

***In vivo* Microperfusion Studies: Validation and Potential Uses in Critical Patients**

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Abstract: Recent studies have suggested an increase in sepsis rates, this increase is due to several factors, such as the increase in the average age of the population, the growing number of invasive procedures and use of immunosuppressive drugs. The increase in sepsis incidence causes localized endothelial damage in various regions, it was this aspect that led us to focus the studies on the endothelium and *in vivo* microperfusion investigations in order to contribute to the scientific knowledge in this field. The present study will meticulously analyze this current and complex issue that affects on a daily basis, everyone involved in critical patient care.

Key words: Endothelium, vascular epithelium, microcircol, endothelium, sepsis, Italy

INTRODUCTION

Tissue perfusion depends on the number of capillaries that receive blood from the progenitor arterioles. Under physiological conditions, blood supply to the organs varies according to functional requirements and cell metabolism. The mechanisms that increase blood flow in an organ include changes in the diameter of the arterioles and the increase of blood flow through the capillaries.

Physiologist A. Krogh (1874-1949) hypothesized that increase in capillary blood flow is due to recruitment of new capillaries that are perfused according to the metabolic requirements of skeletal muscle tissue. At rest, the muscle microvasculature remains, in fact, little perfused as a precapillary sphincter mechanism would be able to reduce the number of perfused capillaries. Krogh's hypothesis has stood the test of time and still is the basis of many interpretations of the increase in perfusion in skeletal muscle and other organs.

The increase in perfusion of an organ is thus linked to an increased number of perfused capillaries compared to the number perfused ones at baseline. Several researchers believe that in different organs, many capillaries are not perfused in basal conditions and that they open only under conditions of increased activity.

Recent studies have shown that in skeletal muscle of many laboratory animals, there are no specific precapillary sphincters but the structure of the arterial terminals, where capillaries originate is such that they act as regulators of capillary perfusion (Debbabi *et al.*, 2010). Arteriolar smooth muscle is endowed with rhythmic contracting and

dilating action, defined as vasomotility. The degree of blood flow to the capillaries depends on the state of activity of the arteriolar smooth muscle that leads to vasoconstriction during the contraction of all arteriolar muscles, leading to a reduction or a cessation of blood flow in all capillaries connected to the progenitor artery.

During muscle relaxation, vasodilation occurs with perfusion of all capillaries that are connected to the progenitor artery and constitute the functional microvascular unit.

Therefore, increase in the perfusion of an organ is due to dilation of the arterial terminals which perfuse all the capillaries that originate from them. The greater the dilation, the greater the perfusion of the organ in question.

The regulation of tissue perfusion occurs in the microcirculation, where the arterioles control blood flow to the capillaries. The arterioles contract and relax, broadly varying their diameter and determining the vascular tone. The variations in diameter are due to the activity of the vascular smooth muscle that responds to various stimuli, such as: blood pressure, nervous (for activation of the autonomic nervous system), hormonal, local metabolic, endothelial, blood flow-dependent.

Pressure stimuli: Distension of a vessel, due to an increase in blood pressure is a key stimulus for contraction of muscle fiber cells of the arterial walls. In this way, the microcirculation manages to maintain a constant blood flow, despite of variations in systemic pressure. This mechanism constitutes the so called autoregulation that is present in all tissues and organs

such as brain, kidney and myocardium. The blood flow remains constant with the blood pressure ranging from 50-150 mm Hg (Ebihara *et al.*, 2009). In 1902, Bayliss introduced the concept of myogenic response based on studies on isolated arteries. Just like skeletal muscle contracts when stretched, vascular smooth muscle responds to stretch by contracting.

On the other hand, a reduction in intravascular pressure is accompanied by arteriolar dilation. The condition of sustained contraction, present in vessels, constitutes the basal vascular tone. The myogenic response is modulated by calcium channels which are stimulated by stretching. The basal tone is not dependent on the endothelium, as it is also present in endothelium-deprived vessels.

Nervous stimuli: Total peripheral resistance is regulated primarily by the activity of the sympathetic nervous system. The nervous control activity is coordinated and integrated by the bulbo-pontine centers among which the vasomotor center. Many organs differ in the extent to which they are dependent on central control, as well as in their autoregulatory abilities: several morphological studies have shown significant differences in the density of sympathetic endings in microvessels of different organs and tissues.

The sympathetic nervous system innervates all the arterioles including the terminal ones that can be defined as the area of the precapillary sphincters. Muscle venules are also innervated. Noradrenaline and adrenaline produce their effects through alpha and beta-adrenergic receptors. The response of smooth muscle cells to stimulation with noradrenaline is complex, as contraction mechanisms of both thick and thin filaments are affected. The contraction of smooth muscle is regulated by two enzyme systems, the Myosin Light Chain Kinase (MLCK) and light chain phosphatase (myosin phosphatase) which act on myosin light chain. Noradrenaline activates the kinase and inhibits the phosphatase through an enzymatic cascade. In human skeletal muscle, sympathetic cholinergic fibres are present that release acetylcholine which in turn induces arteriolar dilatation. Their function is to increase blood flow into muscles during exercise.

