INTRODUCTION

"Shock" is the result of inadequate cellular energy production and is most commonly caused by insufficient oxygen delivery to the tissues. The criticalist’s goal in the treatment of patients suffering from cardiovascular shock is to maximize global oxygen delivery. The parameters commonly used to monitor these patients include heart rate, pulse quality, blood pressure, mucous membrane color, capillary refill time, extremity temperature, mentation, lactate, and central venous pressure. However, the heterogeneous distribution of blood flow that commonly occurs with sepsis is difficult to assess with these parameters. Subsequently, many patients suffering from a systemic inflammatory response syndrome (SIRS) and sepsis develop multiple organ dysfunction syndrome (MODS), multiple organ failure (MOF), and subsequent death, despite a rapid diagnosis and aggressive treatment. Sepsis-associated MOF in people living in industrialized nations is responsible for more intensive care unit (ICU) deaths in than any other disease process. The microcirculation has been termed the “motor of sepsis” and is probably the first organ to fail in the progression of sepsis to MOF. 1 The array of pathogenic factors that occur in sepsis affects virtually every cellular component of the microcirculation, including endothelial cells, leukocytes, erythrocytes, and tissue cells. In humans, microcirculatory distress that is not corrected for 24 hours has been shown to serve as a single independent factor predicting patient outcome. Novel technology is now enabling the clinician to visualize and assess the microcirculation in order to diagnose microcirculatory dysfunction, formulate more accurate prognoses, and treat patients more effectively.

THE MICROCIRCULATION

The microcirculation consists of vessels less than 100μm in diameter and consists of arterioles, capillaries, and venules. It contains the biggest endothelial surface within the body (>0.5 km2), and 10 billion capillaries (diameter 5-9 μm) are responsible for transporting oxygen and nutrients to and removing waste products from all cells of the body, ensuring adequate immunological function, and delivering therapeutic drugs to target cells in disease states. The microcirculation is comprised of endothelial cells lining the inside of microvessels, smooth muscle cells (mostly in arterioles), red blood cells, leukocytes, and plasma components in the blood. The main determinants of flow within the capillary include the driving pressure, arteriolar tone, hemorheology, and capillary lumen patency.

Almost all vascular beds have longitudinal and radial oxygen gradients, resulting in a significantly lower capillary pO2 and hemoglobin saturation compared to arterial values. This is most likely due to the unloading of oxygen directly from the arteriolar network to the tissues and the intrinsic oxygen consumption of the vascular endothelium to sustain endothelial function and vascular tone. The hematocrit is typically lower within the microvasculature than systemically and is heterogeneously distributed. This may be due to a combination of factors. First, the Fahreus effect causes a dynamic reduction of the intravascular hematocrit due to axial migration of the red blood cells near the center of the vessels, making the erythrocyte and plasma velocities different from each other. Additionally, there is a nonlinear distribution of the red blood cells at the vascular branch points. Subsequently, there is a heterogeneous distribution of oxygen along the vascular branch points, combined with the existence of different shunting pathways that make the microcirculation particularly vulnerable to hypoxic insults. For example, the antiparallel juxtaposition of arterioles and venules in the intestinal villi allows for countercurrent diffusion of oxygen away from the villous tip and make these cells susceptible to hypoxic injury.

REGULATION OF THE MICROCIRCULATION

The primary regulators of the microcirculatory perfusion include the myogenic (sense strain or stress), metabolic (regulate according to O2, CO2, lactate, and [H+]), and neurohormonal systems. Based on the oxygen requirements of the tissue cells, both autocrine and paracrine interactions regulate blood flow in the microcirculation. During pathologic conditions, the loss of these autoregulatory safeguards favors the development of microcirculatory dysfunction. Additional insults to the microcirculation, such as activation of inflammatory and coagulation cascades, endothelial and rheologic disorders, and edema formation, commonly occur in critically ill animals.
The autoregulatory mechanisms do not always respond appropriately in animals with sepsis, leading to microcirculatory dysfunction. The derangements lead to heterogenous microcirculatory perfusion; some capillaries are underperfused while others have abnormally high blood flow. The vulnerable microcirculatory units become hypoxic, leading to an oxygen extraction deficit (commonly associated with sepsis) and resulting in a “PO$_2$ gap.” This gap occurs when the microcirculatory PO$_2$ falls below the venous PO$_2$, creating an oxygen gap that is proportional to the severity of functional shunting. Thus, conventional monitoring of systemic macrohemodynamic and oxygen-derived variables is often unable to sense such microcirculatory distress, masking the ongoing abnormality.

