Focus on flow: imaging the human microcirculation in perioperative and intensive care medicine

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Chapter 1

The Microcirculation is a Vulnerable Organ in Sepsis

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The Microcirculation as a Key Organ in Septic Shock

There is now increasing evidence that the microcirculation is one of the key organisms in the pathophysiology of sepsis and septic shock [1, 2]. However, its importance does not seem to be reflected in current clinical practice. In addition, the surviving sepsis campaign, a world wide effort to decrease sepsis related mortality, focuses only minimally on the importance of the microcirculatory organ [3]. By definition, sepsis is initiated by an infectious agent and the ultimate therapeutic strategy will therefore be its removal from the body. However, the systemic hostile inflammatory response that ensues from sepsis is the real culprit of this disease. The microcirculation is severely affected by this inflammatory response. At the same time, it is responsible for maintaining or even fuelling the devastating disease process of sepsis and septic shock. Even in the face of stable systemic hemodynamics, the microcirculation may be at risk giving rise to regional dysoxia, causing multiple organ failure and ultimately death. Monitoring the microcirculation provides sensitive information on the severity of disease and the effect of therapies [4]. In addition, if sepsis is a disease of the microcirculation [5], resuscitating this organ may become as important as antibiotic therapy.

The Microcirculation as a Functional System

The microcirculation is one of the largest organs in the body and by definition comprises vessels with a diameter roughly smaller than 100 μm, i.e. arterioles, capillaries and venules, and the blood flowing in them. The entire length of the organ is lined with endothelial cells, which are surrounded by smooth muscle cells mainly in arterioles. Red blood cells (RBCs) and the various types of white blood cells (WBCs) complete the cellular picture. However, the microcirculation also embraces a large number of other components including platelets, coagulation factors, and a plethora of cytokines and chemokines [6]. Among the many different microcirculatory functions, the delivery of oxygen to tissue is paramount. This is part of the microcirculation's larger function as an exchanger of nutrients and waste products and chemical or cellular signals. Pertaining to sepsis, however, it is also important to realize the pathogenic interplay of WBCs, RBCs, endothelium, and messenger molecules in inflammation and coagulation in the microcirculation [6].

Therefore, it is not surprising that this organ is a highly regulated one. Central to coordinating microcirculatory perfusion, and hence oxygen delivery (DO_2), is the endothelium. In order to meet the oxygen requirements of the cells, the endothelium will ultimately control arteriolar smooth muscle cell tone, both directly and via neurohumoral mechanisms, resulting in altered microcirculatory perfusion. This is achieved by mechanisms such as stress and strain sensing as well as detection of oxygen and metabolic waste products [7]. Endothelium produced nitric oxide (NO) deserves special attention in this context. Apart from its role as a mediator of the inflammatory cascade, the vasodilating properties of NO are important in regulating the distribution of perfusion.

The endothelium, helped by WBCs, platelets, and messenger molecules, is also involved in the regulation of inflammation and coagulation [8]. Interestingly, RBCs are nowadays

considered to regulate perfusion by releasing vasodilators, such as NO [9] and ATP [10], when encountering oxygen deprived environments. In addition it has been shown that deoxyhemoglobin can convert nitrite to NO, causing arteriolar dilatation [11]. Thus, apart from transporting oxygen, RBCs effectively redirect flow and oxygen where it is needed.

Scientific Importance of the Microcirculation

Realization of the importance of the microcirculation is growing, although the concept of microcirculatory disturbances in sepsis is not new. For several decades now, microcirculatory alterations have been recognized as important in pathophysiology [12, 13], and given attention as potential therapeutic targets [14]. One reason why the microcirculation has become an organ of increasing interest in critical care medicine is the validation [15] and clinical introduction [16] of orthogonal polarization spectral (OPS) imaging, which has allowed direct visualization of the human microcirculation in solid organs and mucous membranes for the first time. OPS imaging has revealed the important role of microcirculatory abnormalities in patients with sepsis, confirming results from animal models [17-19]. In addition, we recently validated a scoring system for quantification of microcirculatory abnormalities in sepsis [20] and introduced side stream dark field (SDF) imaging [2, 21] as a successor to OPS imaging.