Hormonal stimuli: Several circulating hormones, such as catecholamines, renin-angiotensin system, vasopressin and atrial natriuretic peptide may have a significant effect on the microcirculation, causing vasoconstriction/vasodilation. The levels of adrenaline released from the adrenal medulla, increase during stress, exercise, hyperthermia and hypoglycemia. The renin-angiotensin-aldosterone system plays a key role in the modulation of

vascular tone, angiotensin II being a potent vasoconstrictor. Endothelial cells have the capacity to synthesize angiotensin converting enzyme, therefore contributing to the formation of active angiotensin II, involved in the regulation of vascular tone. Additionally, synergy between the sympathetic nervous system and angiotensin II has been observed.

Vasopressin plays a role in the general regulation of the peripheral resistance by determining vasoconstriction of the vascular smooth muscle, activating V1 receptors. Atrial natriuretic peptide stimulates soluble guanylate cyclase in vascular smooth muscle, determining the formation of cyclic guanosine monophosphate resulting in vasodilation. Many hormones and neuropeptides are released together with the classical neurotransmitters. Vasoactive intestinal peptide is released together with acetylcholine in postganglionic parasympathetic neurons. Its function is to determine vasodilation via activation of adenylate cyclase. Substance P, distributed in the nerve endings of arterioles, causes dilation, seemingly mediated by the vascular endothelium. The calcitonin gene-related peptide that is released together with substance P has vasodilating effects through a direct mechanism on vascular smooth muscle, as well as an endothelium dependent mechanism. The neuropeptide co-localized with noradrenaline in sympathetic nerve endings has vasoconstricting effects, enhancing those of noradrenaline.

Local metabolic stimuli: Arterioles may respond to metabolic stimuli generated in tissues. Increasing metabolic stimuli leads to an accumulation of catabolic products that can cause vasodilation.

Reactive and functional hyperemia: One of the local perfusion control mechanisms, reactive hyperemia is the transient increase in organ blood flow that occurs following a brief period of ischemia. Massive dilation of small arterioles takes place after arterial occlusion of at least 20, accompanied by a lesser dilation of larger arterioles, resistance vessels and veins, peaking at 120 after restoration of blood flow.

During the restoration phase, an active myogenic component can be observed, this constricts the arterioles and prevents an excessive increase in blood pressure at the capillaries.

Functional hyperemia leads to an increase in organ blood flow following an increase in its metabolic activity. In some organs, such as heart, skeletal muscle and endocrine glands, there is a direct relationship between metabolic activity and blood perfusion. The increase in activity leads to arteriolar dilation which in turn leads to

an increase in capillary blood flow and hence tissue perfusion. This type of hyperemia is typical in skeletal muscle during exercise.

Oxygen and carbon dioxide: Cells need oxygen and produce vasoactive catabolites. Reducing the partial pressure of oxygen results in vasodilation in most vascular districts with the exception of pulmonary circulation where it results in vasoconstriction. The reduction of prostacyclin synthesis by the endothelium, determining vasodilation plays an important role in this mechanism. Under conditions of low oxygen intake in respect to the functional requirements of tissues, several vasoactive substances are produced; hydrogen ions, potassium and phosphate, CO₂, adenosine triphosphate and adenosine monophosphate nucleotides and lactate. An increase in the partial pressure of oxygen causes vasodilation in many tissues, as well as an increase in hydrogen ion and lactate levels.

Potassium ions and adenosine: Increase in potassium ion levels and interstitial fluid osmolarity can cause vasodilation, as observed in physiological conditions of increased tissue activity.

Adenosine is considered one of the most important mediators of vasodilation. It is formed by AMP hydrolysis induced by a 5'-nucleotidase. The effects are mediated by purinergic P1 and 2.

The activation of P1 receptors is followed by vasodilation whereas P2 receptor activation causes vasoconstriction and the release of endothelium-derived relaxing factor and PGI₂. Adenosine is also considered as the main modulator of blood flow both in coronary circulation as well as in the microcirculation of skeletal muscle during exercise.

Kinins, histamine and serotonin: There are several active substances that regulate vascular tone at a local level, such as kinins, histamine and serotonin.

Kallikreins liberate kinins from endogenous precursors such as kininogens. Tissue kallikrein release kallidine which is converted to bradykinin. Kidney, along with vascular smooth muscle is one of the most important kinin formation sites. Arterial dilation induced by bradykinin is facilitated by the release of Nitric Oxide (NO) and Endothelium-Derived Hyperpolarizing Factor (EDHF) released by endothelial cells.

Histamine causes vasodilation through activation of H₂ receptors, whereas serotonin, binding to several kinds of receptors can induce either vasoconstriction, acting on vascular smooth muscle or vasodilation through endothelial cells.

Endothelium: overview and characteristics

Endothelium-dependent stimuli: Endothelium plays a fundamental role in controlling muscle tone in the arterioles and blood flow in tissues. After the observations of R.F. Furchgott establishing that acetylcholine-induced arteriolar dilation is mediated by an endothelium-derived dilating factor, multiple studies have emphasized the importance of endothelial cells in the regulation of the diameter of small (10-100 microM) arteries where the majority of vascular resistance is located (50-80%).