Patients with sepsis commonly have disturbed signal transduction and loss of electrophysiological communication and smooth muscle control in the microcirculatory endothelial cells. A heterogenous expression of inducible nitric oxide synthase (iNOS) in different areas of individual organ capillary beds leads to pathological shunting of blood flow. Subsequently, the microcirculatory units that lack iNOS have less NO-induced vasodilation and tend to become underperfused. Additionally, arteriolar smooth muscle cells may lose their adrenergic sensitivity and tone during septic states. Red cells can become less deformable and more aggregable. Erythrocytes also help regulate microcirculatory blood flow by releasing NO in the presence of hypoxia, leading to vasodilation, but this ability is often deranged with sepsis, further impeding microcirculatory flow and function. Reactive oxygen species generated by stimulated leukocytes can directly disrupt the microcirculation, cellular interactions, and coagulation mechanisms. These changes may lead to altered intercellular barrier junctions and endothelial glycocalyces within the microcirculation, thus predisposing to tissue edema and further oxygen extraction deficits. If the microcirculatory deficits are not corrected in a timely fashion, inadequate oxygenation and nutrient delivery to the tissue cells will lead to organ dysfunction or failure.

Although it is unknown whether the primary etiology of oxygen extraction deficits in septic patients is due to shunted weak, hypoxic microcirculatory units or mitochondrial dysfunction or “cytopathic hypoxia”, it appears that the progression from early to severe sepsis is accompanied or caused by microcirculatory dysfunction that leads to mitochondrial failure over time.

MICROCIRCULATORY AND MITOCHONDRIAL DISTRESS SYNDROME
When regional and microcirculatory distress persists following resuscitation of hemodynamic and oxygen-derived variables, a condition termed “microcirculatory and mitochondrial distress syndrome” or “MMDS” results. This name recognizes the vulnerable physiologic compartment that is masked from the general circulation, but fails to deliver sufficient oxygen and nutrients to dependent organs and thus predisposes to organ dysfunction or failure. The severity of microcirculatory derangement depends on several factors: the nature of the “initial hit” that caused sepsis, the pathogenicity of the organism, coexisting illnesses, individual genetic makeup, previous therapy, and time to treatment.

An understanding of microcirculatory dysfunction may help the clinician to better understand the presence of a persistently increased lactate concentration with deranged acid-base values in the face of macrohemodynamic resuscitation and stability. Microcirculatory distress can occur despite normal, or even supranormal, systemic hemodynamic and oxygen-derived variables. The microcirculatory distress is therefore masked by the systemic circulation via shunting pathways. Monitoring techniques in the critically ill patient should ideally verify that resuscitation strategies are effective for the macro- and microcirculation.

Monitoring the Microcirculation
A number of different techniques have been used in an attempt to assess microcirculatory flow in critically ill patients. There are indirect methods, such as DO$_2$ and VO$_2$, blood lactate levels, intestinal capnography, and mixed venous oxygen saturation. However, these methods evaluate oxygenation “downstream” from the pathological processes in the circulatory network. Direct assessment of microcirculatory perfusion has been investigated in animals using intravital microscopy (IVM), but this technique requires large equipment and potentially toxic fluorescent dyes for contrast enhancement, making it impractical and possibly dangerous for routine clinical use.

