients often develop multi-organ failure (MOF) despite therapeutic maintenance of normal blood pressure and adequate blood oxygenation.\(^1\) Although their systemic circulation is characterized by high cardiac output and low peripheral resistance,\(^2\) peripheral oxygen extraction is low, and the elevated oxygen needs of tissue in septic patients can seemingly no longer be met, as they progress into severe sepsis or septic shock.\(^3\)

Apparently, MOF is not the result of insufficient blood supply to individual organs, and attempts to improve outcome by increasing oxygen delivery have yielded conflicting results.\(^4\) In the kidney, acute renal failure (ARF) in sepsis is associated with marked reductions of glomerular filtration rate and urine output. Nevertheless, renal vascular resistance is low and blood flow seemingly normal, or even elevated, in ARF,\(^5\) suggesting that an overabundance of oxygen somehow fails to support the energy demands of glomerular filtration. Myocardial depression and elevated serum levels of cardiac troponin are common in sepsis, and associated with poor outcome.\(^6,7\) These findings are difficult to reconcile with the normal or even elevated myocardial blood flow found in sepsis,\(^8,9\) Brain function is often affected very early in sepsis, so-called septic encephalopathy, and survivors often show ischemic lesions on magnetic resonance imaging (MRI), suffering severe permanent neurological sequelae.\(^10\) Despite findings of suppressed oxygen metabolism and cerebral blood flow (CBF) in sepsis patients with neurological symptoms,\(^11,12\) their CBF values remain well above the accepted threshold of cerebral ischemia.\(^13\)

Mitochondrial dysfunction is a likely contributor to the profound imbalance between oxygen availability and oxygen utilization in sepsis – see Singer for a comprehensive review.\(^14\) This review addresses functional shunting\(^15\) as another contributor, noting that tissue oxygen tension (\(P_O_2\)) is expected to be normal or elevated in mitochondrial dysfunction, but low if oxygenated blood is shunted through the microvasculature. Microvascular shunting can occur either by anatomical arterio-venular shunts, by rapid oxygen diffusion from arterioles to veins,\(^16\) or by a pathological redistribution of capillary flows such that some cells are deprived of oxygen while some capillaries are perfused in excess of what can be extracted by the tissue.\(^15,17\) Sepsis is indeed linked to profound, generalized changes in the tissue microcirculation,\(^18\) including reductions in the proportion of perfused capillaries, and the appearance of intermittent or sluggish passage of erythrocytes through some capillary paths.\(^19\)

Using the brain, heart, and kidney as examples, we discuss whether pathological redistribution of capillary flow patterns may suffice to explain the apparent mismatch between tissue blood flow and organ function in sepsis. Finally, we discuss diagnostic means of detecting capillary flow disturbance in animal models and in the critically ill patient, and therapeutic strategies to improve tissue oxygenation by improving capillary flow patterns.

### Tissue blood flow, capillary blood flow patterns, and oxygen availability

The relation between tissue blood supply (ml blood per 100 ml tissue per minute) and tissue oxygenation – defined as the maximum metabolic rate of oxygen (ml oxygen per 100 ml tissue per minute) that can be supported by this blood flow – is not linear per se as one might expect. Rather, studies of solute extraction from individual capillaries show that oxygen extraction is less effective at high flow rates because erythrocytes pass through the microvasculature at transit times that are too short to permit complete extraction of their oxygen load – so-called functional shunting. The text-book flow-diffusion equation\(^20\) uses these single-capillary properties to predict the relation between tissue blood supply and its oxygen availability. While the relation is not linear for the reasons stated above, it has two fundamental properties: (i) there is a one-to-one relation between tissue blood supply and tissue oxygenation, and (ii) increase tissue blood supply always improves tissue oxygenation. As illustrated by Fig. 1, these widely held assumptions are only true if all tissue capillaries have identical flow velocities. In the normal tissue, however, capillary flow patterns are highly heterogeneous during rest\(^21-23\) but redistribute to a more homogenous pattern when the flux of erythrocytes...
increases, permitting a more efficient oxygen extraction during hyperemia than one would expect based on the flow-diffusion equation. Failure of this intrinsic capillary flow homogenization during hyperemia can have dramatic consequences, as shown in Fig. 1D: The resulting increase in functional shunting can cause tissue oxygenation to decrease when blood supply...
increases. Conversely, a reduction in blood flow can, paradoxically, improve tissue oxygenation if accompanied by a homogenization of capillary flow patterns.