Vascular endothelium has multiple functions, including prevention of leucocyte and platelet adhesion, production of factors involved in blood coagulation (Von Willebrand factor, plasminogen activators and inhibitors), activation (e.g., of angiotensin) and inactivation of circulating hormones and other plasma constituents (e.g., noradrenaline, serotonin, bradykinin, adenosine 5'-diphosphate). Additionally, vascular endothelium also synthesizes and secretes vasodilating (PGI₂, NO endothelium-derived hyperpolarizing factor) and vasoconstricting (endothelin, thromboxanes, angiotensins, histamine) substances, as well as producing leukocyte adhesion molecules, a heparin like cell growth inhibitor factor, a growth factor that regulates vascular muscle cells, glycosaminoglycans, mucopolysaccharides and fibronectin.

Bacteria and toxins can cause profound alterations in peripheral circulation (Abe *et al.*, 2010; Chuang *et al.*, 2009). The concentration of inflammatory cytokines determines the degree of damage caused to vascular endothelium and target organs. Leukocytes are activated and adhere to the vessel wall and the ability to agglutinate alters blood circulation, potentially leading to significant oxidative injury of the vascular system. Arterial blood pressure can be markedly reduced as a result of an endotoxic state, capable of increasing the permeability of the vascular system resulting in edema. In the most severe cases, consumptive coagulopathy may occur. In most of the cases of mortality due to sepsis, the patients have low levels of anticoagulants, such as protein C and antithrombin and protein replacement therapy may result effective.

A complex interaction evolves between the endothelium, inflammation and coagulation system. Furthermore, the activation of humoral and cellular factors, particularly those involved in endothelium-neutrophil interaction, alters the endothelial barrier and vascular regulation, causing disruptions in oxygen transport and its use by tissues. The coagulation cascade is easily activated in different experimental models in clinical practice, consumptive coagulopathy and

particularly, the phenomena of thrombosis represent a rather common occurrence, capable of worsening the course of sepsis. The presence of intravascular thrombus in peripheral circulation may cause disseminated intravascular coagulation. Proinflammatory cytokines interact with the coagulation system through the activation of tissue factor and are able to profoundly alter the profile of the coagulation cascade.

The depletion of protein C (with concomitant reduction in thrombomodulin levels in the blood) (Baruti *et al.*, 2010) low levels of ATIII and C1 esterase inhibitor, as well as the resulting inhibition of fibrinolysis are all factors that can determine an increased procoagulant action.

There are several interacting factors and thrombin can also lead to upregulation of P and E selectin; this activates the contact factor and stimulates bradykinin production, contributing to hypotension and hypoperfusion. Clinical studies have demonstrated that ATIII and protein C levels are drastically reduced during sepsis, the mortality rate in patients with sepsis is inversely correlated with these two elements.

Hypotension is defined by a systolic blood pressure value <90 mm Hg and mean arterial pressure <60 mm Hg or by a reduction >40 mmHg from normal value in the absence of other causes when the circulatory system is no longer able to ensure adequate tissue perfusion, shock ensues (Hizette *et al.*, 2009).

Septic shock is usually characterized by an initially high cardiac output and low systemic vascular resistance with hypotension, maldistribution of blood flow in the microcirculation and impaired tissue perfusion. Physiopathologically, characteristics of hypovolemic, obstructive, cardiogenic, distributive and cytotoxic shock are present. Septic patients requiring hemodynamic support are by definition unstable (Ilsar *et al.*, 2010). Maldistribution of a normal cardiac output can impair organ perfusion inside an organ, a poor distribution due to compromised vascular resistance exacerbates the dysfunction. Additionally, the impact of sepsis mediators on cellular metabolism leads to inadequate usage of oxygen and other nutrients.

Pulmonary endothelium plays a fundamental role in the physiopathological dynamics of sepsis, since it may originate in the lungs, as well as targeting them. Inflammatory insult to the vascular endothelium results in increased permeability to proteins, as well as damaging the autoregulation system of the arteriolar tone. These events alter the balance between hydrostatic and oncotic pressure, favoring the release of fluids from vessels. This process naturally affects the pulmonary circulation and causes interstitial edema. The key factor that explains

pathogenesis of interstitial edema is the balance between hydrostatic and oncotic gradients between the interstitium and vessels in relation to the degree of capillary permeability. When fluids accumulating in the lung tissue can not be drained (by the lymphatic system) extravascular fluid accumulation occurs. The degree of capillary permeability may differ during sepsis and therefore the gradient of hydrostatic pressure varies in relation to that of the oncotic pressure as the molecules responsible for maintaining the latter cross the endothelial barriers that have become very permeable.