Early in the 21st century a method for observing the microcirculation has been developed called orthogonal polarization spectral (OPS) imaging, which uses reflected light in the tissues to create a high-contrast image. The subject medium is illuminated with light that has been linearly polarized in one plane, while imaging the remitted
light through another polarizer (analyser) oriented in a plane exactly orthogonal to that of the illumination. In order to form the image, the light is collected, passed through a spectral filter to isolate the wavelength region, and linearly polarized. This light is then reflected toward the target by a beam splitter and an objective lens focuses the light onto a region of approximately 1 mm in diameter. Light that is emitted from the target is collected by the same lens and an image of the illuminated region is then formed and contrast is obtained from the absorption of linearly polarized light by hemoglobin in the blood (both oxygenated and deoxygenated). Subsequently, red blood cells in the microcirculation appear black on the white background of the surrounding tissue. A 5x or 10x objective lens is used (with on-screen magnification 326x) during the measurements and data are recorded for later analysis. A second generation model of this technology (MicroScan™, Cytometrics, Philadelphia, PA or Capiscope™, KK Technology, Honiton, UK) uses sidestream dark field (SDF) can create reflectance avoidance so the illuminated light and reflected light travels via different pathways and surface reflections do not interfere with the image quality. With this modality, a light guide is surrounded by green (530nm) light emitting diodes (LEDs). The light from the LEDs is absorbed by the hemoglobin of erythrocytes and results in the ability to observe the flowing cells. The evaluation of parameters such as microcirculatory flow index (MFI), perfused vessel density (PVD), and proportion of perfused vessels (PPV) of various sizes can then be performed, as recommended by a recent consensus statement regarding the evaluation of the microcirculation.2 Recently, a third generation of handheld microscope (CytoCam™, Braedius Medical, Huizen, Netherlands) was introduced using IDF technology. The CytoCam™ system offers video resolutions of 4416 X 3312 pixels, while the MicroScan™ system offers resolutions of 720 X 580 pixels. Automated video analysis software is also provided by the manufacturer (CytoCamTools™, Braedius Medical, Huizen, Netherlands). This device has been shown to enhance visualization of up to 20% more vessels, has improved image quality and better illumination and focus than the second generation video microscope. There are ongoing technical challenges, but progress is continuing with the IDF technology and automated software analysis.

**THE HUMAN EVIDENCE**

Research in septic humans utilizing OPS technology has been validated and shown that the distributive defects associated with sepsis are characterized by decreased capillary density, increased perfusion heterogeneity, and an increased proportion intermittently perfused or non-perfused capillaries.3 Stagnant blood flow in the smallest capillaries with concurrent normal (or near normal) blood flow in the larger microcirculatory vessels is often visualized in these patients.

The sublingual microcirculation in 50 people with severe sepsis was investigated and compared to a cohort of healthy volunteers and noninfected ICU patients. They found a significant decrease in vessel density and a decreased proportion of small-perfused vessels (<20 m), from 90 to 48%, in septic patients compared to volunteers. Nonsurvivors had more severe derangements. Another study prospectively examined 49 septic patients and evaluated the sublingual microcirculation daily from the day of shock onset until its resolution. Although the patients were similar at baseline, there was rapid and more marked improvement in small-vessel perfusion in the survivors compared to nonsurvivors, despite no difference in global hemodynamic measurements between the two groups. In addition, the capillary perfusion following resolution of clinical evidence of shock was closely related to the severity of resultant organ failure.

A human study examined the sublingual microcirculation of 25 people with severe sepsis (SBP<90 or lactate >4) and 5 controls. The septic people were then resuscitated with early goal directed therapy (EGDT) and OPS imaging was repeated daily until resolution of sepsis-induced organ failure or death. Flow velocity score (FVS) and PVD were compared to SBP, MAP, CVP, CI, ScvO2, CV-SOFA score, and lactate. Septic patients had a lower FVS and PVD and the initial indices were lower in nonsurvivors vs survivors. MAP was the strongest correlate for both FVS and PVD.

The microcirculation has also been investigated in disease processes other than sepsis. When 40 patients were evaluated within 48 hours of ICU admission for severe heart failure, a lower proportion of perfused small vessels were seen in the cardiac failure group compared to the control group. The survivors had better perfusion compared to nonsurvivors. Similar findings were also reported for human cardiac surgery patients. OPS technology has also been used to investigate cortical microvessel reactivity in response to hypocapnea in neurosurgical patients suffering from subarachnoid hemorrhage. The cerebral microvasculature of patients undergoing surgery soon after the subarachnoid hemorrhage was compared to patients having surgery later after the subarachnoid hemorrhage. The contractile response of cerebral arterioles was found to be increased and was correlated with a decrease in diameter of the vessels. OPS technology has also been used to study burn and organ transplant patients.
ANIMAL USES
Videomicroscopy has been used experimentally to evaluate the microcirculatory system of septic sheep and pigs with similar results as those found in the human studies. Following resuscitation from endotoxin-induced sepsis in sheep, resuscitation with hydroxyethyl starch normalized mean arterial pressures, cardiac output, superior mesenteric artery flow, and sublingual and serosal intestinal microvascular flow indices, but did not restore intestinal mucosal microcirculatory perfusion.