The oxygenation effects of capillary flow patterns illustrated above underscores the importance of blood rheology and capillary patency in sepsis when considering whether a certain blood supply optimizes organ oxygenation. Such functional shunting effects might indeed add to the mismatch between tissue blood flow and organ function in critical illness.

We extended the classical flow-diffusion equation to take the biophysical effects of capillary transit time heterogeneity (CTH) into account and refer the reader to Jespersen and Østergaard and Østergaard et al. for details on the model and its application to brain and heart. To address renal oxygenation in this report, we extended our model to renal cortex and medulla – see Fig. 2 for details and model parameters.

Matching blood flow to the metabolic needs of kidney, heart, and brain during capillary dysfunction

The combined effect of capillary flow disturbances (elevated CTH), tissue blood flow (TBF), and tissue oxygen tension ($P_{O_2}$), are shown in Fig. 3 for the renal cortex and medulla, the heart, and the brain. The green surface in each plot surrounds combinations of MTT, CTH, and $P_{O_2}$ that, biophysically, can support the metabolic needs of these organs in the resting, awake state. The red plane marks the boundary, left of which vasodilation no longer improves tissue oxygen availability – so-called malignant CTH.

All tissue types share common features: At normal tissue oxygen tension, the metabolic needs of resting tissue can no longer be met if CTH grows beyond a tissue-specific threshold, defined by the full red lines labeled ‘A’ in the figures. For higher CTH values, these metabolic needs can only be met because of parallel reductions in blood flow (to reduce functional shunting) and in $P_{O_2}$, caused by continued oxygen utilization in the tissue and more efficient oxygen extraction. Biophysically, these mechanisms can only support tissue function until $P_{O_2}$ becomes negligible. The corresponding CTH thresholds are indicated as full red lines at zero $P_{O_2}$ in Fig. 3. Note that as CTH approaches this threshold, even small changes – decreases as well as increases – in blood flow can cause critical reductions in tissue oxygenation and thereby affect tissue function.

The dotted black lines (labeled B) in Fig. 3 indicate the ‘optimal blood flow’ in terms of maintaining tissue function during critical CTH increases. Note that the highest degree of capillary flow disturbances can be sustained if blood flow is suppressed considerably compared to resting, normal values. One notable exception is the renal medulla, in which oxygen tension is already low, and oxygen extraction high, in the normal state. The maximum tolerable CTH (red solid line at $P_{O_2}$) is therefore only slightly higher than ‘physiological’ CTH values at normal tissue oxygen tension (labeled A), and sufficient oxygenation can therefore only be maintained within a narrow range of flow values once CTH increases. These properties imply that that once the function of the medulla (such as urine production) is affected by disturbances in the microcirculation (i.e., CTH becomes critically high), then subsequent maintenance of renal function is contingent on the maintenance of renal medullar perfusion within a very narrow range.

The relation between microcirculatory changes, organ function, and hypoperfusion in critical illness

The extended flow-diffusion gives rise to three important predictions with regard to hemodynamics and tissue oxygenation when the microcirculation is disturbed, e.g., in sepsis: First, increases in CTH, even in the absence of capillary occlusions, can reduce oxygen availability below the metabolic needs of the tissue and thereby cause organ failure or even tissue damage. Second, increasing CTH can be compensated for by reductions in tissue blood flow and tissue oxygen tension relative to their values in healthy tissue. Our analysis therefore suggests that attenuated blood pressure and organ perfusion, paradoxically, may facilitate the maintenance of tissue oxygenation in conditions where CTH is elevated. The development of endothelial dysfunction in human endotoxemia and endotoxin tolerance occur in parallel with dis-
turbances in their microcirculation, suggesting that vascular tone may in fact undergo adaptations during changes in CTH to maintain tissue oxygenation. If vascular tone remains intrinsically regulated to optimize tissue oxygenation in severe sepsis, then severely elevated CTH might contribute both to the loss of vasopressor responses in patients, and to MOF and death by critically reducing tissue oxygenation.

Third, the range of blood flow values that can support organ function and tissue survival gradually narrows as CTH increases. When CTH becomes critically elevated, both increases in tissue blood flow above and reductions in blood flow below the threshold shown in Fig. 3 can therefore cause organ failure and tissue injury.