The accumulation of extravascular fluid and plasma protein exudation provoke interstitial edema that leads to ARDS. Pulmonary hypertension in ARDS has a multifactorial origin but it is independent from the underlying cause; patients with a significant increase in pulmonary vascular resistance have a worse prognosis. Perivascular edema which predominates the early stages of ARDS has an important role in the pathogenesis of vascular hypertension. Other important contributing factors include vasoconstriction caused by cellular hypoxia and other vasoactive mediators such as thromboxanes and endothelins, as well as obstructions caused by platelet thrombi. In the evolution of the disease, the progression of hypertension reflects the advancing fibrosis process also responsible for the obliteration of the vascular bed. Although, increase in pulmonary artery pressure is characteristic of ARDS, pulmonary vascular resistance is usually only slightly or moderately elevated, essentially because of a reduced cardiac output. The main cause of sepsis-associated hypoxemia is linked to the intrapulmonary shunt. In ARDS, the shunt is due to the persistent perfusion of atelectatic, fluid filled alveoli, as well as the abolition of the physiological reflex of hypoxic vasoconstriction. After the initial lung damage, a gradient develops along the gravitational axis, dependent portions of the lung (and thus better perfused) become consolidated, causing shunting.

Blood shunting through the nonventilated parts of the lung explains the refractory nature of hypoxemia in ARDS.

Vasodilation: Endothelial cells release cellular NO and PGI₂ in relation to a series of stimuli that are exerted on the cell surface. PGI₂ is derived from arachidonic acid by the action of the cyclooxygenase enzyme. It is released by the endothelium and causes relaxation of the vascular smooth muscle, increasing intracellular cAMP levels. PGI₂ has notable antiadhesive effects on platelets, thus contributing to peripheral tissue perfusion. NO released by endothelial cells induces the activation of a soluble

guanylate cyclase that activates the cGMP formation mechanism in vascular smooth muscle cells. This leads to a reduction in calcium flow and a relaxation of smooth muscle, causing vasodilation. The amino acid L-arginine serves as substrate for NO formation and therefore its administration may increase the formation and release of NO.

Endothelium-derived hyperpolarizing factor is the third factor that contributes to the dilation of arterioles and increase in blood flow. Its biochemical constitution is still unclear in many vascular districts but generally it is released by the endothelium and determines the hyperpolarization of smooth muscle cells resulting in vasodilation.

Vasoconstriction: The factors that cause vasoconstriction include endothelins (ET1-3), thromboxanes which are synthesized from arachidonic acid by cyclooxygenase. Cyclooxygenase-independent constricting factors have also been described. Thus the endothelium can modulate the vasoconstrictor response of vascular smooth muscle acting as a barrier between blood and muscle cells, limiting the amount of active substances on smooth muscle and releasing both NO and releasing factors which cause vasodilation.

Flow-dependent stimuli: Variations in arteriolar blood flow can induce endothelial responses capable of regulating vascular tone. When blood flow is increased in an artery, it dilates, facilitating the reduction of the hydraulic resistance of the system. This vasodilation mechanism activated by an increase in blood flow is due to the release of NO and PG12 induced by tangential forces acting on endothelial cells (shear stress). When shear stress increases, arteriolar dilation takes place, contributing to the quick regulation of resistance during functional hyperemia. There are endothelial receptors sensible to shear stress which respond by releasing NO and expressing nuclear transcription factors, such as nuclear factor kappa B and nuclear protein-1 activation factor, capable of binding to shear stress response elements in genes that respond to mechanical stimulation.

Sepsis and dysfunction of regulatory systems: The aforementioned autoregulatory mechanisms and their microcirculatory functions are often altered during sepsis. The microcirculatory dysfunction is characterized by diverse anomalies in blood flow with the presence of some underperfused capillaries while others have normal or high blood flow rates. Functional microcirculatory units become hypoxic and this explains the impairment in oxygen extraction during sepsis. Under these conditions,

the partial oxygen pressure drops below the venous PO₂. This disparity has been termed the PO₂ gap, reflecting the consequences of functional shunting more severe in sepsis than in hemorrhage (Lee *et al.*, 2010; Legrand *et al.*, 2010).

In sepsis, the microcirculation cannot carry out its regulatory function due to interference from transduction signals and electrophysiological communications.

The nitric oxide system, a central component of the autoregulatory system of the microcirculation is severely impaired in sepsis due to a heterogeneous iNOS expression in different organ parts, resulting in pathologic shunting. Since iNOS is not homogeneously expressed in organ systems, areas lacking iNOS have less NO-induced vasodilation and become hypoperfused. The smooth muscle cells that surround the arterioles and regulate perfusion lose their adrenergic sensitivity and tone during sepsis (Jung *et al.*, 2010; Lee *et al.*, 2010).

Red blood cells become less deformable and more adherent. They also play an important role in regulating microcirculatory blood flow with their ability to release NO in the presence of hypoxia and to cause vasodilation. This regulatory property may be absent during sepsis. The aforementioned defects, together with coagulation impairments, ultimately deteriorate perfusion and microcirculatory function during sepsis. Additionally, the activation of leukocytes by septic inflammation generates reactive oxygen species that damage microcirculatory structures, cell interaction and coagulatory functions. These and other inflammatory mediators alter the microcirculatory barrier, including cell junctions causing tissutal edema and further deficits in oxygen extraction. Microcirculatory dysfunction determines respiratory distress of the parenchymal cells resulting in organ failure.