The Septic Microcirculation

In their landmark clinical study of 50 patients with severe sepsis, De Backer and colleagues showed that functional vessel density and the proportion of perfused vessels smaller than 20 μm were significantly lower than in healthy controls, non-septic patients, and post-cardiac surgery patients [17]. In addition, microvascular deterioration was more severe in non-survivors. A later study by the same group showed that septic patients who did not survive their disease showed no improvement in microvascular perfusion whereas survivors did [18].

Our group reported comparable observations of sluggish microcirculatory perfusion in a small group of septic patients. These observations also independently showed sustained flow in larger vessels confirming that shunting of the capillaries of the microcirculation is a key feature of sepsis [2, 19, 22].

These findings are important because they show that there is indeed a microvascular problem in human sepsis, which is associated with organ dysfunction and death. It also shows the importance of looking at the actual vessels. There has been some confusion in the past, where plethysmography [23], xenon dilution [24], and laser Doppler flux [25] have been used as surrogate markers for microcirculatory perfusion. While observations using these techniques have brought useful data, it should be remembered that they cannot account for any degree of microcirculatory heterogeneity, a characteristic property of sepsis. For this reasons, these techniques should be considered as indicators of regional rather than microcirculatory perfusion.

Of particular note is that the clinical picture of a disturbed microcirculation in sepsis is paralleled by the abnormalities found in various animal models using intravital microscopy and carbon injection. Observations in mice, rats, and dogs invariably show a reduction in perfused capillary density, and stopped flow next to areas of hyperdynamic blood flow, resulting in increased heterogeneity in skeletal and intestinal microvascular beds, despite normotensive conditions [26-29]. It has also been shown experimentally that hemorrhagic shock does not affect microvascular perfusion as much as endotoxic shock for the same degree of hypotension [27].

An increased heterogeneity of the microcirculation was shown to provoke areas of hypoxia and generally impaired oxygen extraction, both mathematically and in a porcine model of septic shock [30]. This means that while some parts of the microcirculation may do relatively well after an insult, there may be other more vulnerable areas that are underperfused. We call these areas microcirculatory weak units [22].

Dysfunction of Individual Microcirculatory Components

To understand the causes of microcirculatory abnormalities in sepsis, the impact of sepsis on the different components of the microcirculation needs to be considered. A common finding has been the decreased reactivity of smooth muscle cells to vasostimulating drugs in experimental sepsis. This applies to both vasoconstrictors [31, 32] and vasodilators [33]. However, observations in humans show that the response to nitroglycerin and acetylcholine is still preserved, at least partially [17, 19]. Vasoconstrictor activity can be improved by inhibiting the formation of NO [34]. This is in agreement with observations of a severely deregulated state of the endothelium in sepsis, in which there is massive overexpression of inducible NO synthase (iNOS). As this expression is not homogeneous within tissues, the resulting heterogeneous vasodilatation may partly explain the variation in microcirculatory perfusion observed clinically [35-37].

Apart from its central role in sepsis, the endothelium also serves a passive role lining the vessel wall. In sepsis, this barrier becomes swollen and leaky allowing fluids to extravasate passively [38]. This leads to edema formation, which is aggravated by a possible impairment in the glycocalyx [39] and a reduction in the anionic charge on endothelial cells [40, 41], allowing charged proteins to pass.

There are numerous interactions of WBCs and the endothelium during sepsis, representing the crossroads between inflammation and coagulation. Essentially a complex defense system against infectious agents, this interaction is responsible for the inflammatory response. Many mediators are released, including tumor necrosis factor α, interleukin (IL) lβ, IL-8, E-selectin, P-selectin, and the intercellular adhesion molecules [6,42]. All are responsible for activating neutrophils, while the latter three, produced both in endothelium and monocytes, are also associated with the initiation of a procoagulant state [43]. While leukocytes themselves become less deformable [44], and have a prolonged capillary transit time [45], potentially blocking microcirculatory flow, the procoagulant state can give rise to a coagulopathy of consumption, disseminated intravascular coagulation (DIC). This coagulopathy

gives rise to microthrombi in the smallest of vessels, again disrupting flow, in addition to the induced risk of bleeding as a result of diminished levels of platelets and clotting factors, both in the micro- and macrocirculation [46].