Taken together, these predictions suggest that disturbances in capillary flow patterns can contribute to the development of ARF, myocardial depression, and septic encephalopathy, despite maintenance of normal blood flow values, and even capillary patency, in these organs.

The origins of capillary flow disturbances or occlusion in sepsis

Systemic inflammation in general, and sepsis in particular, have long been known to cause profound changes in capillary flow patterns due to changes in the size, number, deformability, and endothelial adhesion of blood cells. In the normal state, the endothelium serves as mechanical barrier, regulates microvascular blood flow, and inhibits inflammation and coagulation. During sepsis, the endothelium is activated by bacterial components, toxins, and inflammatory mediators. The release of inflammatory mediators induces morphological changes of the endothelium and its protective glyocalyx.

Endothelial exposure to oxidative stress, oxidized lipoproteins, and hyperglycemia cause further glyocalyx injury which in turn give rise to profound changes in capillary flows and hematocrit. The glyocalyx constitutes a fluid barrier, and degradation of the glyocalyx...
is hence associated with edema and capillary compression.37,38

The endothelial activation in relation to sepsis also triggers increased expression of several adhesion molecules that promote leukocyte rolling and adhesion, and further disturb capillary flows. Red blood cell deformability is also reduced early in the course of sepsis.39,40 Meanwhile, the normal anti-thrombotic state of the endothelium shifts toward a pro-coagulant state, as evidenced by reduced expression of anti-thrombotic factors and increased expression of tissue factors.41 Upon activation, endothelial cells and adhesion molecules are shed. The increased levels of circulating endothelial cells and adhesion molecules in septic patients may therefore reflect factors of importance to parallel changes in the capillary circulation.42–46

The control of pericyte tone remains much less studied than that of arteriolar tone.47 Oxidative stress causes irreversible capillary constriction in cerebral pericytes,48,49 while nitric oxide (NO) is obligatory for pericyte relaxation.57 NO production often require oxygen as a sub-
and the tissue hypoxia associated with sepsis are therefore likely to contribute to the development of capillary dysfunction.

Evidence of changes in capillary density and CTH from direct studies of the microcirculation in sepsis

The microcirculation in the sublingual mucosa of septic patients has been studied by orthogonal polarization spectral (OPS) imaging\(^50\) or side-stream dark-field (SDF) imaging\(^51\) at some institutions, and reported according to the standardized scores.\(^52\) Patients with sepsis show reductions in the proportion of perfused capillaries,\(^53\) and in animal models of normotensive sepsis, the density of capillaries with either intermittent or no flow is elevated, while the normal response to vasodilators is reduced.\(^54,55\)

Notably, death from MOF after septic shock is antedated by reductions in the proportion of perfused sublingual capillaries compared to survivors, in spite of identical systemic hemodynamic and oxygenation variables.\(^56\) With respect to evidence of a separate role of CTH in sepsis, disturbed capillary flow patterns are associated with poor oxygen extraction in models of endotoxemia,\(^15,17\) and early sublingual capillary flow velocities are lower and more heterogeneous in patients who die after severe sepsis or septic shock than in patients who survive.\(^37,58\) Early improvement in the microvascular flow patterns of septic patients seems to be associated with a reduced risk of subsequent MOF.\(^59\)

Therapeutic implications

The effects of capillary flow patterns on tissue oxygenation re-emphasizes the microcirculation as a potential target of therapy in critical illness, either through active control of capillary and arteriolar tone, by maintenance of appropriate blood viscosity, or by protection of the endothelium.\(^60–63\) The prevention of mitochondrial injury, however, remains a crucial goal: Pathological redistribution of capillary flows exposes some cells to oxygen in excess of their metabolic needs while others are deprived of oxygen – both of which are associated with mitochondrial reactive oxygen species production and the initiation of apoptotic pathways.\(^64\)