Sepsis, microcirculation, D.I.C.

Mitochondrial distress: If the primary cause of oxygen extraction deficit in sepsis is due to a weak shunt, microcirculatory hypoxia and mitochondrial incapacity to process oxygen is under intense debate.

The mitochondrion acts as an integration center for apoptotic stimuli. These can be of different nature (caspases, ceramides, kinases) and are able to determine the opening of a proteic complex called the mitochondrial Permeability Transition Pore Complex (PTPC), located at contact sites of the inner and outer mitochondrial membrane. This event causes a voltage drop due to proton release and entry of molecules previously blocked. As a result, the mitochondrion is filled with fluid and the external membrane ruptures, releasing into the cytoplasm apoptosis-stimulating factors, such as AIF (Apoptosis Inducing Factor) that is able to reach the nucleus and

activate a caspase-independent pathway capable of degrading DNA, as well as the cytochrome c and bind to Apaf-1 (apoptotic protease activating factor) proteins, caspase-9 and ATP molecule forming a complex called the apoptosome. Caspase-9 activates other caspases that initiate a mitochondrial cascade that results in DNA degradation by nuclear factors. Mitochondrial permeability alteration processes also involve members of the bcl-2 family, composed of at least 16 proteins that interact with mitochondrial membranes. This proteic family contains both antiapoptotic (such as Bcl-2 and Bcl-XL) and proapoptotic (such as Bax, Bid and Bcl-XS) elements. These elements can merge to form homodimers and heterodimers that have both pro- and antiapoptotic effects. The key event leading to permeability-altering dimer formation is the release of proapoptotic factors in abundance in relation to antiapoptotic factors.

Hypoxic endotoxemia has been observed in some studies on mouse hearts; however in this model no mitochondrial dysfunction was found, as evidenced by the normal response of the energetic state of mitochondria to local hypoxia. During the progression to severe sepsis, mitochondrial dysfunction would probably arise accompanied or even caused by more serious microcirculatory dysfunctions.

Mitochondrial dysfunction effectively plays an important role in sepsis, where the level of severity of respiratory dysfunction of the mitochondrion is correlated with the outcome of the patient. Sepsis-associated mitochondrial failure contributes to respiratory distress, especially in hypoxic areas and can increase tissue distress leading to organ dysfunction (Nef *et al.*, 2010; Nguyen *et al.*, 2010).

Microcirculatory and mitochondrial distress syndrome:

An eventual recovery of a circulatory failure associated with sepsis consists in correcting the hemodynamic system and oxygen-derived variables but when respiratory distress persists, the condition is termed Microcirculatory and Mitochondrial Distress Syndrome (MMDS). This concept was formulated in order to identify the vulnerable physiological compartment masked from the systemic circulation and responsible for oxygen transportation and cellular respiration whose function becomes impaired in sepsis and can lead to organ dysfunction. The elements defined by the type and severity of sepsis include the type of the initial trigger leading to sepsis, comorbidity, individual genetic mapping, previous therapies and treatment time. The duration that the condition has persisted and previous therapies are responsible and modulate the pathophysiology and define the subclasses of the

syndrome. When therapy and duration are included in the definition of MMDS, integrated assessments of these determining mitochondrial microcirculatory and functional factors are necessary for the evaluation of the degree of severity of the condition (Oehmcke and Herwald, 2010; Salgado *et al.*, 2010).

Microcirculation restoration strategies: The presence of respiratory distress, despite resuscitation based on hemodynamics and oxygen derivatives, strongly suggests that the microcirculatory failure is a key factor to interrelate with elevated lactate levels, acid base balance disturbances and high levels of CO₂ sometimes highlighted in these conditions. Microcirculatory failure may occur with the presence of normal or supernormal hemodynamic and oxygen-derived variables with the microcirculatory distress being masked from the systemic circulation by shunting. (Sogawa *et al.*, 2009; Taccone and Backer, 2010). Therefore monitoring techniques for verifying therapy effectiveness and evaluating the microcirculation are essential.

Microcirculation function monitoring for treatment optimization:

Several monitoring methods have been used during circulation failure in surgery and intensive therapy. These include sublingual, buccal and subcutaneous CO₂ monitoring, as well as absorbment, reflectiveness and infrared spectroscopy to measure the hemoglobin saturation in the microcirculation.