The RBC is an underappreciated cell. By virtue of its hemoglobin content, it is responsible for the bulk transport of oxygen. RBCs have to pass through capillaries smaller than the cell itself, meaning that they have to deform to be able to pass in single file through the smallest vessels, where there is an effective capillary hemodilution, with hematocrits far lower than that of arterial blood [47]. In addition, a consistent finding both clinically and experimentally is that RBC deformability is decreased in sepsis. This decrease may be caused by direct binding of endotoxin to the RBC, complement coating of RBCs, membrane alterations associated with intracellular ATP changes or the formation of schistocytes in DIC [48-50]. Of specific interest is that the reduction in RBC deformability has been shown to be NO dependent [51], suggesting that the excessive NO production in sepsis may contribute to RBC dysfunction.

Dysoxia and the Oxygen Extraction Paradox

The factors discussed above lead to a disturbed microcirculation which, if not corrected adequately, is associated with a very poor prognosis [18]. From this perspective, the microcirculation may be considered as the motor of sepsis [2].

The model that fits this viewpoint is that a disturbed microcirculation in sepsis will lead to an uneven distribution of tissue oxygenation leading to regional dysoxia in microcirculatory weak units, loss of cell viability, organ failure and death. It may, therefore, be meaningful to see if there is evidence linking microcirculatory abnormalities and dysoxia.

In terms of clinical practice, it is perhaps surprising that regional monitoring is not more routinely applied. Usually, clinicians rely on global parameters such as oxygen delivery (DO_2), oxygen uptake (VO_2), cardiac output, and arterial and central venous blood pressure. In addition, commonly observed parameters such as urinary output, lactate levels and skin color or temperature are only nonspecific markers of regional perfusion. Circumstantial evidence of abnormal regional perfusion and dysoxia comes from the fact that patients can be dying even in the light of normal or even improving global parameters.

It is a common finding in clinical sepsis that there is a deficit in oxygen extraction rate. This is illustrated by a normal or high mixed venous oxygen saturation (SvO_2). However, trials aimed at maximizing tissue DO_2 did not improve outcome [52, 53]. This means that either the oxygen is not reaching the microcirculation or that cells and their mitochondria are simply not using it. Indeed mitochondrial dysfunction has been found to be associated with the severity and outcome of clinical sepsis [54]. This type of mitochondrial malfunction in the presence of normal to high amounts of tissue oxygenation has been termed cytopathic hypoxia [55]. Postulated mechanisms include reverse cytochrome inhibition by NO and peroxynitrite. One important study supporting the existence of cytopathic hypoxia examined pigs in which oxygen availability, as assessed by Clark electrodes, remained high

while metabolic distress persisted as evidenced by a high intragastric PCO_2 [56]. While cytopathic hypoxia may be one of the causes of metabolic dysfunction, evidence is gathering that microcirculatory blood flow is the main determinant of metabolic disturbance. Microcirculatory PO_2, assessed by palladium porphyrin phosphorescence, was less than venous PO_2 in a pig model of sepsis [22]. This was direct evidence of shunting of oxygen transport from the microcirculation. Further evidence for this theory comes from a recent study by Creteur et al. in which they showed, amongst other findings, that increasing microcirculatory blood flow, as assessed by OPS imaging, with dobutamine, led to an increase in tissue CO_2 levels, confirming that capillary blood flow was an important factor in the metabolic challenge in this setting [57].

Microcirculatory and Mitochondrial Distress Syndrome (MMDS)

The pathophysiology of severe sepsis unresponsive to treatment is determined at the level of the microcirculation and probably at the mitochondrial level. The time factor and the nature of treatment being applied are also important elements. We have termed these deleterious changes, the microcirculatory and mitochondrial distress syndrome (MMDS, figure 1), in which time and therapy are considered as important modulating co-factors [2]. It is important to realize that MMDS is caused by the initial septic hit but then acts to maintain the septic process. Keeping in mind the pathophysiological mechanisms described previously, the microcirculation may be considered a motor of sepsis, effectively shutting down oxygen, nutrient, and medication supply to regions of tissue.