Our analysis shows that the oxygenation of certain organs may improve if their blood supply is lowered relative to their pre-sepsis values, whereas relative increases in organ blood supply, which may not have the desired effect. Interventional studies attempting to increase oxygen delivery to supra-normal levels have indeed not shown beneficial effects on outcomes, and in some cases even the opposite.\(^4\) Notably, not all patients and organs are predicted to tolerate relative reductions in blood flow: The renal medulla seems particularly prone to hypoxic...
injury, especially in patients with pre-existing capillary flow disturbances. Here, kidney function may already depend on blood flow values within a narrow range—whereas any sepsis-related increase in CTH or reductions in RBF are likely to elicit renal failure. A randomized controlled trial comparing a mean arterial pressure (MAP) of 80–85 mmHg to one of 65–70 mmHg in septic patients demonstrated no survival benefit for the group randomized to the higher MAP, but a lower need for renal replacement therapy in patients with prior hypertension.65 We speculate that these findings may reflect the specific vulnerability to hypoperfusion of kidney medulla in patients with pre-existing renal capillary dysfunction. The analysis thus re-emphasizes the need for early goal-directed therapy (EGDT), but suggests that subsequent evaluation of the microcirculation in individual organs may guide organ-specific, optimal macro-, and microcirculatory endpoints for therapy in individual patients.

Blood rheology affects CTH, and thereby tissue oxygenation. Phosphodiesterase (PDE) inhibitors reduce platelet aggregation,66 decrease blood viscosity,67 and increase the flexibility of erythrocytes,67 and would therefore be expected to improve tissue oxygenation in sepsis. Consistent with this prediction, the PDE inhibitor pentoxifylline normalizes the rheology of human blood exposed to endotoxin in vitro68 while improving net oxygen extraction in experimental sepsis.69

Studies of retinal pericytes suggest that these cells react to pharmacological stimuli in much the same way as smooth muscle cells (SMCs). They may constrict in response to mechanical stretch, angiotensin II (via AT1 receptors),70 and endothelin-1 (via ETA receptors),71 by a Ca2+-dependent mechanism.72 Interestingly, pre-hospital prescription of angiotensin II receptor blockers is associated with reduced sepsis-related mortality.73 Studies of the dose-dependent effects of vasopressors on both SMCs and capillary pericyte tone in various organs may help us better understand how they affect tissue oxygenation in vivo. When translating such results into humans, it should be kept in mind that age and cardiovascular risk factors such as hypertension and diabetes are associated with profound changes in capillary morphology, especially in terms of basement membrane thickening.74–76 The microcirculation of patients may therefore respond differently to vasoactive substances than that of animal models with fully functional capillaries, just as patients may tolerate less, sepsis-induced increase in CTH before organ failure or tissue damage ensues.

**Diagnostic implications**

The extended flow-diffusion equation may be useful in converting OPS- and SDF-based observations of erythrocyte velocity distributions, and the fraction of perfused capillaries, into estimates of tissue oxygenation by using tissue-specific nomograms such as those shown in Fig. 2. Knowledge of the tissue oxygen tension is necessary for the absolute quantification of metabolic deficit, in that P O2 reflects the residual oxygen reserve in terms of the blood–tissue concentration gradient. In the experimental setting, such data are available from NAD fluorescence imaging techniques,15 and may in principle be obtained by needle sensors in both animals and human.77 In brain, CTH and MTT can be measured non-invasively by monitoring the clearance of intravascular contrast agents as part of perfusion weighted MRI or CT.78 In organs with substantial leakage of MRI and CT contrast agents, contrast-enhanced ultrasound may be refined for future, bed-side assessment of tissue microcirculation.79

**Other organs and other metabolites**

It should be kept in mind that changes in capillary transit time patterns of the lung affects blood saturation in the same way as they affect blood deoxygenation in other tissues.22,80 Successful strategies for the protection of capillary wall integrity are therefore expected to improve both blood saturation and tissue oxygenation.81 Also, capillary flow disturbances cause a general reduction in the extraction of diffusible solutes,22 including tracers used when quantifying glucose metabolism82 and, we fear, tissue hypoxia.83 We analyzed the effects of capillary flow disturbance on oxygen uptake, glucose uptake, and ATP production in tumor and brain tissue,84,85 and discovered that the uptake of glucose becomes limited, yet remains favored.
over that of oxygen, as CTH increases. Further studies should therefore address whether microcirculatory disturbances contribute to the hyperglycemia, aerobic glycolysis, and lactate production observed in sepsis.

Conclusion

Capillary flow disturbances, such as those observed in sepsis, can cause oxygen availability to fall below the requirements of normal renal, heart, and brain function, although their blood supply remain normal or elevated. Paradoxically, one cannot predict whether reductions in blood flow improve oxygenation or not in organs with severely disturbed microcirculation. Non-invasive methods to quantify capillary transit time characteristics may serve as means to test these predictions, and to examine whether they can guide future strategies to optimize organ oxygenation in individualized sepsis management.

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