Orthogonal Polarizing Spectroscopy (OPS) was introduced for use in surgery and constituted the first means for direct observation of the microcirculation in human internal organs. This technique involves the microscopic visualization of the deep microcirculation and red blood cell flow in the various parts of the microcirculation. In its sublingual application, OPS provides an accurate means for measuring the severity of the distribution impairment in sepsis that cannot be obtained by conventional monitoring of systemic and oxygen-derived hemodynamics. Sublingual capnography combined with OPS imaging has been used to investigate eventual correlations between the microcirculation and metabolic conditions during resuscitation. During cardiac surgery, simultaneous use of spectroscopy and sublingual infrared monitoring in several deep regions of the microcirculation and photo-spectrometry of its superficial regions has been shown to provide integrative information regarding the redistribution of oxygen in the microcirculation. Such approach, monitoring the different functional compartments of the microcirculation is capable of verifying the distribution of oxygen transport during sepsis, septic shock and therapies, lacking in

conventional monitoring of the systemic and oxygen derived hemodynamics. De Backer *et al.* (2010) studied sublingual microcirculation in septic patients with OPS, demonstrating a direct association between the degree of respiratory distress and the severity of the condition together with therapy response. These OPS studies show that the distributive impairment associated with sepsis is characterized by stasis of blood flow in small capillaries and normal blood flow in adjacent larger veins. This observation underlines the necessity to monitor the blood flow in small capillaries.

OPS images are limited, capillaries are sometimes blurred and can not always be identified. Ince and colleagues have developed a new imaging technique for observing the microcirculation called Sidestream Dark Field Imaging (SDF).

SDF consists of a thin guide wire surrounded by 530 nm diodes and LED lights, with a light wave length that is absorbed by the hemoglobin of red blood cells allowing them to be observed as dark cells flowing in the microcirculation. The LEDs in the tip of the guide are optically isolated from the interior of the conducting image and provide a deep light in the tissue illuminating the microcirculation. This illumination applied in the obscured field prevents surface reflections, providing clear images of the microcirculatory structures and blood flow.

Therapeutic options: There are several therapeutic options available to improve microcirculation in septic patients.

Resuscitation volume: If the autoregulatory mechanisms are intact, they will ensure the resuscitation from hypovolemic shock through volume that effectively recuperates the microcirculatory bed. The volume supplied also restores the barrier function of the microcirculation and promotes oxygen transport within the microcirculation. However, it also induces hemodilution that may cause a redistribution of the oxygen distribution (Tsao *et al.*, 2010; Venet *et al.*, 2010). The implications of oxygen redistribution and its role in the pathophysiology of sepsis and resuscitation are still not clear. Blood is considered to be the best oxygen transporter, being more effective than crystalloids and colloids. Yet the age of the red blood cell reserves can modify the properties of blood and this must be taken into consideration. Oxygen transport with hemoglobin, although effective can not be applied in clinical routine.

iNOS inhibitors and steroids: Nitrogen monoxide also called nitric oxide is one of the most powerful biochemical

mediators produced by living organisms; it is worth to note the connection of this substance to the 1998 Nobel prize in Medicine/Physiology awarded to the American researcher Louis Ignarro for his discoveries regarding nitric oxide as a signaling molecule in the cardiovascular system. Six years earlier, the Science magazine elected NO as the molecule of the year. NO is produced from the L-arginine amino acid through a multi-step reaction catalyzed by the nitric oxide synthetase enzyme. The latter exists in several isoforms, some of them constitutive (endothelial cells, platelets, nervous system) and other inducible (macrophages, leukocytes, smooth muscle cells, hepatocytes). NO acts as an important intra and intercellular messenger, regulating numerous functions. Endothelial cells produce NO that spreads in the circulation reducing the adherence of platelets and leukocyte adhesion to the blood vessel walls, as well as reaching the underlying vascular smooth muscle inducing its relaxation. The resulting antiadhesive, anti-inflammatory and antihypertensive effects are highly significant (Viridis *et al.*, 2010; Volakli *et al.*, 2010).

NO has other functions apart from its effect on the endothelium: cerebral (learning and memory control), gastrointestinal (modulation of secretions and motility), respiratory (regulation of bronchial smooth muscle tone). Studies are under way on its possible functions in bacterial infections and tumor growth control.

In sepsis, the autoregulatory mechanisms are often damaged. Simple fluids while effectively capable of correcting systemic hemodynamics can recuperate vulnerable areas of the hypoxic microcirculation. This flow distribution among other mechanisms is related to the heterogeneous expression of iNOS in different regions of the organ vascular beds, resulting in pathologic flow shunts.

It must be noted that deficiencies in iNOS in a mouse model did not cause circulatory dysfunctions associated with endotoxins that typically arise in wild mice, highlighting the importance of controlling iNOS in sepsis. In recent studies on septic pigs, fluid administration combined with iNOS inhibitors recuperated the circulatory bed of the intestine.

iNOS inhibition also protects the barrier function of the microcirculation and can be considered as a microcirculatory recruiting measure. Anti-inflammatory agents, as well as steroids are extremely valid iNOS inhibitors and can prevent endotoxin-induced hypotension. A delayed administration, however does not guarantee iNOS inhibition due to glucocorticoid-induced sepsis. These studies emphasize the importance of early therapy (Ito *et al.*, 2010). Steroids also improve the autoregulatory function as observed in a study on the

autoregulatory properties of an isolated rat heart during sepsis. However, the majority of experimental studies use elevated quantities of steroids which clinical studies advice against in sepsis treatment. Nevertheless, these studies demonstrate that reducing iNOS expression may crucially allow controlling the hemodynamic distribution deficit occurring in sepsis.