In addition, it should be remembered that the intricate process of microcirculatory organ function is very much dependent on the stage of the disease and the therapy given [2]. An intensive care unit (ICU) physician treating many septic patients will only rarely see one in whom at least some form of therapy has not been started, e.g., fluids, vasoactive agents, antibiotics, or steroids. This will also apply to the microcirculation in sepsis, where it would be more correct to take into account time and therapy when defining microcirculatory disorders. Since the microcirculatory organ can now be visualized in humans more readily, it is possible to directly observe the microscopic consequences of sepsis in man.

Monitoring the Microcirculation

The hallmark of global hemodynamics in septic shock is that of a hyperdynamic circulation. This means an increased cardiac output, low arterial blood pressure, and decreased total peripheral resistance. However, this increased flow does not necessarily result in adequate tissue oxygenation in weak microcirculatory beds in vulnerable organs or their compartments. This paradox can be explained by extreme heterogeneity of the microcirculation or massive arteriovenous shunting of blood flow, effectively bypassing at least some microcirculatory areas.

As has been pointed out above, it is very easy to miss regional perfusion and oxygenation deficits if solely relying on monitoring global parameters. Important studies by LeDoux

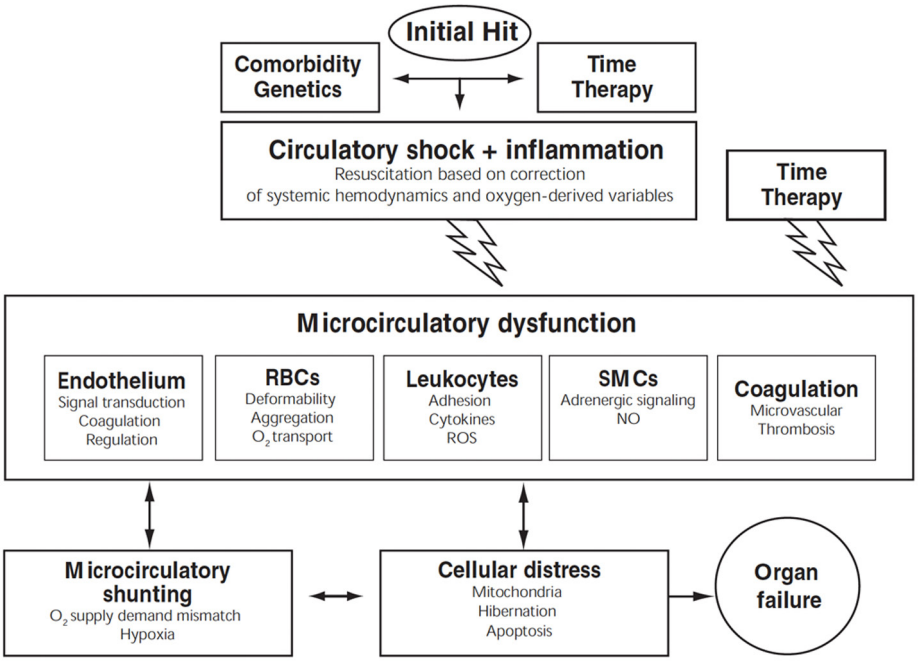

Figure 1. Microcirculatory and rnitochondrial distress syndrome is the condition whereby distributive alterations of microcirculatory control result in shunting and regional mismatch of oxygen supply and demand leading to cellular distress and organ failure. Circulatory failure as a result of sepsis can be initiated by various insults such as trauma, infection, and shock. The treatment of circulatory failure is initially based on correction of systemic variables. In distributive shock, however, systemic variables may be normal and regional hypoxia can persist due to microcirculatory shunting and dysfunction. Here, time and therapy contribute to the definition and nature of microcirculatory and mitochondrial distress syndrome. Left uncorrected, the different cellular and inflammatory components of the distressed microcirculation interact and increase in severity, fueling the respiratory distress of the parenchymal cells and ultimately leading to organ failure (adapted from [2] with permission). RBC, red blood cell; SMC, smooth muscle cell.; ROS, reactive oxygen species; NO, nitric oxide.

et al. [58] and Bourgoin et al. [59] emphasize this idea, showing that resuscitating septic patients to a higher mean arterial pressure (MAP) using norepinephrine actually reduced urinary excretion, increased gastric PCO_2 , and worsened capillary blood flow.