The microvascular perfusion score (MPS) was found to decrease by 53% following the induction of sepsis in pigs. The surviving animals’ scores increased to 81% of baseline, but the nonsurvivors did not regain significant perfusion scores. The vascular density score (VDS) was also found to decrease substantially with sepsis. However, there was no correlation between cardiac output and MPS or VDS, and the microcirculatory perfusion and VDS remained decreased following resuscitation, despite the normalization of hemodynamic variables.

The microcirculation during cardiac arrest and resuscitation has been studied in porcine research models and found useful. Following cardiac arrest, animals that were successfully resuscitated had superior microcirculatory blood flow after 1 and 5 minutes of chest compressions compared to animals who were unable to be successfully resuscitated. Another study also monitored coronary perfusion pressure and found that sublingual microcirculatory abnormalities mirrored changes in coronary perfusion pressures. Additionally, the administration of epinephrine resulted in marked decreases in microcirculatory blood flow and these changes persisted for more than 5 minutes. Research in dogs has assessed the normal microcirculation and changes with hemorrhagic shock, and further research is underway.

THERAPEUTIC INTERVENTIONS
It seems that the ability to monitor the microcirculatory effects of therapeutic interventions in clinical patients might help the veterinarian to understand not only the global effects of these interventions, but also the microvascular changes that are occurring. Rodent and porcine studies have shown that colloid solutions such as dextrans and starch solutions can improve microcirculatory flow. The effects of packed red blood cells have produced inconsistent results. Some research has shown a negative impact on the microcirculation, most likely due to 2-3-DPG depletion, decrease red blood cell deformability, and red blood cell interactions with the endothelium and other blood cells. The effects of storage time and presence of residual leukocytes in the transfused products likely represent important factors to consider when evaluating microvascular changes in response to red blood cell transfusions.

The laser Doppler technique has been used to study perfusion pressure in vasopressor-dependent critically ill humans. Researchers found that the use of norepinephrine to increase the mean arterial pressure from 65 to 85 mm Hg did not improve skin microcirculatory blood flow (there were some serious limitations in this study, however). Additional researchers have examined the use of low dose vasopressin therapy for catecholamine resistant vasodilatory shock and found no deterioration in sublingual or renal blood flow.

The use of vasodilatory agents to manipulate the microcirculation in sepsis has also been studied. Acetycholine and nitroglycerin have been found to reverse sublingual microcirculatory alterations in septic people. The clinical utility and safety of these agents will require further investigation.

LIMITATIONS
Several limitations do exist for the currently used techniques to assess the microcirculatory network. The laser Doppler technique provides only an average estimation of perfusion in ~1mm³ of tissue, without regard for the morphology, type of microvessels being studied, and the direction of flow. It also does not take into account the heterogeneous nature of perfusion changes, a major area of concern within critically ill people or animals. The OPS technique can only investigate the microcirculation in areas of the body that are covered by a thin epithelial layer and therefore internal organ perfusion cannot be assessed outside of the surgery suite. Additionally, movement artifacts can make quantitative analysis difficult. Immediate results are not possible but require offline analysis that can prove cumbersome, although a new software program has greatly decreased the time necessary to do these analyses. However, dogs with pigmented mucous membranes are more difficult to analyze using the computer software. Lastly, velocity measurements are currently limited to low ranges, although the use of a double-flash illumination with a stroboscope may allow measurements in high velocity vessels in the future.
CONCLUSION
As interest in the microcirculation is growing, it appears that microcirculatory disturbances may play an important role in a multitude of pathologic conditions. Our understanding of its role in the development of MODS and MOF in critically ill patients is still in its infancy. Someday, bedside microcirculatory analyses might allow the clinician to evaluate, prognosticate, monitor, and safely treat the microvascular sequelae of conditions known to affect the microcirculation.

References