Vasodilators and vasopressors: Recruitment of microcirculatory perfusion in normovolemia can be achieved by vasodilator therapy because it increases the driving pressure of blood flow at the entrance of the microcirculation. In a septic pig model, NO administered in combination with fluids improved intestinal microcirculatory oxygenation and corrected gastric partial pressure of CO₂, whereas this does not happen when only fluids are administered.

De Backer *et al.* (2010) reported similar microcirculatory anomalies in septic patients. They also showed that the endothelial vasodilatory response was intact, as well as demonstrating that topical application of acetylcholine was effective in the recruitment of the opening/closing mechanism of the capillaries. Sublingual OPS imaging studies in patients with sepsis found that while the driving pressure effectively restored after therapy, the systemic blood pressure did not have a corrective effect on microcirculatory (Wan *et al.*, 2010; Wurzinger *et al.*, 2010). Vasopressors should be applied with care, with microcirculatory monitoring.

In a study by Dubois, the systemic blood pressure was restored by vasopressin treatment in patients with septic shock. Direct observation of sublingual circulation through OPS imaging showed no adverse effects on the microcirculatory perfusion. However, in other patients with septic shock, although, vasopressin increased blood pressure and the amount of eliminated urine, it caused a complete arrest of sublingual microcirculatory flow, reducing regional circulation and leading to death. Animal experiments have also show conflicting results: some studies showed that vasopressin has beneficial effects on renal microcirculation while others showed that vasopressin causes an interruption of the intestinal microcirculatory activity.

Multiple-action therapy: Fluids, combined with vasoactives and inotropic support, effectively recruit the microcirculation, even if their effects on the microcirculations can not be inferred only with the systemic changes. In patients whose microcirculation was not responsible for resuscitation, however, the prognosis is poor (Zimmermann and Williams, 2010). The recruitment of the microcirculation can be accomplished in different

ways and combinations of therapies. In this way, a NO donor agent can open the microcirculation and perfusional therapy can slowly recuperate the microperfusional units while an anti-inflammatory agent or iNOS specific inhibitors reduce the pathological shunt and slowly reroute the blood flow to the microcirculatory units. This may seem paradoxical from a certain point of view but both therapies effectively recruit the microcirculation and could theoretically be combined. It is clear that for a combined therapy application, the efficacy on microcirculatory recruitment needs to be verified in different organ systems. Taking into consideration that resuscitation in different forms of sepsis is multifactorial, drugs with multiple sites of action may provide an effective treatment strategy to recruit microcirculatory functions during sepsis. For example, it has been shown that activated protein C (APC) inhibits iNOS expression and protects against endotoxin-induced hypotension. In addition, through its action on nuclear factor kB and APC also reduces the level of tumour necrosis factor, an effect that is absent when iNOS inhibitors are administered alone. Moreover, APC reduces leukocyte activation and the release of reactive oxygen species, as well as intervening on the coagulation cascade.

Further studies have shown that these multifactorial actions improve microcirculation in animals with sepsis. Protein C triggers a number of effects that can be seen as a strategic liberation for the microcirculatory dysfunction in sepsis. However, several aspects of the mechanism of action of protein C remain unclear:

How does treatment time and dosage affect the variables needed to benefit microcirculation? How does it react in different organs? How does the presence of other therapeutic agents affect the efficacy of APC in liberating the microcirculation? Direct observations and monitoring the microcirculation should help to determine these queries in sepsis treatment.

CONCLUSION

In this review, the researchers discussed the role of microcirculatory dysfunctions in the development, monitoring and treatment of impaired circulatory distribution associated with sepsis.

Traditional systemic hemodynamics and oxygen-derived variables should uncover microcirculatory dysfunctions and responses to therapy. Microcirculatory dysfunctions can cause cellular distress and lead to organ dysfunction. From this perspective, the microcirculation can be seen as the motor of sepsis. Additional monitoring of microcirculatory functions will contribute to diagnostics and treatment of sepsis.