There is already a myriad of techniques to monitor the microcirculation or at least some form of regional tissue perfusion or oxygenation. Although a detailed overview is not

within the scope of this chapter, some methods should be mentioned. The easiest available today is probably SvO_2 [60]. Although classically considered a global parameter, low SvO_2 values are indicative of tissue at risk of anaerobic metabolism. In the absence of a pulmonary artery catheter the clinician may use the central venous or right atrial oxygen saturation, $ScvO_2$ or $SraO_2$.

Interpretation of these latter values should, however, be performed with caution, as they do not correlate with individual SvO_2 values. However, following their trend may be useful in clinical practice [61]. Also of interest is the arteriovenous PCO_2 difference, essentially monitoring whether cells are actually doing their job and receiving the energy to do so, especially when combined with the arteriovenous O_2 content difference [62].

Monitoring regional oxygenation can be done by gastric pH or gastric, sublingual, buccal, esophageal, or tissue PCO_2 measurement, informing us about the splanchnic vascular bed [36, 58, 64, 65]. For measurement of tissue oxygenation the clinician may use methods based on different forms of spectroscopy to measure microcirculatory hemoglobin saturation [36].

For the moment,.the best available monitors of the human microcirculation are SDF and OPS imaging. The SDF imaging technique [21] seems promising as it completely avoids tissue reflectance by illuminating tissue from the side, rendering sharp images of the microcirculation, especially capillaries. An important point to remember, however, is that even though microcirculatory distress, especially measured sublingually, is a serious clinical observation which is associated with a bad prognosis, the microcirculation of other organs may remain unresponsive to therapy and need different recruitment procedures to return to normal function.

It should be noted that images of the septic microcirculation show considerable variation. Again, time and therapy play a very important role here. For example, we observed stagnant capillaries in pressure guided resuscitation in sepsis. In contrast, capillaries with continuous or even hyperdynamic flow may be observed next to capillaries with stopped flow in ongoing fluid resuscitated sepsis. We are currently trying to classify these flow abnormalities in distributive shock based on actual moving pictures. This may be helpful in identifying the causes of these microcirculatory disturbances and perhaps in fine tuning our therapies.

Resuscitating the Microcirculation

Knowledge of the pathophysiology of microcirculatory disturbances in sepsis can be used to resuscitate this organ. Loss of barrier function resulting in edema and the heterogeneity of the microcirculation will cause an effective loss of fluids from the global circulation. In addition, there is a flow redistribution at a regional level, predominantly away from vulnerable organs such as those of the splanchnic region [65]. In order to recruit microcirculatory units that are not adequately perfused, it is important to administer fluids and inotropic agents as a first step in microcirculatory resuscitation. Fluids have been shown to increase tissue oxygenation in an animal model [66]. In addition, dobutamine has been shown to

increase microcirculatory perfusion and oxygenation in humans [57, 67]. However, this may not hold later on in sepsis underscoring the importance of time in MMDS. In addition, fluids are not effective in consolidating pathological shunting and cause redistribution of blood flow due to both hemorheological effects and altered regulatory properties of the vasculature [36].

While normalizing the systemic hemodynamic profile can be considered the first step in rescuing the microcirculation in shock, apparently adequate resuscitation based on systemic variables is not always affective in recruiting the microcirculation. That is why direct monitoring of the microcirculation may be so crucial. Under such conditions other microcirculatory recruitment maneuvers may be considered.

The role of NO in sepsis is complex and incompletely understood [35]. However, it is now generally accepted that nonselective inhibition of NOS is not a good thing as it led to increased mortality in human sepsis as shown by the early termination of a phase III trial [68]. This is perhaps also the basis of ambiguous results of administering steroids, which non-selectively inhibit NOS in sepsis. However, as mentioned before, from a microcirculatory point of view, selective iNOS inhibition could be favorable in redistributing blood flow away from where it is not needed towards dysoxic regions. In fact, in a porcine model of septic shock, selective iNOS inhibition led to improved intestinal tissue oxygenation and normalization of the gastric PCO_2 gap [36]. Still, the need for a more robust understanding of iNOS inhibition, including issues such as the best timing and the degree of blockade, calls for cautiousness in clinical use of this strategy.