REFERENCES

- Abe, R., S. Oda, T. Sadahiro, M. Nakamura and Y. Hirayama *et al.*, 2010. Gram-negative bacteremia induces greater magnitude of inflammatory response than Gram-positive bacteremia. *Crit. Care*, 14: 27-36.
- Baruti, Z., H. Pacarizi, B. Zhubi, L. Begolli and V. Topciu, 2010. The importance of determining procalcitonin and C reactive protein in different stages of sepsis. *Bosn. J. Basic Med. Sci.*, 10: 60-64.
- Chuang, T.Y., C.J. Lin, C.L. Chi, A.Y. Liu and S.W. Lee *et al.*, 2009. Rapidly fatal bacteremic pneumonia caused by *Klebsiella pneumoniae* with K1 hypermucoviscosity phenotype in a previously healthy young man receiving levofloxacin treatment. *J. Microbiol. Immunol. Infect.*, 42: 439-441.
- De Backer, D., P. Biston, J. Devriendt, C. Madl and D. Chochrad *et al.*, 2010. Comparison of dopamine and norepinephrine in the treatment of shock. *N. Engl. J. Med.*, 362: 779-789.
- Debbabi, H., P. Bonnin, P.H. Ducluzeau, G. Lefthérotis and B.I. Levy, 2010. Noninvasive assessment of endothelial function in the skin microcirculation. *Am. J. Hypertens.*, 18: 54-61.
- Ebihara, I., K. Hirayama, K. Nagai, T. Kakita and Y. Miyamoto *et al.*, 2009. Angiotensin balance in septic shock patients treated by direct hemoperfusion with polymyxin b-immobilized fiber. *Ther. Apher. Dial.*, 13: 520-527.
- Hizette, P., C. Simoens, J. Massaut, V. Thill and D. Smets, 2009. Septic shock in digestive surgery: A retrospective study of 89 patients. *Hepato-gastroenterology*, 56: 1615-1621.
- Ilsar, R., C. Chawantanpipat, K.H. Chan, T.A. Dobbins, R. Waugh, A. Hennessy and D.S. Celermajer, 2010. Measurement of pulmonary flow reserve and pulmonary index of microcirculatory resistance for detection of pulmonary microvascular obstruction. *PLoS One*, 5: 96-110.
- Ito, S., T. Hattori, O. Shigeta and H. Katagami, 2010. Steroid replacement therapy for shocks after cardiac surgery. *Kyobu Geka*, 63: 204-207.
- Jung, C., H.R. Figulla and M. Ferrari, 2010. High frequency of organ failures during extracorporeal membrane oxygenation: Is the microcirculation the answer? *Ann. Thorac. Surg.*, 89: 345-346.
- Lee, G.M., J.A. Salomon, C. Gay and J.K. Hammitt, 2010. Preferences for health outcomes associated with group A *Streptococcal disease* and vaccination. *Health Qual. Life Outcomes*, 8: 28-28.
- Legrand, M., E. Klijn, D. Payen and C. Ince, 2010. The response of the host microcirculation to bacterial sepsis: Does the pathogen matter? *J. Mol. Med.*, 88: 127-133.
- Nef, H.M., H. Möllmann, Y.J. Akashi and C.W. Hamm, 2010. Mechanisms of stress cardiomyopathy. *Nat. Rev. Cardiol.*, 12: 67-87.
- Nguyen, H.B., M. Loomba, J.J. Yang, G. Jacobsen and K. Shah *et al.*, 2010. Early lactate clearance is associated with biomarkers of inflammation, coagulation, apoptosis, organ dysfunction and mortality in severe sepsis and septic shock. *J. Inflamm.*, 28: 67-72.
- Oehmcke, S. and H. Herwald, 2010. Contact system activation in severe infectious diseases. *J. Mol. Med.*, 88: 121-126.
- Salgado, D.R., J.R. Rocco, E. Silva and J.L. Vincent, 2010. Modulation of the renin-angiotensin-aldosterone system in sepsis: A new therapeutic approach? *Expert Opin. Ther. Targets.*, 14: 11-20.
- Sogawa, K., T. Nagaoka, N. Izumi, S. Nakabayashi and A. Yoshida, 2009. Acute hyperglycemia induces endothelial dysfunction in retinal arterioles in cats. *Invest. Ophthalmol. Vis. Sci.*, 30: 125-132.
- Taccone, F.S. and D. de Backer, 2010. Is cerebral microcirculation really preserved in shock states? *Crit. Care Med.*, 38: 1008-1009.
- Tsao, C.M., S.J. Chen, M.C. Shih, W.M. Lue, M.Y. Tsou, A. Chen and W.J. Liaw, 2010. Effects of terbutaline on circulatory failure and organ dysfunction induced by peritonitis in rats. *Intensive Care Med.*, 11: 45-54.
- Venet, F., F. Davin, C. Guignant, A. Larue and M.A. Cazalis *et al.*, 2010. Early assessment of leukocyte alterations at diagnosis of septic shock. *Shock*, 9: 21-34.
- Virdis, A., L. Ghiadoni and S.P. Taddei, 2010. Human endothelial dysfunction: EDCFs. *Flugers. Arch.*, 34: 56-70.
- Volakli, E., C. Spies, A. Michalopoulos, J. Groeneveld, Y. Sakr and J.L. Vincent, 2010. Infections of respiratory or abdominal origin in ICU patients: What are the differences? *Crit. Care*, 14: 15-32.
- Wan, Z., S. Sun, G. Ristagno, M.H. Weil and W. Tang, 2010. The cerebral microcirculation is protected during experimental hemorrhagic shock. *Crit. Care Med.*, 38: 928-932.
- Wurzinger, B., M.W. Dünser, C. Wohlmuth, M.C. Deutinger and H. Ulmer *et al.*, 2010. The association between body-mass index and patient outcome in septic shock: A retrospective cohort study. *Wien Klin Wochenschr*, 122: 31-36.
- Zimmerman, J.J. and M.D. Williams, 2010. Adjunctive corticosteroid therapy in pediatric severe sepsis: Observations from the resolve study. *Pediatr. Crit. Care Med.*, 10: 71-80.