As far as the microcirculation is concerned, one should probably be careful with vasopressor therapy in sepsis. Although it is obvious from Ohm's law that at least some perfusion pressure is necessary for blood flow to different organs, resuscitating septic patients to fixed blood pressure endpoints using vasopressor agents may actually jeopardize microcirculatory flow. This was shown by Boerma et al. who administered a relatively high dose of the vasopressin analogue terlipressin to a septic shock patient [69]. While urine output and blood pressure improved, sublingual microcirculation came to a halt and the patient died. When using vasopressors it may be advisable to monitor the microcirculation in some way. This has been done by Dubois et al. who showed that vasopressin at lower doses did not affect the sublingual microcirculation [70].

Vasodilators could resuscitate the microcirculation by improving flow and by raising capillary hematocrit [71]. As previously mentioned, it has been shown that the septic microcirculation is still responsive to acetylcholine [17]. Experimentally, we have shown that the NO donor, SIN-1, improved gastric PCO_2 in a porcine model of fluid resuscitated shock [36]. Commonly used NO donors in intensive care medicine are nitroglycerin and nitroprusside. In septic patients, marked improvement of microcirculatory flow was indeed observed after nitroglycerin infusion [19].

It may be counterintuitive that NO donating vasodilators and iNOS inhibiting agents can both be beneficial for the microcirculation, although theoretically, they can be combined.

This problem can be circumvented, however, by using other vasodilators such as ketanserin, a 5-hydroxytryptamine antagonist. Another potentially useful agent in this respect is prostacyclin, which has been shown to improve oxygen consumption and delivery as well as improve gastric intramucosal pH (pHi) in human studies [72, 73].

The vasodilator pentoxifylline is a phosphodiesterase inhibitor and has multiple modes of actions that could resuscitate the microcirculation. Pentoxifylline has experimentally been shown to improve cardiac output, RBC and WBC deformability and to interfere with leukocyte endothelial interaction, causing less WBC stasis [74-78]. In addition, recent research shows that pentoxifylline may act as an iNOS inhibitor thus possibly correcting microcirculatory perfusion maldistribution in sepsis [79]. Indeed, pentoxifylline improved oxygen extraction in an animal model [80], and in septic neonates it was even shown to induce a survival benefit. However, a large clinical trial, in adults or children, has not been conducted so far [81].

Interest in recombinant activated protein C (APC) started because of its anticoagulant activity, inactivating factors Va and VIIIa and increasing fibrinolysis [42]. As such it could counteract DIC and may help resuscitate the microcirculation. APC is currently the only drug that has shown a survival benefit in human sepsis; trials with other anticoagulant drugs have failed to do so [82]. This finding may be explained by the fact that APC also has anti-inflammatory properties. From a microcirculatory perspective, this is beneficial as APC has been shown to reduce endotoxin-induced leukocyte rolling and adhesion as well as improving small vessel blood flow [83]. In addition, APC is also known to block iNOS, which may be another explanation for the observed microcirculatory improvements [84].

Conclusion

The microcirculation is a vulnerable organ in sepsis. At the same time, the diseased microcirculation fuels sepsis, leading to organ failure. Direct monitoring of the microcirculation itself or at least some indicator of regional perfusion may therefore be useful in assessing the course of disease.

However, it should be noted that the effectiveness of many microcirculatory recruitment maneuvers has not yet been confirmed in appropriate clinical trials. Similarly, although there is strong evidence that an improving microcirculation is associated with a better outcome, this is not necessarily a cause and effect relationship and resuscitation of the microcirculation has not been the subject of clinical investigation at the present time. Nevertheless, it is important to remember that normal or improving global hemodynamics or oxygen-derived parameters do not preclude microcirculatory dysfunction, multiple organ failure, and fatal outcome. The microcirculation may be the much-needed end-point of resuscitation of clinical sepsis and septic shock. In addition to accepted therapies, such as fluid resuscitation and inotropic support, promising microcirculatory resuscitating maneuvers including vasodilatation, iNOS inhibition, and multi-action drugs, such as APC, could complement the armamentarium of tomorrow's ICUs.

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